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Effects of Cholesterol Feeding Periods on Blood Haematology and Biochemistry of Rabbits

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Abstract: The aim of the present study was investigation of the haematological and biochemical abnormalities in rabbits fed high cholesterol and saturated fat diet for feeding periods of 5, 10 and 15 weeks, i.e., to elucidate changes occurred in blood parameters due to feeding of the diet. For this purpose, forty New Zealand white male rabbits (12 weeks old) were purchased, individually caged and divided into control group (10 rabbits) and cholesterol-fed group (30 rabbits). Blood parameters were Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDLC), High-Density Lipoprotein Cholesterol (HDLC), triglyceride (TG), fibrinogen, haemoglobin, hematocrit, White Blood Cells (WBC), Red Blood Cells (RBC), platelets, lymphocytes, neutrophils, monocytes and eosinophils levels. Control rabbits were subjected to blood analysis at feeding period of 15 weeks. The cholesterol-fed rabbits were subjected to blood analysis at feeding periods of 5, 10 and 15 weeks. The results of the present study indicated that TC, LDLC, HDLC, TG, platelets, fibrinogen levels, WBC and lymphocytes percentage were significantly ($p < 0.05$) increased in cholesterol-fed rabbits as compared with control rabbits. On the contrary, haemoglobin, hematocrit, RBC and neutrophils percentage were significantly ($p < 0.05$) decreased in cholesterol-fed rabbits as compared with control rabbits. However, monocytes and eosinophils percentage were not significantly different in the two groups. The present study demonstrates that blood parameters of rabbits fed high cholesterol and saturated fat diet indicated different changes during the cholesterol feeding periods. Moreover, it became evident that blood parameters may can help in diagnosis and monitoring the progression of atherosclerosis in rabbits fed high cholesterol.

Key words: Blood parameters, cholesterol feeding periods, rabbits, atherosclerosis

INTRODUCTION

Atherosclerosis is the disease of large and medium-sized arteries, i.e., carotid artery, coronary artery and arteries of lower extremities. It is characterized by focal lesions of one of the following types; fatty streak, fibrous plaque and complicated lesion. A vast number of hypotheses to elucidate pathogenesis of atherosclerosis have been published. Some of them were lipid hypothesis, thrombogenic hypothesis and endothelial cell injury hypothesis. Controversy still remains as to the relevance of blood haematology and biochemistry during the development of atherosclerosis; some investigators have suggested that some blood parameters of rabbits fed high cholesterol diet increased in oppose to control rabbits, while others suggested the opposite. A great numbers of studies have revealed that chronically elevated lipids levels and cholesterol levels are associated with an increased incidence of atherosclerosis (Mbenza *et al.*, 2007; Abdelhalim *et al.*, 1994). Evidence from

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experimental, clinical and epidemiological studies have been suggested that several hemostatic and hemorheological factors such as fibrinogen levels, plasma viscosity, hematocrit, RBC aggregation and WBC counts might not only play an important role in the evolution of acute thrombotic events, but may also take part in the pathophysiology of atherosclerosis (Tamura *et al.*, 2007; Loimaala *et al.*, 2006; Wolfgang and Edzard, 1992). High TC and LDLC have been correlated with the increased risk of atherosclerosis (Martin *et al.*, 1986). Triglyceride-rich lipoproteins of both intestinal and liver origin were considered atherogenic factor (Zilvermit, 1995; Philips *et al.*, 1993). Activation of leukocytes is obligatory for inflammation and atherogenesis by adhering to the endothelium via specific ligands. There is a TG-specific increase of neutrophil counts and increased activation of monocytes and neutrophils, i.e., a pro-inflammatory situation that may correspond with increased adhesive capacity of these cells contributing to the inflammatory component of atherosclerosis (Van Oostrom *et al.*, 2004). It has been hypothesized that higher neutrophil percentage are associated with an increased incidence of major adverse cardiovascular events in patients with clinically advanced atherosclerosis. In patients with peripheral artery disease, only neutrophils counts, but not eosinophils, basophils, monocytes, lymphocytes, or total WBC count indicated a substantially increased risk for major adverse cardiovascular events (Markus *et al.*, 2005). Although the underlying mechanisms of atherosclerosis and intimal hyperplasia remain unclear, the basal adherence of monocytes only was significantly elevated in atherosclerosis, resulting in increased adherence to endothelial cells (Dovgan *et al.*, 1994). Fibrinogen concentration has been identified as independent atherosclerotic risk factors during the development of atherosclerotic plaques and thrombi (Ernst and Resch, 1993; Danesh *et al.*, 1998). Elevated plasma fibrinogen level has known to progress atherosclerosis and to be one of the risk factors for the occurrence of cardiovascular diseases (Yoshiyasu *et al.*, 1996). Dietary cholesterol increased blood total leucocytes count, serum and liver total cholesterol concentrations, low-density lipoprotein concentration and induction of atherosclerotic plaques in the aorta and coronary arteries (Meijer *et al.*, 1996). Thus, to elucidate changes occurred in blood parameters due to feeding of the diet, rabbits were fed high cholesterol and saturated fat diet for feeding periods of 5, 10 and 15 weeks.

