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Expression of Collagen 1 and Heat Shock Protein 47 in Hereditary Gingival Fibromatosis (Immunohistochemical Study)

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ABSTRACT

Background: The gingiva is part of the masticatory mucosa that acts as a barrier against mechanical stress and pathogen. It is made up of a dense, vascular fibrous tissue with a keratinized stratified squamous epithelium, surrounded by mucous membrane that is attached firmly to the periosteum of the alveolar processes of the maxilla and mandible.

Gingival enlargement is one of most commonly presenting gingival disease. Clinically described as gingival enlargement or overgrowth. Gingival enlargement may be hereditary or acquired.

Hereditary gingival fibromatosis is a rare form of gingival enlargement that effects the mandible and maxilla and is characterized by gingiva that grows slowly and progressively over a period of time.

Excessive accumulation of extracellular matrix components seems to play a role in the pathogenesis of the gingival fibromatosis; however, the biochemical and molecular mechanism that induce such pathological condition remain undefined.

Aim of the study: was to evaluate the expression of collagen 1 and heat shock protein 47 in hereditary gingival fibromatosis (Immunohistochemical) and to evaluate Immunohistochemical expression of collagen 1 heat shock protein 47 in hereditary gingival fibromatosis influenced by age, sex and site.

Materials and methods: This study included 36 formalin-fixed paraffin embedded tissue blocks, eighteen of which were collection of sample from archives of the department of oral pathology, the college of dentistry, University of Baghdad between1972 and 2021 diagnose as hereditary gingival fibromatosis.

The second group were composed of eighteen formalin-paraffin embedded tissue blocks which diagnosed as clinical healthy gingival tissue obtain from patient that will undergo gingivactomy due to esthetic demand such as crown lengthening ,gummy smile or prior to teeth extraction

Result: significant increase in the Immunohistochemical expression of collagen 1 and HSP47 were noted in HGF tissue compared with controls (P value=0.0001 and 0.0001 respectively).moreover, no significant difference of study group (p>0.05) regarding age, sex, and site.

Conclusion: Gingival tissue fibrosis in hereditary gingival fibromatosis was observed to be associated with increased collagen 1 and HSP47 synthesis. collagen 1 together with heat shock protien47 has important role in the pathogenesis of hereditary gingival fibromatosis.

Key words: Collagen 1, Heat shock protein 47, Fibrosis, Hereditary gingival fibromatosis

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INTRODUCTION

one of the genetic gingival diseases, as categorized by Armitage's current periodontal diseases and conditions classification, which has been published in 1999 [1-5]. Although the prevalence of this condition is low (1/175,000), but it's possible that several cases can run within the same family [6].

The gingiva is pink in color, fibrous appearance and stippled, but there are no signs of inflammation. HGF can be generalize or localized with various degree of

severity, while the teeth may be partially or completely covered, but it has no effect on the bone [2,4,5,7,8]. It usually affects speaking, chewing, and closure of the lip. However, it can also be a psychological burden and compromised the patient's self-esteem depending on the age at which it appears [2,5]. HGF usually seems within the permanent teeth eruption, though some cases have been reported within deciduous teeth and even at birth [3].

Collagen 1 is the predominant structural component of underlying gingival connective tissue (C.T) and it is important in connective tissue remodeling and wound healing. However, tissue fibrosis eventually could be resulted from over production and accumulation of COL 1 in the extracellular matrix [9]. The biosynthesis of Col 1 in the endoplasmic reticulum initiated by post translational modification of nascent single procollagen polypeptides that leads to triple helical chains formation and finally secretion and deposition into the extracellular matrix .Heat shock protein 47 is a collagen-specific molecular chaperone required for proper procollagen folding in the endoplasmic reticulum, playing a central role in this process by binding to type 1 pro-collagen peptides to impede it's premature folding and aggregation, consequently inhibiting the secretion of procollagen into the extracellular matrix [10]. In numerous human and experimental fibrotic diseases, there is a strong association between elevated HSP47 expression and excessive collagen accumulation. Enhanced levels of HSP47 in fibrotic disorders hypothesized to contribute to the excessive deposition of collagens in fibrotic areas by assisting in the enhanced assembly of procollagen [11]. Increased production and deposition of extracellular matrix collagen by gingival fibroblasts is the main pathological manifestation of HGF. The processes that induce gingival fibrosis, on the other hand, are not completely understood. As a result, the goal of this study was to investigate the process that lead to increased Col 1 and HSP47 synthesis in HGF.

