D iabetes results in a variety of oral health complications. Among which is its effect on salivary glands that results in decreased salivary flow. Diabetes effect on posterior lingual glands is an issue that deserves proper research. Our aim is to investigate the effect of experimental diabetes on the histopathology of posterior lingual glands as well as on the change in the expression of α-SMA between normal and diabetic rats.

The studied groups included; control (I), un-controlled diabetic (II) & insulin-treated diabetic group (III). Von Ebner salivary gland was obviously affected, while the Weber salivary gland was resistant. The results demonstrated von Ebner salivary gland showing an alteration in its histology represented by inflammation, tissue fibrosis & atrophy. It also showed the abnormal increase in the expression of anti-α-SMA antibody in untreated diabetes. Both histopathological and immunohistochemical findings were nearly corrected by insulin treatment in the insulin treated group. Conclusions; the alteration in the expression of α-SMA which was correlated to the histopathological findings may have an explanatory role in understanding the clinical effect of diabetes, as regards to the decreased salivary flow.

Key words; diabetes, alpha smooth muscle actin, von Ebner salivary glands, rats, Weber salivary glands.

INTRODUCTION

Von Ebner & Weber glands; are serous and mucous glands that open in the troughs of circumvallate and foliate papillae & under the crypts of the lingual tonsil, respectively (15&33). Myoepithelial cells are spindle or stellate cells of epithelial origin, having smooth muscle cell characters (24&38). These cells were demonstrated embracing the secretory end portions and the intercalated ducts of all salivary glands (10). Myoepithelial cells are always found when contractile function is required (3&5). Researchers have dealt with myoepithelial cells from different aspects concerning their structure, immunoreactivity and even their role in the development of malignant lesions (4, 25 & 30).
Alpha-smooth muscle actin (α-SMA) is a contractile protein present in myoepithelial cells of the sweat, mammary, and salivary glands as well as myofibroblasts, vascular smooth muscle and during wound contraction (5,6,23). Its antibody is the most commonly used immunohistochemical marker to demonstrate myoepithelial cells (9,29).

Diabetes mellitus is a chronic endocrine disorder with multiple adverse reactions manifested on all body tissues (8,34). It was demonstrated to result in alterations in salivary glands as regards to the structure & function, which was reflected on the glands’ performance leading to xerostomia (16), swelling, tenderness, inflammation and even atrophy (21,28).

α-SMA was found to be over expressed in diabetes and in some malignant conditions (12,39). The delayed expression of α-SMA was reported to alter vascular contractility leading to disturbance in blood pressure (27). Diabetes was reported to cause alterations in the smooth muscle structure & function in both vascular & non-vascular smooth muscles (31,36). Some researchers demonstrated that the alteration in the smooth muscle in diabetes was related to the alteration in the expression of contractile proteins like actin and myosin (17). On the other hand, others attributed the disturbance to the neural parasympathetic input responsible for contraction (2 & 20).

Till the present time, no much evidence was available from the literature concerning the effect of diabetes on posterior lingual salivary glands, or its effect on the local expression of α-SMA, which lead to the aim of the present study.

MATERIAL AND METHODS

Animals; Thirty adult male Sprague-Dawley rats (150-175gm) were selected and divided into three groups (10 animals each); group I; acted as control, group II; in which diabetes was induced, and group III; in which the diabetes was induced and insulin treatment was started. The animals were housed and caged separately in the animals’ house of the faculty of medicine, Cairo University.

Diabetes induction; Diabetes was induced in the animals of both groups II & III by a single intravenous injection of 60 mg/kg body wt streptozotocin (STZ) (Sigma Aldrich Inc), dissolved in 0.9% NaCl & citric acid (pH 4.5). 24 hours after the induction of diabetes; blood glucose levels of the animals of groups II & III were measured using a colorimetric system (sigma glucose Kit No 115-A). Animals of the experimental groups were considered diabetic when their serum glucose levels were above 300 mg/dl.

