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Histological investigation of the effect of A-Tocopherol on Arterial Wall Structure in Diabetic Rat Model

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Abstract

Cardiovascular diseases are the most important cause of mortality in diabetic individuals. The aim of our study is to investigate histologically whether the use of alpha-tocopherol in diabetic rats would have an effect on the antioxidant system and would provide positive effects on vessel wall damage. For the study, 32 wistar Albino rats were randomly divided into 4 groups. The groups are Control+SF, Control+alpha-tocopherol, DM model, DM+alpha-tocopherol. Arterial tissue specimens were fixed formaldehyde. Routine tissue follow-up was performed and hematoxylin-Eosin (H-E) staining was performed. Arterial wall damage score was calculated. As a result of the evaluation, Kruskal-Wallis, Mann-Whitney U Test and descriptive analyzes were applied for statistical analyzes. In light microscopic examinations; in DM group was determined endothelial cell damage, intracytoplasmic edema and swelling in the smooth muscle cells. In sections of the DM + alpha-tocopherol group, endothelial cells were evaluated as normal. Although there were no differences between DM and DM + alpha-tocopherol groups, there was a significant difference in luminal diameter between Control and DM +alpha-tocopherol in evaluating parameters such as vessel diameter, lumen cap, vessel wall thickness and Tunica media thickness ($p< 0,005$). In our study, the use of alpha-tocopherol in diabetic rats was thought to have an effect on the antioxidant system, which could have a positive effect on vessel wall injury. However, it has come to the conclusion that the therapeutic effect of alpha-tocopherol on cellular damage due to vascular complications in the vascular structure of diabetes is limited.

Introductıon

Diabetes mellitus (DM) is a metabolic disease of carbohydrates, lipids and proteins, defined by the high levels of fasting and postprandial plasma glucose . The prevalence of diabetes in the world has been increasing dramatically [1]. Hyperlipidemia, inflammation and altered antioxidant profiles are expected complications for diabetes mellitus [2]. Cardiovascular diseases are the most important cause of mortality in diabetic patients [3,4]. Cardiovascular complications are mortality reasons 2-5 times more often in diabetic patients in comparison with nondiabetic patients having the similar ages and the same gender. And this is due to the imbalance between reactive oxygen species (ROS) and anti-oxidant systems [5]. If free radical production exceeds the capacity of antioxidant defense, oxidative stress occurs and results in cellular damage. The role of antioxidants in the fight against oxidative stress was found to be important. [6]. The protective effects of antioxidants were presented by experimental, clinical and epidemiological studies and it was thought that antioxidants may be helpful in the treatment of diabetes and its complications. Many studies have been conducted on the use of antioxidants in experimental diabetes models [7]. Antioxidants reduce oxidative stress, reduce peripheral nerve and vascular disorders [8]. Alpha-tocopherol (vitamin E) is a fat-soluble essential vitamin that acts as an antioxidant in the human body, which also benefits in improving diabetic complications on various tissues.[9,10].

Reactive oxygen species due to oxidative stress have been shown to cause ineffective erythropoiesis and increased lysis of erythrocytes in some experimental animals and humans. Several studies on patients with various hereditary hemolytic anemia, chronic kidney disease, early low birth weight infants and apparently healthy people have shown that vitamin E can be therapeutically effective in the prevention and / or treatment of anemia in these subjects. [11]. In this study, the histological evaluation of alpha-tocopherol, which has beneficial effects on the cardiovascular system, on the aortic wall of diabetic rats was evaluated. The aim of our study; The aim of this study was to histologically investigate whether the

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use of alpha-tocopherol in diabetic rats has positive effects on vascular wall damage by acting on the antioxidant system.

Materials and Methods

This study was conducted at Mersin University, School of Medicine, Experimental Animals Research Laboratory (Mersin, Turkey). For animal experiments, approval was obtained from Mersin University Animal Experiments Ethics Committee. Thirty-two Wistar Albino rats were randomly divided into four groups. For the formation of a DM model in half of the experimental groups; streptozotocin (STZ) solution in freshly prepared pH 4.5 citrate buffer was administered to Wistar male rats as a single dose of 40 mg / kg intraperitoneally (i.p). Blood glucose was measured by glucometer (Aquo-Check, Roche) at 48 and 72 hours after the application. For rats forming the diabetes group; those with a blood sugar level above 300 mg / dl on average were considered DM. For antioxidant application; A daily dose of 40 mg / kg (i.p) Alpha-tocopherol was given for 3 weeks. At the end of the experiment, rats were excised under anesthesia, and arterial tissues were removed and histological evaluation was started.

