Significance of Kappa and Lambda Immunohistochemical Expression in the Diagnosis of Oral Plasma Cell Granuloma

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Abstract:
Objectives: To determine the nature of plasma cell proliferation in oral plasma cell granuloma either to be polyclonal or monoclonal via immunohistochemical expression of kappa(k) and lambda(λ) antibodies. This study would confirm the diagnosis of oral plasma cell granuloma and exclude other plasma cell neoplasms.

Material and methods: This study was performed retrospectively on 25 oral plasma cell granuloma cases. Immunohistochemical expression of kappa and lambda light chains was made and the mean percentage of expression was scored semi-quantitatively. The ratio of kappa to lambda mean expression was recorded.
Results: All current study cases revealed positive cytoplasmic expression of both kappa and lambda antibodies in plasma cells. Moreover, the K:λ ratio equaled 2:1 in 44% of cases followed by 1:1 in 36% and 1:2 in the remaining 20%. This revealed a polyclonal nature of plasma cell proliferation. No statistically significant difference was observed in relation to the immunohistochemical expression of both antibodies and the clinical data (age, sex & site) of the cases with p >.05.

Conclusion: Plasma cell granuloma of the oral cavity is a reactive lesion that might resemble plasmacytoma, a malignant plasma cell tumor that affects the oral soft tissue. Polyclonal expression of kappa and lambda light chains enables accurate diagnosis of oral plasma cell granuloma that requires conservative treatment with a favourable prognosis.

Introduction:
Plasma cell granuloma (PCG) is a pseudotumor-like condition characterized by polyclonal proliferation of plasma cells. The features of PCG include sheets, aggregates, and infiltrating groups of plasma cells with intervening fibrous stroma and minimal to absent myofibroblastic proliferation. Plasma cell lesions represent different groups of PCs proliferations either polyclonal or monoclonal. The polyclonal plasmacytic infiltrates are defined as reactive lesions. While monoclonal varieties of plasmacytoma and multiple myeloma (MM) are well-defined as neoplasms.

Oral plasma cell granuloma may be misinterpreted clinically or histologically as many other plasma cell lesions mainly Multiple Myeloma and Extramedullary Plasmacytoma. An accurate differential diagnosis from these plasma cell neoplasms is mandatory hence correct
management and treatment are performed. Multiple myeloma (MM) is a relatively uncommon malignant plasma cell tumor that usually arises within the bone. Whereas plasmacytoma is a soft tissue tumor of MM that may be observed on the gingiva, palate, floor of the mouth, tongue, tonsils, and pillars, as sessile or polypoid reddish masses in the mucous membranes that do not tend to ulcerate. Histologically, it comprises diffuse sheets of neoplastic, variably differentiated, and monoclonal plasma cells. Mitotic activity and amyloid deposition may be present and inflammatory cells are very sparse.

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The immunohistochemical examination recognizes cellular (cytoplasmic light chain) and extracellular (surface light chain) k and λ proteins, in differentiated B-lymphocytes (plasma cells). Individual B-lymphocyte or plasma cell in lymphoid tissue possesses either k or λ light chain, but never both together. Thus, a positive reaction of both k and λ light chains reveals polyclonality of plasma cells i.e. the lesion is reactive, rather than neoplastic in nature showing monoclonal plasma cell proliferation.

This study would be considered a crucial step towards a confirmatory diagnosis of plasma cell granuloma of the oral cavity and exclusion of the more serious neoplasm plasmacytoma and MM that involve significant complications and requires more serious intervention.

**Material and Methods:**

**Specimens:**

Sample size calculation was performed via the website (calculator.net). Data provided was 

power (confidence level) = 95%, margin of error 5%, population proportion 50% and a population size of 26 which resulted in sample size (n) = 25. The current work was operated as a retrospective study of 25 OPCG cases that were diagnosed during the last 7 years (from January 2015 to January 2021). The paraffin blocks and clinical data were taken from the archives of the Oral Pathology Department, Faculty of Dentistry, Mansoura University. Ethical approval was obtained prior to the study from the Ethics Committee, by code no. A05060819.
Each Paraffin block of the study cases was serially cut into three 4μ thickness sections.

**Histopathologic examination:**

Hematoxylin and Eosin (H&E) staining was performed according to the manufacturer structures for one of the tissue sections to confirm diagnosis with the aid of the light microscope, the hematoxylin and eosin (H&E) sections were thoroughly investigated and re-diagnosed histopathologically.

