Aortic Diameter, Intima-media Thickness and Blood Flow Doppler Parameters of Aorta, Renal Artery and Intrarenal Arteries in Hyperlipidemic Male Rabbits

Akeel, AM., Hassan Zwain (PhD. UK)
Assistant Professor, Department of Physiology, College of Medicine, Kufa University.
E mail: drakeel49@yahoo.com

ألخلاصة:

يعتبر تصلب الشرايين المرض القاتل الرئيس على مستوى العالم، أُجريت هذه الدراسة لمعرفة تأثير تصلب سُمْكِ الطبقة الوسطية والداخلية للشريان الأبهر على سرعة جريان الدم في الشريان الأبهر والشريان الكلوي والشرايين الداخل كلوية في ذكور الارانب المعطاة غذاء عالي الدسم يحتوي ٢% كولسترول لمدة اثني عشر أسبوعا .وجد زيادة معنوية (P<0.05) في مستوى الكولسترول الكلي والكلسيريدات الثلاثية والكولسترول واطئ الكثافة والكولسترول واطئ الكثافة جدا والكولسترول عالي الكثافة . كان هناك أيضا زيادة معنوية في عرض وسُمك الشريان الابهر ومقياس درجة مقاومتها ومقياس درجة الانقباضية والانبساطية فيها والسرعة الانقباضية العليا ومقياس درجة المقاومة ومقياس درجة الانقباضية والانبساطية في الشرايين الكلوية.

Abstract

Background: Cholesterol is a lipidic, waxy steroid found in the cell membranes and transported in the blood plasma of all animals. It is an essential component of mammalian cell membranes, where it is required to establish proper membrane permeability and fluidity. Abnormally high cholesterol levels (hypercholesterolemia); that is higher concentrations of LDL and lower concentrations of functional HDL are strongly associated with cardiovascular disease because these promote atheroma development in arteries (atherosclerosis). High cholesterol fed rabbits as an animal "model" has frequently been used to study atherosclerosis .However reports on Doppler study of atherosclerotic effects on blood flow of different size arteries in the rabbit are lacking.

Objectives: to assess the effect of cholesterol enriched diet on aortic diameter and intima-media thickness and blood flow velocities of the aorta, renal artery and intra-renal arteries in male rabbits.

Materials and methods: This study was conducted on 16 male rabbits. The animals were randomly divided into two groups (8 rabbits per group), Rabbits in the first group fed normal chow diet and those in the second group fed with 2% cholesterol enriched diet for 12 weeks.. Blood samples were collected for measurement of serum lipids profile at the start and at the end of the study. Aortic diameter , its intima- media thickness and aortic, renal artery and intra-renal artery blood flow velocities were also measured by a triplex Doppler machine throughout.

Results & Conclusions: Feeding rabbits with 2 % cholesterol enriched diet for 12 weeks results in a significant increase (P<0.05) in serum level of TC, TG, HDL, LDL and VLDL. Also there was a significant increase (P<0.05) in aortic diameter , intima-media thickness, resistive index, pulsatality index, renal artery and intra-renal arteies peak systolic velocity, resistive and pulsatality indices and renal aortic ratio in comparison to the normal control group. There was no significant change (P>0.05) in

aortic peak systolic velocity and aortic, renal artery and intra-renal arteries end diastolic velocities. It was concluded that cholesterol enriched diet increased the vascular resistance and caused development of renal artery stenosis as indicated by the increase in aortic, renal artery and intra-renal arteries resistive and pulsatility indices and increase in renal artery peak systolic velocity and renal /aortic ratio

Key wards: Aorta, Intima-media thickness, renal arteries, Atherosclerosis, Doppler, Rabbit

Abbreviations: IMT: intima-media thickness, AO; aorta, RA: renal artery, TC: total cholesterol, TG :triglycerides, HDL: high density lipoprotein, ,LDL: low density lipoprotein, VLDL: very low density lipoprotein, RI: resistant index, PI: pulsatility index, PSV: peaked systolic velocity, EDV: end diastolic velocity, MV: mean flow velocity, RAR: renal aortic ratio.

Introduction:

Atherosclerosis is a disease of the vessel wall involving lipid accumulation, chronic inflammation, cell death, and thrombosis that causes heart disease and stroke[4].
Atherosclerotic disease including coronary artery disease is still the leading killer in industrial countries [22]. The development of coronary artery disease is a lifelong process; hypercholesterolemia is one of the major risk factors for coronary artery disease. Epidemiological and experimental data have indicated that a high cholesterol–containing diet is highly related to the development of hypercholesterolemia and atherosclerosis [13].

