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Acute and Chronic Effects of Diabetes Mellitus on Telogen Hair Bulge

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Abstract. The hair follicle bulge contains the progenitor cells that differentiate into all skin epithelial linages. The bulge area can be clearly identified in the telogen as the epithelial sac surrounding the hair club. Diabetes mellitus directly causes prematurely stop hair growth in telogen phase, called telogen effluvium. Taken together, this study aimed to examine the duration of streptozotocin induced diabetes affects the histological aspect of telogen bulge components in back skin of rats. In both 4-week acute and 24-week chronic diabetes, increased dark nuclei of bulge cells and hair follicle stem cells in the bulge were exhibited with decreased both numbers in the chronic stage. Dermal sheath cells surrounding the bulge enlarged in both diabetic durations. It was suggested that diabetes accelerated the precursors for neogenic hair follicle defects, that is a common cause of hair loss.

1. Introduction

Hair follicle bulge, a reservoir of multipotent stem cells, maintains the cyclical growth of hair, because this area composes of bulge and hair follicle stem cells (HFSCs) [1]. They are important in not only generation and renewal of continuous cycling hair follicle but also epidermal repair after wounding [2]. Moreover, the dermal compartments of hair follicle, including dermal papilla (DP) and dermal sheath (DS) cells, are required for hair follicle growth during development [3]. DS has been considered as a fully functional DP cell reservoir. Moreover, the dermal signal induces down-growing epithelial cells to develop hair formation [4]. The association between diabetes mellitus (DM) and hair loss has been established in many studies [5], [6]. During diabetes, high blood glucose and poor blood circulation cause hair loss, leading to high impact on the quality of life and self-esteem of patient. An alteration of the normal hair cycle induced by diabetes has been reported, such as telogen effluvium, the early stop growing of hair in the telogen (resting) phase and shedding later [7]. Therefore, this study delineated the effects of acute and chronic diabetes induced by streptozotocin (STZ) on telogen bulges, containing stem cells, in the skin on the back of male Sprague-Dawley rats using routine histological techniques.

2. Materials and methods

2.1 Experimental animals

A total of 13, 5-8 week-old male Sprague-Dawley rats from National Laboratory Animal Center, Mahidol University, Thailand, were randomized into 2 groups, including a diabetic (n=7) and a healthy

age-matched control (n=6) group. Diabetic group was received an intraperitoneal injection of 60 mg/kg body weight STZ dissolved in citrated buffer at a single dose. The age-matched control rats were injected with an equal volume of the buffer only. The development of diabetes was confirmed by measurement of blood glucose levels at 72 hours after STZ administration and before sacrifice.

2.2 Processing of the tissue

At the end of experimental period, rats were sacrificed at 4 and 12 weeks after STZ injection as acute and chronic diabetic conditions, respectively. Skin samples at the back of each rat were removed and fixed in Bouin's solution for the histological analysis. Then, the specimens were processed for paraffin blocks and cut into 10 µm thick, and stained with hematoxylin and eosin (H&E). The histological characteristics were observed under a light microscope (LM; Axiostar plus Jena, Germany).

3. Results

Both acutely and chronically STZ-diabetic rats exhibited hyperglycemia. The skin sample at the back of rats contained the hair follicles that could be classified into three phases; anagen (organized growth), catagen (regression), and telogen, (resting) (Fig. 1). During the anagen, the long hair follicle reached to the bottom of the subcutaneous fat tissue. Well-formed hair follicle bulb surrounding distinct dermal papilla appeared in this phase (Fig. 1A). In the catagen, the dermal papilla separated from the hair root due to the shrinkage of hair bulb (Fig.1B). Telogen follicle showed the appearance of a club hair as the lump of cells on the end of follicle. The bulge area which housed the stem cells was clearly identified in this phase as the epithelial sac surrounding the club hair (Fig. 1C). Unlike the telogen phase, anagen bulge did not consist of any protrusion morphologically. It can be identified adjacent to the attachment site of the arrector pili muscle just below the sebaceous gland (Fig. 1A).

