Haliç Üniversitesi Sağlık Bilimleri Dergisi 2019,2: 59-68

Deneysel Diyabette Testis Dokusunun Histolojik Olarak İncelenmesi

Pınar KÖROĞLU [1]*

[1] Haliç Üniversitesi, Tıp Fakültesi, Histoloji Embriyoloji Anabilim Dalı, İstanbul, Türkiye

Geliş Tarihi: 02.11.2018
***Sorumlu Yazar e mail:** pinarkoroglu@halic.edu.tr **Kabul Tarihi:** 27.12.2018

Özet

Diyabet kronik metabolik bir hastalıktır. Diyabet, sperm sayısında ve sperm motilitesindeki azalmaya bağlı olarak infertiliteye neden olan bir faktördür. Streptozosin (STZ) deneysel diyabet oluşturmak için en sık kullanılan ajanlardan biridir. İntraperitoneal (i.p.), subkutan, intravenöz (i.v.), parenteral verilmesiyle diyabet oluşturulabilir. STZ pankreasta Langerhans adacıklarındaki beta hücresini tahrip ederek, hipoinsülinemik ve hiperglisemik duruma neden olurlar. Bu çalışmanın amacı diyabetin testis dokusu üzerindeki etkisini araştırmaktır. Wistar albino erkek sıçanlar rastgele iki gruba ayrıldı. 1) Kontrol grubu: 0,3 ml serum fizyolojik solüsyonu i.p. olarak enjekte edildi, 2) Diyabet grubu: tek doz STZ (65 mg / kg) i.p. enjekte edildi. Hayvanlar mevcut yiyecek ve su ile beslenerek normal şartlarında tutuldu. Kan örnekleri başlangıçta ve haftada bir kez olmak üzere el glukometresi ile ölçüldü. Dördüncü haftanın sonunda sıçanlar sakrifiye edildi ve kan örnekleri değerlendirildi. Doku kesitleri, ışık mikroskopik seviyesinde testiküler yapıyı değerlendirmek için Hematoksilen Eosin ile boyandı. Periodic asit-Schiff (PAS) reaksiyonu uygulanarak intersisyel bağ doku ve bazal membran değişiklikleri belirlendi. Kontrol grubunda normal bir testis morfolojisi ve düzenli seminifer tübülleri vardı. Diyabetik grupta, regresif ve dejeneratif tubüllerin sayısı kontrol grubuna göre anlamlı olarak artmıştır. Bulgularımız diyabetin testis morfolojisini etkilediğini gösterdi. Sonuç olarak diyabetin birçok dokuda olduğu gibi testis dokusunda da oksidatif stres oluşturmak sureti ile ciddi hasarlar meydana getirdiği saptanmıştır. Diyabet kaynaklı testiste oluşan dejeneratif hasarın seminifer tübüllerin genel yapısında, tübül içerisindeki

spermatogenetik seri hücrelerinin varlığında ve interstisyel alanın görünümü yönünden ciddi mikroskobik hasar yarattığı saptanmıştır.

Anahtar Kelimeler: Deneysel diyabet, testis, histopatoloji.

Histological Investigation of Testes Tissue in Experimental Diabetes

Abstract

Diabetes is a chronic metabolic disease. Diabetes is a factor that causes infertility due to decrease in the number of sperm and the reduction of sperm motility. Streptozocin (STZ) is one of the most commonly used agents to produce experimental diabetes. Intraperitoneal, subcutaneous, intravenous (i.v.), parenteral administration may cause diabetes. STZ destroys the beta cell in the islets of Langerhans and cause hypoinsulinemic and hyperglycemic conditions. The aim of this study is to investigate the effect of diabetes on testicular tissue. Wistar albino male rats were randomly divided into two groups. 1) Control group: 0,3 ml saline solution was injected intraperitoneally (ip), 2) Diabetic group: single dose of STZ (65 mg/kg) was injected ip. Animals were kept under normal conditions of feeding with available food/water. Blood samples were measured by glucometer at the beginning and once a week during the study. At the end of the fourth week rats were sacrified and blood samples were assessed. Tissue sections were stained with Haematoxylin-Eosin to evaluate the testicular structure. It was applied Periodic Acid-Schiff (PAS) reaction to examine the alterations in the interstitial connective tissue and basement membrane. Control group had a normal testicular morphology and regular seminiferous tubules. In diabetic group, the number of regressive and degenerative tubules significantly increased compared to control group. Our results showed that diabetes affect testicular morphology. In conclusion, like many tissues, diabetes also causes serious damage testis tissue by causing oxidative stress. Testicular damage caused by diabetes was determined microscopically in terms of the general structure of the seminiferous tubules, the presence of spermatogenetic series cells in the tubule and the appearance of the interstitial area.

