Comparison of gold standards with common histopathologic evaluations in diagnosis of oral neurofibromas in pathology department of Shiraz Dental School

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Abstract

Introduction: Diagnosis of neurofibroma usually is based on the specific morphology and arrangement of mesenchymal cells in routine Hematoxylin and Eosin (H&E) sections, and detection of mast cells supports the diagnosis. Sometimes definite diagnosis from other mesenchymal lesions may be difficult. The aim of the present study was to compare S100 expression and mast cells count (as Gold Standard) with routine histopathologic diagnosis.

Methods: In this cross-sectional analytical study, all cases of neurofibroma and compatible/consistent with neurofibroma, that had been diagnosed in department of oral & maxillofacial pathology, school of dentistry, Shiraz, from 1986 to 2013, were enrolled. Immunohistochemistry was performed using S100 antibody and slides were stained by Giemsa. S100 labeling index, intensity and distribution as well as mast cells count were evaluated using light microscope.

Results: Mast cells were present in 97% of cases that 56.4 % showed 1-200 cells/10HPF. 82 % of cases were positive for S100 that 40.7% showed 2-30% labeling index and 70.4% had moderate intensity for S100 staining.

Conclusions: The comparison of routine histopathologic examination with gold standard method in Oral Pathology Department of Shiraz Dental School confirmed the routine histopathologic diagnosis in all cases, therefore no more evaluation may be required if a pathologist considers all routine diagnostic criteria.

Keywords: Neurofibroma, S-100 protein, Giemsa stain, Mast cells


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مقایسه استاندارد طلایی شناسایی نوروفيبرومای دهانی با روش تشخیص هیستوپاتولوژیک رایج در بخش پاتولوژی دانشکده دندانپزشکی شیراز

علي دهقان، زهره جعفری اضکونی، نسیه شاملو، زینب منیری

چکیده
تشخیص نوروفيبروما به صورت معمول بر یاد رگ آمیزی هماونکسیلین و اتودین (H&E) و مشاهده ی تغییرات مورفولوژیک و آنژیوپاتی سلول های مرکزی از دلیل حضور آن در سطح مایع می دهد. گاهی یک توجه به اشکالات شایع ترمیمی هیستوپاتولوژیک در گروه کنترل ممکن است، در تشخیص افتراقی نوروفيبروما پترن برای طول قلی نمی گردد. تاکنون تحقیقاتی در این زمینه فراوانی بررسی می شده است. S100 و بررسی سلول ها به عنوان استاندارد طلایی تشخیص برای روش رایج تشخیص بوده است.

مواد و روش ها:
در این مطالعه ی تحقیقی -مقطعی، بیمارانی که تحت رگ آمیزی (H&E) می گردد و نوروفيبروما و یا مشابه نوروفيبروما بوده است، از آزمون یک ماه استخراج گردید. سپس میزان رگ آمیزی گیمسب و اینوتوموهیستوئومیک S100 رگ شناخته شد. با استفاده از میکروسکوپ نوری، شمارش سلول ها و میزان، شدت و توزیع رگ آمیزی S100 مورد ارزیابی قرار گرفت.

یافته ها: 
از بین 33 نمونه مورد بررسی در 79٪ نمونه ها حضور کامل سترا ان با درصد 42٪ و 57٪ ثابت شد که در 97٪ نمونه ها رگ آمیزی گیمسب و اینوتوموهیستوئومیک S100 در 100٪ نمونه ها، در سطح 0.01 یا زیاد، مثبت بود.

نتیجه گیری: 
نگ آمیزی که در این مطالعه در بخش پاتولوژی و مقایسه ی دو روش در گروه پاتولوژی دهانی دانشکده دندانپزشکی شیراز موید هامونکسیلین-هیستوپاتولوژیک و H&E تشخیص رایج است و با استفاده از هیستوپاتولوژیک، تنها در مواردی می تواند در تشخیص نوروفيبروما به عنوان استاندارد طلایی و در صورتی که یک روش تشخیصی به ترتیب رایج را در نظر گیرد، احتمالاً الگوی تناوبی به صورت هنری را ساخته و زمان بیشتر برای تأیید آنها با استاندارد طلایی وجود ندارد.