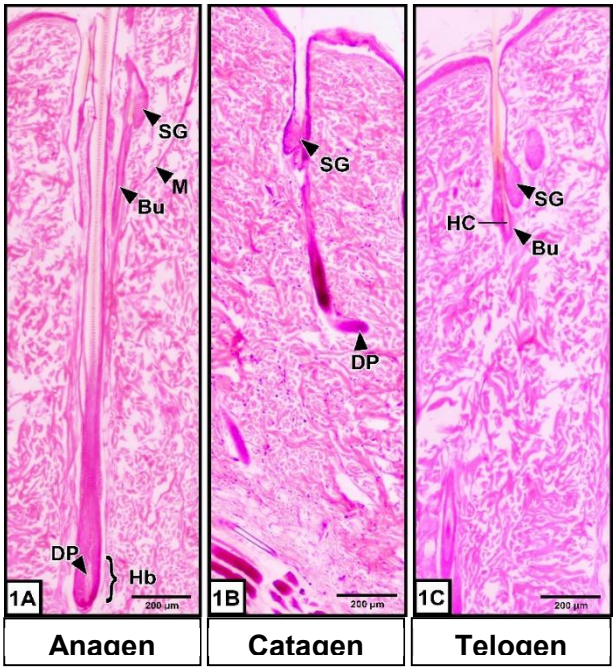

Fig. 1. Histology of hair follicles at different phases in normal skin of rat. In the anagen hair, long tube like structure with hair bulb (Hb) surrounded the dermal papilla (DP) (1A), The catagen hair follicle shrank with detached papilla (1B). The telogen hair follicle was short with a club (HC) at the tip (1C). Sebaceous gland (SG); bulge area (Bu); arrector pili muscle (M). H&E staining.

At the high magnification, the bulge area located at the lowest part of the telogen follicle engulfing a club hair. There were two cell layers, including inner bulge cell layer and outer layer of HFSC. All cells in both layers were cuboidal shape with oval nuclei. Moreover, the spindle-shaped DS cells spread close to the bulge. In the acute STZ-diabetic rats, some of HFSCs and bulge cells contained dark pyknotic nuclei surrounded by pale cytoplasm like clear area (Fig. 2). These changes also continued to appear in the chronic stage. In the chronic diabetes, the decreased cell number of both cell types was observed. Focusing on the bulge cells, some of them contained pale nucleus with large clear area in its cytoplasm (Fig. 3B). Most of them were irregular shape and detached away from the club (Fig. 3C). Furthermore, DS cell surrounding the hair follicle bulge became hypertrophy with a pale-stained nucleus during diabetic development in both periods (Fig. 2,3).

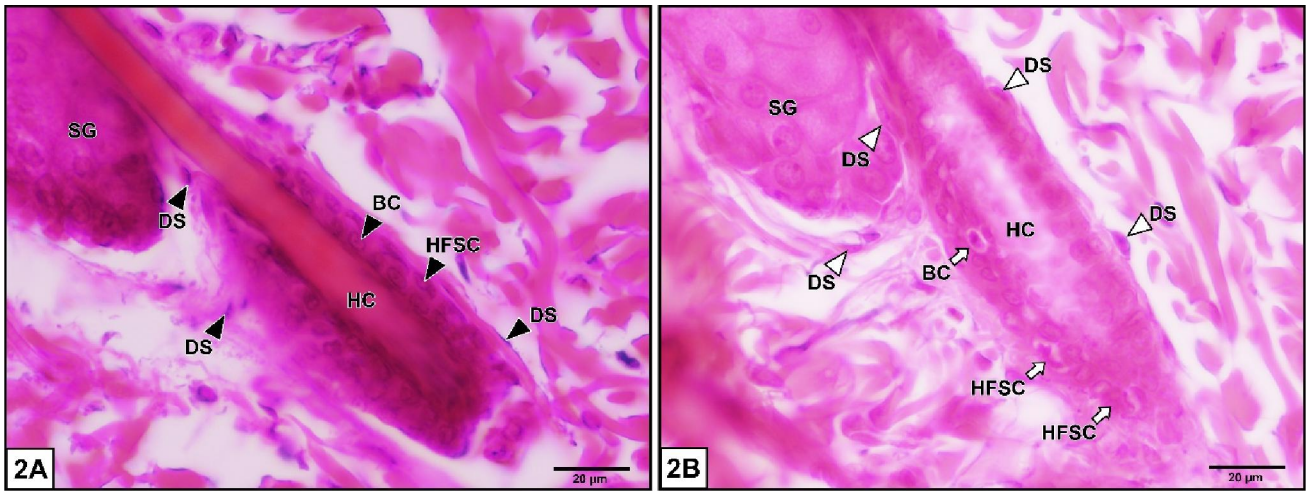

Fig. 2. Histology of telogen bulges in the control (2A) and acute diabetic stage (2B). In the diabetes, pyknotic nucleus with pale cytoplasm (white arrows) in both HFSC and bulge cells (BC). DS cells were hypertrophy and pale staining, when compared to control (white arrowheads). Sebaceous gland (SG); hair club (HC). H&E staining.

