Distribution and localization of ground substance of carbohydrate group in an inflammatory and phenytoin induced gingival enlargement using histochemical method

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ABSTRACT

Background: Gingival enlargement detected as a result of pathological changes or by induction of drugs such as Phenyoitn. Changes in distribution of macromolecules of glycogen, proteoglycan and glycoprotein in gingival enlargement were observed by histochemical method. The aim of the study was to illustrate the localization and distribution of ground substance in an inflammatory and Phenyoitn induced gingival enlargement, using histochemical methods.

Materials and Methods: Twenty two individuals, ten with inflammatory gingival enlargement, other ten with Phenyoitn induced gingival enlargement and two healthy person extraction of impacted 3 rd molar as control .The specimens were stained with periodic acid Schiff reagent (PAS).

Results: In the inflammatory gingival enlargement there is an increase in carbohydrate material production concern to epithelial layer, basement membrane and underneath connective tissue showing reddish purple stain with PAS reaction, while Phenyoitn induced gingival enlargement showed increment in epithelial layer only.

Conclusion: Histochemical method by PAS stain used to show difference in distribution of carbohydrate group in gingival specimens of inflammatory and Phenyoitn induced gingival enlargement.

Key words: Gingival enlargement, Phenyoitn, PAS stain.

INTRODUCTION

Gingival enlargement is a common feature of gingival disease and may be caused by fibrous overgrowth or gingival inflammation or a combination of two. (1) The types of gingival enlargement can be classified according to etiologic factors and pathologic changes as follow: (2)

1. inflammation enlargement
2. Drug induced enlargement
3. Enlargement associated with systemic disease

Inflammatory enlargement, showed inflamed gingival, swollen and consequently hemorrhage due to local factors (bacterial plaque, caries). Histologically gingiva showed epithelial hyperplasia with infiltration of inflammatory cells in lamina propria. (3) Drug induced enlargement as a consequence of administration of some anticonvulsant immuno suppressants drugs, calcium channel blocking agent have been shown clinically and histologically to produce analogous gingival enlargement. (4)

Phenyoitn is an anticonvulsant drug induced gingival hyperplasia in 50%-60% of patients with various levels of inflammation. The degree of inflammation, fibrosis and cellularity depend on the duration, dose and identity of the drug, in addition to individual susceptibility that explain why the induction of the lesion is not of 100% in the patients. (5) Phenyoitn (sodium epanutin, Dilantin) suggested to cause gingival over growth, pronounced in the anterior teeth, histopathological sections showed an increased in thickness of epithelium and in sub epithelial region. (6)

All oral tissues, including gingival, are primarily composed of connective tissue and epithelial linings and associated glands. They posses specific histological matrix includes glycogen, proteoglycan, glycoprotein, mucin, enzymes. These chemical compositions are important in the considerations of the biologic problem, related to oral health. (7)

It is suggested that such intercellular macromolecular substances may play an important role in the maintenance of gingival tissue integrity. (8) Epithelial glycogen is known to increase during inflammation and repair .While glycoprotein showed to be decrease in connective tissues and in basement membrane in cancer disease. (9)Therefore the study was designed to illustrate the localization and distribution of ground substance in Phenyoitn induced and
inflammatory gingival enlargement by histochemical method.

MATERIALS AND METHODS

Twenty two patients participate in the present study, included:

1. Ten patients with gingival enlargement of Phenytoin induced. They were taking drug for 1.5-2 years duration.
2. Ten patients with inflammatory gingival enlargement. Inflammation was assessed by plaque score, bleeding score and gingival depth. Gingival enlargement was assessed on plaster study models by method described by Seymour et al 1985
3. Two normal subjects with extraction of impacted third molar

Histochemical methods

Gingival specimens were taken from all 22 subjects, fixed in 10% buffered formation for 48 hours. Then the periodic acid Schiff (PAS) stain was used for detection of carbohydrate group.

Procedure

1. Deparaffinize section in Xylene and hydrate to water.
2. Oxidize in periodic acid for 5 minutes.
3. Rinse in distilled water.
4. Using Schiff’s reagent for 15 minutes.
5. Rinse in three changes of sulfuric acid 2 minutes each.
6. Washing in running tap water.
7. Counter stain with Harri's hematoxylin for 30 seconds.
8. Dehydration of the section in graded alcohol.

Positive reaction with PAS revealed as reddish purple dye product.

