Diabetes related cataract and histopathological abnormalities of the ocular regions of Wister albino rats

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Abstract

Objective: Diabetes is an important public health disease affected different body organs especially eye. The present study aimed to illustrate the cataractous lenses associated complication on ocular organs.

Research design and methods: Following induction of type 2 diabetes for 6 months (streptozotocin 60mg/kg single dose in combination with nicotinamide100 mg./kg body weight), 6 rats of a colony of 47 individuals developed cataract. Control individuals were used of the similar age. The animals groups were anaesthetized and sacrificed. Ocular regions were incised and subjected for histopathology and comet assay. Transmission electron microscopy was carried out for the optic nerve. In case of meibomian gland, beside histopathological investigations, immunohistochemistry of bcl2, caspase 3 and p53 were carried out.

Results: The present findings revealed the development of cataractous lenses, diabetic retinopathy, thickened cornea and damaged stroma and optic neuropathy explained by demyelinated axons. Single gel electrophoresis explained DNA damage of cells of the retina, ciliary organ and optic nerve. Also, there is a marked reduction of the immunohistochemistry of bcl2, caspase 3 and p53 of diabetic meibomian gland, reflecting cell death.

Conclusion: Finally the authors concluded that the cataractous lenses originated through different pathways of diabetic complication.

Introduction

The Diabetes Mellitus (DM) is characterized by hyperglycemia associated with either decreased insulin production in the body or insulin resistance. It is increased among population reached up to 382 million in 2014 [1] and by 2035 more than 592 million may develop with the disease in a ratio of 1 to 10 individual become diabetic [2]. The disease resulted from impairing of β-cell function, hyperglycaemia and impairment of insulin secretion [3]. Poor diabetic-treatment resulted in impairment of visual acuity [4] and the development of cataract in childhood [5, 6]. Cataractous lenses were also reported in diabetic experimental animal [7]. Diabetic related cataract was associated with damaging corneal endothelial cells and increase foveal thickness [8]. Also, diabetic was found to induce retinopathy [9, 10] and represent the main cause of impaired visual acuity and blindness associated with elevated serum homocysteine level Hcy [11]. Also, it is involved in reduction of both retinal thickness and retinal blood flow, and increased thickening of the retinal choriocapillaries [12]. The disease increased retinal apoptosis, overexpression of VEGF and increased oxidative stress [13, 14] and altered osmoregulation leading to acidic retina [15]. Middle-aged, obese rhesus monkeys, developed diabetes mellitus characterized by hypertension, high levels of triglycerides and serum cholesterol. The disease associated with intraocular hemorrhages, damage choriocapillaries, increased basal laminar deposits and hard drusen of Bruch’s membrane and numerical reduction of photoreceptor inner and outer segments [16]. Diabetic retinopathy characterized by up-regulation of pro-inflammatory interleukin-6 and down-regulation of superoxide dismutase and glutathione (GSH)/oxidized glutathione associated with numerical reduction of retinal ganglion cells and increased glial fibrillary acidic protein expression level [17–18].

Concerning cornea which is the transparent organ important for vision. Diabetes caused marked degeneration of corneal secondary nerve fiber branches [19, 20] and enhanced the development of anterior segment disorders such as corneal erosion and ulcer and persistent epithelial defects [21]. There is discrepancy between authors concerning diabetic interference on corneal thickness. Meanwhile Olsen and Busted (1981) [22] reported increased corneal thickness in diabetic patients, Hashemi et al. (2019) [23] reported no variations of corneal thickness between diabetic and non diabetic individuals. Diabetic keratopathy resulted in reduction of corneal sensitivity [24].

The ocular region guards by eye lid containing glands such as the meibomian glands (MGs) which are arranged in parallel strands within the tarsal plates of the eyelids. It secretes oily meibum by meibocytes and transported through the ductal system by ductule and the central duct towards the free lid margin. The gland possesses a characteristic innervations from sympathetic and parasympathetic
Diabetes was found to induce meibomian gland dysfunction [28, 29] leading to depletion of the lipid secretion associated the instability of tear and development of dry eye disease [27, 30].

Little is known about the diabetes related changes on the histopathological structure of the meibomian glands. Recently, a study from Ding et al. demonstrated that insulin stimulated the proliferation of immortalized human meibomian gland epithelial cells (HMGECs), whereas high glucose was found to be toxic for HMGECs [31]. Diabetes was found to reduce the number of glandular structure impairing its functional activity [32]. Also, diabetes was found to be increased in increase of ciliary body thickness [33] and reduction of both retinal nerve fiber layer and vessel density in the optic disc [34].

The present studies searched for illustrating if the opacity associated with other complication of the eye regions or not and the characteristic features of the histopathological alterations.