واژگان کلیدی: نوروفيبروما، اینوتوموهیستوئومیک S100، رگ آمیزی گیمسب، میکروسکوپ نوری

Introduction
Diagnosis is the most important phase of a patient’s treatment. It is made by combination of mental and practical actions through which the disease is determined and evaluated. One of the common benign neoplasm of peripheral nervous system is neurofibroma. It may appear as a solitary lesion or multiple as a part of neurofibromatosis type 1 syndrome. Its histopathologic feature consists of interlacing fascicles of spindle-shaped cells with fusiform or wavy nuclei. In most cases, presence of mast cells helps the diagnosis. These cells can definitely be diagnosed using Giemsa and toluidine blue staining methods. S100 protein is normally expressed in the nuclei and cytoplasm of cells derived from the neural crest (Schwann and Gelial cells and Melanocytes), fat cells, Myoepithelial cells, macrophages, Langerhans cells, dendritic cells, nevus cells and keratinocytes, chondrocytes, satellite cells of adrenal medulla, adenohypophysis, reticular cells of lymph nodes, interstitial cells of pineal gland and tumors derived from these cells. S100 involves many intra and extra cellular biologic functions, but this protein is usually employed for definitive diagnosis of peripheral nerve sheath and melanocytic tumors.
can show S100 protein in the most cases of neurofibroma which confirms the diagnosis.\textsuperscript{[2,3]} In Karvonen et al. study (2000), S100 was used as the gold standard for identification of new tumors in patients with neurofibromatosis type I.\textsuperscript{[14]}

Karamchandani et al. used S100 for detection of cells with nervous system origin in soft tissue neoplasms.\textsuperscript{[15]} Diagnosis of neurofibroma usually is based on the specific morphology and arrangement of mesenchymal cells in routine H&E sections, and detection of mast cells supports the diagnosis. Sometimes definite diagnosis from other mesenchymal lesions may be difficult because of similarity in histopathologic features and the mast cells may not be detected.

Furthermore, the researchers found no research on comparison between the common H&E method and S100 and Giemsa staining to evaluate the accuracy level of neurofibroma diagnosis. Therefore, the aim of the present study was to compare S100 positivity and mast cells detection as gold standards with routine histopathologic diagnosis.

**Methods**

In this cross-sectional analytical study, all cases of neurofibroma and those compatible/consistent with neurofibromatosis that had been diagnosed in department of oral & maxillofacial pathology School of Dentistry of Shiraz, between 1986 to 2013 were enrolled.

The diagnosis was confirmed by pathologists according to routine histopathologic features. All cases had enough tissue for evaluation. For S100 and mast cell, staining two sections with 4-µm thickness was provided. For Giemsa staining, the sections were deparaffinized and placed in 5% Giemsa solution for one hour, then washed with acid acetic and water. Finally the sections were mounted and evaluated using light microscope.\textsuperscript{[16]}

Mast cell count was evaluated in 10 microscopic fields, at 400 magnification and reported as negative (0), +positive (1-200), ++positives (between 200-1000) and +++positives (>1000). S100 expression was evaluated by immunohistochemistry.\textsuperscript{[16]} The sections were deparaffinized and rehydrated.

Endogenous peroxidase activity was inhibited by 3% H2O2. Then, the sections were incubated with S100, Polyclonal Rabbit antibody (Ready to use, code iR504-DakoLTD) for 30 minutes. 3, 3_di_aminobenzidine (DAB-Code K8004-DAKO LTD) solution was used as chromogen. A section of schwannoma was used as positive control.

Primary antibody was replaced by TBS Buffer in negative control sections.\textsuperscript{[17]} S100 expression was classified in 4 groups: negative (<2%), +positive(2-30%), ++positives(30-80%), +++positives(>80%). Regarding to intensity of expression, the results were categorized in 3 grades: 1: low, 2: moderate, 3: intense.\textsuperscript{[18]} Data were analyzed using SPSS software version 11.

**Results**

33 cases of neurofibroma were evaluated. They were 16-74 years, with mean age of 50 years. 18 cases (54.5%) were male and 15 (45.5%) were female. Regarding to the location, neurofibroma was reported in gingival (42.4%), buccal mucosa (24.2%) and other areas such as retromolar pad, mandibular body, hard palate, tongue and floor of the mouth (33.4%). Giemsa staining demonstrated the mast cells as round, oval or polygonal cells with purple granules (figures 1&2).

