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Collagen turnover induced by cellular connective tissue cytokines of drug induced gingival overgrowth and hereditary gingival fibromatosis (Histological and immunohistochemical comparative study)

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ABSTRACT

Background: Gingival overgrowth (GO) is usually associated with multiple factors including immunosuppressive agents as cyclosporine (CsA) and Tacrolimus (TAC), and hereditary gingival fibromatosis (HGF).
Objective: To compare the expression of TGF-β1, PDGF, TIMP-1 and MMP-9 at the molecular and cellular levels in patients receiving (CsA or TAC) and patients manifested (HGF), to cast some light on the pathogenic mechanism potentially involved in the collagen (COL) turnover of both conditions.
Subjects: and methods: Gingival tissue samples were obtained from patients undergoing therapy with CsA (n = 6), TAC (n = 6), HGF (n = 3) as well as control tissues from systemically healthy control (n = 6). Tissue sections were immune-stained by labeled streptavidin-biotin (DAB) technique, using monoclonal antibodies against TGF-β1, PDGF-β, TIMP-1 and MMP-9.
Results: comparison of type of expression among the studied groups, showed significant diffuse expression of TGF-β1 and PDGF-β in group I and II with P value = 0.58 and 0.38 respectively. The expression of MMP-9 was significantly diffuse in TAC or CsA group when compared to HGF group with P value = 0.38, mean while there was a significant diffuse expression of TIMP-1 in HGF group when compared to TAC or CsA group with P value = 0.38.
Conclusions: In conclusion the biological mechanisms behind the drug induced gingival overgrowth (DIGO) and HGF is targeting COL turnover but in different ways. Also, this may explain the need for periodic surgical correction of the gingival form and architecture in HGF cases, unlike the DIGO which can be overcame by replacement of CsA by TAC with improvement of oral health.

1. Introduction

Gingival overgrowth (GO) is defined as abnormal growth of maxillary and mandibular gingiva. It may causes aesthetic changes and clinical symptoms such as pain, speech disturbances, abnormal tooth movement, dental occlusion problems, enhanced risk of caries and periodontal disorders [1,2].

Gingival overgrowth is also known as gingival hyperplasia or gingival fibromatosis, it is usually associated with multiple factors including inflammation, drug use, neoplasias, hormonal disturbances, uncontrolled diabetes and blood dyscrasias [3–5]. In rare cases (1 in 750,000 people), the overgrowth can be hereditary or associated with unknown pathogenesis, hence the former are described as hereditary gingival fibromatosis (HGF) while the others recognized as idiopathic gingival fibromatosis (IGF) [6,7].

Most HGF cases are probably caused by genetic disorders, and therefore should not be called idiopathic, usually identified by significant family history. HGF can occur as isolated disease affecting only the gingiva or as part of a syndrome or chromosomal abnormality, transmitted as autosomal dominant trait in which two

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gene loci on the short arm of chromosome 2 where identified in a Brazilian family [8,9].

Drug induced gingival overgrowth (DIGO) is a side effect and unwanted outcome of systemic medications and is limited to gingival tissue. This is most obvious in the cases of organ transplant recipient who require continuous therapy with immunosuppressive agents, primarily cyclosporine A (CsA), its clinical use is often complicated by several well documented systemic and oral side effects. In the last years, Tacrolimus (TAC/FK506) have been introduced as a new immunosuppressive agents, it has been successfully used as an alternative to CsA with limited systemic and oral side effects [10,11].

DIGO is a pathology characterized by increased deposition of extracellular matrix (ECM) components particularly interstitial collagen (COL), together with altered (COL) turnover may be the main switch for (GO) development [12,13].

In HGF, the histological characteristics suggest that the expansion of gingival tissue results mostly from increased ECM accumulation, since the tissue is rich in collagen, associated with relatively few fibroblasts. In addition to increased proliferation of epithelial cells may account for the formation of elongated rete pegs [14].

It is well known, that (ECM) is an important regulation to cell functions, and it also serves as storage for various growth factors and participates in the regulation of their activation. Thus, altered abundance or composition of ECM may play an active part in the pathogenesis of GO in both HGF and DIGO [15,16].

In addition, the content of the interstitial collagen 1 (COL-1) which is the major components of ECM is mainly determined by the finely tuned balance between synthesis and degradation mediated by matrix metalloproteinases (MMPs), which are the metabolism of extracellular components. Disturbance in the physiological balance between MMPs and their endogenous serum and tissue inhibitors of metalloproteinases (TIMPs) is implicated in several inflammatory disorders. The major serum inhibitor is α_2-macroglobulin, which covalently crosslink with and inactivates target MMPs while tissue inhibitors of MMPs to their active forms. In general, any derangement in the regulatory mechanism controlling MMPs and their inhibitors collagen may increase, leading to GO [17–19].

