Journal of Research in Medical and Dental Science
2022, Volume 10, Issue 6, Page No:208-212

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Immunohistochemical Expression of HSP47 and CD206 in GCF and Fibroma of Oral Mucosa

Zahraa Naji Shenbooj*, Ban F Al-Drobi

Department of Oral Diagnosis, College of Dentistry, University of Baghdad, Iraq

ABSTRACT

Background: The GCF is a mucosal fibrous mass that differs from other oral fibrous hyperplasia's by a number of characteristics. The cause of this disease, which was initially documented in 1974, is still unknown. The existence of the giant cells with mono or bi-nuclei characterizes the GCF histopathological.

Aim of the study: This study aimed to Immunohistochemical assessment of HSP47and CD206 in GCF in comparison to fibroma.

Method: Formalin-fixed paraffin-embedded tissue blocks of thirty cases (15 GCF and 15 fibroma) were retrieved from the Oral Pathology Laboratory at the College of Dentistry, the University of Baghdad in the period from 2002 to 2020. Four micrometer thick sections were cut and mounted on positively charged slides and stained immunohistochemically with antibodies to HSP47 and CD206. Statistical analysis was performed concerning the evaluation and comparison of the Immunohistochemical in the above-mentioned lesions.

Results: the Immunohistochemical analysis showed a stronger and more diffuse immune-expression of the HSP47 and CD206 in GCF than fibroma. Regarding the score of HSP47, in GCF the majority of cases was score four while in fibroma scored three. Moreover the highest score of CD206 was three in the GCF and fibroma.

Conclusions: Positive staining for HSP47 indicates that GCF has a mesenchymal differentiation that appears as spindle shaped cells forming the stromal tissue and mono-bi or multinucleated giant cells.

Key words: GCF, Fibroma, CD206, HSP47

HOW TO CITE THIS ARTICLE: Zahraa Naji Shenbooj, Ban F Al-Drobi, Immunohistochemical Expression of HSP47 and CD206 in GCF and Fibroma of Oral Mucosa, J Res Med Dent Sci, 2022, 10 (6):208-212.

Corresponding author: Zahraa Naji Shenbooj

e-mail✉**:** ali.mario28@yahoo.com

Received: 02-May-2022, Manuscript No. JRMDS-22-62387;

Editor assigned: 04-May-2022, **PreQC No.** JRMDS-22-62387 (PQ);

Reviewed: 19-May-2022, QC No. JRMDS-22-62387;

Revised: 26-May-2022, Manuscript No. JRMDS-22-62387 (R);

Published: 02-June-2022

INTRODUCTION

The GCF is a mucosal fibrous mass that differs from other oral fibrous hyperplasia in a variety of ways. Its cause is yet unknown. It currently accounts for up to 5% of all oral fibrous proliferations submitted for biopsy. It did not seem to be caused by chronic irritation [1]. The GCF was first presented as a separate entity of fibrous hyperplastic soft tissue lesions by Weathers and Callihan in 1974. It got its name from its large, stellate-shaped, mononuclear, and multinucleated giant cells [2]. The GCF had no gender predilection [3,4]. The GCF has a little female predominance [1,5,6] and however, slight male predilection is reported in review of twenty-one GCF cases in Clinocopathological study of Sivaramakrishnan et al. [7]. Though GCF can affect any age, but it is most common in the first three decades of life [8]. Clinically, it appears as an asymptomatic raised lesion with a boss elated or pebbly surface, usually one centimeter or less in diameter. It may also be found in places other than the gingiva, such as the tongue, palate, and buccal mucosa. Unless traumatized during mastication or oral hygiene procedures, it typically has a normal mucosal color [4].