A number of large epidemiological studies have identified numerous risk factors for the development and progression of atherosclerosis. The risk factors can be divided into modifiable and non-modifiable risk factors. Non-modifiable risk factors include increased age, male gender, family history and some genetic abnormalities [11]. The most important modifiable risk factors include hyperlipidemia, hypertension, cigarette habituation, diabetes mellitus [10].

Atherosclerotic lesions (atheromata) are asymmetric focal thickenings of the innermost layer of the artery, the intima. They consist of cells, connective-tissue elements, lipids, and debris [8]. Blood-borne inflammatory and immune cells constitute an important part of an atheroma, the remainder being vascular endothelial and smooth-muscle cells [23]. The atheroma is preceded by a fatty streak, an accumulation of lipid-laden cells beneath the endothelium; most of these cells in the fatty streak are macrophages, together with some T cells [24].

There exist several reports demonstrating that rabbits develop hypercholesterolemia rapidly after excessive cholesterol feeding with the result of atherosclerotic changes in their aorta and pulmonary artery [16, 25, 30 and 31]. However, until the time of writing this work, studies on Doppler assessment of blood velocities in the aorta and renal arteries in the rabbit are lacking.

Material and methods:

Animals and study design

This study was conducted on 16 local domestic healthy male rabbits of body weight ranging between 1400-1700 grams. Rabbits were housed in a well ventilated cages and allowed to drink tap water ad libitum, and fed with standard chow (oxiod) diet ad libitum. A 2 weeks period was allowed to elapse without interference for acclimatization. They were then randomly divided into 2 groups (8 animals per group),

the normal control group and atherosclerosis induced group. Rabbits in the normal control group were kept on standard chow diet and tap water throughout the duration of study and those in the atherosclerosis induced group were kept on atherogenic diet (a 2% cholesterol-enriched diet made by the addition of cholesterol powder to the oxiod pellets)[31] and tap water throughout the duration of study (12 weeks)

   All rabbits were examined by Colored Doppler ultrasound machine at the start and at the end of the study. Doppler examination was performed at the morning after an overnight fasting to prevent the occurrence of intestinal gases which may prevent optimal visualizing of the arteries under study. The left lateral side was used for insonation, for technical reasons. Rabbits were sedated by administration of 5mg/kg body weight diazepam intra-peritoneal [21] and their left lateral side and abdomen were shaved . A 7.5 – 10 MHz probe was used to insonate the aorta and renal arteries. The aorta was first visualized and   identified by its characteristic pulsation and by using color flow Doppler; aortic diameter, its intima- media thickness (at the origin of renal artery) were measured. Care was taken to insonate all vessels under study at an angle of 0° and not to exert too much pressure on the flank or abdomen. The aortic blood flow parameters (peak systolic velocity, end diastolic velocity, resistive and pulsatility indices) were measured electronically by the soft ware device built in the Doppler machine. The renal artery and intra renal arteries (Blood flow changes in the interlobar artery at mid pole used in the current study) of the left kidney were visualized and identified using color flow mapping and their blood flow parameters (peak systolic velocity and end diastolic velocity) were also measured as mentioned earlier.

 The mean blood flow velocity (MV) is calculated using following equation [26]:

MV = diastolic velocity +1/3(peak systolic velocity – diastolic velocity)

Renal Aortic Ratio (RAR) calculated from following equation [26]:

$$RAR = \frac{\text{PSV in renal artery}}{\text{PSV in aorta}}$$

   Blood sampling were taken from central ear artery at the start and at the end of the study in order to measure serum lipids levels. The animals were then sacrificed and the aorta was excised for histomorphometric analysis

Measurement of serum lipids level

   From each rabbit blood samples were collected from the central ear artery without using heparin after an overnight fasting. The blood sampling was done at the start of the study i.e. at zero time and at the end of the study i.e. at 12 weeks. The blood samples were allowed to clot at 37 C and centrifuged at 3000 rpm for 15 min. , Sera were removed, and analyzed for determination of serum total cholesterol, triglycerides and HDL- C by enzymatic methods using reagents supplies by BIOLABO SA. LDL-C and VLDL-C were calculated by the following equations [14].

