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Distribution of spleen connective tissue fibers in diabetic and vitamin C treated diabetic rats

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ABSTRACT

We investigated the distribution of connective tissue fibers in diabetic and vitamin C treated diabetic rat spleen. Rats were divided into three groups: group A, control; group B, diabetic; group C, vitamin C treated diabetic. Diabetes was induced by streptozotocin. Vitamin C was administered intragastrically for 21 days. Spleen tissues were examined by light microscopy after staining with Masson's trichrome, Gomori silver impregnation and van Gieson. In group B, we found accumulation of collagen fibers in the trabeculae, in the capsule and around the central artery and splenic sinusoids. Splenic cord thickening due to fibrosis was observed. Reticular fibers accumulated principally in the white and red pulps of the spleen and focal reticular fiber thickening was observed in the dense fiber areas. Partial elastic fiber rupture was observed among the fibers of the elastic lamina of the arteries in the hilum. By contrast, the distribution of collagen fibers in group C was similar to group A. Collagen fiber accumulation was decreased in group C compared to group B. We found little reticular fiber thickening in group C and elastic fibers maintained their integrity and were better organized than in group B. Our findings suggest that *appropriate* doses of vitamin C may exert beneficial effects on the structure of the connective tissue fibers in the diabetic spleen.

KEYWORDS

Connective tissue; diabetes; fibers; spleen; vitamin C

According to the International Diabetes Federation (IDF) (Snouffer 2018), the incidence of diabetes mellitus is increasing worldwide. Diabetes mellitus is a common disease that affects all organs of the body and quality of life adversely (Rubin and Peyrot 1999). Diabetes is characterized by chronic hyperglycemia caused by impairment of insulin secretion due to the destruction of insulin-secreting beta cells in the pancreatic islets or inadequate function of insulin (Eiselein et al. 2004). Oxidative stress also causes diabetes (Ceriello 2000; Kayama et al. 2015). Chronic hyperglycemia increases reactive oxygen species (ROS) production, which causes oxidative stress (Chowdhury et al. 2016). Lipid oxidation is increased in various tissues owing to diabetes (Manna et al. 2010; Bicer et al. 2012). Oxidative stress (Jakus 2000) participates in the pathogenesis of the various complications of diabetes (Giugliano et al. 1996; Ruggiero et al. 1997; Ceriello 2000; Hurrell and Hsu 2017).

Streptozotocin (STZ) is widely used to induce type I diabetes in animal models (Hayashi et al. 2006); STZ destroys the beta cells of pancreatic islets (Ikebukuro et al. 2002).

The spleen is a secondary lymphoid organ that contributes to immunity and serves as a stem cell reservoir; some stem cells behave like precursors of beta cells and produce insulin (Kabelitz et al. 2008).

Thorvaldson et al. (2003) postulated that beta cell destruction that causes type 1 diabetes is due to the imbalance of cytokines produced by T helper 1 and 2 lymphocytes. There is a strong association between diabetes and elevated levels of pro-inflammatory cytokines as well as oxidative stress from ROS (Fatima et al. 2016).

Vitamin C (ascorbic acid) is an antioxidant that inhibits free radical production (Bendich et al. 1986; Pham-Huy et al. 2008). In diabetics, vitamin C supplementation decreases lipid oxidation in the plasma and normalizes the levels of plasma thiobarbituric acid reactive substances (TBARS) (West 2000; Padayatty et al. 2003). Vitamin C could also be used as a preventive measure for type 2 diabetes mellitus (Garcia-Balio et al. 2011). Little is known about the correlation of morphological changes to the lymphoid organs and the effect of vitamin C on diabetes. We investigated the structure of the spleen and the distribution of splenic collagen, reticular and

elastic fibers in diabetic and vitamin C treated diabetic rats.

