LIPID LOWERING BY STATIN; EFFECT OF ADMINISTRATION ON BETA THROMBOGLOBULIN; A MARKER OF PLATELET REACTIVITY IN HIGH FAT DIET INDUCED OBESE MALE AND FEMALE SPRAGUE DAWLEY RATS.

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ABSTRACT… Objectives: To observe the effect of lipid lowering by atorvastatin administration on platelet reactivity by measuring serum beta thromboglobulin concentration in male and female Sprague Dawley rats. Study Design: Randomized control trial (RCT). Place of study: The study was conducted at Physiology department, Army Medical College, Rawalpindi. Animal handling, obesity induction, drug administration and sample collection were 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: 12 months. Material and Methods: Ninety healthy Sprague Dawley (male and female) rats were randomly selected and divided into three equal groups. Group I rats (normal control) were given normal chow diet for three weeks. Group II rats (obese control) were given high fat diet for three weeks to induce obesity. Group III rats (obese treated) were given atorvastatin for three weeks in a dose of 10 mg/kg/day orally by gavage method after obesity induction. Terminal blood sampling was done at the end of the study by intra-cardiac puncture. Blood was centrifuged to obtain serum and serum beta thromboglobulin was measured by using Enzyme Linked Immunosorbant assay. Results: There was a significant (p < 0.05) increase in serum B-TG concentration in obese rats as compared to normal control rats. Atorvastatin administration to obese rats significantly (p < 0.05) reduced serum B-TG concentration. Conclusions: Obesity increases and statin administration decreases platelet reactivity in high fat diet induced obese Sprague Dawley rats as reflected by serum B-TG concentration.

Key words: High fat diet, obesity, beta thromboglobulin, statins, platelet reactivity.

INTRODUCTION

Obesity is a condition in which there is either abnormal or excessive accumulation of fat that may result in impairment of health. Over the past few decades, the burden of obesity has increased considerably. According to World Health Organisation, the prevalence of obesity has more than doubled since 1980 in the whole world. Obesity affects multiple systems in the body, alters their functioning and acts as a risk factor for various chronic diseases especially cardiovascular diseases, type II diabetes mellitus and metabolic syndrome.¹ Multiple factors contribute towards the development of obesity, most important being genetic predisposition followed by consumption of high fat diet, inactive lifestyle and socioeconomic factors.² Westernized dietary patterns that are rich in fats and low in fiber implicate a higher risk of obesity.³

Obesity has been implicated to affect platelet function, and is reflected by various parameters. In a study, platelet sensitivity to the antagonists was investigated in relation to obesity and platelets were found resistant to physiological anti-aggregating agents in obese subjects. This resistance was reversed upon weight loss⁴ when platelet activation was studied in obese women using a noninvasive marker i.e. 11-dehydro-thromboxane B2 (thromboxane A2 enzymatic metabolite) in urine, it was found to be raised. Weight loss in these women resulted in decrease in thromboxane synthesis.⁵ Obese individuals have also been found to have higher mean platelet volume (MPV), which was positively correlated with body mass index in obese.⁶
As platelet reactivity is manifested by various parameters, it also affects beta-thromboglobulin. Beta-thromboglobulin (β-TG) is a group of homologous and immunologically cross reactive proteins that are derived from platelets and differ in the length of their terminal amino acids. Two variants of these proteins comprise of the platelet basic protein and the connective tissue-activating peptide III. β-TG is formed by proteolysis of platelet basic protein and the connective tissue-activating peptide-III during activation of platelets.7

Platelets after being activated, immediately release mediators stored in dense and alpha granules that perform a wide array of functions. The best known chemokines of platelets are (β-TG) and platelet factor 4 (PF-4), released from alpha granules. These chemokines, have been considered as the markers of platelet activation.8 Platelet functional dynamics in vivo is reflected by β-TG and its measurement seems to aid in the diagnosis of thrombotic diseases, and determination of the effect of antiplatelet drugs.8 When there is no platelet activation, β-TG levels have been found normal and increased platelet activation results in higher level of plasma β-TG.10