Mast cells were found in 97% of the cases, the mast cell count in 56.4% (18 patients) of the cases was found one positive (+), 21.8% (7 patients) two positive (++) and 21.8% (7 patients) three positive (+++). In S100 positive immunoreactions, mesenchymal cells were found with brown nucleus and cytoplasm (figure 3). S100 expression is shown in table 1. The diagnosis was confirmed in the cases that were positive for Giemsa, S100 or both of them (table 2).

**Figure 1.** Bundles of fusiform mesenchymal cells with elongated and wavy nuclei in a neurofibroma lesion beside the presence of mast cells (Asterisk) (H&E magnification, 400X).
Giemsa and S100 immunostaining in oral neurofibroma

**Figure 2.** Polygonal mast cells in a neurofibroma lesion with blue nuclei and basophilic abundant granules in its cytoplasm (Giemsa staining, 1000 X)

**Figure 3.** Brown S100 staining in nuclei and cytoplasm of mesenchymal cells with diffuse pattern (IHC staining, 400X)

**Table 1. Quantity, intensity and distribution of S100 in neurofibroma cases**

<table>
<thead>
<tr>
<th>Positive cases</th>
<th>S100 staining</th>
<th>S100 staining intensity</th>
<th>Pattern of S100 staining</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td>27</td>
<td></td>
<td>Grade1</td>
<td>Grade2</td>
</tr>
<tr>
<td>11</td>
<td>9</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>Grade3</td>
<td>focal</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>diffuse</td>
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<tr>
<td>4</td>
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</tr>
<tr>
<td>4</td>
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<tr>
<td>15</td>
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</tr>
<tr>
<td>44.5%</td>
<td>55.5%</td>
<td></td>
<td></td>
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</tbody>
</table>

**Table 2. Comparison of H&E staining with special staining methods in this study**

<table>
<thead>
<tr>
<th>Total cases</th>
<th>H&amp;E diagnosis confirmation with Giemsa</th>
<th>H&amp;E diagnosis confirmation with S100</th>
<th>H&amp;E diagnosis confirmation with both gold standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>32</td>
<td>27</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>97%</td>
<td>82%</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Discussion**

Histopathologic features of all neurofibromas and the similar lesions in this study were interlacing fascicles of spindle-shaped cells with fusiform or wavy nuclei based on H&E sections (figure 1) beside many mast cells scattered among them. According to the study of Leclere et al., Giemsa staining for detection and confirmation of mast cells is considered more appropriate because it had manifested less expense and more convenient application among the other four staining methods.

In this study, 97% of mast cells were stained by Giemsa and proved their existence in H&E slides (figure 2). Their count also showed vast spectrum of their presence in neurofibroma lesions from less than 200 cells/10HPF to more than 1000 cells/10HPF; 56.4% of the cases were under 200 cell/10HPF, half of the remaining cases were from 200 to 1000 and half of the other had more than 1000 cells/10HPF. S100 normally exists in nucleus and cytoplasm of cells derived from neural crest (Schwann and gelial cells and melanocytes) and tumors derived from them. In the present study, the neural origin of the majority of the cases was confirmed by IHC staining for S100 (82% of patients) (figure 3). In a study, 49 patients with peripheral nervous system tumors showed S100 positivity in all neurofibroma cases, while in another study, S100 was positive in 95% of the cases.

Other study also showed S100 staining in about half of the skin tumors of 9 patients suffered from neurofibromatosis type1. The labeling index of S100 in this study was 1 positive in 40.7% (11 patients).
Conclusions

Neurofibroma is a benign tumor with neural origin, its common diagnosis of which is based on H&E staining and the pathologists report this tumor when they detect interlacing fascicles of spindle-shaped cells with fusiform or wavy nuclei and also the presence of mast cells. Gold standard staining and the comparison between the two methods in the present study showed that the current diagnosis was totally confirmed in Oral Pathology Department of Shiraz Dental School, therefore no more evaluation may be required for future cases if a pathologist considers all routine diagnostic criteria.

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