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Original Article

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 نوروفیبروموای دهانی با روش تشخیص هیستوپاتولوژیک رایج در بخش پاتولوژی دانشکده دندانپزشکی شیراز

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چکیده

مقدمه: تشخیص نوروفیبروما به صورت معمول بر پایه رنگ آمیزی هماتوکسیلین و ائوزین (H&E) و مشاهده ی مورفولوژی و آرایش خاص سلول های مزانشیمی درکنار بررسی وجود ماست سل ها صورت می پذیرد. گهگاه با توجه به اشتراکات نماهای هیستولوژیک در ضایعات مختلفی که در تشخیص افتراقی نوروفیبروما مطرح می شوند به طور قطعی نمی توان تشخیص نوروفیبروما را تایید نمود، هدف این مطالعه مقایسه بیان نشانگر S100 و بررسی ماست سل ها به عنوان استاندارد طلایی تشخیص با روش رایج تشخیص بوده است.

مواد و روش ها: در این مطالعه ی تحلیلی-مقطعی، بلوک های مرتبط با تمامی پرونده های موجود در بخش پاتولوژی دهان و فک و صورت دانشکده دندانپزشکی شیراز که تشخیص هیستوپاتولوژیک آن ها از سال ۱۳۶۵ تا ۱۳۹۲بر اساس رنگ آمیزی H&E، نوروفیبروما ویا منطبق بر نوروفیبروما بوده است، از آرشیو بخش استخراج گردید. سپس لام ها با رنگ آمیزی گیمسا و ایمنوهیستوشیمی S100 رنگ شدند. با استفاده از میکروسکوپ نوری، شمارش ماست سل ها و میزان، شدت و توزیع رنگ پذیری S100 مورد ارزیابی قرار گرفت.

یافته ها: از بین ۳۳ نمونه موجود، در ۹۷٪ نمونه ها حضور ماست سل ها تایید شد که میزان حضور آن ها در۵۶/۴ ٪ موارد (۱۸نفر)، کمتر از ۲۰۰ سلول در ۱۰ فیلد میکروسکوپی با بزرگنمایی بالا High power field (HPF) ثبت گردید. از میان نمونه های موجود ۸۲٪ بیماران به وسیله ی مارکر S100 رنگ گرفتند که میزان رنگ پذیری سلول ها در ۴۰/۷٪ بیماران (۱۱ نفر) ۲ تا ۳۰ درصد و شدت رنگ پذیری آن ها با S100 در ۷۰/۴ ٪ بیماران (۱۹ نفر)، متوسط بود.

نتیجه گیری: رنگ آمیزی های استاندارد طلایی و مقایسه ی دو روش درگروه پاتولوژی دهان دانشکده دندانپزشکی شیراز موید هماهنگی میان تشخیص هیستوپاتولوژیک رایج با H&E و استانداردهای طلایی در تمامی نمونه ها بود که این نشان می دهد در صورتی‌که یک پاتولوژیست تمامی معیار های تشخیصی رایج را در نظر بگیرد، احتمالا دیگر نیاز به صرف هزینه و زمان بیشتر برای تایید آن ها با استاندارد طلایی وجود نخواهد داشت.

واژگان کلیدی: نوروفیبروما، پروتئین S-100، رنگ آمیزی گیمسا، ماست سل

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. [1] One of the common benign neoplasm of peripheral nervous system is neurofibroma.[2] It may appear as a solitary lesion or multiple as a part of neurofibromatosis type 1 syndrome.[3] Its histopathologic feature consists of interlacing fascicles of spindle-shaped cells with fusiform or wavy nuclei.[2,3] In most cases, presence of mast cells helps the diagnosis. These cells can definitely be diagnosed using Giemsa and toluidine blue staining methods.[2-5] S100 protein is normally expressed in the nuclei and cytoplasm of cells derived from the neural crest (Schwann and Gelial cells and Melanocytes) [4,6,7], fat cells, Myoepithelial cells, macrophages, Langerhans cells, dendritic cells, nevus cells and keratinocytes [4,6,7], chondrocytes [4,8], satellite cells of adrenal medulla[9], adenohypophysis[10], reticular cells of lymph nodes, interstitial cells of pineal gland[11] and tumors derived from these cells.[6,7,12,13] S100 involves many intra and extra cellular biologic functions [4], but this protein is usually employed for definitive diagnosis of peripheral nerve sheath and melanocytic tumors.[4] Immunohistochemistry

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can show S100 protein in the most cases of neurofibroma which confirms the diagnosis.[2,3]

In Karvonen et al. study (2000), S100 was used as the gold standard for identification of new tumors in patients with neurofibromatosis type1.[14]

Karamchandani et al. used S100 for detection of cells with nervous system origin in soft tissue neoplasms.[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 neurofibroma 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 were 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.[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.[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.[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.[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).

