The Effect of Swimming Exercise on Experimental Diabetic Myopathy in Rats

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Abstract: Regular exercise is known to increase insulin sensitivity and improve the glucose uptake and lower body adiposity is a powerful non-pharmacological tool for prevention and treatment of Diabetes mellitus and therefore is highly recommended in the type I and type II diabetics. Diabetic myopathy is the main cause of muscular system failure. The purpose of this study was to evaluate the effect of swimming exercise on diabetic myopathy. In this study, 56 wistar rats with approximate age of 12 weeks and 200-300g weight were allocated into two equal groups (treatment and control). For induction of diabetes, these two groups were intraperitoneally injected by streptozotocin (50 mg/kg). The treatment group were kept in normal conditions of management with swimming exercise for 12 weeks; 5 days in a week, an hour every day. control group had normal conditions (food and place) without any physical activity/swimming exercise. After 12 weeks muscles tissues were sampled in both groups and 5-6 micron tissue sections were prepared through H and E staining method. Histopathological study of representative sections in control group showed muscular atrophy, degeneration, necrosis and leukocyte infiltration. Mild pathological changes in treatment group were observed. Differences of histopathological changes between the experimental groups were significant. It was concluded that swimming exercise is capable in reduce of pathological changes and it improved improvement of diabetic myopathy to near normal condition.

Key words: Diabetic myopathy • Diabetes and swimming exercise • Diabetes mellitus

INTRODUCTION

Diabetes mellitus is the most common metabolic disease characterized by hyperglycemia resulted from relative or absolute deficiency of insulin Over 150million individual in world and nearly 3million people in Iran are suffering from diabetes and it is expected that this number reached to 221million in 2010 [1]. According to the prediction of world health organization (WHO) adult wise, this number will reach to 300 million in 2025. while, the reduction of blood glucose in these patients with standard procedures and use of chemical drug are not still sufficient for prevention of diabetes complications such as muscular and arthrocardiac disorders ophthalimic disease, neuropathy and renal failure and it appears that for treatment of diabetes which is now considered as a common epidemy another methods should be researched for. Three types of lesions in diabetes myopathy is considered: 1-cellular lesions 2-atrophy 3-myositis [1]. the most important cellular lesions is atrophic changes and cellular degeneration which is consisted of obvious reduction of diameter and length of muscular fibers and formation of vacuoles in sarcoplasm of muscular cells.

Generally, it seems that there are two processes which play role in creating muscular tissue lesions. One manner is metabolic defect that occurs in all patients and probably it is related to advanced glycosilated products which are responsible for atrophic changes and cellular degenerations. Another manner is increasing oxidative stress which is the most important factor for cellular death. Muscular cells in diabetic patients may suffer from degenerative changes due to over accumulation of glycogen. Sometimes in acute hyperglycemiacconditions, smolality of muscular cell increase and causes

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degenerative changes in cells. The above-mentioned collection demonstrates the importance of diabetes in expression of muscular lesions. How ever, one of the therapeutic and preventive ways for complications of this deficiency is regular daily physical activities and that is why, practice is proposed as an important therapeutic program for the treatment of diabetes disease [1].

The goal of this work was to study the protective role of swimming in experimental diabetic myopathy in rats in order to characterize whether regular practices of swimming could effect on reduction of muscular complications in diabetic patients.

MATERIAL AND METHODS

A total number of 56 rat of three month old from vistar strain weighted 250-300 g weight was randomly selected and distributed into two groups of 28. Feeding and maintenance conditions were the same for the groups. Single-dose streptozotocin (50 mg/kg as intraperitoneal injection) was used for induction of diabetes. 218 hours after injection, to ensure creation of diabetes in mice, one drop from tail vein blood was collected and glucose level was determined by Glucometer system (boehringer mannheim Indianapolis IN). The blood glucose level over 300 mg/dl was considered as the index of suffering from diabetes.

Swimming was performed for 12 weeks, 5 days per week and one hour per day. At the end of 12th week, both group’s members were killed by cervical vertebra dislocation and tissue samples of gastrocnimus muscle were obtained and after fixing in 10% buffered formalin, section were prepared with 5-6 µm thickness and stained with hematoxilin and eosine [1, 2]. Results are analysed using SPSS software 13th edition and the mann-whitney Utest. The differences with P<0.05 are considered significant.

