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## OBESITY;

### EFFECT OF HIGH FAT DIET FOLLOWED BY ATORVASTATIN ADMINISTRATION ON SERUM INTERLEUKIN-6, WHITE BLOOD CELL AND PLATELET COUNT IN MALE AND FEMALE SPRAGUEDAWLEY RATS.

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**ABSTRACT... Objective:** To study the effects of atorvastatin administration on serum IL-6, WBC and platelet count in obese male and female animal models. **Study Design:** Randomized control trial (RCT). **Place and duration of study:** The study was conducted at Department of Physiology, Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad and Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi for funding, blood sampling and biochemical assays respectively. **Material and Methods:** Ninety healthy male and female Sprague Dawley rats were selected and randomly divided into three equal groups. Group I rats were fed normal diet for a period of three weeks. Group II rats were fed high fat diet for a period of three weeks to induce obesity. Group III rats were administered atorvastatin 10 mg/kg/day orally by gavage method for three weeks after obesity induction. Terminal sampling by intra-cardiac puncture was done at the end of study. Whole blood was used to perform blood complete picture by KX 21 Sysmex Hematology Analyzer which includes platelet count and WBC count and serum was used to measure IL-6 levels by Enzyme Linked Immunosorbant Assay (ELISA). **Results:** There was a significant decrease ( $p < 0.05$ ) in serum IL-6 levels and WBC count, whereas platelet count was not significantly ( $p > 0.05$ ) affected by atorvastatin administration. **Conclusions:** Although atorvastatin reduces obesity related inflammation by decreasing serum IL-6 levels and WBC count, it has no effect on platelet count in male and female obese animal models.

**Key words:** Obesity, Interleukin-6, WBC count, platelet count, atorvastatin

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## INTRODUCTION

Obesity is abnormal or excessive fat accumulation that may impair health. It is an emerging epidemic and has raised concerns worldwide as a major global health problem. According to World Health Organization (WHO), in 2008 approximately 1.5 billion adults were overweight out of which approximately 500 million were obese.<sup>1</sup>

Lower BMI cut off values are suggested for Asian population owing to causal association of obesity and comorbid conditions at lower BMI. Using the revised criteria of BMI for Asian population, an individual having BMI 23 kg/m<sup>2</sup> or more is overweight and an individual having BMI 27 kg/m<sup>2</sup> or more is obese, and the prevalence of obesity in Pakistan comes out to be 10 percent. However, there is no agreement on the lower cut off values of BMI for Asian population.<sup>2</sup>

Obesity is known to cause an increase in WBC count and serum IL-6 concentration. The suggested mechanism for an increase in WBC count is exposure of bone marrow to higher concentrations of leptin, which increases the proliferation of myeloid stem cells, resulting in higher WBC counts.<sup>3</sup>

Adipose tissue is a major source of IL-6. Approximately 30 percent of IL-6 in circulation comes from adipose tissue and its levels are positively correlated with obesity.<sup>4</sup> It is a multifunctional cytokine and plays an important role in regulation of platelet production. Among the factors required for megakaryocytopoiesis, IL-6 seems to be the most significant maturational promoter. Due to its potent effect on thrombopoiesis, IL-6 has also been suggested to act as thrombopoietin.<sup>5</sup>

Several clinical trials have been carried out to explore the thrombopoietic potential of IL-6. In a study, where primates were administered different doses of recombinant human IL-6, platelet counts increased two to three times normal. The fact that increase in platelet count, was caused by IL-6, was confirmed when IL-6 administration was stopped and platelet count returned to the normal limits.<sup>6</sup>

Effect on platelet count in obesity has revealed variable results. An adequate number of platelets is required to progress the hemostatic process. The individuals, who have pathologically higher platelet count (thrombocytosis), are at a greater risk of thrombotic complications.<sup>7</sup>

However, increased risk for cardiovascular events and mortality from coronary heart disease in individuals having platelet counts towards the higher range of the normal is thought provoking. A positive correlation was found between high normal platelet count and mortality due to coronary heart disease in apparently healthy men of middle age.<sup>8</sup>

Statins are 3 hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. They competitively inhibit the key rate limiting step in cholesterol biosynthesis, that is, conversion of HMG CoA to mevalonate. This results in up regulation of LDL receptors, and contributes to the decreased circulating levels of LDL cholesterol.<sup>9</sup>

These drugs provide additional benefits by mechanisms which do not involve cholesterol lowering (pleiotropic effects) and include stabilization of atherosclerotic plaque, reduction of oxidative stress, decreased inflammation, improved endothelial function and inhibition of thrombogenic potential.<sup>10</sup>

This study was therefore designed to observe the effect of high fat diet induced obesity on IL-6 release, WBC and platelet count and effect of atorvastatin administration on these parameters.