Materials and methods

We used 29 200 – 220 g male Wistar albino rats. Our study was approved by the Animal Experimentation Ethics Committee at Gazi University. The rats were provided standard rat food and tap water *ad libitum*. Animals were divided into three groups: group A, control group (n = 9); group B, diabetic group (n = 10); group C, vitamin C treated diabetic group (n = 10).

Streptozotocin (STZ), (35503; Serva, Heidelberg, Germany) was dissolved in 1 ml 0.1 M cold citrate buffer, pH 4.5. A single dose of 45 mg/kg STZ was injected intraperitoneally (i.p.) into rats of groups B and C to induce diabetes (Szkudelski 2001; Heidari et al. 2003). The fasting blood glucose level of the rats was monitored to confirm the onset of diabetes. Blood was drawn from the tail vein of the rats 48 h after injection of the STZ and serum glucose was measured with a glucose meter (Accu-Chek; Glucotrend, West Sussex, UK). Blood glucose levels ≥ 200 mg/dl were considered diabetic. Group C animals were given 20 mg/kg intragastric vitamin C (McLennan et al. 1988) for 21 days; tap water was given to group A in the same way.

After 21 days, all experimental groups were decapitated under 50 mg/kg sodium thiopental anesthesia. The spleens were removed, fixed in 10% formalin solution, dehydrated through increasing alcohol concentrations, cleared in xylol and embedded in paraffin. Sections were cut at 5 – 6 μ m. Sections were stained with Masson's trichrome for collagen fibers, Gomori's silver impregnation for reticular fibers and van Gieson for elastic fibers (Bancroft and Gamble 2008). The distribution of the connective tissue fibers

in the spleen were examined histologically using a light microscope.

Results

Masson's trichrome staining of collagen fibers

In group A, the collagen fibers were localized in the trabeculae, capsule, around the central artery and around the splenic sinusoids (Figure 1a, b). In group B, the splenic cord was thickened. Accumulation of collagen fibers (Figure 2a–d) and dilated venous sinuses (Figure 2b) were observed. In some sections from group B, we observed lipid infiltration in the trabecular vein (Figure 2d). Group C was similar to group A with scarce evidence of fibrosis (Figure 2e, f).

Gomori's silver impregnation of reticular fibers

In group A, reticular fibers were distributed homogeneously between the red and white pulps of the spleen. In group B, the characteristic thin network of reticular fibers aggregated into thick bundles in the white and red pulps of the spleen. We found focal reticular fiber thickening in the fiber-dense areas (Figure 3a). Unlike group B, in group C we found scarce reticular fiber thickening and the structure was near normal (Figure 3b).

van Gieson staining of elastic fibers

For both groups B and C, elastic fibers were observed in the arteries of the hilum region of the spleen. In group B, the internal elastic lamina exhibited atypical and degenerative elastic fibers and the elastic lamina was discontinuous in some regions (Figure 4a). In group C, the elastic lamina appeared normal (Figure 4b).

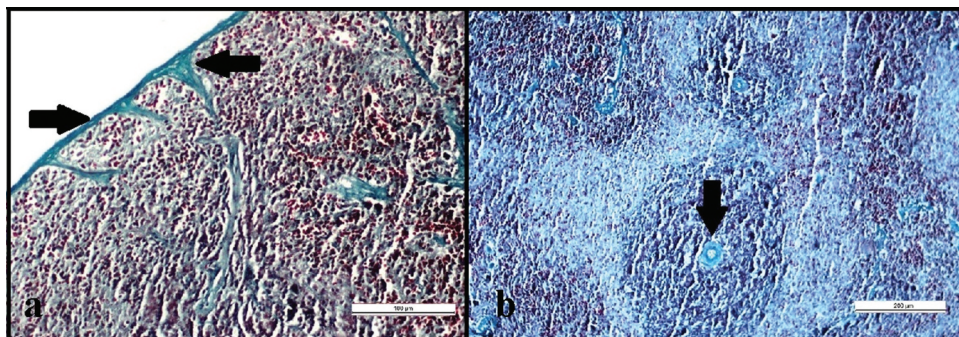


Figure 1. Normal structure of group A. a) Collagen fibers in the capsule and trabeculae (arrows) are stained in blue. Masson's trichrome. Bar = 100 μ m. b) White and red pulps of spleen in the control group. Collagen fibers appear around the central artery (arrow) and sinusoids. Masson's trichrome. Bar = 200 μ m.

