Kowsar Medical Journal Spring 2011, Volume 16, Issue 1; 33-37

Effects of high sodium chloride intake on big arterial wall

Ja`farpour M.[*] ***PhD***, **Mahmoodian A. R.**[1] ***PhD***, **Ja'farpour S.**[1] ***MD***

[*]*Department of Anatomy & Biology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran;*
[1]*Department of Anatomy & Biology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

Abstract

Aims: Uncontrolled salt intake may increase arterial blood pressure. In many patients, high blood pressure is associated with big arterial wall injuries. This study was performed with the aim of evaluating the probable changes of arterial wall in laboratory mice after a period of oral salt intake.

Materials & Methods: In this experimental study, 20 male and 20 female laboratory mice weighing 30-35 grams with 2 months of age and Balb/c ethnicity were randomly divided into two experimental and control groups. There were 10 male and 10 female mice in each group. The experimental group received normal saline as 18g NaCl solved in 1000cc tap water for 12 weeks and the control group received tap water in the same period. All mice were anesthetized by chloroform and killed and the aorta and carotid arteries were excised and placed in 10% formalin solution for fixation. Sections were obtained from the paraffinized blocks and stained by Hematoxylin & Eosin. Stained sections were observed microscopically. Elective specimens were photographed and findings were statistically analyzed.

Results: Atheromas were observed in big arteries' intima in experimental mice but this phenomenon was not seen in control group. Cholesterol deposition and vacuolated macrophage infiltration were other abnormal changes in arterial wall of mice in experimental group that did not exist in control group ($p<0.001$).

Conclusion: Abnormal changes in the experimental group which are mentioned above indicate atherosclerosis in these laboratory models. Therefore, probably high salt intake induces atherosclerosis.

Keywords: Salt, Big Arteries, Atherosclerosis

Introduction

Excessive increase of arterial blood pressure is one of the biggest health problems that can trigger other crippling disorders including renal, cardiac and cerebral complications. Arteries of various body organs are troubled with disorders and structural damages caused by high arterial blood pressure in the path of creating disorders in organs and various parts of the body. Regardless of the issue that what factors in body are responsible for the increase of blood pressure, this phenomenon will create some complications in the arteries wall, especially large and medium sized that finally will result in atherosclerosis and calcification in the site of atheroma formation. Of course, in some patients, the reason for hypertension is not clear, yet in various cases, the researchers have mentioned some reasons.

One of the suspicious factors in this issue is the uncontrolled salt intake. Salt intake more than 5g per day is considered as the risk factor in the increase of blood pressure. This amount of salt includes available salt in all foods and excessive salt that people use. Although the amount of salt excretion from body depends on the structure and body physiology, the danger of salt should not be considered insignificant in anybody, and a few percentages of people are safe from dangers of high salt naturally. Moreover, it should also be mentioned that Chlorine and sodium ions both play some roles in developing hypertension [1, 2, 3, 4]. The issue that how sodium chloride creates this phenomenon, has been less studied. Some researchers have considered the vessel walls inflammations and atherosclerotic damages as the causes of hypertension. Atherosclerosis and arteriosclerosis resulting from it, lead to losing the flexibility of arteries and decrease the diameter of them. Following this complication, increasing the blood pressure will happen in compensation [5, 6].

Since both phenomena of vessel walls (including inflammation and atherosclerosis) and high salt intake have been recognized to be effective in hypertension, this fact may come to the mind that there can be a relationship between high sodium chloride intake and vessel walls complications. Medical sciences have clearly shown that pathophysiology of a clinical problem can help a lot to prevent and treat it. This recognition helps researchers to produce such drugs that create the best therapeutic results with the least side effects. Based on this idea, we studied pathology of artery walls and its relationship with high intake of sodium chloride. The study of probable changes of blood vessel walls in patients who consume salt a lot, has less been considered by researchers. Therefore, the

* Correspondence; Email: jafarpurmokhtar@yahoo.com
Received 2010/08/22; Accepted 2010/09/25

aim of this study was studying probable changes of large artery walls using a laboratory model and salt intake by them.