Insulin treatment was started after the detection of diabetes to the animals of group III in the form of a daily subcutaneous injection of human insulin mix suspensions (VACSERA) (1U/100gm body weight) for the whole study period which extended for eight weeks. (13) During the course of the experiment the animals were allowed to free access to food and water. Animals’ weights as well as their blood glucose levels were monitored on weekly bases. By the end of the experiment; all rats were sacrificed by cervical dislocation. Tongue specimens were dissected, fixed with 10% calcium formole, treated with alcohol, and then embedded in paraffin wax. Sections of 6µ thickness were made for routine histopathological examination with hematoxylin & eosin. For immunohistochemistry; mouse monoclonal anti-body against α-smooth muscle actin (clone 1A4) was used at a dilution of 1:500 in phosphate buffered saline (PBS). The technique specifications were supplied by the product’s data sheet and the exact procedure was provided by Skalli et al, 1986.

RESULTS

Along the course of the experiment the animals showed the clinical signs of diabetes including weight loss, polyurea and Xerostomia which was manifested by the animals’ increased water consumption.

Histopathological results;

Control group I; von Ebner salivary glands were demonstrated as small aggregations of rounded pure serous acini intermingling with the lingual muscle fibers, separated by delicate connective tissue septa (Fig.1).
The acini contained pyramidal cells that were arranged around the lumen & contained highly basophilic cytoplasm. The nuclei were darkly stained with abundant nucleoli that were evident on higher magnifications. Occasionally, in few of the acinar cells solitary unstained vacuoles were seen (Fig.2). Some sections revealed flattened nuclei around few acini demonstrating the myoepithelial cells (Fig.3).

Weber salivary glands were demonstrated as larger mucous acini with less cytoplasmic density. The nuclei were hardly seen as they were compressed against the basal border of the mucous cells (Fig.4).

Experimental group II (uncontrolled diabetic group): von Ebner salivary gland exhibited a less intact structure with marked increase in the fibrous connective tissue, separated acini & atypical nuclear arrangement & morphology. Inflammatory cell infiltration was detected in between the acini and lingual muscle fibers. Some acini showed degenerative changes that progressed into destruction of their cells (Fig.5).

Noticeable decrease in nuclear basophilic stain was noticed, intra cellular vacuolation was also increased. Striated duct cells exhibited an abnormal swelling of the nuclei with apparent increased thickness of the duct lining (Fig.6).
Experimental group III (controlled diabetic): Inflammatory cell infiltration was still evident however to a lesser extent. Some acini approached the appearance of control group. Decrease in the intercellular vacuoles was also noticed (Fig.7&8).

Weber salivary glands didn’t show significant changes compared to the control group with respect to the histopathological results of groups I & II.

Immunohistochemical results;

In the control group: α-SMA exhibited a strong stain of the myoepithelial cells bordering the acini of von Ebner salivary glands & few scattered acini of the Weber salivary gland. The serous & mucous secretory cells as well as those cells of the ducts were all non-reactive (Figs.9&10). Vascular smooth muscles were positive (that served as positive control), and the tongue striated muscles were negative (Fig.11).
Experimental group II (uncontrolled diabetic group): The expression of α-SMA showed noticeable changes, as it was not only restricted to the myoepithelial cells but it extended to involve the whole acini with a heterogeneous appearance. The majority of von Ebner gland tissue showed an intense reaction alternating with fewer areas exhibiting a strong to moderate reaction with very few non reactive spots. The positive reaction also extended to the duct lining while the tongue striated muscles remained non reactive (Fig.12).

Experimental group III (controlled diabetic):

Insulin treatment brought about some change in the image; as the reaction was intermediate between those of groups I & II. The reaction to α-SMA was still involving the entire gland tissue in von Ebner salivary gland. However, to a lesser extent than that noticed in group II (Fig.14). The expression in Weber salivary gland did not differ from that of the control group (Fig.10). The connective tissue stroma was non reactive among the three studied groups.
DISCUSSION

Streptozotocin (N-methyl-N-nitroso-urea streptozotocin) (STZ) is a broad spectrum antibiotic with anti-tumor and diabetogenic properties. It is widely used for induction of diabetes in experimental animals. It is thought to act via alkylation of DNA and proteins so it inhibits glucose-stimulated insulin secretion in islets of Langerhans (20).