Materials and Methods

1. Induction of diabetes

Experimental type 2 diabetes mellitus was induced in all the rats by a single interperitoneal injection of streptozotocin (60 mg/kg) in citrate buffer (0.05 M) (pH 4.5) and 100 mg/kg nicotinamide [35]. Hyperglycemia was verified by measuring the blood glucose within 240–280 mg/dl were selected for the study.

2. Experimental animals

Following investigating a colony of 47 individual diabetic rats (*Rattusnorvegicus*) after 8 months of streptozotocin-induction and weighing approximately 200–250g body weight, 6 rats exhibiting cataractous lenses either unilateral (4/6) or bilateral (2/6). Control healthy individuals (n = 6) were selected. They were housed in good ventilation on a 12-h light and dark cycle. The studied cataractous rats were observed in about 47 individual diabetic rats after 6 months of induction the disease reaching incidence of about 13%. The cataractous group was separated and a similar control non-diabetic group was also selected. Free excess water and food were allowed ad Libitum. The studied groups were sacrificed by light diethyl ether anesthesia and dissected and ocular region, upper eye lid and optic nerves were separated and processed for the following investigations.

3. Histological investigation

Retina, cornea, proximal optic nerve, ciliary organ specimens of the studied proximal optic nerve close to the retina of the studied groups were immediately fixed in 10% neutral phosphate buffered formalin (pH 7.4) containing 75 mmNaCl and 24 mM NaH, ethylenediaminetetraacetic acid (EDTA), pH 13. Six μL of the homogenate were suspended on 0.5% low melting agarose and sandwiched between a layer of 0.6% normal-melting agarose and another of 0.5% low melting agarose on fully frosted slides and kept on ice for the polymerization. The slides were the immersed in a lysis solution (1% sodium sarcosinate, 2.5 M NaCl, 100 mM NaEDTA, 10 mm Tris-HCl, 1% Triton X-100, and 100 mM DMSO) at 4°C. After 1 h, they were placed in electrophoresis buffer (0.3 M NaOH, 1 mM NaEDTA, pH 13) for 10 min at 37°C to allow DNA to unwind. Electrophoresis was carried out for 10 min at 300 mA and 1 V/cm, followed by staining with 20 mg/mL ethidium bromide. Each slide was analyzed using a Leitz Orthoplan (Wetzlar, Germany) epifluorescence microscope. One hundred cells were analyzed on each slide using the Comet assay II automatic digital analysis system. Perspective tail length (mm) is the distance of DNA migration from the center of the body of the nuclear core and is used to evaluate the extent of DNA damage. Tail length was measured automatically by image analysis software [36].

4. Immunohistochemistry for caspase 3

Histological 5 μm thick sections of upper eye lid were cut and mounted onto super frost plus glass slides (Fisher Thermo Scientific, Nepean, Ontario, Canada). The tissue sections were retained at normal room temperature and processed for antigen retrieval by digestion in 0.05 % trypsin (pH 7.8) for 15 min at 37°C and incubated with antibodies against bcl2, caspase 3 and P53 (dilution 1:100 Thermo Fisher Scientific, Fremont, CA, USA; Cat. No. A1–70007) for overnight at 4°C. Then treated with a horseradish peroxidase streptavidin detection system (Dako), followed with DAB plus Chromagen to detect the immunoactivity by counterstaining with hematoxylin (Sigma). Sections incubated with 1% non-immune serum phosphate buffer solution (PBS) solution served as negative controls. Specimens were observed with a Leica BMS000 microscope (Leica Microsystems, Wetzlar, Germany) and photographed. For assessments of the percentages of positive immunoreactive areas were determined by investigating slides using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, using 40 X objective. The result images were analyzed on Intel® Core i5® based computer using Video Test Morphology® software (Russia) with a specific built-in routine for area, % area measurement and object counting.

5. Transmission electron microscopy

Specimen of proximal optic nerve close to the retina of the studied groups were immediately fixed in 0.1M cacodylate buffer (pH 7.4) containing 2.5 % glutaraldehyde and post-fixed in 1 % osmium tetroxide, dehydrated in ascending grades of ethyl alcohol, and embedded in epon–resin. Ultrathin sections were cut with a diamond knife on a LKB Ultratome IV (LKB Instruments, Bromma, Sweden) and mounted on grids, stained with uranyl acetate and lead citrate, and examined under a Joel JCM transmission electron microscope (Musashino 3-chome, Akishima, Tokyo 196–8558, Japan).