Histopathological, the GCF is covered by a thin layer of stratified squamous epithelium that is either parakeratinized or orthokeratinized, consists almost entirely of avascular moderately dense fibrous connective tissue with a fibromyxomatous stroma present in certain areas. Surface epithelium has elongated, small, and sometimes pointed rete processes, which are often accompanied by surface papules or boss elations, the existence of large giant cells usually with mono or bi-nuclei is considered as a consistent and diagnostic feature [9]. A number of authors have questioned whether the GCF should be classified as a distinct entity from the fibroma. They reached their

conclusions based on the presence of the giant cells at different stages of the lesion's maturation, as well as the fact that other histological features aren't distinctive to support recognition as a separate entity [3,8,10].

MATERIALS AND METHODS

Thirty cases of GCF and fibroma were retrospectively retrieved as formalin fixed-paraffin embedded tissue blocks (excisional biopsies). Tissue sections (5µm) were cut, mounted on positively charged slides, and immuno-histochemically stained with monoclonal antibodies to HSP47 (ab109117, 1:300), CD206 (ab64693, 1:1000) using EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit (Abcam®, ab236466; 15ml).

The presence of a brown granular DAB staining pattern within the particular tissue compartment for a specific antibody in positive control tissue sections, but not in negative control tissue slides, was used to demonstrate Immunohistochemical signal specificity, according to manufacturer's datasheets.

All primary antibodies were observed and scored microscopically with a 400X objective in 5 representative fields for each tissue section; the average percent of the 5 high power fields was determined for each marker. All of the instances were evaluated blindly, with no prior knowledge of the other variables.

The Immunohistochemical staining for HSP47, CD206 antibodies was measured semi-quantitatively and assigned into categories for each one, as follows:

Hsp47 scoring: –, none, +, <10%, ++; 10-25%, +++, 26–70%, ++++, 71% [11].

CD206 scoring: 0, none; 1, less than 5%; 2, 5-25%; 3, 25-50%; and 4, more than 50% [12].

RESULTS

Immunohistochemical findings

HSP47 expression is seen as brown cytoplasmic staining of stromal spindle and multinucleated giant cells in Figure 1. The distribution of study sample by HSP47 score, in GCF group 8 cases (53.3%) are scored four and 6 cases (40%) are scored three. In fibroma group, 10 cases (66.7%) are scored three and 3 (20%) are scored four (Figure 2).

The comparison in mean percentage of HSP47 between GCF group and fibroma group revealed that there is a statistically significant difference in mean percentage of HSP47 between the studied groups. Mean percentage of HSP47 is significantly higher in GCF group compared with fibroma group (69.4% versus 53.2%, P=0.015) (Table 1).

CD206 expression is seen as brown cytoplasmic staining of stromal spindle and multinucleated giant cells in Figure 3. The distribution of study sample by CD206 score revealed that the highest proportion of patients in both groups were with score three of CD206, 11 (73.3%)

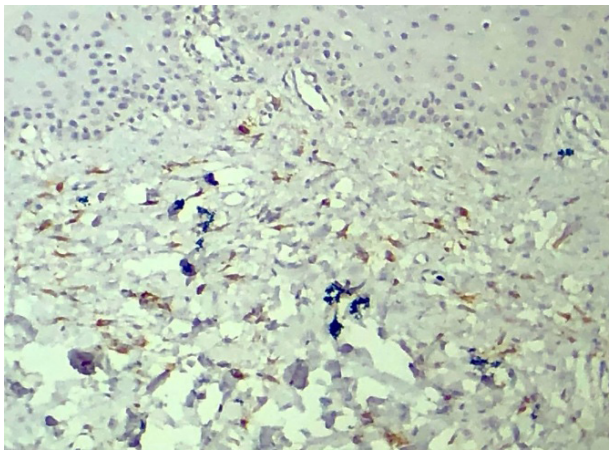

Figure 1: HSP47 in GCF.