LDL = Total cholesterol – (HDL + VLDL)

VLDL = serum triglyceride / 5

Histopathological procedure

   Rabbits were killed by administration of high dose of Phenobarbital intravenously. Aortic arch with about 1 cm of the descending aorta was resected. Sections were then examined by a microscope under magnification power of (×4, ×10 and ×40) , the histological changes were determined   according to the American Heart Association classification of atherosclerosis[24] which divides atherosclerotic lesions into six types as follows: Type I (initial) lesion : Isolated macrophage foam cells . Type II (fatty streak) lesion: intracellular lipid accumulation. Type III (intermediate) lesion: Type II changes

and small extracellular lipid pools. Type IV (atheroma) lesion: Type II changes and core of extracellular lipid. Type V (fibro-atheroma) lesion: lipid core and fibrotic layer or multiple lipid cores and fibrotic layers. Type VI (complicated) lesion: complicated fibro-atheroma with hemorrhage or thrombus

Statistical analysis

The data were expressed as mean ± SEM. Statistical analysis were done by using computer program (SPSS) . In all tests P value <0.05 was considered to be statistically significant. Analysis of Covariance (ANCOVA) was used for comparison of differences among the groups.

<u>Results</u>

1-Effect on serum lipids level

There was a significant increase (P<0.05) in serum level of total cholesterol, triglyceride, LDL, VLDL, and HDL in the atherosclerosis induced group after 12 weeks of atherogenic diet feeding in comparison to the normal control group as shown in table(1).

2-Effect on aortic diameter, intima media thickness and blood flow velocity parameters

There was a significant increase (P<0.05) in aortic intima-media thickness, diameter, resistive and index, pulsatility indices after 12 weeks of atherogenic diet fed rabbits (atherosclerosis induced group) in comparison to the normal control group, but there was no significant difference in aortic peak systolic velocity and end diastolic velocity between the atherosclerosis induced group and normal control group as shown in table (2), and in figures (1, 2); figure (3) demonstrates Doppler spectrum wave form parameters of rabbit abdominal aorta. Figure (6) shows normal rabbit aortic wall layers; figures (7, 8 and 9) illustrate different stages of atherosclerotic changes in hyperlpidimic rabbit aortic wall layer

3-Effect on renal artery blood flow velocity parameters and renal- aortic ratio

There was a significant increase (P<0.05) in renal artery peak systolic velocity, resistive index, pulsatality index and renal-aortic ratio after 12 weeks of atherogenic diet fed rabbits (atherosclerosis induced group) in comparison to the normal control group, but there was no significant difference in renal artery end diastolic velocity between the atherosclerosis induced group and normal control group as

shown in table(3). An example of renal artery Doppler spectral wave form is shown in figures (4 , 5)

4-Effect on intra-renal arteries blood flow velocity parameters

There was a significant increase (P<0.05) in intra-renal arteries peak systolic velocity, resistive index and pulsatality index after 12 weeks of atherogenic diet fed rabbits (atherosclerosis induced group) in comparison to the normal control group, but there was no significant difference in intra-renal arteries end diastolic velocity between the atherosclerosis induced group and normal control group as shown in table (4). An example of intrarenal artery(Interlobar artery at mid pole of the kidney) Doppler waveform is shown in figure(5)

Table (1): Effect of cholesterol enriched diet on rabbit serum lipid profile in comparison to the normal control group

	Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
At zero time	Normal control	54.3±2	44.5±0.56	16.4±0.24	29±1.8	8.9±0.11
After 12 weeks	Normal control	57.2±1.3	46.5±0.92	16.3±0.31	31.6±1.4	9.3±0.18
	Dietary induced	721.3±9.4*	187.8±8*	19.3±0.73*	664.4±8.7*	37.6±1.6*

* P<0.01 (means of dietary induced group after 12 weeks versus means of normal control group after 12 weeks)

Table (2): Effect of cholesterol enriched diet on rabbit aortic diameter, intima media thickness (IMT), PSV, EDV, PI, and RI in comparison to the normal control group

	Groups	Aortic IMT in mm	Aortic diameter in mm	Aortic PSV in cm/s	Aortic EDV in cm/s	Aortic RI	Aortic PI
At zero time	Normal control	0.3±0	2.22 ±0.09	31.3±2.4	3.33 ±0.56	0.84±0.018	1.93±0.1
After 12 weeks	Normal control	0.32±0.02	2.28 ± 0.09	31.8±3	3.67±0.62	0.83±0.022	1.87±0.11
	Dietary induced	0.87±0.05**	2.78±0.11*	33.5±3.1	3.33±0.36	0.9±0.012*	2.24±0.079*

* P<0.05, ** P<0.01 (means of dietary induced group after 12 weeks versus means of normal control group after 12 weeks)

Table (3): Effect of cholesterol enriched diet on rabbit renal artery PSV, EDV, PI, RI and renal artery to aortic PSV ratio in comparison to the normal control group