Statins belong to a heterogeneous group of molecules that inhibit the activity of key enzyme in cholesterol synthesis i.e. 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA reductase). The discovery of this drug occurred during search of novel antimicrobial substances as a by-product, but initially the statin obtained was hepatotoxic and hence had a limited use.11 Later lovastatin became a successful statin with effective clinical usage.12 It was confirmed by Scandinavian simvastatin survival study that statins decrease general mortality in atherosclerotic patients as well as that caused by cardiovascular events.13

Apart from lowering lipids, statins also exert various pleiotropic effects (effects other than lipid lowering) which can either be beneficial or harmful. Statins can improve endothelial function, can act as antioxidative, antiproliferative and anti-inflammatory agents.14,15 The aim of the present study was to observe the effect of atorvastatin on serum beta-thromboglobulin levels in high fat diet induced obese male and female rats.

MATERIAL AND METHODS
The study was a randomized control trial. It was conducted at Physiology department, Army Medical College, Rawalpindi. The duration of study was 12 months. 90 Sprague Dawley rats (half male, half female) having an average weight of 220 ± 30 grams were randomly selected from National Institute of Health, Islamabad. The rats who developed disease during the course of the study were excluded from the study. All the rats were acclimatized initially by giving normal chow diet and water ad libitum for five days. 23 ± 5 °C of room temperature and equal hours (twelve) of light and dark photoperiod was maintained. Rats were randomly divided into three equal groups.

Group-I: Rats in this group were given normal chow diet and water ad libitum for duration of three weeks.

Group-II: Rats in this group were given high fat diet and water ad libitum for duration of three weeks. The composition of high fat diet was 59% fats, 20% carbohydrates, and 21% proteins.16 Twenty percent or more weight gain in these rats compared to their initial weight at the start of the study was considered as obesity.17

Group-III: Rats in this group were first made obese similar to group II rats and later administered atorvastatin in a dose of 10 mg/kg/day orally by gavage method for three weeks along with high fat diet.

Blood sampling was done by means of intra cardiac puncture in anesthetized rats at the end of the study. For group I and group II rats, it was done at the end of three weeks and for group III rats it was done at the end of six weeks. Approximately 5 ml of blood was obtained from each rat. The blood was transferred to serum gel and clot activator tubes which were later centrifuged in the Eppendorf centrifuge machine (5810R) for 10 minutes at the speed of 3000 rpm to obtain serum. Serum was pipetted out of the serum gel and clot activator tubes by means of micropipette
and transferred into the Eppendorf storage tubes. These tubes were stored at a temperature of -80 °C in CREAM lab, Army Medical College for estimation of β-TG later.

Data was analyzed by SPSS version 22. Value of quantitative variables was expressed as mean ± standard deviation. To compare among groups, One-way Analysis of Variance (ANOVA) was used and for individual comparisons post hoc tukey’s test was used. Intragroup gender comparison was done by independent sample t-test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

In normal control rats, the mean value of serum β-thromboglobulin was 43.69 ± 18.13 pg/ml in males and 49.21 ± 18.60 pg/ml in females. The value was significantly (p<0.05) greater (114.11 ± 39.44 pg/ml in males and 121.78 ± 37.62 pg/ml in female) in 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 serum β-thromboglobulin levels (69.83 ± 28.09 pg/ml in male and 75.40 ± 21.84 pg/ml in female rats) as shown in Table II and III.

Although atorvastatin administration significantly decreased serum β-thromboglobulin levels, but it could not bring it back near the baseline value. There was a significant difference in serum β-thromboglobulin levels, when any of the two groups were compared using post hoc Tukey’s test. Serum β-thromboglobulin levels were greater in female subgroups in all the three groups, but the difference was insignificant as mentioned in Table-I and Figure-1.

