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Bcl-2 Expression in Skeletal Muscle in Diabetic Rats

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Abstract: ***Diabetic myopathy is a complication characterized by a decrease in muscle mobility and strength, which varies according to the severity of diabetes.). In this study, histopathological changes and Bcl-2 expression in skeletal muscle in diabetic rats were shown. Rats were randomly assigned to two groups as control and diabetes group. Control group was allowed to feed at libitum for chow and water for 8 weeks. Single dose STZ (Streptozotocin 55mg/kg) was dissolved in sodium citrate buffer and intraperitoneally administered to diabetic rats. Glucose values of both groups were compared. Rats in diabetic groups has significantly higher glucose concentration (p<0.05). Atrophic areas with degenerative changes in muscle fibers were observed in the diabetes group. It is thought that the high number of picnotic nuclei induces nuclear apoptosis and may cause angiogenesis negatively with vascular dilatation and congestion. In our study, the increase in Bcl-2 expression in the nuclei located in the periphery of muscle fibers, although the degenerative effect of the nuclear structure was seen as a sign of the severity of diabetes, only induced a significant change in muscle structure. The involvement of extracellular matrix and connective tissue cells between the muscle fibers of diabetes has been considered as an important inducing effect in the pathology of diabetic myopathy.***

Keywords: Diabetic myopathy, rat, Bcl-2 antibody

1. Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by chronic hyperglyce-mia leading to long-term damage, dysfunction and failure of various organs, especially pancreas, heart, skeletal muscle and blood vessels. T2DM is initially caused by peripheral insulin resistance syndrome, i.e., the inability of insulin to stimulate glucose absorption in peripheral tissue, in association with the progressive failure of the pancreatic cells to supply a sufficient amount of insulin [1].

Skeletal muscle plays a crucial role in the development of insülin resistance because it is one of the major organs participating in the assimilation, storage and utilization of glucose provided by food intake(2). However, the skeletal musculature is also significantly involved in diabetic complications, that is, contractile weakness, fibre-type changes, decreased oxidative activity and peripheral insulin resistance (3) Muscle is the most important insulin-dependent glucose sink in the body(4), therefore, impaired hormonal signaling has a deleterious effect on glucose uptake.

Studies on insulin-resistance / Type-2 diabetes mellitus animal models showed that muscle regeneration deteriorate skeletal muscle plasticity, leading to serious changes to SC function.

Therefore the authors justify their outcomes by this central mechanism. As discussed, diabetes mellitus impinges on skeletal muscle health.

Uncontrolled diabetes causes characteristic muscle atrophy. The reason for it is elevated proteolysis and incapability to fix damaged skeletal muscle. Loss of skeletal muscle, with enhanced protein breakdown, has been demonstrated in rats with experimental diabetes (6, 7). Alongwith the increased proteolysis, the inability to repair damaged skeletal muscle is a characteristic feature of uncontrolled diabetes. (8)

Since hyperglycemia causes elevated free radicals and reduced antioxidant levels since it disrupts prooxidant and antioxidant balance. There is enough evidence that oxygen radicals contribute to the progression of diabetes and its complications (9), and promising strategies using antioxidant compounds to prevent oxidative damage in diabetes have been proposed (10, 11). As far as skeletal muscle metabolism is concerned, it has been demonstrated that oxidative stress affects the expression of the redox-sensitive genes involved in protein synthesis (12) and that in vitro H2O2 inhibits myogenesis at the level of muscle-specific protein expression (13). Type 2 diabetes mellitus leads to hyperglycemia, disrupts homeostasis, prevents inflammatory response and generates reactive oxygen species. Apoptosis is a cellular event that plays role in development and tissue process. Level of apoptosis varies in diabetes and depends on pro-apoptotic Bax and anti-apoptotic Bcl-2 (14). In this study, histopathological changes and Bcl-2 expression in skeletal muscle in diabetic rats were shown.

2. Material and Method

The present work was conducted in accordance with the guidelines for the Care and Use of Laboratory Animals from the Dicle University. The study was conducted as per approval of the Animal Experiments Local Ethics Committee, Dicle University. Experimental Animal Research Center. 20 adult male Wistar rats were randomly divided into 2 groups. Control group (n=10) was fed standard rat chow and drinking water for 8 weeks. Diabetic Control (DC) group (n=10):Single dose STZ (55 mg/kg), was dissolved in sodium citrate buffer (0.1 M, PH 4.5) and carried out intraperitoneal injection. The experiment subjects fasted for 12 hours, then started to be fed with standard rat chow and drinking water after 4 hours. After 2

days, 12 hours of fasting after the application from the tail end with the capillary blood glucose meter (Contour TS Bayer) hand blood glucose levels were measured and the value 250 mg / dl or above was taken to diabetic group. At the end of the experimental period, rats were anesthetized under ketamine+xylazin and sacrificed by cardiac puncture. Blood samples were taken for biochemical tests of rats. The animals were sacrificed by decapitation. Samples were collected included limbs skeletal muscle Specimen's from rats muscles were exiced and cut to small pieces .The muscle tissue were fixed with neutral buffered formalin solution Samples were directly dehydrated in a graded series of ethanol and embedded in paraffin wax. Next, 4–6 µm sections were cut with a microtome (Rotatory Microtome, Leica, RM 2265, Germany) and mounted on coated slides. The sections were stained with Haematoxylin and Eosin for observation by light microscopy (Nikon Eclipse 80i)