	Groups	Renal artery PSV in cm/s	Renal artery EDV in cm/s	Renal artery RI	Renal artery PI	Renal artery / Aortic ratio
At zero time	Normal control	44.5±4.6	22.83±2.4	0.483 ± 0.024	0.72±0.057	2.13 ± 0.24
After 12 weeks	Normal control group	46.2±5.6	24±2.8	0.480 ± 0.01	0.71±0.021	2.18 ±0.25
	Dietary induced group	78.5±8.5**	30.5±4	0.607 ± 0.043**	1.06±0.13**	2.39 ±0.17*

* P<0.05, ** P<0.01 (means of dietary induced group after 12 weeks versus means of normal control group after 12 weeks)

Table (4): Effect of cholesterol enriched diet on rabbit intra-renal arteries PSV, EDV, PI, and RI in comparison to the normal control group

	Groups	Intra-renal arteries PSV in cm/s	Intra-renal arteries EDV in cm/s	Intra-renal arteries RI	Intra-renal arteries PI
At zero time	Normal control	42.7±6.7	21 ± 4.2	0.52 ± 0.022	0.81± 0.051
After 12 weeks	Normal control	43.2±6.6	21.3 ±3.7	0.52 ±0.018	0.79±0.043
	Dietary induced Untreated	63.7±7.8*	24.8 ±2.8	0.61 ±0.031*	1.03±0.084**

* P<0.05, ** P<0.01 (means of dietary induced group after 12 weeks versus means of normal control group after 12 weeks)
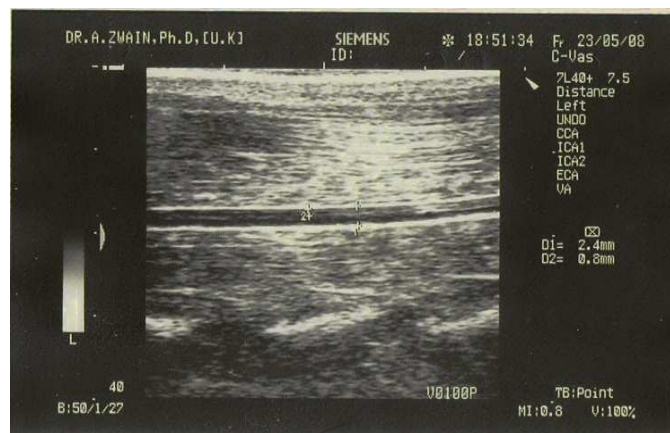
Figure (1): A B-mode ultrasound image showing a rabbit abdominal aorta with normal aortic wall thickness, D1 is the intima-media thickness = 0.3 mm , D2 is the aortic diameter = 2.1 mm
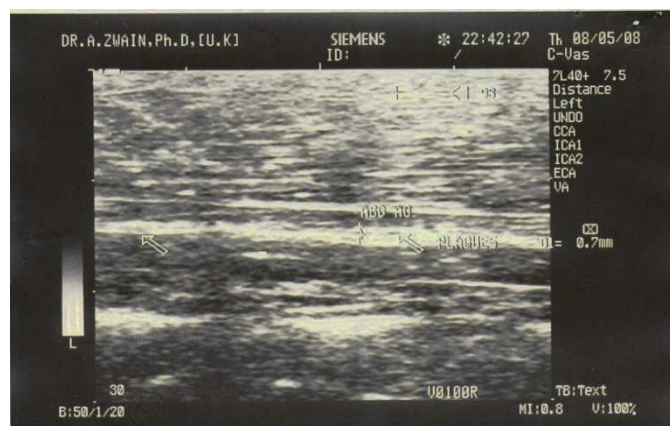

