Histological and immunohistochemical study of apoptotic changes in pulp and periodontal ligaments of normal and diabetic adult albino rats

Thesis

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By

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بسم الله الرحمن الرحيم

" و ما أوتيتم من العلم إلا قليلاً "

صدق الله العظيم

دراسة هستولوجية و هستوكيميائية مناعية للتغيرات التي تحدث عند موت الخلية فسيولوجيا في لب الأسنان والأربطة المحيطة بها في الفئران البيضاء البالغة الطبيعية والمصابة بمرض السكر

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To My Parents

And To My Lovely wife

Keryn

Key words: Diabetes, Bcl-2, Rat, Immunohistochemistry, Pulp, Periodontal ligament, Alloxan monohydrate.

Abstract:

A histoligical and immunohistochemical was conducted to study the effect of diabetes on apoptotic changes in pulp and periodontal ligament of adult albino rats were used and divided into two equal groups, a control one and an experimental group. The experimental animals were given daily intrapeitoneal of 140mg/kg body wight for one week. When diabetes was successfully induced and maintained for three weeks, the jaws of the animals were taken prepared and examined. The results of the present study revealed a higher apoptotic rate and cell changes in the diabetic rats' pulp and periodontal ligament. The immunohistochemical examination also revealed a decreased Bcl-2 immunoexpression. In addition the results proposed the presence of multinucleated giant cells suggesting resorptive changes induced by diabetes and an increased inflammatory cell infiltrate. The results of the present study proved the destructive effect of diabetes was more pronounced on the periodontal ligament more than the pulp.

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Introduction

Diabetes mellitus represents a group of diseases of heterogeneous etiology, characterized by chronic hyperglycemia and other abnormalities in carbohydrate, lipid and protein metabolism which are caused by deficiency of the insulin effect ***(Kuzuya et al., 2002).***

Diabetes has a destructive effect on various oral tissues including the periodontal ligaments leading to periodontitis. ***Lalla et al., 2007*** found that periodontal disease was the first clinical complication of diabetes mellitus. ***Taylor and Borgnakke, 2008*** identified periodontal disease as a possible risk factor for poor metabolic control in people with diabetes mellitus. This bidirectional relationship between periodontal disease and diabetes mellitus makes diabetes a disorder of importance to dentists, dental hygienists and patients seen in the dental office.

Apoptosis has been reported to be an important mechanism of cell death and cell self-destruction. Apoptosis is modulated by several inducer or suppressor molecules ***(Schwartz and Osborne, 1993). Graves et al., 2006*** reported that in addition to the robust inflammatory response, enhanced apoptosis (the sequence of programmed events leading to cell death) may contribute to the pathogenesis of periodontitis as a complication of diabetes.

Among these regulatory molecules, the protein product of the proto-oncogene Bcl-2 inhibits apoptosis, preventing several of its stages including blebbing, volume contraction, and endonuclease DNA cleavage. ***(Cohen, 1993) (Williams and Smith, 1993) (Krammer et al., 1994) .***

There are two classes of regulatory proteins in Bcl-2 family that substantiate opposite effects on apoptosis: the antiapoptotic members including Bcl-2 and Bcl-xL which protect cells against some forms of apoptosis, and proapoptotic members including Bax, bak, and Bcl-xS which progress programmed cell death. Apoptotic signals converge toward a common death pathway, for which caspases perform apoptosis and the Bcl-2 family proteins regulate it ***(Hengartner, 2000).***

Thus, this study has been carried out to investigate the effect of diabetes on apoptotic cell changes in the pulp and periodontal ligaments of adult albino rats, using Bcl-2 as an anti-apoptotic marker.

Aim of the study

The aim of the present work is to study the apoptotic changes within pulp and periodontal ligaments of normal and diabetic adult albino rats through:

1) Histopathological examination.

2) Imunohistochemical detection of : Bcl-2 in pulp tissue and periodontal ligament.

Review of literature

Cell Death

Necrosis has always been confused with apoptosis, several studies were carried out to differentiate between them.***Renvoize et al., 1998 and Martin, 2001*** reported apoptosis and necrosis as two distinct forms of cell death. It was proved by ***D'Amours et al., 2001 and Kelly et al., 2001*** that apoptosis is a genetically controlled, energy-dependent method of cellular deletion without inflammation**,** whereas necrosis is associated traditionally with inflammation**.**

Uezozno et al., 2001 stated that apoptosis occurs in response to any mild injury, whereas necrosis is said to occur in response to more severe forms of the same types of injury. Necrosis is difficult to prevent, whereas the apoptotic pathway can potentially be modulated to maintain cell viability. It is assumed that necrosis is 'ordinary' cell death with the characteristics of a passive process while apoptosis is a 'special' form of cell death with the characteristics of an active process ***(Denecker et al., 2001 and McLaughin et al., 2001).***

Apoptosis is a form of cell death characterized by morphological as well as biochemical criteria and can be considered as a counterpart of mitosis ***(kerr , 1971).*** Morphologically the cell shrinks and becomes