DISCUSSION

In our study, the effect of high fat diet induced obesity followed by atorvastatin administration on a marker of platelet reactivity i.e. beta thromboglobulin were studied. Correlation of obesity with platelet reactivity has been studied widely using different parameters and has revealed varying results. In our study, obesity resulted in increased platelet reactivity using serum beta thromboglobulin as a marker.
A study conducted by Gaglia MA et al., in predominantly male, obese and non-obese patients found no association between obesity measured as body mass index and platelet reactivity measured by various platelet function assays like LTA: light transmission aggregometry, VASP: vasodilator- stimulated phosphoprotein phosphorylation, P2y12 and Verify now aspirin assays. The study was conducted in patients of elective PCI who were given antiplatelet therapy consisting of clopidogrel and aspirin and their platelet reactivity was assessed within 24 hours after PCI. The authors concluded to follow the same regimen of antiplatelet therapy in obese and non-obese subjects based on no difference in platelet reactivity.\(^{19}\)

In a study conducted by Bordeax BC et al., obese subjects were found to have more ex vivo platelet activation to various agonists, which include collagen, Adenosine diphosphate and arachidonic acid. Platelet activation in vivo was also more in obese subjects as quantitatively assessed by 11-dehydro-thromboxane B\(_2\) excreted in urine.\(^{20}\)

Obesity was found to be positively correlated with platelet reactivity in Shabbir F et al., study where the results showed significantly increased serum thromboxane B\(_2\) levels in high fat diet induced obese Sprague Dawley rats.\(^{21}\)

A positive correlation was also found by Jennifer N. Cooper et al., in their study where they enrolled normotensive overweight and obese subjects. They found that obese subjects had increased arterial stiffness that predicts increased platelet activation as measured by raised beta thromboglobulin levels.\(^{22}\)

Statins effects are widely studied nowadays and various new aspects are being revealed with the ongoing research. Statins are known to be vasoprotective and anti-atherothrombotic. In our study, statin administration resulted in significant decrease in platelet reactivity. In a study conducted by Laufs U et al., where additional antithrombotic effect of statins apart from lipid lowering in causing stroke prevention was studied in mice, it was found that statins cause up regulation of platelet endothelial nitric oxide synthase and decrease platelet activation by decreasing beta thromboglobulin and platelet factor 4 levels. These effects were suggestive of thrombosis and stroke prevention by prophylactic treatment with statins.\(^{23}\)

Gertz K et al., studied the antithrombotic effect of statins in causing stroke prevention in another way. They observed the beneficial effects of statin treatment given for 14 days in mouse models having cerebral ischemia and infarct. However, withdrawal of statin treatment showed increased levels of beta thrombogloulin and increased risk of stroke and the effects were significant as early as two days of withdrawal of treatment.\(^{24}\)

Sivri N et al., studied the effect of two classes of statins on platelet activation. They studied the effect of atorvastatin and rosuvastatin on platelet activation using mean platelet volume as the marker and found that both statins decreased platelet reactivity. The decrease in platelet activity was independent of cholesterol lowering effects.\(^{25}\)

Undas A et al., compared the anti-inflammatory and anti-thrombotic effects of simvastatin and fenofibrate. The results showed a decrease in beta thromboglobulin levels by simvastatin only after 3 and 28 days of treatment. This effect was independent of lipid lowering effect.\(^{26}\)

Hence although the effect of obesity on platelet reactivity is not clear but statin treatment has shown promising results in decreasing platelet reactivity as evident by our as well as majority of other studies.

**CONCLUSION**

High fat diet obesity in Sprague Dawley rats results in an increase in serum B-TG concentration which decreased on treatment with atorvastatin. Hence obesity increases and statin administration decreases platelet reactivity in high fat diet induced obese Sprague Dawley rats as reflected by serum B-TG concentration.
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“The best way to destroy an enemy is to make him a friend.”

Abraham Lincoln (1861-1865)