Figure (2): A B-mode ultrasound image showing a rabbit abdominal aorta with increased aortic thickness (sclerotic wall), there also dissemination of plaques and thrombi inside the aorta , D1 is the intima media thickness = 0.7 mm
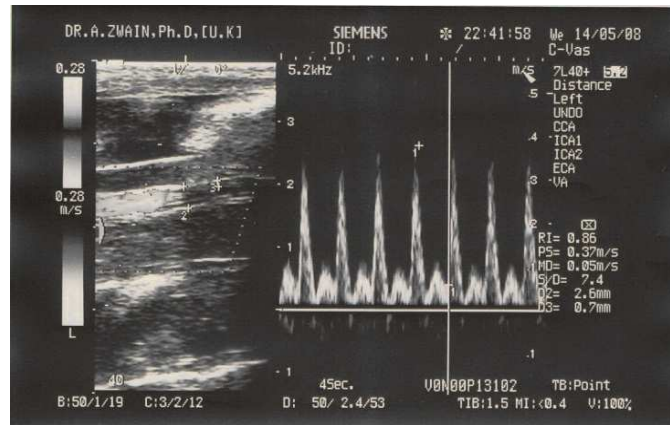
Figure (3): A Doppler wave form spectrum showing a rabbits aortic blood flow velocity, PSV=0.37 m/s , EDV=0.05 m/s RI=0.86 , S/D ratio=7.4
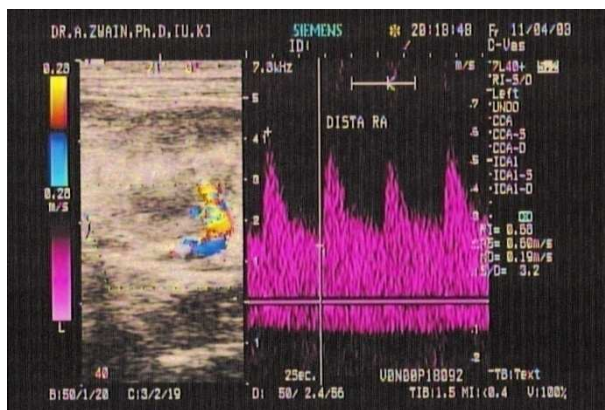

Figure (4): A Doppler wave form spectrum showing a rabbit distal renal artery blood flow velocity, PSV=0.60 m/s, EDV=0.19m/s, RI=0.68, S/D ratio=3.2
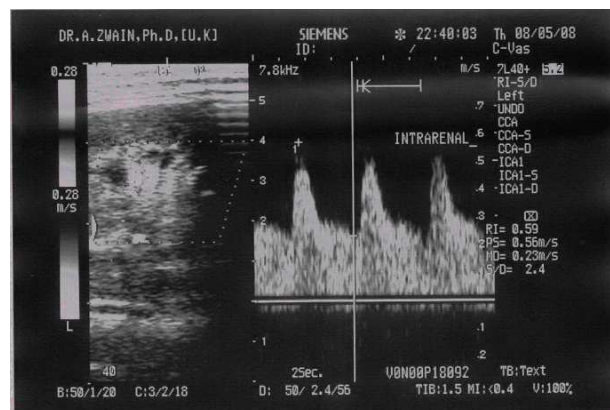
Figure (5): A Doppler wave form spectrum showing a rabbit intra-renal artery blood flow velocity, PSV=0.56 m/s, EDV=0.23m/s, RI=0.59, S/D ratio=2.4

Figure (6): A cross section of normal rabbit aorta shows the normal appearance of arterial wall layers: Lumen , Intact continuous endothelium (Intima), regularly arranged smooth muscle fibers (media) and adventitia . The section stained with haematoxylin and eosin (×4)

Figure (7): A cross section of hypercholesterolemic rabbit aorta demonstrating many lipid laden macrophages (Foam cells) that represent an early atherogenic event (fatty streak), the fatty streak affecting mainly the intima (Type-2 atherosclerosis). The section stained with haematoxylin and eosin (×10)
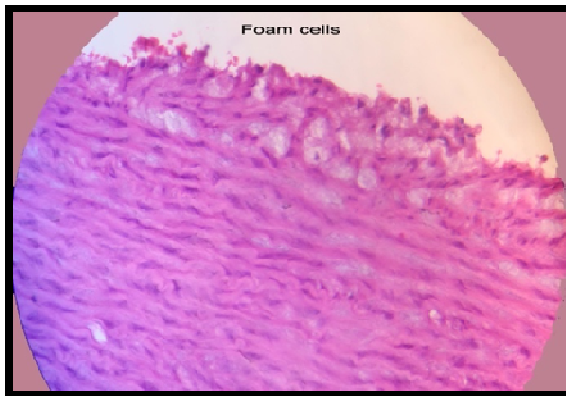
Figure (8): A cross section of hypercholesterolemic rabbit aorta demonstrating an early atherogenic event (fatty streak) affecting mainly the intima (Type-2 atherosclerosis). The section stained with haematoxylin and eosin (
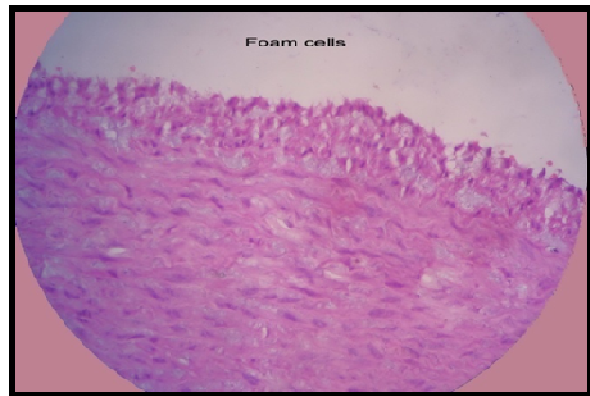