RESULTS

Studies of Tissue Pathology: Atrophic changes were observed in different parts of muscular tissue of control mice. Pathologically, these changes were as obvious reduction in size of muscular cell associated with vacuolization of sarcoplasm. Above mentioned changes were very mild and often insignificant at treated group. (Figure 1/5). Some times, accumulation of inflammatory mononuclear cells (macrophages and lymphocytes) associated with infiltration of adipose tissue and fibrosis in muscular tissue were sometimes seen in the control mice which were not seen in treated group (Figure 2/3).

Fig. 1: Microscopic profile of gastrocnimus muscular tissue of blank mice in which degenerative changes and degradation of muscular cells(arrow 2). Associated with Zinker Necrosis (arrow 3) and mononuclear infiltration in the interstitial space muscular tissue (arrow 1) are obvious. Specially, note the denaturation of sarcoplasm proteins of muscular cells.(hematoxilin-eosin staining / magnification × 100)

Fig. 2: Microscopic profile of gastrocnimus muscular tissue of blank mice in which the adipose infiltration (arrow 1) and deposition of extra cellular matrix (arrow 2) are observable (hematoxilin-eosin staining, magnification × 10)

Fig. 3: Microscopic profile of gastrocnimus muscular tissue of blank mouse in which infiltration of mononuclear cells and collagen deposition that is initiation of formation of fibrosis (arrow 2) and muscular tissue atrophy (arrow 1). (hematoxilin-eosin staining, magnification × 100)
Fig. 4: Microscopic profile of muscular tissue of treated mice. In some regions, edema in interstitial space (arrow 2) associated with very mild accumulation of inflammatory cells (arrow 1) in much less number in comparison with the blank group is still observable. (hematoxilin-eosin staining, magnification × 20)

Fig. 5: Microscopic profile of muscular tissue of treated group in which edema of interstitial space (arrow 1) and sometimes necrosis of muscular fibrils (arrow 2) associated with very mild infiltration of mononuclear cells in comparison with blank group is observable. (hematoxilin-eosin staining, magnification × 100)

Fig. 6: Necrosis of muscular fibriles (arrow 2) associated with very mild infiltration of mononuclear cells in comparison with blank group is observable. (hematoxilin-eosin staining, magnification × 100)

Fig. 7: Microscopic profile of muscular tissue of treated group which indicates normal histologic structure and in comparison with control group there is not tissue injuries. (hematoxilin-eosin staining, magnification × 100)

Cellular degenerative changes were obvious as formation of vacuoles in sacroplasm (Figure 3). Cellular necrosis was frequently observed as zinker of coagulation necrosis with the characteristics of kariopycnosis and

Diagram 1: The graduated mean of histopathologic changes of muscular tissue of treated and blank group (n = 28). Data expressed as mean ± SEM. P<0.01 and P<0.001 in comparison with blank group.
Table 1: Mean of tissue changes in muscular of mice in treated group data express as mean ± SEM

<table>
<thead>
<tr>
<th>Dependent parameters</th>
<th>Error deviation</th>
<th>Standard deviation</th>
<th>Error Deviation ± mean</th>
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<tbody>
<tr>
<td>Degeneration</td>
<td>0/2134</td>
<td>0/6749</td>
<td>1/3 ± 0/2134</td>
</tr>
<tr>
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<td>0/6749</td>
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<tr>
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<td>Extra cellular matrix deposition</td>
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<td>0/6325</td>
<td>0/8 ± 0/2</td>
</tr>
<tr>
<td>Mononuclear infiltration</td>
<td>0/2</td>
<td>0/6325</td>
<td>0/8 ± 0/2</td>
</tr>
<tr>
<td>Interstitial tissue edema</td>
<td>0/2211</td>
<td>0/6992</td>
<td>1/4 ± 0/2211</td>
</tr>
<tr>
<td>Muscular atrophy</td>
<td>0/2108</td>
<td>0/6667</td>
<td>1 ± 0/2108</td>
</tr>
</tbody>
</table>

Table 2: mean of tissue changes in muscles of mice in blank group data expressed as mean ± SEM

<table>
<thead>
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<th>Dependent parameters</th>
<th>Error deviation</th>
<th>Standard deviation</th>
<th>Error Deviation ± mean</th>
</tr>
</thead>
<tbody>
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<td>Degeneration</td>
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<td>0/7071</td>
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<tr>
<td>Mononuclear infiltration</td>
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<td>Interstitial tissue edema</td>
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<td>0/3162</td>
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<tr>
<td>Muscular atrophy</td>
<td>0/2211</td>
<td>0/6992</td>
<td>2/4 ± 0/2211</td>
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kariolysis. These mentioned cellular injuries were often observed in soleous and gastrocnimus muscles. Microscopic examinations demonstrated that above cellular injuries were larger and much more evident in non treated group.