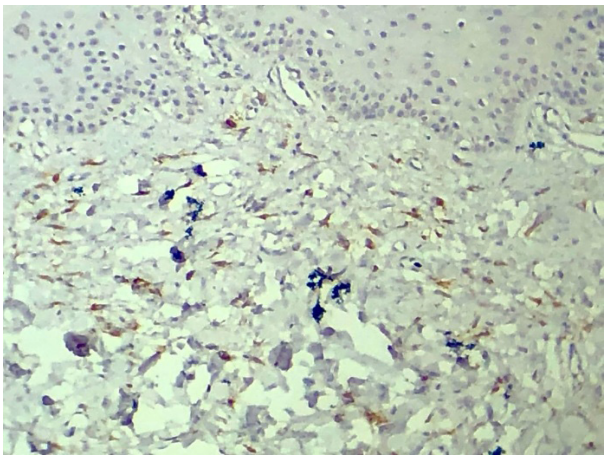

Figure 2: HSP47 expression in fibroma.

of GCF group and 8 (53.3%) of fibroma group (Figure 4). In this study the GCF group has significantly higher mean percentage of CD206 compared with those in fibroma group (67.5% *vs.* 55.2%, P=0.023) (Table 2).

Correlations among immunohisto-chemical markers

In this study, we found a significant moderate positive correlation between percentage of HSP47 and percentage of CD206 of study groups (r=0.493, P=0.006) (Table 3).

DISCUSSION

The GCF is a mucosal fibrous mass with many special characteristics that distinguish it from other oral fibrous hyperplasias [1]. HSP47, a collagen-specific molecular chaperone with a mass of 47 kDa, is found in the endoplasmic reticulum. During the folding, assembly, and transport of procollagen from the endoplasmic reticulum, HSP47 interacts with it transiently [13]. CD206 is a 175 kDa type I transmembrane glycoprotein that interacts with glycoproteins and collagen ligands and internalizes them. CD206 is expressed by cardiac resident macrophages, peritoneal macrophages, adipose tissue macrophages [14], placental macrophages, and skin macrophages [15].

Table 1: Comparison in mean percentage of HSP47 between the studied groups.

HSP47 Score (%)	Study Groups		P - Value
	GCF (Mean ± SD)	Fibroma (Mean ± SD)	
	69.4 ± 20.1	53.2 ± 13.5	0.015

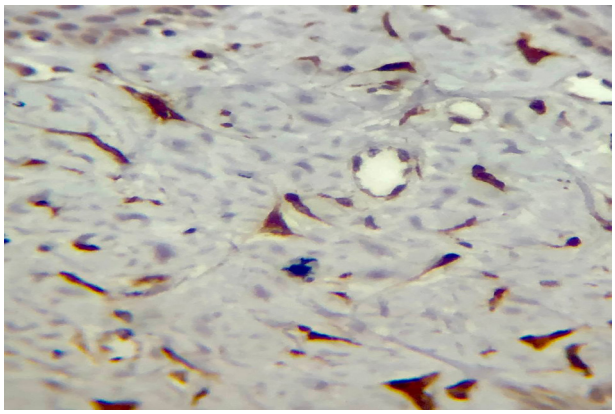

Figure 3: CD206 expression in GCF.

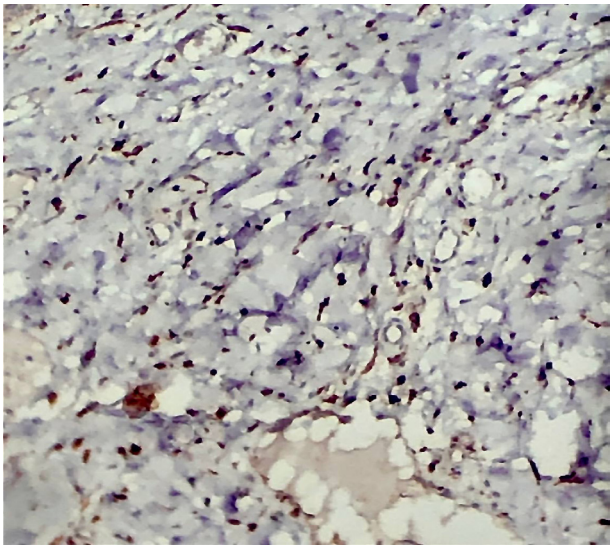

Figure 4: CD206 expression in fibroma.