Materials & Methods

In this experimental study, 20 male and 20 female laboratory mice weighting 30-35grams with 2 months of age and Balb/c ethnicity were randomly prepared from medical college animal house and were placed randomly in 2 experimental and control groups. The number of male and female mice was assigned the same in each group, as in each of the experimental and control groups, 10 male and 10 female mice were placed. Male and female mice were placed separately in 5-item cages. Animal room had enough light and dark for 12 hours, temperature about 24°C, saturation about 55%, and enough water and food. Salt liquid was given by 18gram in 1000cc as the running water to the experimental animal group, while the control group used ordinary running water. This process lasted for 12 weeks [5]. After passing the considered time, the mice of both groups were anesthetized with chloroform, and by opening the chest, the inferior vena cava was cut in order to kill the animal. Then, aorta and carotid arteries were dragged out from the chest and were rinsed for 5 minutes in physiologic serum. In the next stage, the mentioned arteries were kept in formalin 10% for 72 hours to get tissue stability and after that the arteries were placed in alcohol with incremental degrees for dehydration. Then other stages of tissue preparation such as clarifying with xylol and embedding with paraffin were done. 7Microne pieces were prepared of Paraffin blocks containing considered tissue, as serial sections using a Microtome (Olympus; Japan) of research laboratories of anatomical Sciences and cellular biology. Then the slices were stained by Hematoxylin and eosin. From each mouse, 10 pieces and totally 200 pieces of each stained group were studied microscopically. Observation and analysis with multiplayer educational microscope and by three people were done blindly, and the results were done as abnormal changes in largeartery walls were registered and compared in separate tables. Selected samples, has been photographed. The results of 2 groups were analyzed through Chi-square.

Results

Cholesterol and lipid accumulation were observed clearly in the acerate grooves created in intima and even media of large arteries in all pieces obtained from large arteries of experimental group. Tissue cuts related to 10 male and 10 female mice of the experimental group show the above damages. Despite the differences in shape grooves created in intima were observed and saved in all experimental cases by the three observers. These grooves included whole intima in some cases. The sizes of grooves were different and the site of their creation was close to each other curly almost in one way and crescentic around the arterial duct. Some grooves were very big and some were very tiny (Figure 1). Grooves including the mentioned cholesterol in some cuts prepared from large arteries of experimental group mice were extended to media (Figure 1). In control group including male and female mice, these grooves were not observable, and intima layers, media, and adventitia had a completely natural appearance (Figure 2 and 3).

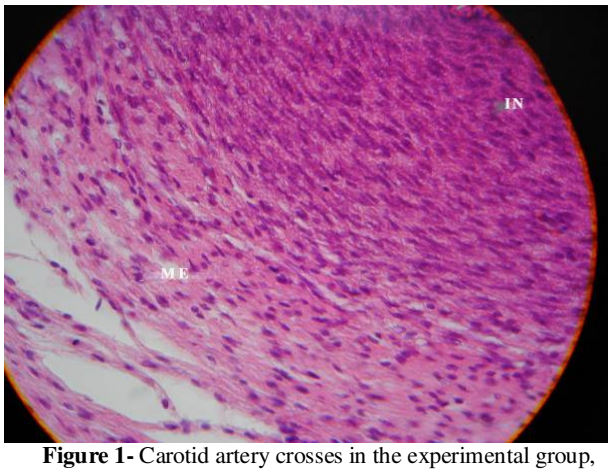

Figure 1- Carotid artery crosses in the experimental group, cholesterol accumulation (flash), intima (IN), media (ME), Zoom 40×10, painting H & E.

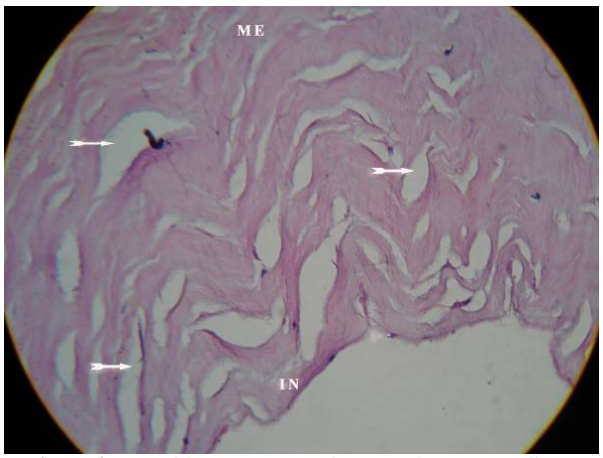

Figure 2- Carotid artery crosses in control group, media (ME), intima (IN), Zoom 40×10, painting H & E.

In statistical analysis the comparison of change cases of Lipid and Cholesterol accumulation in large artery intima layers including aorta and carotid showed a

significant difference in experimental and control groups (Table 1).