Deposition of extracellular matrix and adipose infiltration were observed in muscular tissue of blank group (Figure 3) which were not seen at treated group.

Statistical analyses of data in both groups revealed that the difference of histopathologic changes such as degeneration, necrosis, vacuolization of sarcoplasm-deposition of extra cellular matrix-infiltration of mononuclear cells and edema in treated and blank groups were significant (diagram 1). Data resulted from statistical analyses presented as Mean ± SEM in Tables 1 and 2.

**DISCUSSION**

Diabetic myopathy as a very important lesion has been studied by many scientists and attempt to reduction of muscular tissue lesions in diabetic patients has been a dream for many researchers all over the world for so many years. Several drugs have been used in this field, but have not been able to reduce complications resulted from diabetes in muscular tissue till now but recently physical activities and regular practices are suggested in this regard. This study investigated the effectiveness of regular practice such as swimming in reduction of diabetic myopathy lesions such as degenerations, necrosis, deposition of extra cellular matrix (muscle tissue fibrosis) and so on. The effects of swimming on reduction of severity of myopathic complication has been reviewed by several researchers.

Camerone et al. [3] reported that muscular injuries induced by diabetes disease put these cells into the risk of cellular death and transformation. This finding coincide with the present result of the control group. Also, other mechanisms may contribute to induction of cellular death among which oxidative stresses. Factors such as free toxic oxygen resulting from oxidating stress / inactivation of extra cellular signal _ regulated kinase (ERK 1, 2) and avtiation of C_jun aminoterminal kinase_1 [3-5] (C_Jun/ C_Jun/ AP_1) can be initiation of cellular death over accumulation of extra cellular matrix can be affect muscular tissue atrophy. This cellular change probably affect induce programized death that referred as apoptosis. This is a question that Challiss et al. [6] answered and it was in harmony with results of present survey. They described that inhibitory role of glycogen synthase kinase (Gsk 3ß) is suppressed with initiation of cellular death by TNF.a. path way by over accumulation of glycogen in the muscular cells and are involved with P65 phosphorylation and expression of NF.KB gene [6, 7]. Thus, major factor of cellular injury in the diabetic myopathy is oxidative stress which can be reduced by swimming, in general and this is the same subject that Cotter et al. [6, 7]. It was described that practice is a very important factor at the reduction of injuries induced process by diabetes disease. Also, with comparing the results of treated and blank groups in this study, the mentioned issue is observable [8].
The level of expression of insulin-like growth factor-II (IGF-II) gene increases in muscular tissue after physical activities. Since this factor has a protective role in muscular cells and is affective on improvement of hypoglycemia and increase of insulin sensitivity in muscles and muscular tissues, the positive role of practice in reducing of diabetic complications and correlation of the results of this study with the result of other studies were confirmed. It should be considered that IGF-II is an agonist of insulin and can be important in induced muscular carcinosarcoma of diabetic myopathy for supplying very large consumed glucose by cancer cells. Therefore, it was concluded that this factor does not always have a positive role and it is better to start physical activities at early stages of the disease and permanent and regular basis. However, if the patient is affected by neoplastic changes, unfortunately this program won't have an affective role [1].

Ehrlich et al. [11] for the first time referred to glycogen accumulation in cells nucleus and described that this case has occurred not only in diabetes, but also in tuberculosis, septisemia, hepatitis and autoimmune diseases. Moreover, they described that over accumulation of glycogen can cause irreversible cell injuries. Induced injuries by over accumulation of glycogen with formation of fibrosis tissue following abundant deposition of extra cellular matrix is very clearly obvious [9, 10]. Physical activities cause reduction in glucose in take by muscular cells with inducing hypoglycemia and the result is that the occurrence of glycogen storage disease reduces [11]. Therefore, the positive role of practice is characterized by reduction of complications for diabetes which is confirmed through the results of the present study [5].