MATERIALS AND METHODS

Animals

Forty New Zealand white male rabbits (12-weeks old), were purchased from Kitayama Lab. Ltd., Kyoto, Japan, individually caged and divided into either control group or cholesterol-fed group. The control group (n = 10) was fed on 100 g day⁻¹ of normal diet, ORC-4 (Oriental Yeast Co. Ltd., Tokyo, Japan) for 15 weeks. The cholesterol-fed groups (the experimental group was divided into 3 groups; n = 30) were fed on high cholesterol and saturated fat diet of ORC-4 containing 1% cholesterol plus 1% olive oil (100 g day⁻¹) for feeding periods of 5, 10 and 15 weeks.

Collection of Blood and Preparation of Serum

Blood samples of 2 mL were obtained from the rabbits via venepuncture of an antecubital vein. Blood was collected into two polypropylene tubes viz., one for serum and one for plasma. The blood for plasma was collected in heparin. Serum was prepared by allowing the blood to clot at 37°C and centrifugation at 3000 rpm for 10 min. Fibrinogen levels were measured by the thrombin clotting method using a Coagulex 700 analyzer (International Reagents, Kobe, Japan). TC, HDLC, LDLC, TG, haemoglobin, RBC, WBC, platelets, lymphocytes, neutrophils, monocytes and eosinophils levels were measured in the clinical laboratory centre of King Khaled University Hospital (KKUH, 2007) with ADVIA 120 Haematology System (Bayer Medical, Tarrytown, New York, USA).

Statistical Analysis

Statistical analysis was performed in among all groups. The results were expressed as mean±SE. To assess the significant differences between control group and cholesterol-fed group of rabbits, statistical analysis was performed using One-Way Analysis of Variance (ANOVA) for repeated measurements with significance assessed at 5% confidence level.

RESULTS AND DISCUSSION

Table 1 shows that TC, LDLC, HDLC, TG, platelets, fibrinogen levels, WBC and lymphocytes percentage were significantly increased in the cholesterol-fed rabbits as compared with the control rabbits. On the contrary, haemoglobin, hematocrit, RBC and neutrophils percentage were significantly decreased in the cholesterol-fed rabbits as compared with the control rabbits. However, monocytes and Eosinophils percentage were not significantly different in control and cholesterol fed groups.

The present study demonstrates that feeding rabbits high cholesterol and saturated fat diet for feeding periods of 5, 10 and 15 weeks induced abnormalities in the blood parameters of rabbits. A high-cholesterol diet elevated the level of plasma TC, LDLC, HDLC, TG and platelets to be incorporated into the atherosclerotic plaques. The elevation of TC, LDLC, HDLC and TG concentrations may be associated with the pathophysiology of the atherosclerotic process in hypercholesterolemic rabbits and may be responsible for the risk of cardiovascular diseases. Thus, the effects of hypercholesterolemia are not only confined to induce abnormalities in the blood parameters of rabbits, but it increases the deposition of lipids in the atherosclerotic lesions, produces primary endothelial injury and elevates blood viscosity (Gregory, 1999; Lee *et al.*, 1998). Moreover, softening the aortic wall structure (e.g., tunica media of aortae underlying plaques showed marked disruption with loss of collagen and elastin fibres) (Toz *et al.*, 2004; Abdelhalim *et al.*, 1994). When LDLC is oxidized by macrophages in lesions, it becomes toxic to the endothelium and thereby could injure endothelial cells. It has been reported that serum LDLC and HDLC levels have effects on blood viscosity and correlate with the increased risk of atherogenesis (Sloop and Garber, 1997). Low HDLC levels are associated with an elevated blood viscosity and this rheological abnormality contributes to cardiovascular risks (Thomas and Robert, 1999). It has been reported that serum hypercholesterolemia accelerates atherogenesis and contributes to symptomatic atherosclerosis by increasing blood viscosity