6. Comet assay

Retina, cornea, optic nerve, ciliary organ specimens of the studied groups were immediately fixed in chilled homogenizer buffer, pH 7.5, containing 75 mMNaCl and 24 mM NaH, ethylenediaminetetraacetic acid (EDTA), pH 13. Six μL of the homogenate were suspended on 0.5% low melting agarose and sandwiched between a layer of 0.6% normal-melting agarose and another of 0.5% low melting agarose on fully frosted slides and kept on ice for the polymerization. The slides were the immersed in a lysis solution (1% sodium sarcosinate, 2.5 M NaCl, 100 mM NaEDTA, 10 mm Tris-HCl, 1% Triton X-100, and 100 mM DMSO) at 4°C. After 1 h, they were placed in electrophoresis buffer (0.3 M NaOH, 1 mM NaEDTA, pH 13) for 10 min at 37°C to allow DNA to unwind. Electrophoresis was carried out for 10 min at 300 mA and 1 V/cm, followed by staining with 20 mg/mL ethidium bromide. Each slide was analyzed using a Leitz Orthoplan (Wetzlar, Germany) epifluorescence microscope. One hundred cells were analyzed on each slide using the Comet assay II automatic digital analysis system. Perspective tail length (mm) is the distance of DNA migration from the center of the body of the nuclear core and is used to evaluate the extent of DNA damage. Tail length was measured automatically by image analysis software [36].

7. Statistical analysis

Data are presented as means ± standard deviation (SD). The statistical analysis was performed with multi-variant analysis of
variance (MANOVA) using the SPSS (version 13) software package for Windows, comparing the multivariations between diabetes and control groups. Significance was determined at p < 0.05.

Results

1. Cornea & Retina

The cornea is composed of outer lining epithelium, followed by Bowman's membrane, stroma, descemet's membrane and corneal endothelium. Regard to the control (Fig. 1A), the diabetic group revealed that the corneal epithelium was comparatively thickened with vesicular vacuolar degenerated nuclei. The stroma showed patches of hyaline degenerated collagenous fibrils and infiltrated by necrotic regions. Vacuolated keratocytes were detected in between the collagen fibrils of the stroma. The corneal endothelium lining the descemet's membrane attained considerable thickening (Fig. 1A1).

Regarding to the normal structure of the retina (Fig, 1B ), the diabetic group showed comparative reduction of the retinal thickness. There was comparative numerical reduction of the inner and outer nuclear cells. The nuclei of these cell layer were electron-dese manifesting clumping of the nuclear chromatin. The photoreceptor layer was comparatively atrophied. The ganglion cells were markedly missing and the nerve fiber become thin, fragile and vacuolated (Fig.1B1). Diabetic retina showed detached retinal cells manifesting damaged DNA (Fig. 1C1) compared to non-changed in control (Fig.1C). The whole retina, inner and outer nuclear layer and photoreceptors attained considerable reduction in diabetic group in comparison with the control (Fig. 2).

2. Ciliary organ and optic nerve

The ciliary body occupied the interface between the iris and the choroids. It is made up of two ring-shaped components: the pars plicata and the pars plana. The pars plicata detected in the anterior region of the ciliary body, at the scleral spur. The ciliary body composed of branched ciliary processes. It is lined by two layers of columnar cells. The outer one is pigmented, mean while the inner lining cells lack pigmentation. The lumen is enclosed with fine collagenous network (Fig. 3 A). Following application of comet assay the control ciliary cells are round (Fig.3A1).

Experimental diabetic group revealed missing of the ciliary processes and comparative thinning of their lining epithelium. Congestion of their blood capillaries, widened lumen and distortion of their collagenous network were observed (Fig. 3B). Following single gel electrophoresis (comet assay), revealed DNA segregation assessed by formation of detached tail region manifested DNA damage (Fig. 3B1).

Optic nerve

Concerning the optic nerve, light microscopically, these are formed of densely aggregated and enclosed in-between cells with basophilic nuclei suspected the oligodendrocytes and astrocytes (Figs. 3 C&C1). Ultrastructurally, the nerve axons are densely packed and composed of myelinated and non-myelinated axons of varying small size. The axon enclosed by several layers of myelin sheath. Abundant mitochondria are distributed the cytoplasm of the inner compartment of the axons (Fig.3 C2).

Diabetic–treatment revealed widespread of vacuoles and fragility of the nerve fibers, and comparative reduction of the oligodendrocytes. Many of them possessed vacuolated axoplasm (Fig. 3D). Ultrastructurally, vacuolation and demyelination of the theaxonal sheath were detected (Fig. 3 D1). Following application comet assay, there was apparent increase of apoptic cells with detached tail region (Fig.3D2) compared to non-changed in control (Fig.3C3).
The present findings supported the work of Olsen and Busted [22] and Hashemi et al. [23] that reported increased corneal thickness in diabetic patients. Diabetic keratopathy was also, mentioned by Bikkova et al. [24] which impaired the corneal sensitivity. Alterations of diabetic cornea may contribute to damage of the corneal nerve fibers [20, 44] and progress the development of anterior segment disorders [21] resulting in impairing the vision.