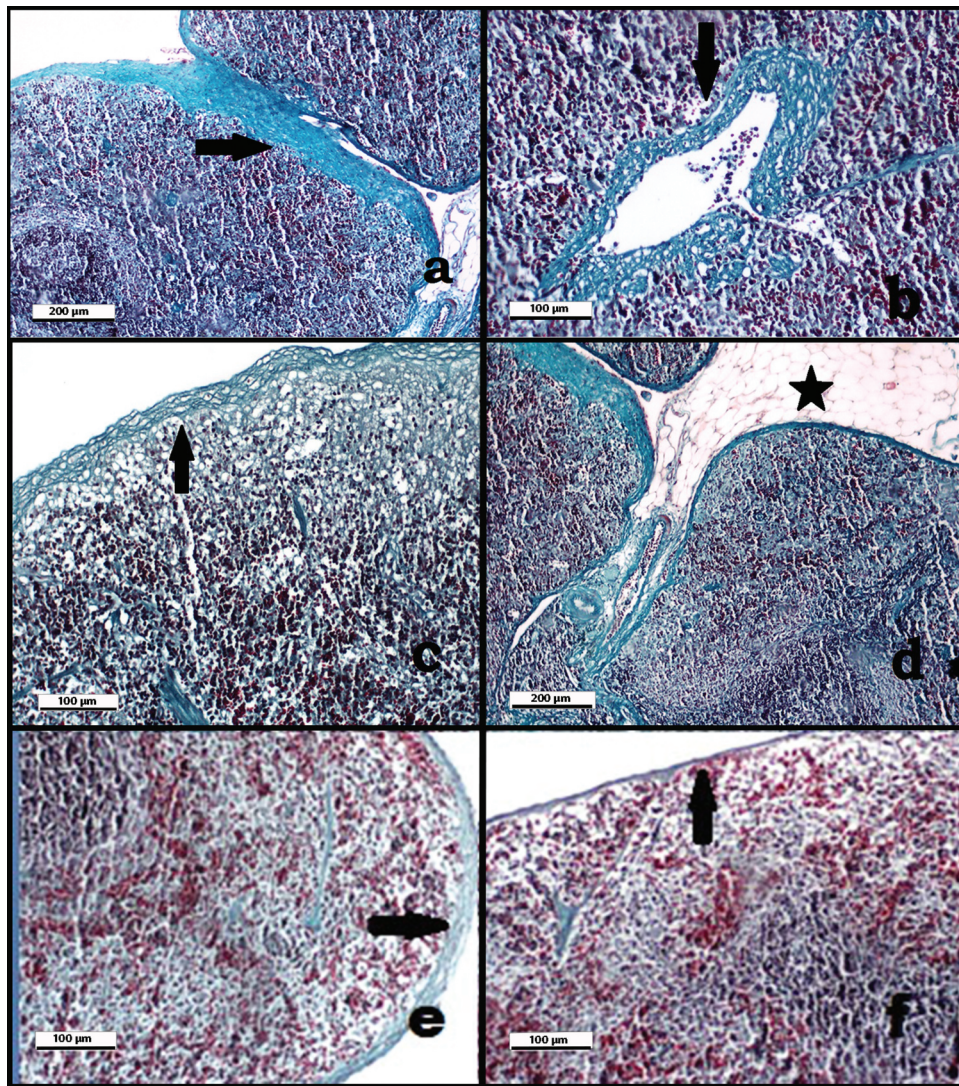


Figure 2. Masson's trichrome staining of collagen fibers. a) Fibrosis (arrow) in the trabeculae of group B. Bar = 200 µm. b) Collagen fiber accumulation (arrow) is evident around the dilated venous sinus in group B. Bar = 100 µm. c) Disorganized collagen fibers (arrow) in the capsule in group B. Bar = 100 µm. d) Lipid infiltration (star) and fibrosis in group B. Bar = 200 µm. e) The capsule (arrow) of the group C. Bar = 100 µm. f) No fibrosis (arrow) was observed in group C animals. Bar = 100 µm.