Table 1: Effect of different treatment groups on the haematological and biochemical parameters of rabbits

| Parameters | Groups | | | |
|---|--------------------------|-------------------------|---------------|---------------|
| | Control 15 weeks (10) | Cholesterol-fed rabbits | | |
| | | 5 weeks (10) | 10 weeks (10) | 15 weeks (10) |
| Total Cholesterol (TC; mg dL ⁻¹) | 54.625±16.4 | 841.30±45.4 | 837.400±21.5 | 708.70±14.8 |
| Low density lipoprotein (LDLC; mg dL ⁻¹) | 36.000±10.1 | 677.70±38.5 | 677.200±11.01 | 608.70±24.1 |
| High density lipoprotein (HDLC; mg dL ⁻¹) | 18.000±6.6 | 131.30±5.0 | 77.800±26.09 | 26.00±4.0 |
| Triglyceride (TG; mg dL ⁻¹) | 50.100±3.6 | 161.50±4.2 | 410.200±54.9 | 406.00±120.03 |
| Hematocrit (%) | 43.820±1.04 | 38.74±1.7 | 40.225±2.6 | 35.50±5.1 |
| Platelets (K UL ⁻¹) | 358.790±85.75 | 471.00±27.5 | 490.750±60.5 | 419.66±78.8 |
| Fibrinogen (mg dL ⁻¹) | 248.330±30.8 | 347.00±0.01 | 186.000±8.0 | 264.66±35.02 |
| Hemoglobin (g dL ⁻¹) | 13.360±0.3 | 12.22±0.5 | 12.550±0.7 | 10.83±1.5 |
| WBC (K UL ⁻¹) count | 13.780±1.76 | 13.34±2.52 | 13.750±3.21 | 20.93±4.51 |
| RBC (K UL ⁻¹) count | 6.210±0.12 | 5.70±0.28 | 4.900±0.30 | 4.28±0.93 |
| Lymphocytes (%) | 39.400±8.96 | 34.80±5.54 | 57.750±3.27 | 60.00±2.0 |
| Neutrophils (%) | 57.800±9.94 | 62.40±6.79 | 41.000±4.88 | 36.33±18.83 |
| Monocytes (%) | 2.330±0.33 | 2.12±0.11 | 1.500±0.64 | 2.33±0.88 |
| Eosinophils (%) | 2.000±0.57 | 2.15±0.3 | 2.660±0.66 | 1.33±0.66 |

() is the number of rabbits; the blood parameters were estimates as Mean±SE

and disturbing the mechanical fragility of atherosclerotic plaques making them vulnerable to rupture and thrombosis (Gregory, 1999; Abdelhalim *et al.*, 1994). Hemorheological-hemodynamic theory suggests that the increased blood viscosity associated with serum hypercholesterolemia accelerates atherogenesis (Sloop, 1996). The present study demonstrates an increase in fibrinogen levels in rabbits fed high cholesterol diet which in turn may induce reduction in blood flow through an increase in plasma viscosity, red cell aggregation and leukocyte activation. Fibrinogen levels are elevated in familial hypercholesterolemia patients and fibrinogen concentration is weakly, but positively associated with LDLC (Koenig *et al.*, 1992). Another possible role for hypercholesterolemia is the modulation of homeostasis by altering the expression and/or function of thrombotic, fibrinolytic and rheologic factors. In the present study, the increase in platelets and fibrinogen levels due to the high cholesterol diet may induce binding of fibrinogen to platelets through ADP resulting in faster aggregation and formation of larger platelet aggregates. The present study demonstrates increase in WBC counts and lymphocytes levels in rabbits fed high cholesterol diet. This in turn may increase the adherence of WBC and lymphocytes to the endothelium which represents one of the early responses to injury. On the contrary, the adherence of neutrophils to the endothelium during the early responses to injury is decreased. It has been reported that the basal adherence of monocyte only was significantly elevated in atherosclerosis resulting in increased adherence to endothelial cells (Dovgan *et al.*, 1994). In conclusion, these results strongly suggest that hypercholesterolemia elevates TC, LDLC, HDLC, TG, Fibrinogen, WBC, platelets and lymphocytes during the cholesterol feeding periods which in turn may impair blood rheology. On the contrary, it reduces haemoglobin, hematocrit, RBC and neutrophils. The progress of atherosclerosis may due to change in cholesterol metabolism and to increase in rheology of blood. The elevation of plasma viscosity is considered a predictor of atherosclerotic vascular disease as well as a potential mechanism for increasing the risk of cardiovascular diseases. Thus, further studies are required to investigate whether lipid lowering therapies may improve impaired rheology in rabbits with hypercholesterolemia to prevent cardiovascular disorders.

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