**Immunohistochemical (IHC) examination:**

The other two sections were mounted on electrically charged Opti plus slides obtained from Bio GEMEX Laboratory. Immunostaining was performed using the Avidin-Biotin complex (ABC) method according to the manufacturer's instructions. For an affinity purified rabbit polyclonal antibodies in a ready-to-use form of Kappa Light Chain and Lambda Light Chain (λ)(Quartett, Germany, concentrated antibody in TRIS (Hydroxymethyl) aminomethane (PH 7.4) with < 0.1% in sodium azide). Tonsillar tissues were used as positive control. For negative control, sections were run with the test and the step of polyclonal antibody was replaced by addition of non-immune serum. This negative control slide is important to assess the background staining.

**Evaluation of the IHC staining:**

Assessment of k and λ light chain markers individually in each tissue section was detected specifically in plasma cells(PCS). The mean count of positive PCs in 5 selected hot spots at high power magnification (x400) in each section was performed and scored semi-quantitatively as follows: 0 = percentage of positive PCs = zero, 1 (low) = percentage of positive PCs = 1–20%, 2 (intermediate) = percentage of positive PCs = 21–49%, 3 (high) = percentage of positive PCs = > 50%. Finally, the ratio of K: L in each OPCG was determined and assessed. If
expression percentage of both markers was approximate i.e. $k: \lambda \approx 1:2-2:1$ this indicates a polyclonal or reactive proliferation. Meanwhile divergent expression percentage of both markers i.e $k: \lambda \approx 1:10-10:1$ means a monoclonal or neoplastic proliferation.\(^6,^8\)

**Statistical analysis:**

Data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 24.0. In the statistical comparison between different groups, the significant differences were tested using Chi-square test. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level. A $P$ value $<0.05$ was considered statistically significant.

**Results:**

**Clinical findings:**

The detailed clinical data was mentioned in an earlier study of us on the same cases.\(^9\)

**Histopathologic findings:** All cases of the current study were represented as a gingival lesion that histologically revealed a fibrovascular connective tissue stroma enclosing variable groups of non-atypical plasma cells with the characteristic cartwheel appearance (Fig. 1).

**Immunohistochemical findings:**

**The IHC staining for $k$ and $\lambda$ light chain among the current OPCGs:**

All cases of the current study revealed positive cytoplasmic reaction of both $k$ and $\lambda$ antibodies (Fig 2. a&b). Percentage of positive plasma cells was high in all cases except for two cases only that represented low percentage in both antibodies. Lymphocytes, fibroblasts, endothelial cells (Fig. 2.c) as well as the superficial epithelium showed negative reaction (Fig 2 a & b). Regarding the ratio of k: $\lambda$ mean expression percentage, mostly, the current
studied OPCG revealed approximate expression percentages of k: λ ratios $\approx 2:1$ in 44% of cases, $\approx 1:1$ in 36% of cases and $\approx 1:2$ in the remaining 20%.

Statistical correlations performed between immunohistochemical expression of both antibodies, and the clinical data revealed no statistically significant difference in between (Table 1 & 2).
Fig 1: Hematoxylin and eosin stained section showing a: A case of OPCG showing fibrovascular connective tissue stroma containing abundant groups of plasma cells. b: High power magnification showing mature non-atypical plasma cells entrapped within the fibrous septa. c: Oil Lense magnification revealing the characteristic cartwheel nuclei. (H&E a: X 100 b: X400, c: X1000).
Fig 2: Positive immunohistochemical expression of plasma cells in a: OPCG tissue section stained with kappa, b: and lambda antibodies. Note: Negative stain of superficial epithelium with both antibodies. c: Strong cytoplasmic expression of plasma cells (violet arrows), negative expression in fibroblasts (black arrow), negative endothelial cells (red arrow) and negative lymphocytes (yellow arrow). (DAB a&b X100, c: X400).
Table 1: The relation between clinical data and IHC expression of k light chain of studied OPCGs.