Immunohistochemical staining

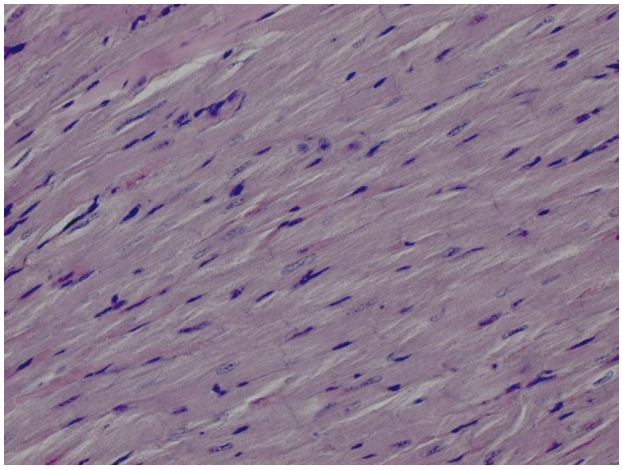
An antigen-retrieval process was performed in citrate buffer solution (pH 6.0) two times: first for 7 min, and then for 5 min in a microwave oven at 700 W. They were allowed to cool to room temperature for 30 min and washed in distilled water for 5 min twice. Endogenous peroxidase activity was blocked in 0.1% hydrogen peroxide for 15 min.Ultra V block (Histostain-Plus Kit, Invitrogen, Carlsbad, CA) was applied for 10 min prior to the application of the primary antibody (Bcl-2 antibody, mouse monoclonal, 1/200, Santa Cruz Biotechnology) overnight. The secondary antibody (Histostain-Plus Kit, Invitrogen, Carlsbad, CA) was applied for 20 minutes. Then the slides were exposed to streptavidin-peroxidase for 20 min. Diaminobenzidine (DAB, Invitrogen, Carlsbad) was used as a chromogen. Control slides were prepared with same procedure but no primary antibodies. After counterstaining with Harris Hematoxylene stain, slides washed in tap water for 5 min, and in distilled water for 2×5 min, mounted.

3. Results

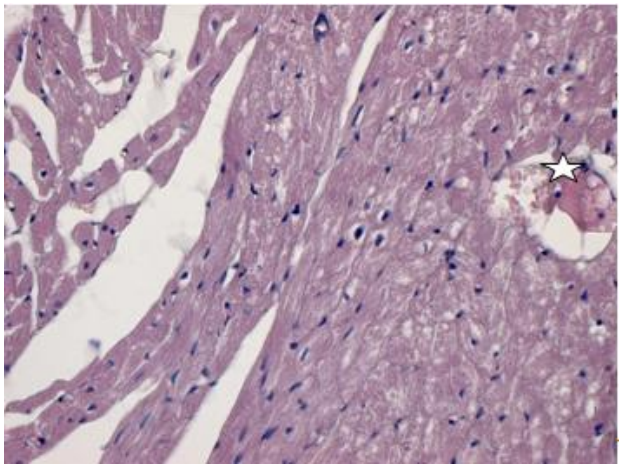
The serum glucose levels were found statistically significant between the two groups (Table I). The blood glucose levels of diabetic group showed significantly higher compared to control group. The blood glucose concentration in diabetic rats was significantly increased (p<0, 0001).

Table 1: Glucose levels of diabetic control group

Groups Average of blood glucose concentration (mg/dl)	Mean	Standard Deviation	Difference	Test statistic	
Non-diabetic group	96, 322	5, 246	301, 34	30, 044	**p<0, 0001**
Diabetic group	397, 122	27, 774			