Experimental groups: Control + SF, Control+alpha-tocopherol, DM model, DM + alpha-tocopherol. Hematoxylin-Eosin stained arterial cross-sections were measured in each of the widest and narrowest two axes by measuring arterial diameters and artery lumen diameters. In addition, arterial wall thickness and media thickness were measured at two different points and mean arterial wall thickness and mean media layer thickness were calculated: Vascular diameter, lumen cap, vessel wall thickness, Tunica media thickness were measured and comparisons were made between the groups.

Histological Tissue follow-up Stages: Arterial tissue samples were fixed for 48 hours with 10% neutral phosphate buffered formaldehyde. Tissue samples were passed through ethanol series (50-99 %) and dehydrated. Transparency was performed by passing through xylene series and infiltration was completed by passing through melted paraffins (62 °C), then it was embedded in paraffin blocks with luminal surfaces. 6 µm thick sections were taken on the slides with a microtome from the paraffin blocks. Hematoxylin eosin (H-E) staining was applied to the sections after deparaffinization and rehydration procedures. All sections were examined by Nikon Eclipse Ni-U light microscope with Nikon NIS-Elements Documentation 4.5 image analysis system, measurements were made and micrographs were taken with the Nikon DS-Fi2 camera (Nikon Corporation, Tokyo, Japan). Hematoxylin–Eosin stained artery cross-sections were measured at the widest and narrowest two axes, and arterial diameter and arterial lumen diameters were measured, and mean arterial diameters and lumen diameters were calculated. In addition, by measuring the thickness of the arterial wall thickness and media layer from two different points; mean arterial wall thickness and mean media layer thickness were calculated. Endothelial damage, smooth muscle cell damage and elastic lamina damage were scored in these sections (no = 0, focal areas-mild = 1, moderate-to-severe = 2, widespread-severe = 3, maximum total score = 9.

Statistical Analysis

Descriptive analyzes were used for statistical analysis, Kruskal-Wallis test was used to test the significance of the differences between the averages of the groups and Mann-Whitney U Test was used to compare the independent data.

Results

When light microscopic examinations of arterial tissue of Control+SF group were performed; the endothelial cells were sturdy and intact, overall, with H + E-stained artery cross-sections. The tunica intima, Tunica media and Tunica adventisia layers of the aortic vein were observed in normal histological structure. At the same time, no pathology was observed in the endothelial and subendothelial layer. The alignment of muscle cells and the morphology of elastic laminae were normal (Fig.1A). Control + Alpha Tocopherol group; Hematoxylin-Eosin-stained arterial sections, endothelial cells, smooth muscle cells and elastic laminae were evaluated in normal structure (Fig. 1B). In the arterial sections of the DM group; In some areas, endothelial cell damage, intracytoplasmic edema and swelling in smooth muscle cells, deformation in elastic laminae and separation of fibers were determined. A decrease in smooth muscle cell nuclei was observed in the aortic group in tunica media (Fig. 1C). DM + alpha-tocopherol group: Hematoxylin-Eosin-stained arterial sections were evaluated for normal endothelial cells. In some of the media layer smooth muscle cells, small perinuclear transparent areas were evaluated as intracytoplasmic edema. Intracytoplasmic edema and swelling in smooth muscle cells rarely detected (Fig. 1D). At the same time, in Table 1; the group's definıtıon statıstıcs are seen.

Statistical Analysis

For statistical analysis, Kruskal-Wallis, Mann-Whitney Test and descriptive analyzes were performed (Table 1). As a result of the evaluation of the parameters related to the vascular structure: vessel diameter, lumen cap, vessel wall thickness, Tunica media thickness: DM and DM+ alpha-tocopherol groups were not observed any difference ($p > 0.05$). There was a significant difference between the Control and DM +alpha-tocopherol groups in terms of vessel diameter and lumen diameter ($p<0.05$).