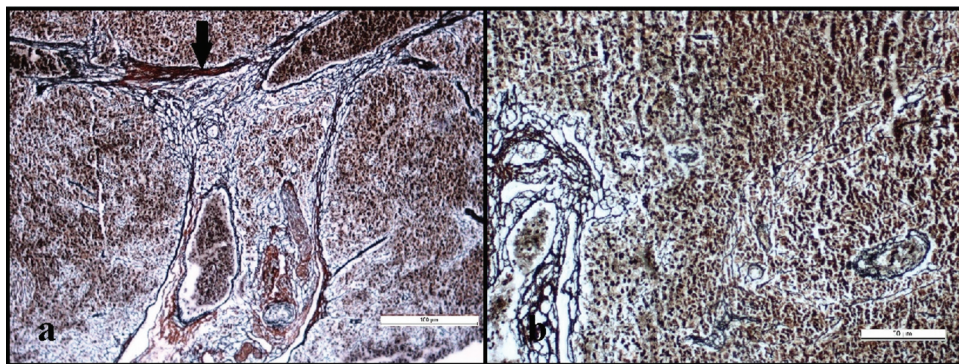


Figure 3. Reticular fibers stained with the Gomori's silver impregnation. a) Focal disorganized reticular fiber thickening in the fiber-dense areas in group B rats (arrow). Bar = 100 µm. b) Distribution of reticular fibers in group C. Treated animals resemble normal tissue. Bar = 100 µm.

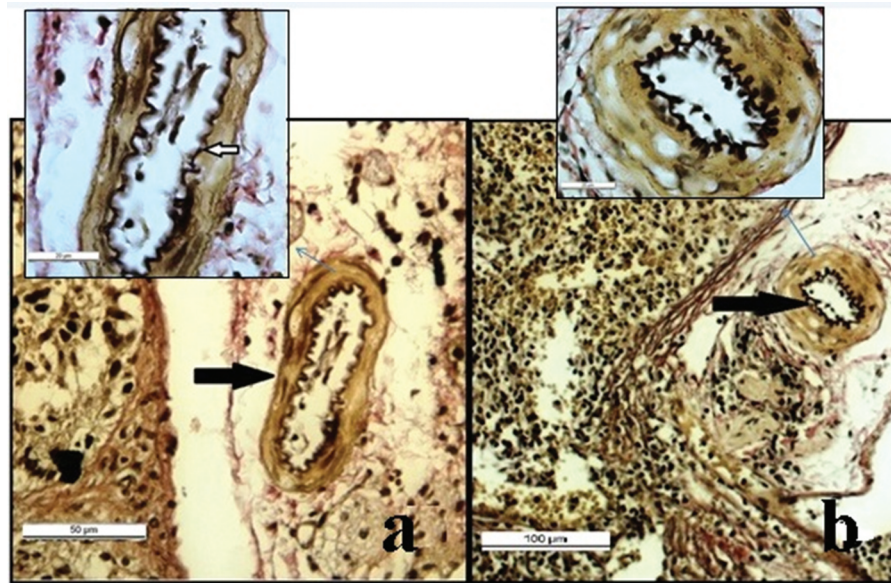


Figure 4. Elastic fibers (arrow) stained with van Gieson. a) Partial elastic fiber rupture (white arrow) in the internal lamina of splenic artery in group B animal. Bar = 50 µm. Inset, bar = 20 µm. b) Normal elastic fibers (arrow) group C animal. Bar = 100 µm. Inset, bar = 50 µm.