In fact, connective tissue turnover is largely controlled by chemokines and cytokines secreted by inflammatory cells such as macrophages and lymphocytes and to a lesser degree by fibroblasts. It has been shown that in Go tissues, there are abnormally high levels of specific cytokines including interleukin-6 (IL-6), (IL-Iβ), Transforming Growth factor (TGF-β1), Platelet Derived Growth Factor-B (PDGF-B), Fibroblast Growth Factor-2 (FGF-2) and Connective Tissue Growth Factor (CTGF) [20,21].

In this tightly regulated mechanism, TGF-β1 is the major mediator influencing collagen turnover. There are three isoforms of TGF-β family (β1, β2, β3) that are expressed in humans to stimulate fibroblast proliferation and deposition of ECM, it also regulate various functions of epithelial cells, including cell migration, proliferation and gene expression. The functions of different isoforms are distinct, since TGF-β1 and TGF-β2 are involved in the development of fibrosis, while TGF-β3 appears to prevent it. Almost all human cells synthesize TGF-β1and have receptors for it, alpha granules of platelets and macrophages are the most concentrated source of TGF-β1 [22,23].

Platelet derived growth factor (PDGF) is a dimeric polypeptides consisting of A and B chains, in homodimer (AA,BB) or heterodimer (AB) combinations. The A chain is believed to play a minor role in cell proliferation and tissue repair, while the B chain act as a potent mitogen for cells of mesenchymal origin. Additionally, it has been demonstrated that PDGF-B is both mitogenic and chemotactic for periodontal ligament cells in vitro. It is a major chemoattractant for fibroblasts stimulating fibroblasts proliferation and synthesis of glycosamine-glycans, proteoglycans, fibronectin and collagen. PDGF-B is released from platelets, macrophage, endothelial cells and fibroblasts. In terms of proliferative activity, the release of tissue proliferation associated with fibroblast activity. Thus, its increased level in gingival tissue may be responsible for fibroblast proliferation and/or fibroblast production of extracellular matrix constituents in hyperplastic tissues [24,25].

Up to now, no detailed in vivo study has investigated and compares the difference in cellular and molecular components of the gingival connective tissue (CT) of both DIGO due to immunosuppressive agents and HGF. Therefore, we sought to analyze and compare the expression of TGF-β1, PDGF-b, MMP-9 and TIMP-1 at the molecular and cellular levels in the gingival connective tissue of patients receiving immunosuppressive agents (CsA or TAC) as well as patients manifested HGF, to cast some light on the pathogenic mechanisms potentially involved in the collagen turnover of both conditions.

2. Subjects and methods

The present study was conducted between Jan 2012and Dec 2014. The study populations were grouped into three groups: ***group I*** (HGF gp) consisted of three females' patients (2 sisters and their mother) was brought to the department of periodontology of Misr University for Science and Technology with the chief complaint of a slow growing, non tender gingival enlargement. There was no history of epilepsy or long term medication for any ailments. ***Group II*** (TAC gp and CsA gp) consisted of twelve male patients aged between (40–55years), having undergone liver transplantation at the hospital of Ain Shams University, Wadi-ElNeal Hospital and liver transplantation center of El Maadi Military Hospital. 6 patients taking TAC while the other 6 patients taking CsA. Finally ***group III*** included the healthy controls, selected from the outpatient clinic of Future University, and they were free from any systemic illness and receiving no medications for at least 6 months prior to any investigations. As regard patients of group II, they were at least 6 months with no graft rejection; none of them received multiple transplantations, and their liver function tests were stable.

Smokers, those treated with hydantoin, nifedipine, amlodipine, verapamil and diltiazem and/or with associated systemic diseases that could have a known effect on the gingival tissues such as thrombocytopenic purpura, leukemia or diabetes mellitus were excluded from the study. All patients of group II were required to have at least 10 anterior teeth. All patients were willing to participate in the research after being informed about the nature of the study and all written forms and consents were explained to the patient and signed accordingly. Our research has been conducted in full accordance with the World Medical Association Declaration of Helsinki of 1975, as revised in 2000, and the study has been independently reviewed and approved by an ethics committee review board at Future University.

Patients and controls received full mouth scaling and instructions upon oral hygiene measures, gingival tissue samples were not harvested until complete subsidence of inflammation as recorded by the gingival and plaque indices [26,27]. The gingival overgrowth was assessed using the scale established by Sasaki et al., [28] and the selected patients showed scale [2,3] or [4].

paraffin embedded gingival samples were obtained from all groups and paraffin embedded tissue blocks were cut into 4–6 micron thick, tissue sections were stained with Haematoxylin and Eosin for histopathological examination of all studied cases.