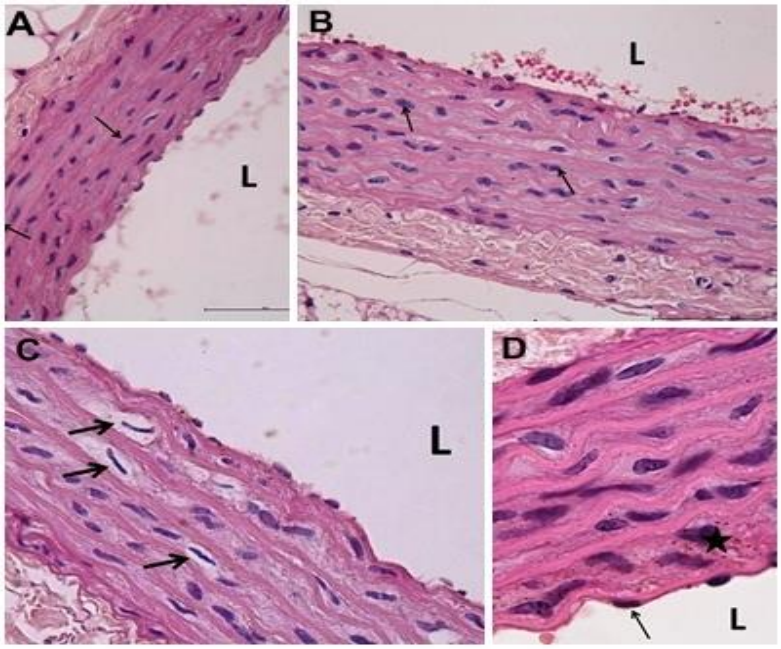

Fig.1: Fig.1A-Control +SF group, Fig.1B-Control+Alpha-tocopherol. Fig.1C-DM group; Fig.1D; DM+ Alpha-tocopherol. Arterial lumen (L), artery media layer, smooth muscle cell nuclei (thin arrow) intrastoplasmic edema and swelling (thick arrow), intima ve media tabasında lipofuscin granülleri (yıldız). H&E, Scale bar = 100 µm

Tab.1: Descriptive Statistics of Groups (Mean± Std. Deviation)

Mean± Std. Deviation	N	VESSEL DİAMATER	LUMEN DİAMATER	VESSEL WALL THICKNESS	T. MEDIA THICKNESS
CONTROL	8	1181,8217± 81,80941	991,9225± 66,42591	89,7008± 14,39775	64,2592± 9,63392
CONTROL+A TOCOPHEROL	8	993,2658± 128,51002	822,4150± 170,47577	101,4183± 24,53467	76,1967± 22,57690
DIABETES MELLİTUS	8	1011,0600± 57,14975	893,2125± 32,09107	86,7575± 22,41240	63,6692± 10,39268
DIABETES MELLİTUS +A TOCOPHEROL	8	1003,3871± 87,54695	852,0893± 52,51561	78,0050± 11,62636	62,9693± 9,18370

Discussion

DM is a disease with long-term microvascular and macrovascular complications. Data from clinical and experimental studies indicate that DM is an important factor predisposing to atherosclerotic diseases and especially Coronary Heart Disease (CHD). In DM, the incidence of cardiovascular disease is higher in both sexes and all ages. Atherosclerotic complications such as coronary artery disease, peripheral vascular disease and cerebrovascular disease are the most common causes of morbidity and mortality in diabetic patients. [12]. Recent evidence suggests that hyperglycemia may be effective in the early stages of vascular cells, that it promotes the development of vascular complications in the future, and that this effect is mediated by oxidative stress. Evidence suggests that this "memory " can occur even when a good glycemia control is achieved. This phenomenon was called the metabolic memory and it has been suggested that glucose release may be very harmful to the vascular structure. This evidence raises many questions about the management of diabetes in patients with diabetes. In particular, the presence of metabolic memory necessitates early and aggressive treatment of hyperglycemia. Long-term follow-ups show that; A more challenging therapeutic strategy may be necessary to maintain glycemia in a normal-narrow range. Some evidence has suggested that several different aspects of hyperglycemia may contribute to the development of diabetic complications more than HbA1c levels. [13,14]. Endotelyum is a large organ that plays a role in the development of cardiovascular disease in diabetic people. [15]. Therefore, how these different aspects of the vessel wall of hyperglycemia affect and contribute to cardiovascular risk in diabetes attracts considerable attention.