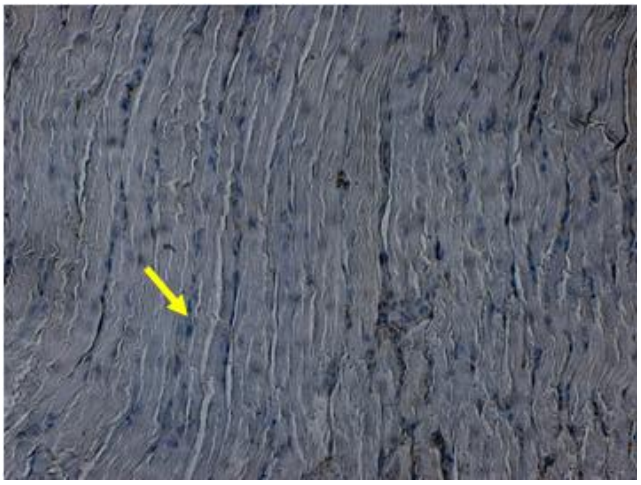


a

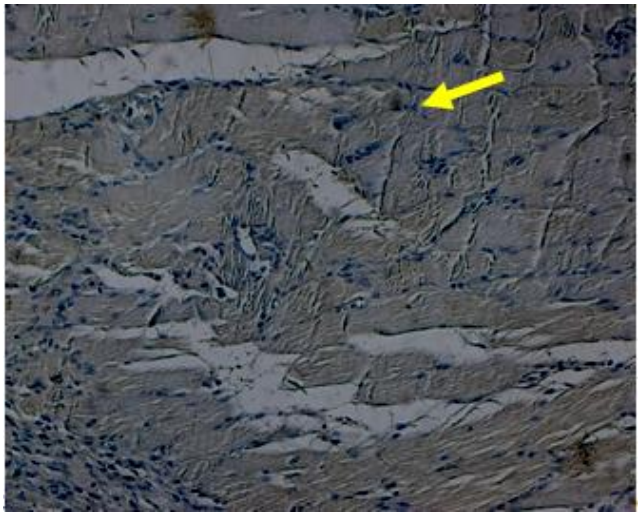


b

In the control group sections; fusiform nuclei between the longitudinal muscle fibers were found to be arranged in parallel.There was no change in the amount of extracellular matrix in which the fibroblast cells settled regularly in the connective tissue between muscles.Blood vessels were seen in flat vision of regular endothelial cells(Figure1a).**In the diabetes group sections;** Picnosis and vacuolar areas were observed in the nuclei located between the muscle fibers.Degenerative changes and atrophic areas between muscle fibers were detected in some areas.Excessive dilatation of blood vessels between muscle fibers and congestion(star) were observed in erythrocyte infiltration in the free state outside the vessel.



c



d

Control group: Negative Bcl-2 expression was observed in the nuclei located in the periphery of the longitudinally extending muscle fibers, while Bcl-2 expression was positive in some fibroblast cells.**Diabetes group;** Expansion of extracellular matrix between muscle fibers, blood vessels endothelial cells and nucleus of muscle cells and also some of the muscle fibers showed positive Bcl-2 expression.

4. Disscussion

Diabetic myopathy is a complication characterized by a decrease in muscle mobility and strength, which varies according to the severity of diabetes. Diabetes affects intracellular PH activity, resulting in decreased muscle strength in skeletal muscles(15).

Inhibition of extracellular matrix (ECM) turnover causes an elevation in plasma PAI-1 level, which is related to impaired diabetic regeneration. Due to this mechanism, macrophages and SC cannot migrate to damaged/necrotic area of injured muscle. Although PAI-1 is increased, muscular pattern showed impaired regeneration (16). Souza et al (17) elevated ECM levels have been demonstrated in a variety of diabetic tissues.The improper turnover of ECM proteins may also hinder growth factor signaling, further impeding myogenesis(18). In our study, the amount of extracellular matrix increased with detachment of muscle fibers(Figure1b). it was thought to induce diabetic myopathy.

In a streptozotocin induced rat model, the expression of MuRF1, E3 ubiquitin ligase, a mediator of skeletal muscle wasting in various skeletal muscle atrophy models, its expression is upregulated by oxidative stress in gastrocnemius muscle was detected by immunohistochemistry (19). In our study, atrophic areas with degenerative changes in muscle fibers were observed in the diabetes group. It is thought that the high number of picnotic nuclei induces nuclear apoptosis and may cause angiogenesis negatively with vascular dilatation and congestion.

Aragno et al (8) stated that oxidative stress reduces myogenesis. Their study revealed that expression of prominent myogenic factors (MyoD, myogenin and Jun D) was lower in STS-diabetic rodents than that of non-diabetic rodents in response to muscular injury. They also measured decreased expression level of muscular creatine kinase and myosin. Any interruption seen in early stages of regeneration (i.e. satellite cell functionality) initates a cascade eventually leading to muscular harm. In another study, it was shown that oxidative stress starts adipogenic transformation of muscle SCs (20).

Hyperglycemia induces apoptosis, causing damage to many organs and systems, including the reproductive system(21).Oxidative stress due to hyperglycemia has been reported to play a major role in the onset of apoptosis(22, 23).DM induces apoptosis by regulating signaling molecules such as Bcl-2 / Bax / Caspas-9 in the apoptosis pathway. Bcl-2 is a protein located in the inner membrane of mitochondria and blocks apoptosis. Transition of cytochrome c from mitochondria to cytoplasm inhibits free radical production. Galkowska et al demonstrated that apoptosis in the endothelial cells of diabetic ulcers and overexpression of Bcl-2(24). In our study, the increase in Bcl-2 expression in the nuclei located in the periphery of muscle fibers, although the degenerative effect of the nuclear structure was seen as a sign of the severity of diabetes, only induced a significant change in muscle structure.Separation of muscle fibers and increase in connective tissue, positivity of Bcl-2 expression in fibroblast cells in connective tissue areas induced apoptotic development. In particular, the involvement of extracellular matrix and connective tissue cells between the muscle fibers of diabetes has been considered as an important inducing effect in the pathology of diabetic myopathy.

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