<table>
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<th>Clinical variable</th>
<th>Low</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
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<tr>
<td>Age</td>
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<tr>
<td>&lt;25 y n=4</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<tr>
<td>Lower anterior gingiva</td>
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<td>16.6%</td>
<td>5</td>
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<tr>
<td>Lower posterior gingiva</td>
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<td>9.1%</td>
<td>10</td>
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Frequency (n=number of OPCG -%=percent). y: years of age. P: Probability. Significance where P value <0.05. Test used: Chi-square test.

Table 2: The relation between clinical data and IHC expression of λ light chain of studied OPCGs.

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<td>Lower posterior gingiva</td>
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<td>9.1%</td>
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Discussion:

Plasma cell granuloma of the oral cavity represents a rarely encountered entity with undetermined etiology. It was important to note that the histological examination of the current studied OPCGs revealed proliferation of uniform, small and mature PCs with no signs of atypia. This agreed with most case reports in the English Literature.\textsuperscript{8, 10} Meanwhile, it was well known that the immature, plasmablastic, and pleomorphic plasma cells were not found in the current study while has been reported by others as features suggestive for a plasma cell neoplasm.\textsuperscript{11}

In addition, most of the OPCGs in the current research showed high immune reactivity of k and λ which might indicate overproduction of both antibodies by the plasma cells. This was in accordance with other research \textsuperscript{10, 12} The low immune reactivity of both antibodies observed in only two cases might represent low production of kappa and lambda by plasma cells in the same lesion. These findings represented absence of overproduction of either antibody than the other which denied a monoclonal neoplastic proliferation and confirmed the polyclonality of plasma cells indicating a reactive nature. Remarkably, these observations certified the importance of immunohistochemistry in determining the nature of plasma cell lesions.

Besides, the expression ratio of both k and λ light chain markers among presented studied OPCGs, revealed positivity of k more than λ light chain in most of the cases in which K/λ expression ratio was approximately 2:1. A finding that might be due to the step-by-step formation process of heavy and light chains. In this process, the locus encoding for the heavy chain portion has been reported to undergo rearrangement first. Then, if successful, it would be followed by a rearrangement of the kappa light chain locus (field of genetics and
biostatistics, where the term would express the specific location occupied by a particular gene on a chromosome. The variants of genes occupying the same locus on chromosomes were called alleles). Only if the k light chain rearrangement for both alleles was ineffective, so λ light chain rearrangement might occur. This finding was in agreement with other previous studies. The close ratios would strongly confirm the polyclonality of such lesions indicating a reactive nature.

Moreover, k and λ light chain IHC markers were stained to diagnose and assert plasma cells. In the current study, it was noticed that the expression pattern of both k and λ immunostaining was cytoplasmic in OPCGs. This was supported by other reports related to the main function of plasma cells in production of immunoglobulins (Igs), that are composed of both k, λ light chain peptides, within the endoplasmic reticulum of the cytoplasm of plasma cells. These Igs have been reported to be accumulated in Russel bodies as large cytoplasmic eosinophilic globules positive with k and λ light chains immunostaining.

On the other hand, It was remarkably observed that lymphocytes in the stroma of the current OPCGs were mainly negative for both k and λ immunostaining as reported also by Rimsza et al. These lymphocytes could be attributed to other white blood cells than B lymphocytes which might be T lymphocytes or NK cells. All of these cell types were known to be originated from the same stem cells. Therefore, the negative reaction of these lymphocytes can occur. They were justified by the lack of Igs that have been formed only in plasma cells. Meanwhile, the plasma cells has been known with their capability to synthesize large amounts of proteins, mainly Ig proteins of k, λ, denoting the expected defense mechanisms of PCs against extracellular microbes or other non-studied stimuli.
At last, as there was no statistically significant difference found between IHC expression of k and λ light chain markers the clinical data of OPCGs of the present study reflecting absence of the impact of clinical presentation upon the immunohistochemical features of this lesion.

**Conclusion:**

- Plasma cell granuloma of the oral cavity is totally a reactive lesion.
- Similarity to other neoplastic plasma cell neoplasms necessitates a comprehensive histopathologic examination supported with immunohistochemical analysis specifically of k and λ light chains to enable accurate diagnosis of OPCG and hence to apply the proper management.
- Further studies upon larger number of cases is recommended to maximize knowledge about this rare oral lesion.

**1-Data Availability:** Data supporting the findings are available from the corresponding author upon reasonable request.

**2-Funding:** This study was not supported by any funding

**3-Conflict of interest:** The authors declare that they have no conflicts of interest

**References:**