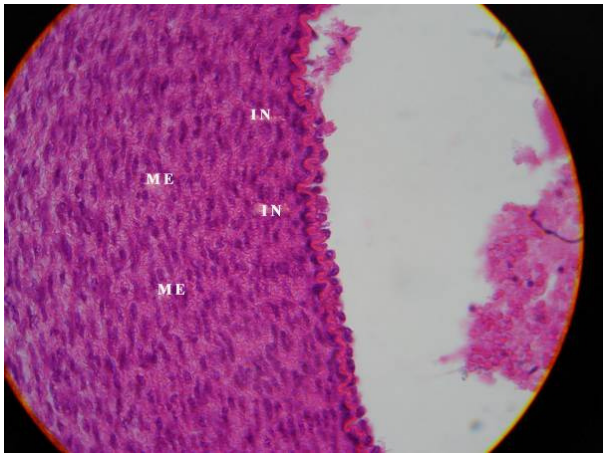

Figure 3- Carotid artery crosses in control group, media (ME), intima (IN), Zoom 40×10, painting H & E.

Table 1- The comparison of control group with the experimental group regarding the cholesterol deposits

Group→ The availability of cholesterol deposits↓	Control		Experimental		Significance Level
	Number	Percent	Number	Percent	
Hasn't	20	100	0	0	<0.001
Has	0	0	20	100	

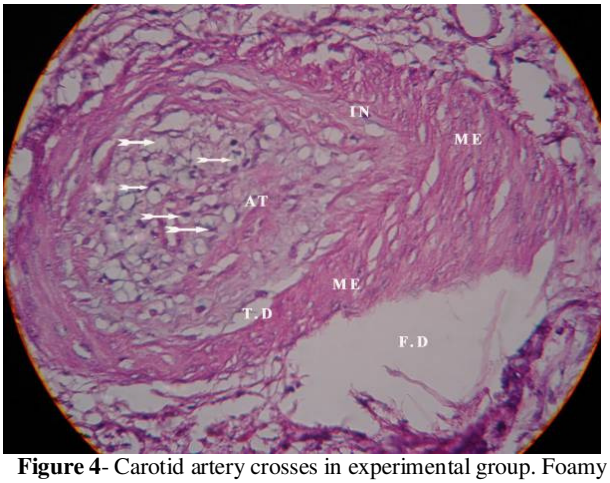

Figure 4- Carotid artery crosses in experimental group. Foamy cell (flash), blocking atheroma (AT), media (ME), intima (IN), false duct (F.D), true channel (T.D), zoom 40×10, painting H & E

Another change that was observed in the wall of large arteries of male and female mice of the experimental groups was the wide infiltration of turgid macrophages (foamy cells) into the intima. These macrophages seemed vacuolated and containing fat. Vacuolated macrophages were mostly focused in one area and created a large mass. This cellular mass was so large in some cases that it had blocked the arterial ducts completely. In these cases, arterial tissue shoed a degenerative process and the disorganization was clear (Figure 4). This phenomenon was observed in intima in addition to grooves containing cholesterol. This abnormal change was not observed in any of the large artery walls of control group mice and the large blood vessels in this group seemed healthy (Figure 2 and 3). The statistical analysis of this result showed a significant difference in comparison of experimental group large vessels with those of the control group (Table 2).

Table 2- The comparison of control group with the experimental group regarding the availability of infiltration of macrophages in intima.

Group→ Macrophage infiltration↓	Control		Experimental		Significance Level
	Number	Percent	Number	Percent	
Hasn't	20	100	0	0	<0.001
Has	0	0	20	100	

Discussion

Uncontrolled intake of sodium chloride had such results as cholesterol accumulation in large arteries intima. Cholesterol accumulation in created acerate grooves was observed in large arteries intima and even inside the media layer. This phenomenon was clear in all the used experimental models in this research. Moreover, infiltration of turgid microphages containing Cholesterol and lipid in arteries intima was another result of uncontrolled salt intake and this phenomenon is one of the results of this research. Most of macrophages were appeared as foamy cells indicating cholesterol and lipid swallowing by these cells. Some of the foamy cells were observed dispersedly in all layers of large arteries walls (Figure 4). Various reports have been registered on the fact that in patients suffered from arthrosclerosis, needle slots have been created in intima and even media, and that these slots contained cholesterol and lipid. In some areas of arterial walls in which there are the accumulation of cholesterol and lipid, macrophages swarm and come into foamy cells by swallowing available cholesterol and lipid in the area and it is the attribute specifies of these types of macrophages. The above phenomenon results in focal narrowing arteries and hypertension creation [7, 8]. Some researchers have known the uncontrolled salt intake as one of the most important factors in creating arterial hypertension. In other various cases, the researchers have recognized high arterial blood pressure as the result of atherosclerosis [9, 10].

