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Detection of PNI using S100 protein in Oral Squamous Cell Carcinoma

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ABSTRACT

Objective: The 5th edition of the recent WHO classification of Head and Neck Tumors mentioned perineural Invasion (PNI) as one of the histopathological factors that are associated with a worse prognosis in Oral Squamous Cell Carcinoma (OSCC). The subjectivity in PNI assessment & histological simulations may contribute to underestimation of this ominous behavior of such tumors. This study was performed to detect the role of S100 as a neural marker to enhance PNI detection in OSCC.

Design: A collection of 54 formalin-fixed paraffin embedded tissue blocks of OSCC were immunostained with S100 protein to facilitate PNI identification.

Results: PNI detection was strikingly doubled upon staining with S100 protein. The number of cases showing PNI were raised from 22 to 42. Cases with hidden PNI foci or even with endoneural invasion were easily recognized upon the use of such immunostain.

Conclusion: PNI in OSCC can be easily recognized upon the use of S100 immunostain with better identification of PNI positive small nerves as well as intraneural invasion pattern.

Keywords: *Oral Squamous Cell Carcinoma, Perineural Invasion, S100 immunostain.*

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1. INTRODUCTION

Perineural invasion (PNI) is a significant kind of tumor spread that is strongly approved to be correlated with recurrence and worse outcome as tumor cells may reach to the brain stem and into other nerves (1,2). Historically, Batsakis defined PNI in the head and neck cancers as “the invasion of tumor cells in, around, and through peripheral nerves” (1985) (3). Other researchers have stated that the presence of tumor cells in the perineural space of nereves is considered as PNI (4). Liebig’s definition was the most accepted and widely distributed in literature to identify PNI, by which reserving the original definition produced by Batsakis and/ or the addition of the criteria of a tumor lies in close proximity to a nerve and surrounding at least one third of its circumference can be even regarded as PNI (5). Dunn et al. showed that pathologists should have a conscious look while examining tumors that are characterized by their neurotropic nature, especially those affecting pancreas, prostate, aerodigestive tract as well as head and neck tumors (6). In the latter, the incidence of PNI can be ranging from 27-82% of the cases, by which Adenoid Cystic Carcinoma (ACC) has the highest tendency for PNI, followed by Squamous Cell Carcinoma. (7).

S100 is a member of a large family of calcium binding proteins, into which at least 25 members were identified (8). It is a soluble protein that is used for the detection of schwann cells in different tissues (9). Several studies demonstrated that S100 protein can be included in several biological events that may be involved in tumor progression (8).

The use of S100 protein in immunohistochemistry revealed its ability for detection of small nerves within tissues and also differentiating them from other structures that may seem alike in conventional H&E sections. The use of this marker to delineate neural structures was an easy, effective method for pathologists to be used for accurate detection of neural reacting structures as they provide a notable contrast in comparison to the associated counter stain (10).

This study was performed to highlight the role of S100 as a neural marker to enhance the detection of PNI in Oral Squamous Cell Carcinoma (OSCC).

2. MATERIALS & METHODS

Fifty-four radically resected paraffin embedded tissue blocks of OSCC were retrieved from the archives of the department of Oral & Maxillofacial Pathology/College of Dentistry/University of Baghdad dated from the period 2012-2017. Sections of 4µm thickness of each case were cut & mounted on normal glass slides, processed and stained with hematoxylin and eosin (H&E), then histopathologically re-evaluated for the presence of PNI, other tissue sections were done & mounted on positively charged glass slides, then immunohistochemichally stained with S100 protien for histopathological evaluation for PNI.

3. RESULTS

Almost one third of the study sample showed the presence of PNI (22 cases) in H&E sections, nevertheless this percentage was elvated to about two thirds upon the use of S100 protien immunostain (42 cases). In terms of PNI status (presence, absence), a significant association was found between PNI status in H&E and S100 immunostained sections *p=(0.01)*, (Table 1).

Table (1): Comparison of PNI status between H&E and S100 sections

		S100 PNI			***P value§***
		Present	***Absent***	***Total n=54***	
H&E PNI	*Presence*	21	1	22	p=0.01*
	Absence	21	11	32	
Total n=54		42	12		

§Chi square test; *significant relation; PNI: perineural invasion.

The use of immunohistochemical stain was valuable in the identification of all nerves with small calibers showing PNI that usually cannot be identified in routine H&E sections, (Figure 1). Added to that, the use of such stain was able to identify intraneural (endoneural) invasion pattern that also may unfortunately be missed while examining H&E sections, (Figure 2).