RESULTS

Histochemical stain revealed that normal gingival tissue showed faint pink (negative stain) of PAS. All epithelial layers including basal, spinosum, granulosum and keratinized layers. Underneath connective tissue (papillary and reticular) showed negative stain figure 1.

| Figure 1: normal gingival tissue showing: epithelial layers, Basal cell (BC) Spinous cell (SC), granulosum cell (GC) and keratinized layer (KL), Connective tissue 2 parts papillary (P), reticular (R), PAS stain X 10 |
| Figure 2: Gingivectomy specimen of phenytoin induced gingival enlargement showing long and thin rete pegs, positive PAS reaction in epithelial layer concerning spinosum layer (arrow) while underneath connective tissue shows negative stain. PAS stain X 10 |
| Figure 3: High power view showing spinosum cell positively react with PAS stain. No histochemical reaction illustrates in basement membrane (BM) and in lamina propria (LP). PAS stain X 20 |
| Figure 4: Spinousum cell stained with PAS (arrow) PAS stain X 20 |
Figure 5: Inflammatory gingival enlargement showing positive PAS reaction in keratinized epithelia (K), in granular layer (G) and in some layer of spinosum(S). Basement membrane (BM) shows positive stain too. PAS stain X 10

Figure 6: High magnifying view of inflammatory gingival enlargement showing negative PAS reaction in basal cell (BC) and Spinosum Cell (SC) while basement membrane and Lamina propria show positive reaction (arrow) PAS stain X 20

Figure 7: Inflammatory gingiva showing positive PAS stain in Basement membrane (BM) and in Lamina propria (LP). PAS stain X 20

Specimens from gingival of Phenytoin induced drug showed localization of positive PAS reaction in spinosum layer while negative stain showed in basement membrane and in connective tissue. (Figures 2-4).

Histopathological slides for specimens of inflammatory gingival stained with PAS showed deep reddish purple stain in keratinized layer, granulosum layer and includes some layers of spinosum, basement membrane shows positive stain (figures 5,6). Figure 7 showed band of reddish purple stain in connective tissue underneath the epithelium.

DISCUSSION

The gingival tissue consist of epithelial cells, collagenous fibers, connective tissue cells, intercellular substance (ground substance) which includes glycogen, glycoprotein, proteoglycan and mucin. Capillaries, arterioles and venules as well as lymph vessels and nerves are also present.

The macromolecular carbohydrate components in epithelium and connective tissue of gingival are secreted by epithelial cell and fibroblast cells (respectively). The carbohydrate group includes glycogen, glycoprotein and proteoglycans detected by periodic acid Schiff (PAS) method.

Changes in epithelial glycogen showed during inflammation and repair and even variation in keratinization may reflect the glycogen content of tissue. Thus the present results showed an intense reaction of PAS stain in area of keratin and granular layer of inflammatory gingival which related to an increase of production of glycogen by epithelial cells basement membrane exhibit high PAS reaction which may relate to increase in fibronectin and lamain (glycoprotein). As basement membrane separates between the epithelium and connective tissue, fibronectin secretes by fibroblast in connective tissue while lamain secreted by epithelium cells.

Both proteoglycans and glycoprotein in connective tissue undergo alteration in various pathological state, therefore during inflammation there is an increase in both glycoprotein and proteoglycan level correlated with pathological
behavior, induce fibroblast cell to secrete glycoprotein like fibronectin and proteoglycan, so the result illustrated the PAS reaction in basement membrane and in the connective tissue.\(^{(14)}\)

Results for induced Phenytoin gingival enlargement showed reddish purple stain of spinosum cell surface. This is contribute to the ability of histochemical dye (PAS) stain to bind to epithelium surface which constituents mainly glycoprotein and glycosamine glycan.

In connective tissues PAS stain showed no reaction indicated of absences of increment in the ground substance in which fibroblast is responsible for synthesis it.

Many studies use PAS method to investigate clinical signs related to injury and disease. Qualitative observations were corroborated by quantitative histochemistry of sections stained with PAS. In soft tissue injury showing high glycogen investigated with increased PAS stain when compared with healthy control.\(^{(15)}\) Huang et al 1993\(^{(16)}\) demonstrated glycogen in tumor cell by PAS stain. Sexton and white in 1996\(^{(17)}\) used PAS stain for identification of glycoprotein in primary Ewings sarcoma. While Lee et al 2002\(^{(18)}\) used PAS stain to investigate progression of oral malignant tumor.

REFERENCES