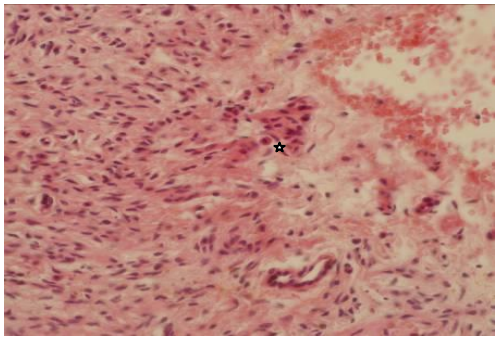

Figure1. Bundles of fusiform mesenchymal cells with elongated and wavy nuclei in a neurofibroma lesion beside the presence of mast cells (Asterisk) (H&E magnification, 400X)

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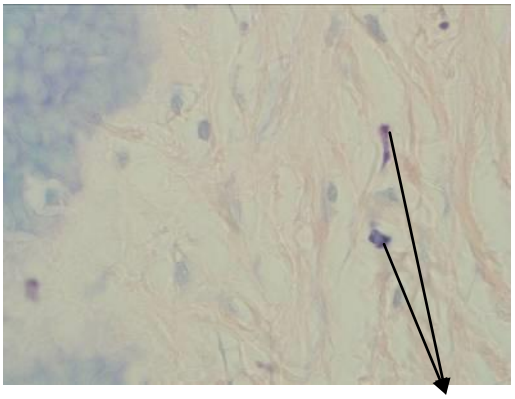

Figure2. Polygonal mast cells in a neurofibroma lesion with blue nuclei and basophilic abundant granules in its cytoplasm (Giemsa staining, 1000 X)

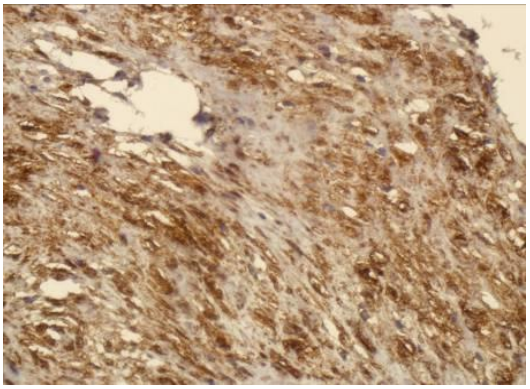

Figure3. Brown S100 staining in nuclei and cytoplasm of mesenchymal cells with diffuse pattern (IHC staining, 400X)

Table1. Quantity, intensity and distribution of S100 in neurofibroma cases

Positive cases	S100 staining			S100 staining intensity			Pattern of S100 staining	
	1+	2+	3+	Grade1	Grade2	Grade3	focal	diffuse
27	11	9	7	4	19	4	12	15
	40.7%	33.4%	25.9%	14.8%	70.4%	14.8%	44.5%	55.5%

Table2. Comparison of H&E staining with special staining methods in this study

Total cases	H&E diagnosis confirmation with Giemsa	H&E diagnosis confirmation with s100	H&E diagnosis confirmation with both gold standards
33	32	27	33
	97%	82%	100%

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 (figure1) beside many mast cells scattered among them. [2] 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. [5]

In this study, 97% of mast cells were stained by Giemsa and proved their existence in H&E slides (figure2). 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.[6,7,12,13] 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[18], while in another study, S100 was positive in 95% of the cases.[15]

Other study also showed S100 staining in about half of the skin tumors of 9 patients suffered from neurofibromatosis type1.[14] The labeling index of S100 in this study was 1 positive in 40.7% (11 patients), 2

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positives in 33.4% (9 patients) and 3 positives in 25.9% (7 patients) which suggests diversity in the quantity of S100 expression. As a result, it may not help in differential diagnosis of neural tumors although the confirmation of this issue needs more research. In the present study, the intensity of S100 staining was also reported the same as previous immuno-histochemistry studies.

Nevertheless, considering the fact that the staining intensity is a subjective matter and quantitative parameters are more significant in data analysis. The intensity was gradually eliminated from these studies and its report can only show staining errors.[19,20] The evaluation of the S100 staining pattern can be helpful in the differential diagnosis of neural tumors too, as in the present study focal staining was observed in 44.5% of the patients and diffuse distribution in 55.5%. Ghilusi et al. stated that the focal pattern of S100 in all cases.[18] Weiss and et al. reported S100 staining only in a group of cells because neurofibroma had different cellular population.

Therefore, S100 staining is distributed in these lesions in various patterns.[21] Karamchandani et al. also have compared S100 staining pattern in a number of soft tissue neoplasms except neurofibroma.[15] Therefore, it is suggested to evaluate and compare staining patterns in studies with higher number of cases and in particular in those associated with neurofibroma and/or neural tumors. Taking into account the confirmation of mast cells in almost all H&E samples, and the positive results for S100 expression in 82% of the cases, this study proved the harmony between the current histopathologic diagnosis (H&E) and gold standards. In this study, 18% of the cases (6 patients) were reported negative for S100, considering re-staining of negative cases beside positive controls throughout the procedure, so it is possible to associate this phenomenon to the absence of S100 expression in some neural tumors.

Yet, expression of other neural markers is probable in these lesions. So for these cases, diagnosis of neurofibroma is confirmed to consider the morphology of cells and also the presence of mast cells in them, too.

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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