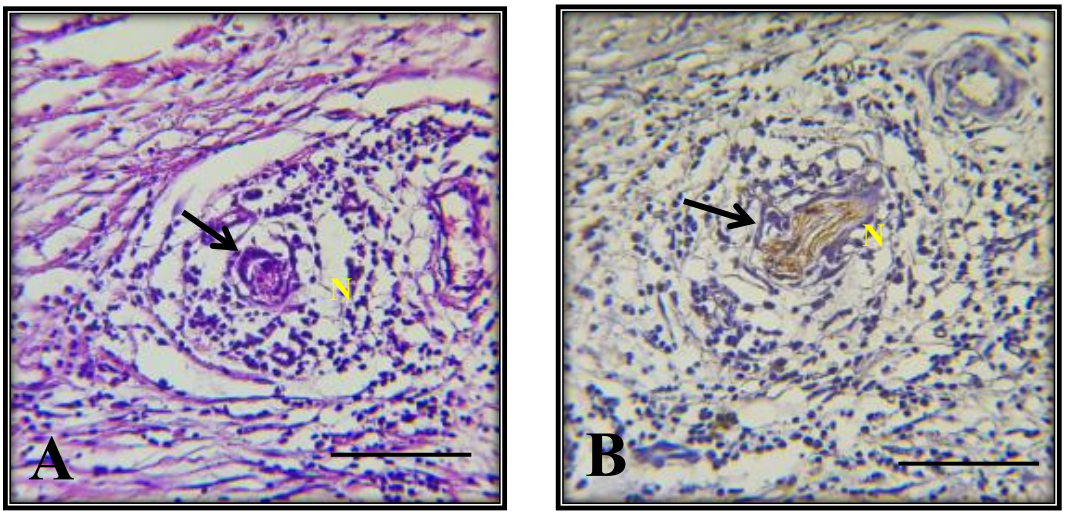

Figure (1): A photomicrograph of PNI (arrows pointing to tumor cells) in OSCC: (A) affecting small nerve that was unidentifiable in routine H&E sections. (B) The same nerve after serial sectioning and immunohistochemical staining with S100; (original magnification 40X); N: nerve bundle; PNI: perineural invasion; Scale bar =100µm.

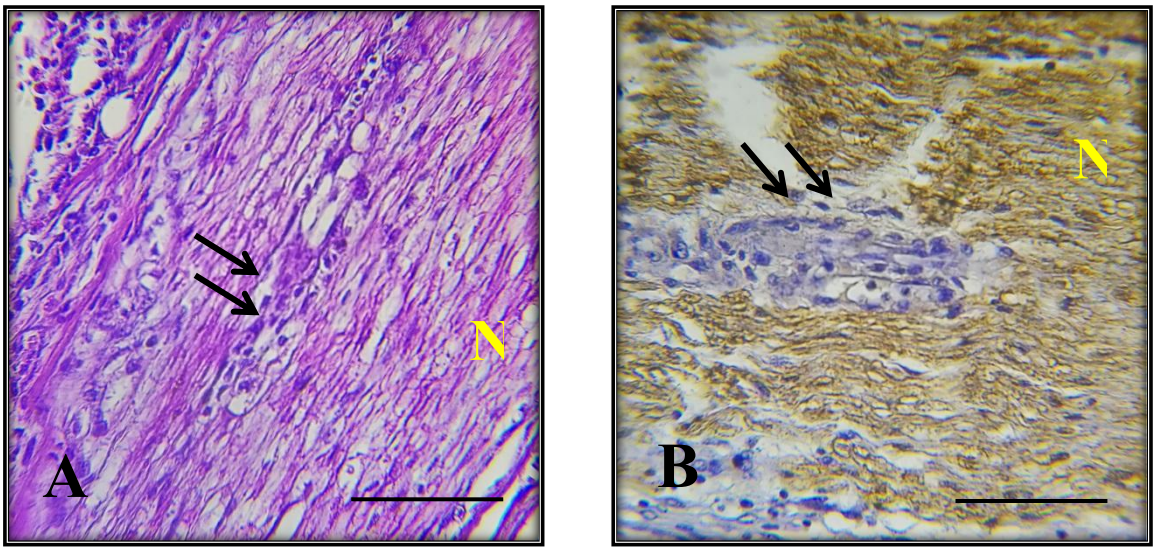

Figure (2): A photomicrograph of intraneural invasion (arrows pointing to tumor cells) in OSCC: (A) affecting a large nerve, however it was unidentifiable in routine H&E sections. (B) The same nerve after serial sectioning and immunohistochemical staining with S100; (original magnification 40X); N: nerve bundle; Scale bar =100µm.