Discussion

Diabetes causes many histopathological changes in many organs; it is possible that changes of the fiber distribution in organs contribute to the pathogenesis of the disease. Because the spleen is the largest secondary lymphoid organ (Cesta 2006; Wluka and Olszewski 2006; Bronte and Pittet 2013; Tarantino et al. 2013), examination of the distribution of fibers in the spleen could clarify the grading of splenic tissue damage due to diabetes.

Collagen accumulation causes pathological disruption of organs (Sakakibara et al. 2011; Bailey et al. 2012; Park et al. 2012) and fibrosis (Binks et al. 2017; Abdullah and Jin 2018; Koo et al. 2018; Tian et al. 2018; Macoska et al. 2019). Juan et al. (1997) reported that chronic expression of murine flt3 ligand following bone marrow transplantation caused splenic necrosis with evidence of fibrosis in the splenic parenchyma and adjacent to the capsule. Necrosis and atypical cell infiltrates also were reported. Goodman et al. (1984) reported fibrosis in the splenic parenchyma and/or the capsule, and fatty infiltration in the spleen of male and female F344 rats fed a carcinogenic diet. Spleens removed from patients with advanced hepato-splenic schistosomiasis exhibited dilated venous sinuses, collagen accumulation and thickened splenic cords (Freitas et al. 1999).

Splenic cord thickening due to collagen accumulation may indicate organ dysfunction. Ebaid et al. (2015) reported that STZ induced diabetes

causes dilated blood vessels, collagen deposition, increased capsule thickness and fibrosis in spleen tissue. We observed accumulation of collagen fibers in group B, especially in the splenic capsule and around the trabeculae and dilated venous sinuses. Our findings are consistent with earlier reports. Also, lipid accumulation was observed in some sections from the diabetic group.

Reticular fibers in some pathological cases were increased (Harju et al. 2010), thickened (Gonçalves et al. 2015) or accumulated in small focal areas of the red pulp (Freitas et al. 1999; Egeblad et al. 2010). These fibers exhibited a disrupted network structure (Chyatte et al. 1990). It has been suggested that reticular fiber staining is a reliable method for diagnosis of challenging hepatocellular carcinoma cases (Hong et al. 2011). We found reticular fiber thickening in focal areas of rat spleen.

Trabecular thickening in mutant mouse spleen due to increased elastic fibers was found in SLC2A10 gene mutated mice compared to wild type mice. Also, an age-related increase in elastic fiber fragmentation and elastic lamina disruption were observed in the mutant mice (Cheng et al. 2009). Aging causes elastic fibers to shorten, fragment, thicken and decrease in number (Rodriguez et al. 1999). We observed partial fragmentation of elastic fibers in the internal lamina of splenic artery in our diabetic group.

Vitamin C deficiency leads to collagen accumulation in various tissues (Chang et al. 1980; Lubec et al. 1997; Sajithlal et al. 1998; Babu et al. 2006). These findings

suggest that exogenous vitamin C is important for preventing tissue damage due to fibrosis in diabetic patients.

Vitamin C exhibits beneficial effects in diabetics (Garcia Balio et al. 2011; RaFigurehi et al. 2013; Rochette et al. 2014; Valdés-Ramos et al. 2015). Vitamin C is an antioxidant that participates in synthesis of collagen and elastic fibers (Arrigoni and De Tullio 2002; Humbert et al. 2003). We found that the collagen fiber structure of group C was similar to group A. Group C exhibited no fibrosis and no lipid infiltration, and the integrity of the elastic fibers and reticular fiber organization were preserved. The dose of vitamin C that we used is protective under conditions of toxicity (Shaban and Said 2011; Viswanatha Swamay et al. 2011), cytogenetic damage (Assayed et al. 2010) and diabetes (Shivavedi et al. 2019).

Vitamin C exhibits beneficial effects on the distribution and structure of the connective tissue fibers in the diabetic spleen.

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Disclosure statement

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