## MATERIALS AND METHODS

The study was a randomized control trial. 90

Sprague-Dawley rats were purchased from National Institute of Health, Islamabad. Healthy Sprague Dawley rats having a weight of  $220 \pm 30$  grams were selected for the study whereas diseased rats at the time of study or rats developing disease during the course of study were excluded. Animals were given regular diet for five days prior to the start of experiments. Daily photoperiod of 12 hours light and 12 hours dark was ensured along with maintenance of room temperature at  $23 \pm 5$  °C. Body weight of all the rats was recorded twice weekly for the whole study duration. Rats were randomly divided into three groups. Group I rats were given normal diet and water ad libitum for a period of three weeks, group II rats were given high fat diet and water ad libitum for a period of three weeks whereas group III rats were given atorvastatin in a dose of 10 mg/kg/day by oral gavage method after obesity induction for three weeks along with continuation of high fat diet. Terminal sampling by intra-cardiac puncture was done at the end of three weeks in group I and II rats and at the end of six weeks in group III rats.

Approximately 5 ml of blood was obtained from each rat. 1-1.5 ml blood was transferred into the potassium EDTA tubes and stored at room temperature for assessing blood complete picture by Sysmex KX-21N Hematology Analyzer. 3.5 ml of blood was transferred to the serum gel and clot activator tubes for measuring IL-6. Tubes were placed in the eppendorf centrifuge machine (5810R) and centrifuged for 10 minutes at the speed of 3000 rpm, serum was pipetted out and transferred into the eppendorf storage tubes. These tubes were stored at a temperature of -80°C in CREAM lab, Army Medical College, Rawalpindi for estimation of IL-6 by Enzyme Linked Immunosorbant Assay (ELISA).

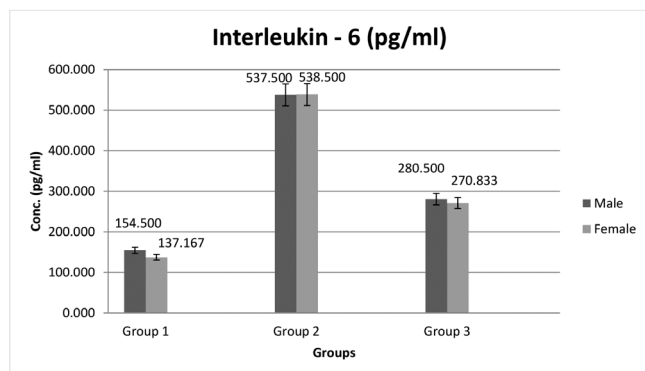
Data was analyzed using SPSS version 19. Quantitative variables were expressed as mean  $\pm$  standard deviation. One way Analysis of Variance (ANOVA) was used for comparison of quantitative parameters among groups followed by post hoc tukey's test for individual comparisons. Independent sample t-test was

used for comparison of various parameters among both the sexes. A p-value of less than 0.05 was considered statistically significant.

**RESULTS**

**IL-6**

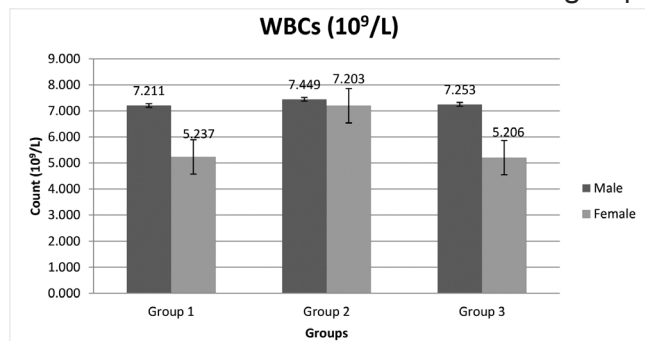
IL-6 levels significantly increased in the obese group (p=0.000). Atorvastatin administration resulted in a significant (p=0.000) decrease in IL-6 levels. IL-6 levels were comparable among the male and females in each group.