Keywords: Experimental diabetes, testes, histopathologic, STZ.

1.Introduction

Diabetes directly affects men in a large number of reproductive age and leads to severe reproductive disorders. However, the underlying mechanisms still remain unknown (1). Diabetes is a metabolic

disorder known for its acute and chronic complications that have adverse effects on the male reproductive system. There are experimental and clinical studies on diabetes related male infertility. Approximately 90 % of diabetic patients have been reported in several studies with a decrease in sexual functions (erection, ejaculation and libido), testicular structural and functional disorders as well as spermatogenesis disorders. It has been suggested that diabetes with several mechanisms induces testicular damage especially cell death and apoptosis, but the actual mechanism has not yet been clarified. One of the possible mechanisms is hyperglycemia that can increase the ROS overproduction and cause cell apoptosis in testis (2). Many adversities in male patients due to diabetes lead researchers to solve the unknowns related to the use of experimental diabetes models. Studies on this subject are increasing day by day. Several studies have shown that histopathological changes and abnormal spermatogenesis have occurred in testicular tissues of diabetic men, with a decrease in testosterone levels and the contribution of apoptosis (3,4,5).

2. Materials and Methods

2.1. Animals

Adult male Wistar Albino rats weighing 250–350 g were housed individually in a light- and temperature-controlled room on a12 h/12 h light–dark cycle and fed a standard pellet lab chow. All experimental protocols were approved by the Animal Care and Use Committee of the Marmara University, School of Medicine, Istanbul, Turkey (20.11.2011-55. 2001. mar) (the number of decisions ethic committee).

2.2. Experimental procedures

(I) Control Group (n:6): 1 ml of saline solution was daily injected intraperitoneally (i.p.) for 5 days, (II) STZ Group (n:6): a single dose

of STZ was injected i.p. Diabetes was induced by 65 mg/kg intraperitoneal injection of streptozotocin (65 mg/kg, freshly dissolved in 1 ml of saline solution). After 72 h, animals with blood glucose concentrations of ≥250 mg/dLwere considered to be diabetic and included in the study. Animals were weighed before and after experiment. At the end of the experimental study, all animals were sacrified under ether anesthesia. Testes tissue samples were taken to be processed for light microscopy.

2.3. Histopathological evaluations

For light microscopic investigations, samples from the left testes were immersed in 10% formalin solution and processed routinely following embedding in paraffin. Tissue sections (5 micrometer) were stained with hematoxylin and eosin (H&E) for histopathological scoring and Periodic Acid-Schiff (PAS) reaction to examine the alterations in the interstitial connective tissue and basement membrane. Stained sections from all animals were analyzed. Five similar areas were selected randomly and examined at ×200 magnification. Histopathological scoring was done according to Hess's data and organized as normal, regressive, degenerative or atrophic tubules (6). Normal tubules depict normal morphology of spermatogenesis and intercellular junctions. Regressive tubules possess seminiferous tubules with one or more abnormalities; however, intercellular tight junctions are normal, the cellular organization is loose and disrupted (picnotic nucleus, granular eosinophilic cytoplasm, and karyolysis). Degenerative tubules represent irregular arrangements of both germ cells and Sertoli cells with disrupted intercellular tight junctions, and atrophic tubules represent either Sertoli cells or a few germ cells and Sertoli cells with loss of tight junctions. All light microscopic sections were observed and photographed with a digital camera (Olympus C-5060, Tokyo, Japan) attached to a photomicroscope (Olympus BX51, Tokyo, Japan).

2.4. Statistical analysis

All statistical analyses were done with SPSS (IBM, Newyork, USA) and Graph-Pad Prism 3.0 (GraphPad Software, San Diego, CA, USA). Within the groups, comparisons were carried out by paired t-test and comparisons between the groups were performed using independent t-test. Data were expressed as mean ± standard deviation (SD). Significance of differences was taken as the level of $p < 0.05$.