Regarding, glycosilated hemoglobin reduction after swimming glucose chemically links to amine groups and proteins without enzyme activities by accuracy of non-enzymatic glycosilation and glycosilated products are produced. These products may rearrange and create early and stable glycosilated products so-called amadory type. Rating of non enzymatic glycosilation is directly dependent on blood glucose level. Instead of destroyling, new glycosilated products on the collagen and other long life protein in intirstial tissues and membrane of blood vessels are more involved in slow chemical changes and are rearranged which at least, irreversible and advanced glycosilated end products are created, referred as Advanced Glycosylated Hemoglobin(AGE). AGE formation is accured on proteins, lipids and nucleic acids. These materials cause cross-reaction between collagen molecule poly peptides and capturing of plasma or interstitial proteins. In large vessels for the example, low density lipo proteins capturing causes the emerging from membrane occurs slowly and Cholestrol deposition in intima increases and thus atherogenesis accelerates. Plasma proteins such as albumin are linked to glycosilated base membrane and this manner is to some extent responsible for thickness ancreasing of base membrane which is characteristic of diabetic microangiopathy. Proteins that cross-reaction with AGE, are resistant to proteolytic digestion are resistant. Therefore, cross-reaction linkages not only cause reduction of proteins in take, but also cause accumulation of them. Also cross-reaction linkages of AGE with collagen type 4 of base membrane may disturb reactions between collagen and other components of interstitial substances (laminin, proteoglycans) and lead to structural and functional defects of base membrane and interstitial tissue. AGE are linked with many receptors on cells surface (endothelium, monocytes, microphages, linnphocytes and mesenchimal cells). These linkages cause activation of a numerous series from biologic activities consisting of monocyte migration, releasing of cytokins and growth factors from macrophages, increasing of endothelial permeability, increasing of coagulative property of endothelial and macrophages surface and increasing of production and proliferation extra cellular matrix by fibroblasts and smooth muscle cells. Regarding above mentioned collection and gained results from this study, the reduction of vessels lesions can follow the reduction of AGE formation after swimming [1, 12, 13].

Stephen et al. [19] described the role and effect of diabetes disease on muscular tissue structure. The results of their studies demonstrated that injuries of vessels such as microangiopathy were due to increasing of oxidative stress, high density lipo proteins level reduction, increasing of proteins glycosilation, Hyperglycemia and increasing of fats peroxidation and 8-iso PgF2a production. In addition to vessels changes, increasing of connective matrix of interstitial tissue of muscular cells is also observable. Studies of Doustar et al. [1], Delissio et al. [14] Chiasevra et al. [15], Stephen et al. [19] and Zineman et al. [20] showed the role of regular practice in reduction of diabetes complications which correlated with gained results by present study in most of the cases and well explained significant result in it [14, 15]. One of the very important growth factor which is transforming growth factor beta (TGF-ß) which has a role in diabetic myopathy. Hyperglycemia is one of the most important inducing factors of production and releasing of TGF-ß from
myofibroblast cells differentiated from muscular cells. Severe increasing of blood glucose causes stimulation of myofibroblast proliferation and thus, producing high level collagen type 1, 4 and TGF-ß, physical activities can prevent myoblast cells proliferation by increasing of insulin sensitivity and inducing of hypoglycemia [3, 6, 16]. By this way, physical activities suppress production and releasing of TGF-ß and collagens type 1, 4 [2, 12, 17, 18]. Practice certainly causes suppressing of production and releasing of TGF-ß with blood glucose controlling and reduces process of muscular tissue fibrosis in diabetic patients. In this study, at fibrosis occurrence in muscular tissue there was a significant difference between treated and blank groups which is in accordance with above mentioned findings [1, 14, 15, 19, 20].

Due to overpresence of calcium ions throughout cell membrane, cytosol and mitochondria, activation of calpain factor and more expression of Bax protein in diabetic patient, cell death occurs while regular Performing of physical activities establishes the equilibrium at level of calcium ion in muscular cell sarcoplasm which helps to prevent effects of diabetes on cellular injuries [1, 12, 21-23]. In general swimming with different mechanisms has positive and effective role in improvement of diabetic myopathy.

It was concluded that swimming can be a treatment and preventive way for diabetes complications in patients muscles, but exploring more effective reasons about swimming role and it is impression on diabetic myopathy necessitates more expansive studies.

REFERENCES