**Figure-1. Comparison of interleukin-6 among the three groups and within each male and female subgroup.**

**WBC count**

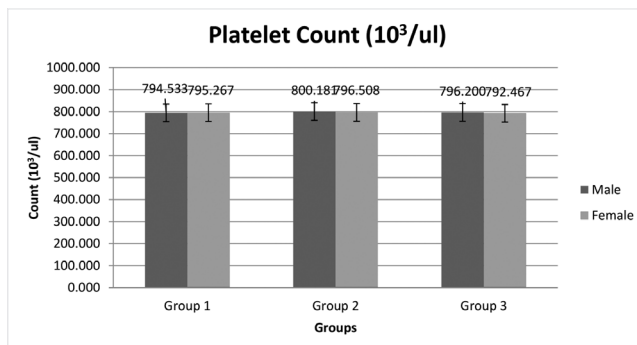
High fat diet resulted in a significant increase (p=0.000) in WBC count in obese group. The increase was observed in both sexes but more in female subgroup. There was a significant difference in WBC count in male and female rats of normal group. A greater increase in WBC count in females resulted in comparable values of WBC count in male and female subgroups of obese rats. Atorvastatin administration resulted in a significant (p=0.00) decrease in WBC count. This decrease was also more in female subgroup.



**Figure-2. Comparison of WBC count among the three groups and within each male and female subgroup**

**Platelet count**

Platelet count was not affected significantly either by obesity or after atorvastatin administration. The difference in platelet count was insignificant between normal and obese rats (p=0.36) as well as between obese and atorvastatin treated rats (p=0.26). Platelet count was also comparable between each of the male and female sub group.



**Figure-3. Comparison of platelet count among the three groups and within each male and female subgroup.**

**DISCUSSIONS**

In the present study, IL-6 was investigated as an adipocytokine released from adipose tissue and its secretion was found to be increased in obesity with increasing fat mass. This corresponds with the findings in animal obese models as well as humans.

IL-6 levels were elevated in high fat diet induced obese Wistar rats in a study conducted by Cano et al. Obesity has been considered as the state of low grade inflammation of adipose tissue. IL-6 and TNF-α secreted in increased quantities from the adipose tissue in obese subjects are believed to produce local as well as systemic effects.<sup>11</sup> IL-6 levels were also found elevated by Hernandez et al in obese lactating female Wistar rats. The inflammation in this animal model as indicated by increased IL-6 gene expression was suggested to be caused by obesity as well as mammary gland involution.<sup>12</sup>

Our study demonstrated a significant decrease in IL-6 levels after atorvastatin administration, which corresponds with the results in animal obese and hyperlipidemic models.<sup>13,14</sup>

Khan et al revealed anti-inflammatory effects of statins in genetically obese mice. Simvastatin administration in a dose of 40 mg/kg/day for 2 to 4 weeks resulted in significant reduction in IL-6 levels. This reduction was the part of anti-inflammatory effects produced by statins due to decrease induction of IL-6 by liver.<sup>13</sup> Zhao and Zhang also observed decrease IL-6 levels in high fat diet induced hypercholesterolemic rabbits after atorvastatin administration (1.5 mg/kg/day for 2 weeks). The proposed mechanism for reduction in IL-6 levels in hypercholesterolemic rabbits was due to the up regulation of messenger ribonucleic acid expression of peroxisome proliferator activated receptor (PPAR) gamma which is a key regulator of energy homeostasis. PPAR regulates inflammation and genes involved in fatty acid uptake by the cells, and PPAR gamma inhibits many factors associated with endothelial degeneration such as TNF- $\alpha$  and IL-6.<sup>14</sup>

Studies conducted in humans also manifested similar results. Dimitrow and Jawein et al studied the anti-inflammatory effect of atorvastatin in patients with aortic sclerosis (transaortic flow velocity less than 2.5 m/s on transthoracic echocardiography) or mild aortic stenosis (transaortic flow velocity between 2.5 and 3.0 m/s on transthoracic echocardiography) independent of hypercholesterolemia. 17 hypercholesterolemic and 16 non hypercholesterolemic subjects were selected for the study and given atorvastatin 20 mg/day for the period of 4 weeks. The anti-inflammatory effects indicated by decreased levels of IL-6, CRP and monocyte chemo attractant protein 1 were independent of lipid levels in the body as they were observed in both hypercholesterolemic and non hypercholesterolemic subjects. Hence the anti-inflammatory effect of atorvastatin observed was also pleiotropic in nature.<sup>15</sup>

Platelet count in our study did not change significantly in the obese group as compared to healthy controls. There was no effect of obesity on platelet count in obese animal models.<sup>16,17</sup> The results in human studies however are different and show increased platelet count in obese individuals.<sup>18</sup>

Mohamed et al conducted a study on platelet reactivity in cafeteria diet induced obese rats. Cafeteria diet induced obese rats although showed increased platelet reactivity, increased tendency for aggregation and resistance to anti aggregating agents but platelet count did not vary among the obese and control groups. Administration of cafeteria diet to rats in 3rd week of their life was unable to produce obesity associated dyslipidemia as the rats were perhaps adapted to the energy changes in their diet as compared to the old rats that manifested significant dyslipidemia.<sup>16</sup>