3. Results

Testes and body weights Total body weight of rats from STZ Group significantly was reduced at the end of the experiment comparing to the first day of the experiment ($p < 0.05$) (Fig. 1). Moreover, it was significantly reduced different from the Control Group at the end of the experimental procedure ($p < 0.05$). There was a decrease in testicular weight of experimental ani-mals of STZ Group compared to Control Group ($p < 0.001$). Testicular weights and body weights of the animals at the first day and final day of the experimental procedure were given in Table 1.

Table 1: Body weight and testis weight for each group:

	Control group	Diabetic group
Body weight (g)	305 ± 20	260 ± 8.94
Testes weight (g)	3.20 ± 0.24	1.98 ± 0.31
Values were expressed as mean±SD, n = 6 for each group		

Testis tissue of the Control Group showed a normal testicular morphology and regular seminiferous tubules with normal spermatogenesis process. Testis of STZ Group has represented disrupted with regressive, degenerative and atrophic seminiferous tubules, cellular debris in the lumen and damaged spermatogenic cells. Damaged basal membranes of seminiferous tubules presented a decrease in PAS positivity (Figure 1).

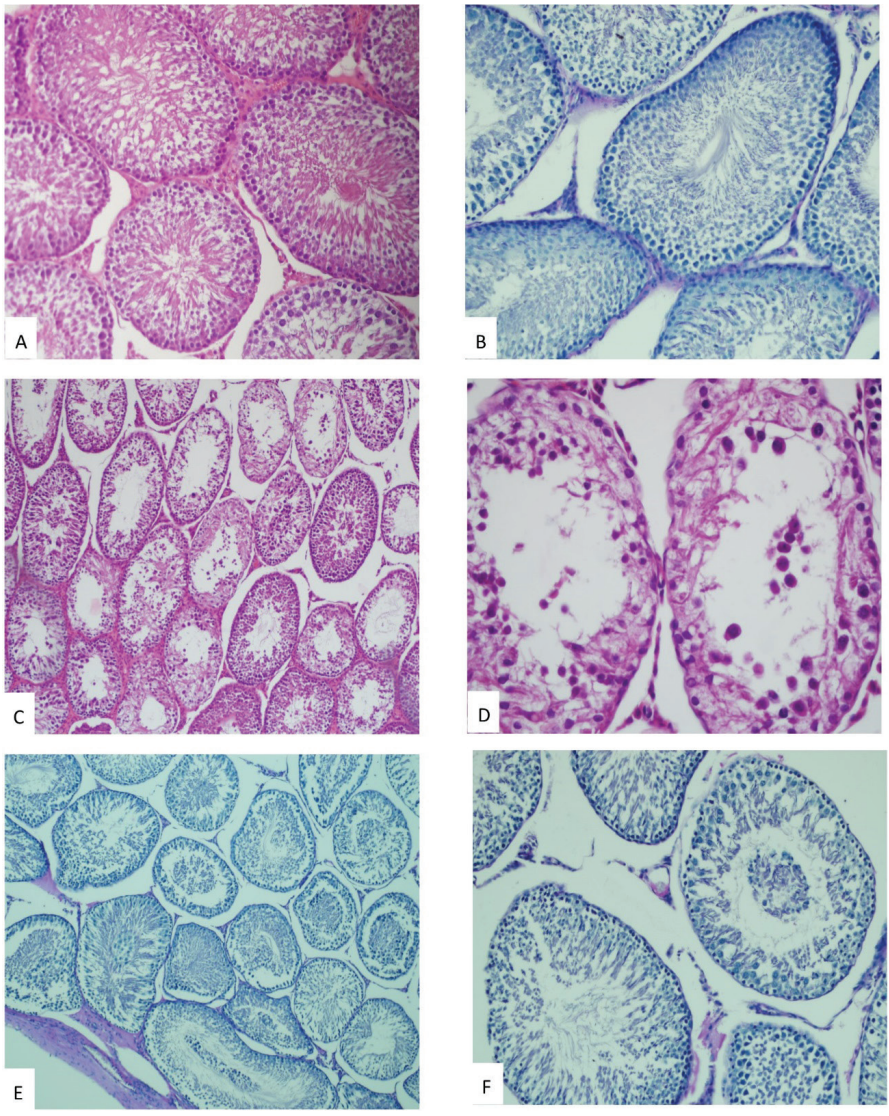

Figure 1: Control Group: (A) Normal morphology in seminiferous tubules, spermatogenic cells and spermatozoa in lumen of the seminiferous tubules, (B) intensive PAS (+) reaction in seminiferous tubules and regular basal membrane, **Diabetic Group:** (C, D) Regressive, degenerative and atrophic seminiferous tubules, cellular debris in the lumen and damaged spermatogenic cells, (E, F) Decreased PAS reaction in seminiferous tubules. **A, C, D:** H&E staining; **B, E, F:** PAS reaction., 200× magnification; bar indicates 50 µm. 400× magnification; bar indicates 20 µm.

4. Discussion

There is an increasing focus on association between male infertility and metabolic disorder such as diabetes. Diabetes is a metabolic disease characterized by disorders of carbohydrate, lipid and protein metabolism known for its acute and chronic complications of insulin secretion and chronic hyperglycemia (7). Diabetes is an endocrine disorder which is closely related to 347 million people worldwide in 2008 and is expected to increase twice as much as 2050. Diabetes can cause varios complications in all organs, especially in the male reproductive system. Some mechanisms may declare he reproductive system damage observed in patients with diabetes mellitus (8). Oxidative stress is responsible for the initiation of various diabetic complications such as vascular diseases, kidney damage and reproductive dysfunction. Diabetes leads to reproductive system dysfunction, such as testicular dysfunction in both humans and animals. Diabetes also induces