Also, the diabetic retina attained a comparatively reduced thickness; numerical reduction of inner and outer nuclear cells and atrophied photoreceptors coincides with increased detached retinal cells manifested DNA damage.

The observed diabetic retinopathy supported the work of El-Sayyad et al. [37], Kim and Yu [33], Yao et al. [12] and Li et al [10]. The authors mentioned that the retinopathy impaired the visual acuity and the development of blindness. Diabetic retinopathy related to blindness affected about 800 cases each year [45].

At the same time, the detected atrophy and damaged photoreceptors supported the work of Johnson et al. [16] and El-Sayyad et al. [37].

Diabetic retinopathy was found to exhibit over expression of inflammatory markers, down-regulation of antioxidant enzymes associated with increased retinal cell death [13, 14, 17].

The present data revealed that diabetes caused abnormal non-branched biliary processes and reduced thickness of their lining epithelium parallel with single strand DNA damage. The present findings agree with [33] following investigating diabetic patients.

Ciliary organ is important for contraction and relaxation of lens allowing it for accommodation. Damage of ciliary epithelium and defect of collagenous fibrils of the ciliary lumina interfered with ciliary functions [46].

The present findings revealed neuropathy of optic nerve of diabetic rat assessed by fragility and degeneration of nerve fibers, demyelinated axons and decrease of neuronal cells coincides with single strand DNA damage of astrocytes. Similar findings of optic neuropathy were detected in aging rats which exhibited similar diabetic deterioration of the neuronal cells [47].

Diabetes associated microvascular abnormalities may facilitate the progress of ischemia of the anterior optic nerve [48].

On the other hand the meibomian glands are important for ocular function through secretion of oily meibum which lubricate the ocular surfaces and protecting against tear evaporation [49].

The present findings revealed that diabetes induced atrophy and degeneration of meibomian glands. Following immunohistochemical staining, decreased expression of bcl2 and overexpression of caspase3 and p53, the markers of cell death were detected.

Similar findings of diabetes associated meibomian gland dysfunction were reported [28, 31, 32]. Decreased lipid secretion was found to be associated with the instability of tear and progress of dry eye disease [27, 30].

Finally the authors concluded that the cataractous lenses originated through different pathways of diabetic complication.
Figure 3A-B1. Photomicrographs of histological sections of ciliary organ. A. Control showing branched ciliary organ. A1. Comet assay of control showing normal round cells (single star). B&B1. Diabetic rat showing atrophied ciliary organ (B) with detached damaged cells post comet assay (double star) (B1).

Figure 2C-C1. Photomicrographs of histological sections of control optic nerve showing attachment with retina in optic disc and compacted axons and contents of neuronal cells (dark arrow head). C2. Transmission electron micrographs of control showing compacted myelinated axons. C3. Comet assay of control showing round cells (Single star). D-D2 Diabetic group showing fragile histological section and vacuolated neurons (white arrow head) (D) and demyelinated electron micrograph (D1) and detached neuronal cells of optic nerve after comet assay (D2). Abbreviations: C, connective tissue; CE, ciliary epithelium; DCE, damaged ciliary epithelium; DMA, degenerated mitochondrial axons; LI, lymphocytic infiltration; M, mitochondria; MA, mitochondrial axons.
Figure 4. A. Photomacrograph of normal (A) and diabetic cataract (B). A1-A3. Photomicroph of histological sections of control meibomian gland showing normal branched acinar gland. B1-B3. Diabetic rat showing atrophied and degenerated meibomian gland (B1&B2) and degenerated germinativum of epidermis (B3) illustrated by arrow head. Abbreviations; DHF, degenerated hair follicle; DMG, damaged meibomian gland; E, epidermis; HF, hair follicle; MG, meibomian gland. HE.
Figure 5. Photomicrographs of formalin fixed histological sections of meibomian glands immunohistochemically stained with Bcl2 (A&B), Caspase 3 (A1 &B1) and P53 (A2 &B2). Note decreased expression of Bcl2 (B), increased expression of caspase 3 (B1) and overexpression of p53 (B2) in diabetic group compared to control (A-A2). Arrow head pointed to the immunostaining activity.
Figure 6. Chart illustrating the image analysis of percentages immunoreactive area of diabetic group in comparison with the control. Each result represent the mean SD (n = 5). Diabetic is significant at P < 0.05.

References