Figure (9): A cross section of hypercholesterolemic rabbit aorta demonstrating a more advanced fatty streak affecting the intima and extending down to the media (Type-2 atherosclerosis). The section stained with haematoxylin and eosin (×40)
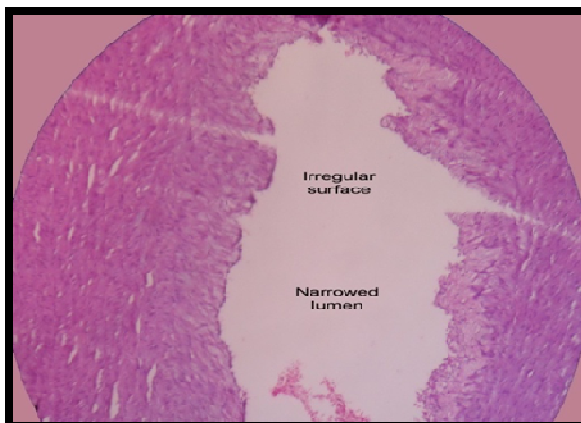

Figure (10): A cross section from aorta shows wide spread fatty streak with irregular surface (diffuse intimal thickening and narrowing of lumen) (Type-3 atherosclerosis). The section stained with haematoxylin and eosin (×10)

Figure (11): A cross section from aorta shows an advanced wide spread atheromatous plaque involving both intima and media with early separation in the media (Type-4 atherosclerosis). The section stained with haematoxylin and eosin (×10)

243

Figure (12): A cross section from rabbit aorta shows a fibrous atheromatous plaque with focal ulceration (Type-5 atherosclerosis). The section stained with haematoxylin and eosin (×40)
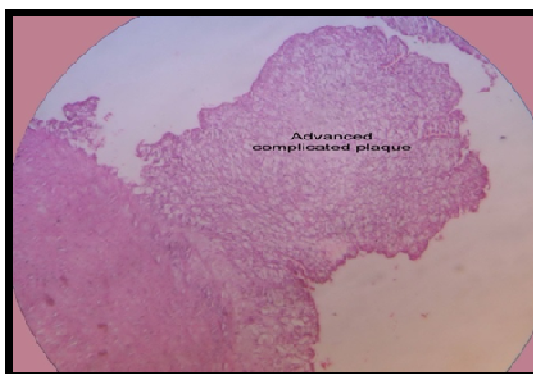
Figure (13): A cross section from aorta shows an advanced complicated atheromatous plaque with marked organization and sloughing (Type-6 atherosclerosis). The section stained h haematoxylin and eosin (×10)

Discussion

It has already been shown that arteriosclerosis of the coronary and peripheral vasculature is the leading cause of death among men and women in the United States and worldwide [22]. The present investigation was aimed to demonstrate in the rabbit the effect of hyperlipedemia induced atherosclerosis in different size arteries on blood flow velocities as calculated by colored Doppler equipment

In the current study, a significant increase in serum TC, TG, HDL, LDL and VLDL levels was found in rabbits fed with cholesterol enriched diet as compared with that in the normal control group. These results are consistent with those previously reported by Alipour *et al*.[1], Bauersachs *et al*. [3] , Howard and Culley [9] ,Sun YP et al [25] Mohammadi *et a.l*[16] and Zhu BQ et al [30.] ,

Intima-media thickness (IMT) is currently reported as an important index of atherosclerosis progression. It has been reported that baseline IMT can predict coronary heart diseases such as angina and non fatal myocardial infarctions [12]. Zulkhairi et al [31] and McConnell *et al*.[15] reported that rabbits fed with cholesterol enriched diet showed significant increase in the aortic IMT, the results obtained from the present study have confirmed these observations .

Further, a significant increase in aortic diameter was found in rabbits fed with cholesterol enriched diet as compared to their values at zero time. This observation can be explained by the expansive (enlargement) arterial remodeling which was recognized as an important determinant in vascular pathology in which narrowing of the lumen is the predominant feature[15]. The present findings are in line with the observation of Armstrong *et al*.[2] who reported that radial enlargement of vessels occur in macaque monkeys fed with cholesterol enriched diet due to progressive plaque growth.