structural changes in testes and spermatozoa and may stimulate germ cell apoptosis, impaired sperm parameters, and hormonal changes that cause infertility (9). Male reproductive disorders and associated pathological disorders have been found to have a significant increase in the population. Life style and environmental toxic factors are the leading causes of male reproductive disorders (10). This study was designed to assess the effect of diabetes on testicular damage in STZ diabetic rats and to determine the possible involvement of histopathological damage, oxidative stress, inflammation and apoptosis.

STZ induced diabetic model is widely used to evaluate the adverse effects of diabetes on spermatogenesis and testicular steroidogenesis. However, the actual mechanism of infertility in diabetic males has still not clear (11). Diabetes mellitus is a severe metabolic disorder. STZ, as an diabetogenic agents, induces DNA strand breaks in pancreatic beta-cell through the formation of free radicals. Diabetic male infertility is a remarkable research topic in recent years. Subfertility or infertility

in diabetic male has become a major problem recently (12, 13,14). Zha et al. also supported this finding by diabetic. 40 mg/kg STZ after being fed a high-fat diet for 8 weeks, histological changes in the testes were determined. It was suggested that degeneration and disruption of seminiferous tubule structure were observed in diabetic rats (15).

5. Conclusions

In this study, the results demonstrated that diabetic male rat has severity of testicular damage histopathology. Testicular weight was significantly reduced in diabetic group compared to the control group. In addition, STZ adversely affected sperm parameters. Especially, it could cause testes basal membrane damage. Our results suggest that diabetes affects testicular damage significantly. In conclusion, diabetes is one of the major causes of male infertility. In experimental animal models, STZ, has several negative effects on male infertility and diabetes. Diabetes could increase percentages of abnormal sperm morphology and testicular damage in STZ induced model.

References

(1). Du, Z., Xu, S., Hu, S., Yang, H., Zhou, Z., Sidhu, K., Miao, Y., Liu, Z., Shen, W., Reiter, R.J., Hua, J., Peng, S.,. Melatonin attenuates detrimental effects of diabetes on the niche of mouse spermatogonial stem cells by maintaining Leydig cells. Cell Death Disease, 20; 9 (10):968-983. Doi: 10.1038/s41419-018-0956-4. **2018**

(2). Keyhanmanesh, R., Hamidian, G., Alipour, M.R., Ranjbar, M., Oghbaei, H.,. Protective effects of sodium nitrate against testicular apoptosis and spermatogenesis impairments in streptozotocin-induced diabetic male rats. Life Science, S0024-3205(18)30564-2. Doi: 10.1016/j.lfs.2018.09.019. **2018**