14. Bahçeci et al. reported that DM causes angiopathic changes in a very important vessel such as the aorta even in the very early period. [16]. The possible sources of oxidative stress in diabetes are the autooxidation of glucose, diminishing tissue concentrations of antioxidants such as reduced glutathione and alpha-tocopherol, and poor activity of antioxidant defense enzymes. [17,18]. Özkan et al. also investigated the effect of triple antioxidant (alpha-tocopherol, C vit., Alpha lipoic acid) on the brains of diabetic rats. Antioxidants prevent lipid peroxidation and reduction of endonural blood flow in the cell membrane by reducing oxidative stress. In this way, they reduce peripheral nerve and vascular disorders. [8]. Paolisso et al. Type 2 diabetes patients taking alpha-tocopherol supplements show that this reduces insulin resistance and ameliorates glucose intake [19]. When diabetic patients were given 600 or 1200 mg alpha-tocopherol a day for a period of 2 months, it was seen that glycated hemoglobin and other proteins were clearly decreased [20]. In our study; alpha-tocopherol that is thought to reduce the tissue concentration, depending on the oxidative stress was given and the fact that this can generate positive results on vascular disorders has been emphasized. The protective effects of antioxidants are presented by experimental, clinical and epidemiological studies and it is thought that antioxidants can help in the treatment of diabetes and its complications. [21,22]. When 2000 IU of alpha-tocopherol per kg for 8 weeks was given to rats that got diabetes by Streptozotocin (STZ), it has been reported that they provided significant protection in DM-induced cardiac disorders. In another rat study, protective effects against oxidative stress and inflammation were seen when C, E and D were given after diabetes was formed by giving STZ [23]. Both vitamin E deficiency and oxidative condition were found to be associated with prediabetes in healthy looking rats [24]. In our study; alpha-tocopherol has been ensured to evaluate the positive morphological changes in the cardiovascular system of diabetic rats, especially in the arteries. Intracytoplasmic edema and swelling of smooth muscle cells in diabetic rat artery media layer, as well as pyknotic nuclei, elastic lamina degeneration, endothelial damage, presence of lipofuscin granules in the intima and media layer are important for showing the tissue damage of diabetes. (Fig.1C). It has been detected that tissue damage is fairly decreased and artery walls have features similar to the control group in Alpha-tocopherol given DM group (Fig.1A-1D)This morphological difference observed has been construed as a favorable effect of alpha tocopherol on tissue. also vessel diameter, lumen cap, vein The fact that there was no significant difference between DM and DM + alpha-tocopherol groups in terms of wall thickness and Tunica media thickness, but a significant difference in luminal diameter between Control and DM + alpha-tocopherol groups strengthened the idea that the beneficial effect of alpha tocopherol was related to dosage and duration ($p < 0{,}005$). In maintaining the structural and functional properties of the vascular wall; smooth muscle cells and integrity of endothelial cells have been observed to play a critical role (Fig.1)

To the best of our knowledge, similar studies have not been found in the literature to compare the results of this study.

Meanwhile, in the literature alpha tocopherol studies that contradict the positive results are also mentioned. Evaluating the role of antioxidant micronutrients in cardiovascular events or development in DM patients In Sarmento et al. (2013), reported high and inadequate quality of information due to high clinical heterogeneity among studies [25]. In addition, it has been suggested in some clinical studies that antioxidant supplementation (vitamin C, alpha-tocopherolve β carotene) does not provide a significant benefit in preventing cardiovascular events and may even cause mortality. [7,26]. Cai et al. also emphasized that vascular pathology is not responsible for primary cardiomyopathy. In this study, the role of micro-nutrients such as alpha-tocopherol as an antioxidant in the presence or development of cardiovascular events in diabetic patients was evaluated. However, it has ben observed that the results of the studies include high clinical heterogeneity.

Conclusion

In conclusion; in our study, as a result of histological examination of vascular structure in rats with diabetes, the use of alpha-tocopherol is thought to have an effect on the antioxidant system and may have a positive effect on vascular wall damage. However, it has ben surmised that the healing effect of alpha-tocopherol on cellular damage depending on the complications that occur in vascular structure, is limited. And it has been concluded that the dose-dependent efficacy should be evaluated by further investigation.

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