Concerning Doppler indices: There was no significant increase of aortic peak systolic velocity (PSV); in contrast, significant increase of renal artery and intra-renal arteries PSVs were found in rabbits fed with cholesterol enriched diet as compared to that of normal control group. These results are in agreement with that reported by Yamashita *et al*.[28], who found that the peak systolic velocity of atherosclerotic iliac arteries of rabbits increased significantly (2.1 times) in comparison to normal iliac arteries.

The increase in renal artery and intra-renal arteries PSVs is due to the increased wall thickness and development of plaques in the arterial wall by cholesterol enriched diet feeding. When plaques protrude in to the blood vessels, narrowing of vascular lumen occurs which leads to very high blood flow velocity through the stenosed region[6, 29]. Moreover, the increase in blood flow PSV may be due to the fact that the atherosclerotic vessels are hardened and exhibit stiff and non compliant state[27].

The finding that the aortic PSV was not increased significantly can be explained by the fact that the aortic lumen is larger and it is difficult for plaques to cause stenosis in such artery with a wide lumen to affect blood flow velocity.

Another observation to be mentioned is that the aortic PSV in some rabbits was found to decrease. This may be due to a possible development of heart failure which perhaps caused reduction in blood pumping capability of the heart and consequently reduction in aortic peak systolic velocity [17].

Cholesterol enriched diet feeding to rabbits does not results in a significant change in aortic, renal artery and intra-renal arteries end diastolic velocities (EDV). These results may be explained as that the end diastolic velocity is not necessarily increased in stenosed arteries (normal or even low end diastolic velocity does not exclude critical vascular stenosis). EDV may be increased only in high degree of stenosis[18,19].

Cholesterol enriched diet caused a significant increase in aortic, renal artery and intra-renal arteries RIs and PIs. These results can be explained by the fact that RI and PI are related to vascular resistance; and such increase in RI and PI is associated with arterial stiffness (which reflects systemic atherosclerosis) [5, 18,].

There was a significant increase in renal aortic ratio (RAR) in rabbits fed with cholesterol enriched diet as compared to the control normal control group. This result may be explained as there was a significant increase in the PSV of renal artery and non significant increase in the PSV of aorta, this leads to significant increase in the RAR which is regarded as an important Doppler index used in the diagnosis of renal artery stenosis[7].

In conclusion, Cholesterol enriched diet caused increase in IMT of the aorta of variable degrees, increased vascular resistance, and development of renal artery stenosis as indicated by the increase of RIs, PIs of aortic, renal artery and intra-renal arteries and increase in renal artery PSV and renal artery /aortic ratio.

Since it is possible to interrogate blood flow of the aorta and renal arteries in the rabbit, using Doppler technique, this work was extended further to study the effect of certain drugs such as statines and sildenafil on aortic and renal blood flow of hyperlipedaemic rabbits .

References
1. Alipour A., Mohammadi M., Zarghami N. and Ahmadiasl N. Influence of chronic exercise on red cell antioxidant defense, plasma malondialdehyde and total antioxidant capacity in hypercholesterlemic rabbits. Journal of sport science and medicine. 2006; **5**:682-691
2. Armstrong ML., Heistad DD., Marcus ML., Megan MB., Piegors DJ. Structural and hemodynamic responses of peripheral arteries of macaque monkeys to atherogenic diet. Arteriosclerosis 1985; **5**: 336-346
3. Bauersachs J., Hiss K., Fraccarollo D., Laufs U. and Ruetten H. Simvastatin improves left ventricular function after myocardial infarction in hypercholesterolemic rabbits by anti-inflammatory effects. Cardiovascular Research 2006; **72**: 438-446

4. Berliner JA., and Andrew D. Watson AD. A Role for Oxidized Phospholipids in Atherosclerosis. N Engl J Med. 2005; **353**:9-11

5. Bude RO., and Rubin JM. Relationship between the Resistive Index and Vascular Compliance and Resistance. Radiology 1999; **211**:411-41

6. Catherine M. Otto Valvular Heart Disease: second ed. 2004 chapter 3 page 65. printed in Spain by Churchill Livingstone (An imprint of Elsevier Science Limited)

7. Dubbin P. Renal artery stenosis – duplex Doppler evaluation . British j. of radiology. 1986; **59**: 225-229

8. Hansson GK. Inflammation, Atherosclerosisand Coronary Artery Disease. N Engl J Med. 2005; **352**:1685-1695

9. Howard HT. and Culley NC. Accumulation of low density lipoprotein associated cholesterol in calcifying vesicle fractions correlates with intimal thickening in thoracic aortas of juvenile rabbits fed a supplemental cholesterol diet. Lipids Health Dis. 2006; **5**: 5-25