Weathers and Callihan (1974) speculated that the giant cells could be melanocytes or Langerhans cells when they first reported GCF. In 1982, Houston added his supported the theory of weather and callihan. Several scientists' negative staining for S-100 [16-18] ruled this theory out. The presence of alpha-smooth muscle actin in the cells ruled out myofibroblastic origin. Negativity for CD68, LCA, and HLA-DR [18] overrides the macrophage-monocyte lineage.

The most accepted hypothesis is the fibroblastic lineage of giant cells that investigated by many authors and most of these authorities suggest the fibrous nature of GCF by 100% positivity of vimentin in their series, accordingly they stated that the characteristic giant cells are a giant fibroblast, the present study is the first study that used the HSP47 in GCF, it revealed an intense positivity of HSP47 in cases of GCF in comparison to fibroma which is in agreement with all previous Immunohistochemical studies that used the vimentin as a marker of fibrous

Table 2: Comparison in mean levels certain biochemical markers between study groups.

CD206 Score (%)	Study Groups		P - Value
	GCF (Mean ± SD)	Fibroma (Mean ± SD)	
	67.5 ± 15.6	55.2 ± 11.9	0.023

Table 3: Correlation between HSP47 percentage and CD206 percentage.

HSP47 Percentage	CD206 Percentage	
	R	P – Value
	0.0493	0.006

differentiation [16,17,19-22], the comparison in mean percentage of HSP47 between GCF group and fibroma group revealed that there is a statistically significant difference in mean percentage of HSP47 between the studied groups. Mean percentage of HSP47 is significantly higher in GCF group compared with fibroma group (69.4% versus 53.2%, P=0.015). CD206, the mannose receptor, is a widely utilized macrophage marker. Although the CD206 is frequently used to recognize M2 macrophage subsets [23-26], its expression by other cell types (including satellite cells) has been documented [27].The present study is the first study in the evaluation of Immunohistochemical expression of CD206 in GCF lesions in which all cases of GCF were highly positive for CD206. In this study the GCF group has significantly higher mean percentage of CD206 compared with those in fibroma group.

As conclusions positive staining for HSP47 indicates that GCF has a mesenchymal differentiation that appears as spindle shaped cells forming the stromal tissue and mono-bi or multinucleated giant cells. Positive staining for CD206 in GCF and fibroma indicate that the marker is not specific for histiocytes differentiation and can stain other types of cells like fibrocyte so considered as fibrohistiocytic marker, and as a conclusion the GCF may considered to have a fibrous differentiation as the maker stain the fibrocyte cells in fibroma.

ETHICAL APPROVAL

All experimental protocols were approved by the College of Dentistry, Dentistry, University of Baghdad. All experiments were carried out following the approved guidelines. (Ref no. 274 on 25/3/2021).

FINANCIAL SUPPORT

There was no financial disclosure.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