Through comparing the results of the present research

with the studies that have been mentioned above, one can prove the consistency of these results together. Cholesterol accumulation and macrophage infiltration in large arteries intima showed vascular inflammation (that proves the availability of macrophages). Moreover it showed narrowing of blood vessels that can create hypertension. Most of the researchers have considered the increases of blood pressure associated with atherosclerosis. Both phenomena have augmented each other and are observed together in patients. Hypertension causes the atherosclerosis, and the latter phenomenon causes the increase of hypertension [11, 12, 13]. The fact that which phenomenon starts the vicious cycle is controversial. In the present study, uncontrolled intake of salt has been introduced as a probable factor of atherosclerosis creation. Therefore, in the above-mentioned vicious cycle, it is one of the participant factors in salt. According to some researchers, salt can cause inflammation and endothelial disorders regardless of its effect in hypertension increase in the beginning [11, 12, 13, 14, 15]. Therefore, one can suggest that uncontrolled intake of salt causes vessels inflammation, atheroma creation, and atherosclerosis and lead to hypertension finally. Two last phenomena i.e. arterial hypertension and atherosclerosis augment each other as a vicious cycle and increase the intensity.

Conclusion

Cholesterol accumulation and macrophage infiltration and atheroma creation, in the large artery walls in mice experimental group in comparison with the control group have a significant difference, and these abnormal differences in the experimental group indicate the creation of atherosclerosis in the laboratory models. Therefore, uncontrolled intake of salt may cause creation of atherosclerosis.

References

1- Nakano A, Inoue N, Sato Y, Nishimichi N, Takikawa K, Fujita Y, et al. LOX-1 mediates vascular lipid retention under hypertensive state.J Hypertens. 2010;28(6):1273-80.

2- Szybinski Z, Jarosz M, Hubalewska-Dydejczyk A, Stolarz-Skrzypek K, Kawecka-Jaszcz K, Traczyk I, et al. Iodine-deficiency prophylaxis and the restriction of salt consumption. Endokrynol Pol. 2010;61(1):135-40.

3- Tsuda K. Nutritional recommandation for hypertension. Nippon Rinsho. 2008;66(8):1547-51.

4-Nakandakare ER, Charf AM, Santos FC, Nunes VS, Ortega K, Lottenberg AM, et al.Dietary salt restriction increases plasma lipoprotein and inflammatory marker concentrations in hypertensive patients. Atherosclerosis.2008;200(2):410-6.

5- Dinarello CA. Hyperosmolar sodium chloride, p38 mitogen activated protein and cytokine-mediated inflammation. Semin Dial. 2009;22(3):256-9.

6- Weiss D, Taylor WR. Deoxycorticosterone acetate salt hypertension in apolipoprotein E-/- mice results in accelerated atherosclerosis: The role of angiotensin II. Hypertension. 2008;51(2):218-24.

7- Henke N, Schmidt-Ullrich R, Dechend R, Park JK, Qadri F, Wellner M, et al. Vascular endothelial cell-specific NF-kappaB suppression attenuates hypertension-induced renal damage. Circ Res. 2007;101(3):268-76.

8- Yazdanpanah M, Aulchenko YS, Hofman A, Janssen JA, Sayed-Tabatabaei FA, van Schaik RH, et al. Effects of the renin-angiotensin system genes and salt sensitivity genes on blood pressure and atherosclerosis in the total population and patients with type 2 diabetes. Diabetes. 2007;56(7):1905-12.

9- Ketonen J, Merasto S, Paakkari I, Mervaala EM. High sodium intake increases vascular superoxide formation and promotes atherosclerosis in apolipoprotein E-deficient mice. Blood Press. 2005;14(6):373-82.

10- Larrousse M, Bragulat E, Segarra M, Sierra C, Coca A, Sierra A. Increased levels of atherosclerosis markers in salt-sensitive hypertension. Am J Hypertens. 2006;19(1):87-93.

11- Schulman IH, Zhou MS, Raij L. Interaction between nitric oxide and angiotensin II in the endothelium: Role in atherosclerosis and hypertension. J Hypertens Suppl. 2006;24(1):45-50.

12- Feletou M, Vanhoutte PM. Endothelial dysfunction: A multifaceted disorder. Am J Physiol Heart Circ Physiol. 2006;291(3):985-1002.

13- Tobe SW, Burgess E, Lebel M. Atherosclerotic renovascular disease. Can J Cardiol. 2006;22(7):623-8.

14- Anderson JL. Lipoprotein-associated phospholipase A2: An independent predictor of coronary artery disease events in primary and secondary prevention. Am J Cardiol. 2008;101(12):23-33.

15- Nakase T, Mizuno T, Harada S, Yamada K, Nishimura T, Ozasa K, et al. Angiotensinogen gene polymorphism as a risk factor for ischemic stroke.Neuroscience. 2007;14(10):943-7.