10. Hopkins PN, Williams RR. A survey of 246 suggested coronary risk factors. Atherosclerosis 1981; 40: 1-52

11. Jerrard-Dunne P.; Markus HS.; Steckel DA.; Buehler A.; Kegler S.; Sitzer M. Early Carotid Atherosclerosis and Family History of Vascular Disease, Specific Effects on Arterial Sites Have Implications for Genetic Studies. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003; **23**:302-306

12. Kazuyuki O.,Takaaki K.,Ryuta I. et al. The Anti-Atherosclerotic effects of lipid lowering with atorvastatin in patient with hypercholesterolemia. Journal of Atherosclerosis and Thrombosis 2006; **13**:216-219

13. Knopp RH. Drug Treatment of Lipid Disorders. N Engl J Med. 1999; 341:498-511

14. Lepper PM., Schumann C., Triantafilou K., Rasche FM., Schuster T. and Frank H. Association of Lipopolysaccharide-Binding Protein and Coronary Artery Disease in Men. J Am Coll Cardiol. 2007; **50**: 25-31

15. McConnell MV.; Aikawa M.; Maier SE.; Ganz P.; Libby P.; Lee RT. MRI of Rabbit Atherosclerosis in Response to Dietary Cholesterol Lowering . Arteriosclerosis, Thrombosis, and Vascular Biology. 1999; **19**: 1956-1959

16. Mohammadi M., Mirzaei F., Badalzadeh R. Effect of amlodipine on blood and aortic tissue concentration of endothelin in male rabbits receiving atherogenic diet. Indian J Pharmacol. 2007; **39**:276-280

17. Nitta N., Homma K. and ShiinaT. Pressure Gradient Estimation Based on Ultrasonic Blood Flow Measurement. Jpn. J. Appl. Phys. 2006; **45**: 4740-4748

18. Ohta Y, Fujii K, Arima H, et al. Increased renal resistive index in atherosclerosis and diabetic nephropathy assessed by Doppler sonography. J Hypertens. 2005; **23**: 1905-1911

19. Olin JW., Piedmonte M., and Young JR. Duplex Scanning of Renal Arteries for Stenosis . Annals of internal medicine. 1996; **124**: 370-371

20. Pasterkamp G., Kleijn DPV. and Cornelius-Borst C. Arterial remodeling in atherosclerosis, restenosis and after alteration of blood flow: potential mechanisms and clinical implications. Cardiovascular Research 2000; **5**: 843-852

21. Quesenberry KE. and Carpenter JW. Ferrets, Rabbits and Rodents: Clinical Medicine and Surgery, Saunders. 2004: St. Louis.

22. Rosamond WD, Chambless LE, Folsom AR, et al. Trends in the incidence of myocardial infarction and in mortality due to coronary heart disease, 1987 to 1994. N Engl J Med. 1998; **339**: 861-867

23. Stary HC, Chandler AB, Dinsmore RE, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. Circulation 1995; **92**:1355-1374

24. Stary HC, Chandler B, Glagov S, et al. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis: a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 1994; **89**:2462-2478

25. Sun YP, Zhu BQ, Sievers RE, Glantz SA, Parmley WW. Metoprolol does not attenuate atherosclerosis in lipid-fed rabbits exposed to environmental tobacco smoke. Circulation 1994 **89**:2260–2265

26. Victor PM., Hulst V., Baalen J., Kool LS. Renal Artery Stenosis. Endovascular Flow Wire Study for Validation of Doppler U.S. Radiology 1996; 200: **165**-168

27. Willens HJ. Relationship of Peripheral Arterial Compliance and Standard Cardiovascular Risk Factors. Vascular and Endovascular Surgery. 2003; 37: 197-206.

28. Yamashita A.; Asada Y.; Sugimura H., et al. Contribution of von Willebrand Factor to Thrombus Formation on Neointima of Rabbit Stenotic Iliac Artery Under High Blood-Flow Velocity. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003; **23**:1105

29. Yoganathan AP. Fluid mechanics of aortic stenosis . Eur Heart J. 1988; **9**: 13- 217

30. Zhu BQ, Smith DL, Sievers RE, Isenberg WM, Parmley WW. Inhibition of atherosclerosis by fish oil in cholesterol-fed rabbits. J Am Coll Cardiol 1988 **12**:1073–1078.[Abstract])

31. Zulkhairi A., and Hasnah B., and Zaiton Z., and Jamaludin M., and Zanariyah A., and Khairul K.A.K., and Taufik H.M., (2006) Atheromatous Plaque Formation in Rabbit Aorta Fed with High Cholesterol Diet. Malaysian Journal of Nutrition, **12** (2). pp. 213-220. ISSN 1394-035X.