1. Ismael FJ, Abdullah BH. Clinicopathological study of oral giant cell fibroma. Indian J Forensic Med Toxicol 2020; 14:929.
2. Weathers DR, Callihan MD. Giant-cell fibroma. Oral Surg Oral Med Oral Pathol 1974; 37:374-384.
3. Bakos, Lester H. The giant cell fibroma: A review of 116 cases. Annals Dent 1992; 1:32-35.
4. Lukes SM, Kuhnert J, Mangels MA. Identification of a giant cell fibroma. Am Dent Hygienists Assoc 2005; 79:9.
5. Houston, Glen D. The giant cell fibroma: A review of 464 cases. Oral Surg Oral Med Oral Pathol 1982; 6:582-587.
6. Wang Z, Levy B. Clinico-pathological study on giant cell fibroma of oral mucosa. Chinese J Stomatol 1995; 30:332.
7. Sabarinath B, Sivaramakrishnan M, Sivapathasundharam B. Giant cell fibroma: A clinicopathological study. J Oral Maxillofac Pathol 2012; 16:359.
8. Savage N, Monsour PA. Oral fibrous hyperplasias and the giant cell fibroma. Australian Dent J 1985; 30:405-409.
9. Kuo RC, Wang YP, Chen HM, et al. Clinicopathological study of oral giant cell fibromas. J Formosan Med Assoc 2009; 108:725-729.
10. Reibel J. Oral fibrous hyperplasias containing stellate and multinucleated cells. Eur J Oral Sci 1982; 90:217-226.
11. Kuroda K, Tajima S. HSP47 is a useful marker for skin fibroblasts in formalin-fixed, paraffin-embedded tissue specimens. J Cutaneous pathol 2004; 31:241-246.
12. Adisa AO, Udeabor SE, Orlowska A, et al. Evaluation of tumour associated macrophages and angiogenesis in ameloblastoma. J Clin Diagn Res 2017; 11:ZC33.
13. Nagata K, Yamada KM. Phosphorylation and transformation sensitivity of a major collagen-binding protein of fibroblasts. J Biol Chem 1986; 261:7531-7536.
14. Zeyda M, Farmer D, Todoric J, et al. Human adipose tissue macrophages are of an anti-inflammatory phenotype but capable of excessive pro-inflammatory mediator production. Int J Obesity 2007; 31:1420-1428.
15. Svensson-Arvelund J, Mehta RB, Lindau R, et al. The human fetal placenta promotes tolerance against the semiallogeneic fetus by inducing regulatory T cells and homeostatic M2 macrophages. J Immunol 2015; 194:1534-1544.
16. Odell EW, Lock C, Lombardi TL. Phenotypic characterisation of stellate and giant cells in giant cell fibroma by immunocytochemistry. J Oral Pathol Med 1994; 23:284-287.
17. Magnusson BC, Rasmusson LG. The giant cell fibroma a review of 103 cases with immunohistochemical findings. Acta Odontol Scandinavica 1995; 53:293-296.
18. Campos E, Gomez RS. Immunocytochemical study of giant cell fibroma. Braz Dent J 1999; 10:89-92.
19. Regezi JA, Courtney RM, Kerr DA. Fibrous lesions of the skin and mucous membranes which contain stellate and multinucleated cells. Oral Surg Oral Med Oral Pathol 1975; 39:605-614.
20. Souza LB, Andrade ES, Miguel MC, et al. Origin of stellate giant cells in oral fibrous lesions determined by immunohistochemical expression of vimentin, HHF-35, CD68 and factor XIIIa. Pathology 2004; 36:316-320.
21. Okamura K, Ohno J, Iwahashi T, et al. Giant cell fibroma of the tongue: report of a case showing unique S-100 protein and HLA-DR immunolocalization with literature review. Oral Med Pathol 2009; 13:75-79.
22. Vergotine RJ. A giant cell fibroma and focal fibrous hyperplasia in a young child: A case report. Case REPOR dent 2012; 2012.
23. Lolmede K, Campana L, Vezzoli M, et al. Inflammatory and alternatively activated human macrophages attract vessel-associated stem cells, relying on separate HMGB1-and MMP-9-dependent pathways. J Leukocyte Biol 2009; 85:779-787.
24. Ambarus CA, Krausz S, van Eijk M, et al. Systematic validation of specific phenotypic markers for in vitro polarized human macrophages. J Immunol Methods 2012; 375:196-206.
25. Italiani P, Boraschi D. From monocytes to M1/M2 macrophages: phenotypical *vs.* functional differentiation. Frontiers Immunol 2014; 5:514.
26. Rőszer T. Understanding the mysterious M2 macrophage through activation markers and effector mechanisms. Mediators Inflam 2015; 2015.
27. Jansen KM, Pavlath GK. Mannose receptor regulates myoblast motility and muscle growth. J Cell Biol 2006; 174:403-413.