4. DISCUSSION

On histopathological basis, the identification of PNI is routinely detected on H&Estained sections, particularly when tumor cells are seen around or within nerves (11). However, the use of certain immunohistochemical markers (e.g., S100 & neurofilament proteins) may enhance nerve fibers detection specifically in equivocal cases (12,13). In fact, PNI may have histopathological mimics that perhaps be mistaken as neural infiltration, these include: peritumoral fibrosis, re-excision PNI, reparative perineural proliferation, and epithelial sheath neuroma (6). The diversity in PNI estimation among pathologists together with different presented definitions for it had its impact for the assessment of this process (14). In an experiment done by Chi et al. using different images of OSCC showing PNI concluded that there was only a moderate agreement between the participating examinees in assessing PNI with minimal approval of the suggested definitions in literature (15).

In the present study, immunohistochemical evaluation of PNI status using S100 was surprisingly doubled compared to routine histopathological assessment. This was actually in agreement with the study of White et al. on rectal cancer that revealed on reviewing of H&E stained sections, the incidence of PNI was about 31% and the addition of the S100 immunostain has increased further detection up to 60% (16). Again, in this study, the incidence of PNI detection was raised to 78% of the cases, which was in agreement with a study done by Shen et al. performed on OSCC, the latter study showed 51% of the cases were having PNI using S100, and by application of Z proportion test a significant relation was found (p=0.034) (17).

The use of special stains that are specific for neuronal cells is widely mentioned in literature to demonstrate nerves with a better clarity and specificity. These specific stains actually are not regularly used on histological sections; therefore, the true incidence of PNI in neurotropic cancers may extensively be missed (18). One of the research studies undertaken for PNI detection in colorectal cancer has declared that despite the effort, cost and time consuming while performing immunohistochemical procedure for better identification of PNI, the incidence of the latter may be upraised 3 times more than routine examination of PNI on H&E sections (19). Therefore; collectively, from a practical point of view immunohistochemical markers can be used for PNI detection, notably although S100 may also stain tumor cells of SCC, but still the intensity by which it stains nerves contribute to their smoothly identification.

One of the interesting findings that was accomplished by the use of immunohistochemical stains for PNI detection in this study was that of identification of intraneural (endoneural) invasion pattern, which was previously considered to reflect a more aggressive and painful biological course of the tumor (20,21).

The accuracy of immunohistochemical stains has been questioned as it is associated with a little exaggeration in the percentage of foci involved by PNI in previous researches as well as the lack of specific precise definition of PNI in literature (22). The overestimation in the number of the involved PNI foci that is identified by the use of special stains can be explained by their ability to reappear even those nerves having very small diameters. Another interesting fact, that when the H&E slides were reexamined after evaluation of PNI using immunohistochemical markers, the process was much easier and the detection of the multiple foci was performed with simplicity. However, it was mentioned that despite the efficacy of S100 to highlight small nerves but yet, it does not support interobserver agreement and the clinical outcome was failed

to relate to PNI. Such poor disagreement with S100 staining would suggest a problem whether its use is reliable for routine examination for PNI facing its subjectivity in PNI reporting (16).

Bur et al. found that the presence of PNI in the head and neck tumors affecting large nerves as well as the presence of multiple foci is associated with a worse prognosis (23). Another study also mentioned that despite the ability of neuronal markers to detect further PNI foci (specifically those of small nerves) compared to standard use of H&E stains, the benefit of discovering such small positive PNI foci may not affect patients' survival rates compared to large nerves showing PNI. Actually, it was proposed that despite that small PNI foci may not end with the same poor outcome compared to large nerves showing PNI, yet it was concluded that nerve-tumor distance is associated with a poorer outcome even when small nerves showing PNI are encountered (24).

Generally speaking, that despite the use of immuno stains (e.g S100) may propagate the detection of PNI in OSCC, yet the use of such stains may affect patient's outcome even when PNI positive small nerves were found, precisely those PNI foci that are lying within the tumor or even with a close proximity to it. Therefore, the use of such immunohistochemical markers is definitely preferred when an offensive neurotrpic behavior is expected in such tumors.

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