(3). Jain, G.C., Jangir, R.N., Modulation of diabetes-mellitus-induced male reproductive dysfunctions in experimental animal models with medicinal plants, Pharmacognosy Review, 8(16):113-121. **2014**

(4) Jangir, R.N., Jain, G.C., Diabetes mellitus induced impairment of male reproductive functions: a review, Current Diabetes Review, 10 (3):147-157. **2014**

(5). Alves, M.G., Martins, A.D., Rato, L., Moreira, P.I., Socorro, S., Oliveira, P.F., Molecular mechanisms beyond glucose transport in diabetes-related male infertility, Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease,1832 (5):626-635. **2013**

(6) Hess, R.A., Linder, R.E., Strader, L.F., Perreault, S.D.,. Acute effects and long term seque-lae of 1,3-dinitrobenzene on male reproduction in the rat II. Quantitative andqualitative histopathology of the testis, Journal of Andrology, 9:327–342. **1988**

(7). Ismail, O.O., Isaac, J.A., Ugochukwu, O., Oluwatosin, O.O., Aniekan, P.I., Edwin, N.C.S., Onyemaechi, A.O.,. Impaired expression of testicular androgen receptor and collagen fibers in the testis of diabetic rats under HAART: the role of Hypoxis hemerocallidea, Folia Histochemica et Cytobiologica, 55(3):149-158. Doi: 10.5603/FHC.a2017.0016. **2017**

(8). Rezaei, N., Mardanshahi, T., Shafaroudi, M.M., Abedian, S., Mohammadi, H., Zare, Z. Effects of l-Carnitine on the Follicle-Stimulating Hormone, Luteinizing Hormone, Testosterone, and Testicular Tissue Oxidative Stress Levels in Streptozotocin-Induced Diabetic Rats. Journal of Evidence-Based Integrative Medicine, 23:2515690X18796053. Doi: 10.1177/2515690X18796053. **2018**

(9) Mao, C.F., Zhang, X.R., Johnson, A., He, J.L., Kong, Z.L., Modulation of Diabetes Mellitus-Induced Male Rat Reproductive Dysfunction with Micro-Nanoencapsulated Echinacea purpurea Ethanol Extract. Biomed Research International, ,30:4237354. Doi: 10.1155/2018/4237354. **2018**

(10) Samie, A., Sedaghat, R., Baluchnejadmojarad, T., Roghani, M., Hesperetin, a citrus flavonoid, attenuates testicular damage in diabetic rats via inhibition of oxidative stress, inflammation, and apoptosis. Life Science, 1;210:132-139. **2018**

(11) Sampannang, A., Arun, S., Burawat, J., Sukhorum, W., Iamsaard, S.,. Testicular histopathology and phosphorylated protein changes in mice with diabetes induced by multiple-low doses of streptozotocin: An experimental study. International Journal of Reproductive BioMedicine, 16 (4):235-246. **2018**

(12) Zha, W., Bai, Y., Xu, L., Liu, Y,, Yang, Z,, Gao, H,, Li, J.,. Curcumin Attenuates Testicular Injury in Rats with Streptozotocin-Induced Diabetes. Biomed Research International, 7468019. Doi: 10.1155/2018/7468019. **2018**

(13) Guneli, E., Tugyan, K., Ozturk, H., Gumustekin, M., Cilaker, S., Uysal, N. Effect of melatonin on testicular damage in streptozotocin-induced diabetes rats. European Surgical Research;40(4):354–360. Doi: 10.1159/000118032. **2008**

(14) Sainio-Pöllänen, S., Henriksén, K., Parvinen, M., Simell, O., Pöllänen, P. Stage-specific degeneration of germ cells in the seminiferous tubules of non-obese diabetic mice. International Journal of Andrology, 20(4):243–253. Doi: 10.1046/j.1365-2605.1997.00061. **1997**

(15) Zha, W., Bai, Y., Xu, L., Liu, Y., Yang, Z., Gao, H., Li, J.,. Curcumin Attenuates Testicular Injury in Rats with Streptozotocin-Induced Diabetes. BioMed Research International, ,7468019. Doi: 10.1155/2018/7468019. **2018**