SUBJECTS AND METHODS

A total of thirty-six formalin-fixed paraffin embedded tissue blocks divided into two groups, the first group consist of eighteen formalin-fixed paraffin embedded tissue blocks were previously diagnosed as gingival fibromatosis obtain from histopathological archives of oral pathology department -college of dentistry, University of Baghdad from 1972 to 2021 .Re-evaluation and conformation of each case was made by specialized pathologist.

The second group consist of eighteen formalin-paraffin embedded tissue blocks were diagnosed as clinical healthy gingival tissue which obtain from patient that will undergo gingivactomy due to esthetic demand such as crown lengthening ,gummy smile or prior to teeth extraction which were chosen on the basis of the following criteria: The absence of gingival inflammation, gingival overgrowth or any systemic disease. Furthermore, there was no medicine being taken by any of the healthy volunteers.

Each case of clinically healthy gingiva was histopathological diagnosed by hematoxylin and eosin stains by a specialized pathologist. Tissue preparation was done by fixing all Tissue specimens in 10% buffered formalin for at least 24 hours, and it was routinely processed into paraffin blocks. Serial sections from each paraffin embedded tissue block (samples and controles) were cut; 4mm thick sections were placed on glass slides, stained with hematoxylin and eosin, and re-evaluated histopathological.

At least four tissue sections of 4um thickness were cut and mounted on positively charged slides for Immunohistochemical staining with collagen 1 and heat shock protein 47.

Immunohistochemical (IHC) signal Specify has been established by the lack of immuno-staining in the negative control slides and its presence in the positive controls slides . Brownish staining of Fibroblast cells were considered as positive Immuno-staining , while the fibroblast cells without brown stain were considered as a negative. Blindly assessment for all slides without prior knowledge of the Clinocopathological parameters was done; a specialized pathologist calibrated the histological examination.

Statistical analysis

It was assessed by using P (Chi squared test), P (fishers exact test) were used to measure association between categorical variable while ANOVA test was used for qualitative variable. Correlation between two markers was calculated by Spearman correlation coefficient test; both descriptive and inferential statistical approaches were undertaken. Level of statistical significance was set to be less than 0.05.

RESULTS

The demographic results of cases group and controls group included in this study are presented in Table 1; there was a female's predominance among study groups when compare to males. Furthermore, site frequency of hereditary gingival fibromatosis group, divided in to two site or distributions: generalized and localized were summarize in Table 2.

The current results revealed a significant statistical difference of collagen 1 and heat shock protein 47 score in hereditary gingival fibromatosis group as compared to clinical healthy gingival group ($p < 0.05$) as shown in Tables 3 and Table 4.

Table 1: Demographic characteristics of the study group.

Sex	Frequency	Percent
Male	13	36.10%
Female	23	63.90%
Age (Year)	Mean± SD	Median (Min.-Max.)
	19.83± 9.76	16.50 (4-48)
Total	36	100

Table 2: Site frequency of hereditary gingival fibromatosis group.

Site	Frequency	Percent
Localized	8	44.40%
Generalized	10	55.60%
Total	36	100%

Table 3: Comparison of collagen 1 score between studies groups (Chi square test).

Collagen 1	Category		Total	P value
	Cases	Control		
1	1	14	15	Chi2= 22.67; P- value= 0.0001
	5.60%	77.80%	41.70%	
2	6	4	10	
	33.30%	22.20%	27.80%	
3	11	0	11	
	61.10%	0.00%	30.60%	
Total	18	18	36	
	100.00%	100.00%	100.00%	

Table 4: Comparison of heat shock protein 47 score in study group.

Heat Shock Protein 47 score	Category		Total	P value
	Cases	Control		
1	0	2	2	P-value= 0.0001
	0.00%	11.10%	5.60%	
2	0	4	4	
	0.00%	22.20%	11.10%	
3	6	11	17	
	33.30%	61.10%	47.20%	
4	12	1	13	
	66.70%	5.60%	36.10%	
Total	18	18	36	
	100.00%	100.00%	100.00%	

Table 5: Spearman's correlations between collagen 1 and heat shock protein 47.