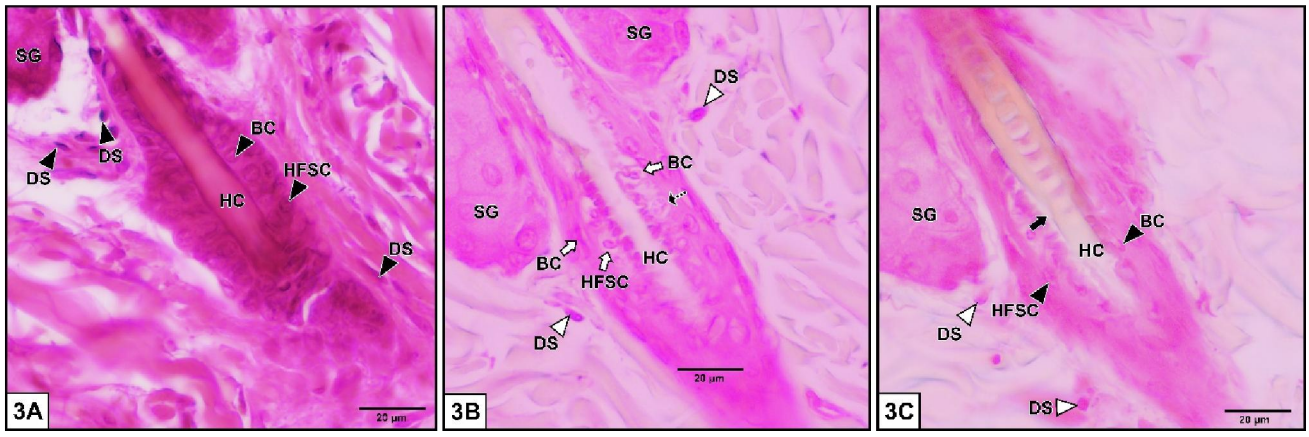

Fig. 3. Histology of telogen bulges in the control (3A) and chronic diabetes (3B-C). The pyknotic nucleus with pale cytoplasm (white arrows) in both HFSC and bulge cells (BC) were investigated in 3B. Both of them decreased their numbers. Irregular shaped bulge cell with a pale nucleus and large clear cytoplasmic area around the nucleus was observed (dash arrow) (3B). Moreover, detached bulge cells (black arrow) from hair club (HC) was also found (3C). Hypertrophic DS cells with pale-stained nuclei (white arrowheads) appeared in prolonged diabetes (3B-C). These changes did not occur in control telogen bulge. Sebaceous gland (SG). H&E staining.

4. Discussion

Both acute and chronic diabetic stages, pyknotic nuclei with pale cytoplasm like clear area in both HFSCs and bulge cells in telogen hair follicle were identified as apoptotic cells. It is known that reactive oxygen species (ROS) increases under hyperglycemia via NADPH oxidase by activation of advance glycation end products [8]. ROS generates mitochondria dysfunction through lipid peroxidation, leading to release of cytochrome C and finally caspase3 activation [9]. Then, there is a release of endonuclease, and DNA in the nuclei of these cells are destroyed, as dark stained chromatin clumping in this study. Moreover, lipid peroxidation causes not only mitochondrial dysfunction, but also rER disruption. The damage of these two organelles release Ca^{2+} into the cells to activate calpain [10]. Next, protease and lipase are released to degrade cell membrane, organelles, and cytoskeleton [11]. Therefore, the pale-stained cytoplasm of both HFSCs and bulge cells were observed [12]. In this investigation, the numbers of bulge cells and HFSCs in acute diabetes did not change, because it was suggested that the recovery of HFSCs can still function. However, decreased numbers of HFSCs and bulge cells were shown. Under diabetic condition, increased β-catenin stabilization via hexosamine pathway [13] inhibits epidermal growth factor signaling in the HFSCs. As a result, reduction of protein involving in migration, such as matrix metalloproteinase-2 and proliferation, cyclin D, E, CDK 2, and 4, occurs [14]. Next, proliferation and migration of HFSCs decrease to lower amounts of both HFSCs and bulge cells, resulting in deceleration of repairing process. In addition, severe alteration in telogen bulge formation was demonstrated in the chronic diabetes, such as irregular shape with pale-stained nucleus and large cytoplasmic area of bulge cells, as well as detachment of the cells from the club. As mentioned above, increased β-catenin during DM in the bulge cells binds to coactivator associated arginine methyltransferase to inhibit glucocorticoid signaling [15]. Next, keratin 6 (K6) decreases, a major keratin type of bulge cell for cell cytoskeleton [16], that causes irregular shape of cells. Because of reduction of K6, the clear cytoplasm was seen in bulge cells. Furthermore, K6 is a keratin cytoskeletal filament in desmosome between bulge cells and hair club [17]. Consequently, the desmosome was damaged. As described earlier, increased ROS leads to cytoskeletal degradation. Moreover, ROS also decreased RhoA in bulge cells [18], that disassociates two components of desmosome, desmoplakin and plakophilin [19]. In addition, induced ROS phosphorylates E-cadherin, a transmembrane protein in desmosome to disconnect with desmocollin and desmoglein between two cells [20]. Finally, the desmosome disrupted. During diabetes, increased transforming growth factor-β1 in stress HFSCs and bulge cells activates DS cells to differentiate into myofibroblasts. These cells were hypertrophy with pale-stained nuclei, which were found in the DS around bulge area of both acute and chronic DM. The myofibroblast synthesizes the extracellular matrix for process of reepithelization [21]. The long stay in this form of DS cells could be develop fibrosis under the diabetic condition [22] causing the malformation of diabetic telogen hair bulge.

5. Conclusion

In the present study, it was concluded that the levels of histological changes of the progenitor cells for hair follicle in the telogen bulge area were closely related with the duration of diabetes.

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