Youssef et al has documented insignificant difference of platelet count and platelet aggregation in obese and healthy control Wistar rats when rats were made obese by feeding high fat diet. They suggested increased insulin secretion associated with obesity responsible for platelet hyper reactivity that could not be achieved in their rat model of obesity.<sup>17</sup>

Charles et al studied the correlation between indices of obesity and hematological parameters among male and female subjects belonging to the same occupation. The data revealed a positive correlation between platelet count and BMI in female subjects. In male subjects, however no anthropometric variable was related to the platelet count. Increase platelet count with obesity in females alone was suggested to be due to an increase in leptin concentration in females.<sup>18</sup> Platelet count in our study remained unaffected after atorvastatin administration. The study of the effect of statins on platelet count has revealed varying results. Kadikoylu et al compared the effect of two statins i.e. atorvastatin and simvastatin on hemostatic parameters. They found that both the drugs significantly reduced serum LDL and total cholesterol levels, while platelet count was decreased insignificantly in hypercholesterolemic patients receiving either of the two statins.<sup>19</sup> Mayer et al observed the reduction in platelet aggregation in hypercholesterolemic patients after lovastatin administration; platelet count however did not change significantly.<sup>20</sup>

In our study there was an increase in WBC count in obese rats. This increase was greater in females. This result is comparable to the results of other human studies where obesity was found to be positively correlated with WBC count. Herishanu Y et al conducted a cross sectional study to find out cause of leukocytosis in obese individuals. A total of 327 patients having persistent leukocytosis were investigated during 1999-2005. Among them 15.3% were obese and without symptoms, most being females having persistent neutrophilia and raised acute-phase reactants.<sup>21</sup>

Charles et al studied the correlation of different anthropometric indices with hematological parameters in police officers and found WBC

count to be positively correlated with abdominal height although the correlation was statistically insignificant.<sup>18</sup>

In our study there was a decrease in WBC count in obese Sprague Dawley rats after atorvastatin administration and the decrease was more in female rats. The decrease in WBC count after statin administration was also observed in the U.S national Health and Nutrition Examination Survey 1999-2004 conducted by Yoon et al. The purpose of the survey was to assess the nutritional status of U.S population by various means including questionnaires, medical examinations and laboratory tests.<sup>22</sup>

Groups	Sex	Platelet Count (10 <sup>9</sup> /μl)	WBC Count (10 <sup>9</sup> /μl)	IL-6 (pg/ml)
Group 1 (Control)	Male	794.53 ± 10.08	7.21 ± 0.19	154.50 ± 61.56
	Female	795.26 ± 9.02	5.23 ± 0.21	137.16 ± 122.28
	p-value	0.38	0.000 *	0.33
Group 2 (HFD)	Male	800.18 ± 9.17	7.44 ± 0.28	537.50 ± 78.37
	Female	796.50 ± 10.78	7.20 ± 0.26	538.50 ± 97.39
	p-value	0.20	0.01 *	0.48
Group 3 (HFD+ATV)	Male	796.20 ± 9.12	7.25 ± 0.20	280.50 ± 38.17
	Female	792.46 ± 10.73	5.20 ± 0.26	270.83 ± 60.78
	p-value	0.19	0.000 *	0.33

**Table-I. Intra group comparison of platelet count, WBC count and IL-6 between male and female Sprague Dawley rats at the end of the study.**

\* Significant ( $p < 0.05$ )

Group Comparisons		Platelet Count (10 <sup>9</sup> /μl)	WBC Count (10 <sup>9</sup> /μl)	IL-6 (pg/ml)
Group 1 (Control)	Group 2 (HFD)	0.36	0.000 *	0.000 *
	Group 3 (HFD+ATV)	0.97	0.98	0.000 *
Group 2 (HFD)	Group 3 (HFD+ATV)	0.26	0.000 *	0.000 *

**Table 2. Comparison of platelet count, WBC count and IL-6 between the three groups at the end of the study by one way ANOVA.**

\* Significant ( $p < 0.05$ )

## CONCLUSIONS

The elevated serum IL-6 levels and WBC count were decreased by atorvastatin administration, whereas platelet count did not change significantly in the treatment group. Hence atorvastatin has anti-inflammatory effects as manifested by a decrease in serum IL-6 and WBC count.

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“One thorn of experience is worth  
a whole wilderness of warning.”

James Russell Lowell



## AUTHORSHIP AND CONTRIBUTION DECLARATION

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3	Dr. Tausif Ahmed Rajput	Paper writing, lab assays, statistical analysis	