	Category		Heat Shock Protein 47
Patient	Collagen 1	R	0.437
		P value	0.07
Control	Collagen 1	R	-0.236-
		P value	0.346

Furthermore, concerning the correlation between COL 1 and HSP47 there were no significant statistical association between two marker, neither in the case nor in the clinical healthy gingival group (p>0.05) as shown in Table 5.

DISCUSSION

The goal of this study was to evaluate the expression of collagen 1 and HSP 47 in hereditary gingival fibromatosis with the expression of the same markers in clinical healthy gingiva (control group). Collagen 1 was chosen because it is the predominant structure of the gingival C.T which is involved in C.T remodeling and wound healing processes , however, its over production lead to extracellular matrix accumulation and, ultimately, tissue fibrosis [11].

As a result, unregulated accumulation of ECM components, particularly Col 1,is the primary cause of gingival overgrowth in HGF [12]. Following that, in 2006, Bitu published a study suggested that the presence of thick layers of collagenous fiber in the gingival C.T in hereditary gingival fibromatosis donors as well as significant increase in Col 1 expression by HGF gingival fibroblasts in vitro, which is consistent with prior finding [13]. Previous research has found that type 1 collagen and HSP47 mRNA and proteins levels are significantly in fibroblast cells derived from patients with HGF than in normal gingiva [14], implying that HGF fibroblasts are metabolically more active than normal fibroblasts.

Heat shock protein 47 is a specific chaperone involved in the synthesis, folding and secretion of collagen molecules. Increased HSP47 production in various fibrotic tissues, such as liver, lung and tendon adhesions have been highlighted the critical role of this chaperone in maintaining fibrotic processes [15]. In the current study, eighteen case of the HGF 47 show positive expression and the majority of cases occupied higher score (2 & 3) and this result was consistent with previous findings [16].

HSP47 expression was shown to be considerably higher in HGF gingival tissue, which is consistent with earlier research [4]. Believing that increased HSP47 levels is required to maintain the interaction between the two molecules, which is required for appropriate folding and overproduction of procollagen polypeptide in hereditary gingival fibromatosis. HSP47 expression was higher in HGF and the clinically healthy gingiva. This finding may be attribute to the multiple clinical, genetic and biological heterogeneity of HGF. This heterogeneity can be illustrate by several findings, as mentioned by Sullivan et al who found that genetic sequencing uncovered a mutation in the gene responsible for amelogenesis imperfect associated with HGF [17]. The expression of HSP47 in both study groups (hereditary gingival fibromatosis and clinical healthy gingiva) indicates a significant statistical difference (0.0001) in the intergroup comparison of HSP47 score, and these findings agree with [18]. Otherwise, enhanced heat shock protein 47 synthesis could be a defense mechanism used by HGF fibroblasts to avoid premature folding of nascent type I procollagen peptides, procollagen chain aggregation and ultimately, cell protection from endoplasmic reticulum stress-induced apoptosis [19]. Although further molecular and biochemical research are needed to identify the mechanisms underlying heat shock protein 47 overexpression, these finding clearly show that up regulation of HSP47 expression is associated with increased collagen 1 synthesis and play a role in accumulation of fibrotic fibrils in gingiva. Therefore, it is important to note that the data presented herein may not fully represent the pathological processes associated with gingival fibromatosis because some limitations of this study, such as the small number of available patients due to the low prevalence of gingival fibromatosis in the general population, as well as the genetic heterogeneity of this condition. Our findings of enhanced heat shock protein 47 and collagen 1 synthesis, in particular, further

examination is require in study groups with larger people of hereditary gingival fibromatosis patients.

Our research, on the other hand, provides a widespread analysis of the processes regulating gingival tissue enlargement that are deregulated in gingival fibromatosis patients, potentially leading to the development of new, non-invasive hereditary gingival fibromatosis treatment strategies aimed at restoring gingival tissue homeostasis in these patients in the future.

CONCLUSION

In summary, fibrosis in hereditary gingival fibromatosis tissue is related with increased collagen 1 and heat shock protein 47 synthesis. An in vitro investigation found that the HGF gingival fibroblasts produced considerably more COL1, HSP47 than control fibroblasts.

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