In the present study, both histopathological and immunohistochemical results of the control group were in accordance with those demonstrated by several authors (6,10,11&22).

Histopathological results of the present study in group II revealed tissue destruction, fibrosis and inflammatory infiltration findings which coincided with those reported in submandibular salivary glands of diabetic rats (14) & in the kidney glomeruli in cases of diabetic nephropathy (37). They related these changes to the derangement of metabolic regulatory mechanisms resulting in increased lipolysis and fatty-acid oxidation, gluconeogenesis and ketogenesis which give rise to a variety of morphological abnormalities. The increased intra cellular vacuolation which were seen in group II were in accordance with Anderson, (1990) who related this to the lipid accumulation within acinar cells of parotid gland in diabetic rats.

In the herein study, findings in group II showed an increase in the fibrous connective tissue as well as a marked increase in the α-SMA expression (Figs 5&12) that was in accordance with Graoma, (1998), who demonstrated that the accumulation of α-SMA would result in extracellular matrix accumulation and interstitial fibrosis resulting in fibrotic lesions in many organs. More over, diabetes was proved to produce an increase in the expression of α-SMA in the kidney glomeruli & the accumulation of collagen type IV resulting in renal fibrosis & nephropathy (1,18&26). Furthermore, increase in the expression of contractile proteins was also demonstrated by other researchers in the mesangial cells in diabetic nephropathy, resulting in mesangial matrix expansion (19). In addition, Xie et al, (2006) concluded that increased blood glucose level would result in decreased smooth muscle/collagen ratio leading to erectile dysfunction.

Our results also coincided with that of Suzuki et al, (2001), who proved the increase accumulation of α-SMA in the lesions of artherosclerosis of diabetic hypercholesteremic pigs. On the other hand Schildmeyer et al, (2000) correlated the vascular contractile dysfunction to the absence of α-SMA in α-SMA-null mice.

Various controversies have been reported about the assumed effect of diabetes on the contractile function of the myoepithelial cells in salivary gland. Lee & Ragonia, 2006 related the decrease in smooth muscle cell contraction in diabetes to an alteration in the myosin light
chain protein and not to the actin over or under expression. Other researchers attributed the defect in salivary flow rate to defects in the neurological function (2&20). Recently, Silva et al, (2009) proved that the alteration in the salivary flow rate can be related to factors other than α-SMA expression alteration. They reported that the reduction in the contractility of myoepithelial cells in salivary glands was related to the increased expression of the Sodium-glucose transport proteins (SGLT1) in the luminal and ductal cells of salivary glands, leading to an increase in the water re-absorption and decrease in the salivary influx.

In the present research, the increase of α-SMA, as well as the increase of fibrous tissue revealed in the immunohistochemical and histopathological results respectively of group II, might lead to altering of the contractile ability of the myoepithelial cells as attributed by other authors (7, 18 & 37).

The histopathological results in the insulin treated group of the herein study revealed an improvement in the condition of salivary glands; in the form of retained nuclear basophilia, decreased tissue destruction, regression of the inflammatory infiltration and a decrease in the intra cellular vacuoles. These findings may indicate that the effect of insulin on salivary gland is rapid both in its reversibility (2).

Our immunohistochemical results of both group II & III were also in accordance with Sanai et al, (2000) who proved that diabetes induced an increased expression of α-SMA & vimentin in the kidney glomeruli of diabetic rats. They also noticed that the situation was corrected by insulin treatment which attenuated or reversed the abnormal expression of the cytoskeletal proteins.

**IN CONCLUSION**

Uncontrolled diabetes induced an alteration in the structure of von Ebner salivary glands. The alteration was in the form of massive inflammatory infiltration, loss of the glands normal architecture and fibrosis. The immunohistochemical results showed an abnormal increase in the expression of the contractile protein (α-SMA). Both histopathological & immunohistochemical results were nearly corrected by insulin treatment. Weber salivary glands appeared to be more resistant to the effect of diabetes along the course of the study.

**RECOMMENDATION**

The effect of diabetes on posterior lingual salivary glands appears to be unique and accepts further investigation. It is also important to solve the puzzle concerning the resistance of Weber salivary glands to such condition.

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