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Perineural invasion in oral squamous cell carcinoma: Incidence, prognostic impact and molecular insight

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Abstract

Background: The objective of this study was to characterise the incidence and prognostic correlation of perineural invasion (PNI) in oral squamous cell carcinoma and determine whether nerve growth factor and its receptor tyrosine Kinase A expression could be used as biological markers for PNI.

Methods: A retrospective review of pathology reports of 430 patients with oral squamous cell carcinoma who were treated from 1992 to 2014 in Tayside, Scotland, was carried out. The expression of nerve growth factor and tyrosine kinase A was assessed with immunohistochemistry in 132 tissue sections of oral squamous cell carcinoma.

Results: Perineural invasion was identified in 17.4% of oral squamous cell carcinomas. High expression of nerve growth factor and tyrosine kinase A was seen in 84% and 92% of oral squamous cell carcinoma, respectively. Tumours with PNI expressed nerve growth factor and tyrosine kinase A with a greater frequency than tumours without PNI. PNI and high expression of nerve growth factor were significantly associated with pain. PNI was significantly associated with stage IV tumours and poor disease-specific survival.

Conclusions: A higher level of expression of nerve growth factor and tyrosine kinase A may predict PNI and therefore may be considered as biological markers for PNI in oral squamous cell carcinoma. PNI and nerve growth factor overexpression may contribute to the pain generation in oral cancer patients. PNI and nerve growth factor expression can predict the aggressiveness and prognosis of oral squamous cell carcinoma patients.

KEYWORDS

nerve growth factor, oral squamous cell carcinoma, perineural invasion, tyrosine kinase A

1 | INTRODUCTION

The oral cavity, including the lip, is the sixth most common cancer in the world.[1] Oral squamous cell carcinoma (OSCC) accounts for more than 90% of all oral cancer.[2] OSCC is associated with a poor 5-year survival rate of <50%, and this is due to the spread of OSCC to regional and distant sites.[3] One of the significant features of cancer cells facilitating spread is their ability to detach from the epithelium, break through the basement membrane and access lymphatic, vascular and nerve tissues.[4] Perineural invasion (PNI), also called

perineural spread and neurotropism, is used to describe the process of cancer cells "invasion in, around and through the nerves".[5] PNI in cancer is also defined as "tumour in close proximity to nerve and involving at least 33% of its circumference or tumour cells within any of the 3 layers of the nerve sheath".[4] There is considerable variability in the reported incidence rates of PNI in OSCC worldwide. At sites such as the tongue and/or floor of the mouth, PNI was detected in up to 70% of OSCC, whereas cancer of the lower lip has a lower rate of PNI at 5.2%.[6] PNI exhibited by some OSCC has been known as an independent predictor of poor prognosis and an indicator of aggressive behaviour.[7-9] The presence of PNI is significantly correlated with advanced T and N tumour staging, extranodal extension, poor tumour differentiation, lymphovascular invasion and increased depth of invasion, and therefore, PNI seems to be critical to prognosis in OSCC.[10,11] PNI is known to be implicated in pain generation in patients with adenoid cystic carcinoma, pancreatic cancer and OSCC.[12-14] A number of neurotrophic factors such as nerve growth factor (NGF) and its high affinity tyrosine kinase A (TrkA) receptor are likely to be involved in the possible molecular mechanism of PNI.[15] Few reports have revealed the association between the NGF and/or TrkA with PNI in OSCC.[16-20] Given the paucity of information on the role of these factors in PNI and their possible role in the development of pain in OSCC, this study was undertaken to characterise the incidence and prognostic correlation of PNI in OSCC and to determine whether NGF and its receptor TrkA expressions could be used as potential markers for PNI in ex-vivo OSCC.

2 | MATERIALS AND METHODS

2.1 | Patients and tissue collections

A retrospective review was undertaken of the histopathology reports of 430 patients with OSCC from 1992 to 2014 in Tayside, Scotland, who were reviewed or a minimum of 5 years or until death. The study was approved by the NHS Tayside, Scotland (Caldicott/CSAppHA1350), and Tayside Medical Science Centre Research and Development (14/ES/0015(HIC). Subjects were excluded if they were under 18 years of age, had histological findings other than primary OSCC or any patients diagnosed with oropharyngeal SCC.

Archival OSCC specimens were selected from 430 patients diagnosed with primary OSCC for immunohistochemical (IHC) analysis. Tumours with evidence of PNI histopathologically were selected as the study group whilst the control group comprised patients with no histopathological evidence of PNI. Both groups were matched for the following factors: age, gender and tumour site. Those who had received pre-operative radiotherapy or chemotherapy were excluded. In order to ensure an equivalent number of specimens in each group, a systematic sampling method was used to randomly select the samples for the control group.[21] In total, 132 of formalin-fixed paraffin-embedded (FFPE) tissue blocks comprising 61 PNI-positive and 71 PNI-negative OSCC were requested from Tayside Tissue Bank following approval (TR000388).

Baseline data were obtained from patient's medical records such as age, gender, smoking and alcohol history, clinical presentation, treatment modalities and survival status. Histopathological diagnostic findings (degree of differentiation, invasive front, tumour size (pT), nodal status (pN), depth of invasion (DOI) of 4 mm or above, lymphovascular invasion, PNI and bone invasion) had been reported according to the Royal College of Pathologists, dataset for histopathology reporting of mucosal malignancies of the oral cavity.[22] TNM staging was determined in accordance with the UICC, 7th edition.[23]

2.2 | Immunohistochemistry

The FFPE tissue blocks were sectioned at 5 µm, deparaffinised in xylene and then rehydrated in serial ethanol solutions, before washing in distilled water for 5 minutes. Haematoxylin and eosin (H&E) staining of all sections was performed, and the sections were examined for evidence of PNI in OSCC sections (Figure 1). Then, 132 OSCC samples were probed with NGF (#365944) and TrkA (#7268) mouse monoclonal antibodies according to the manufacturer's instruction (Santa Cruz biotechnology, Inc). After deparaffinisation and

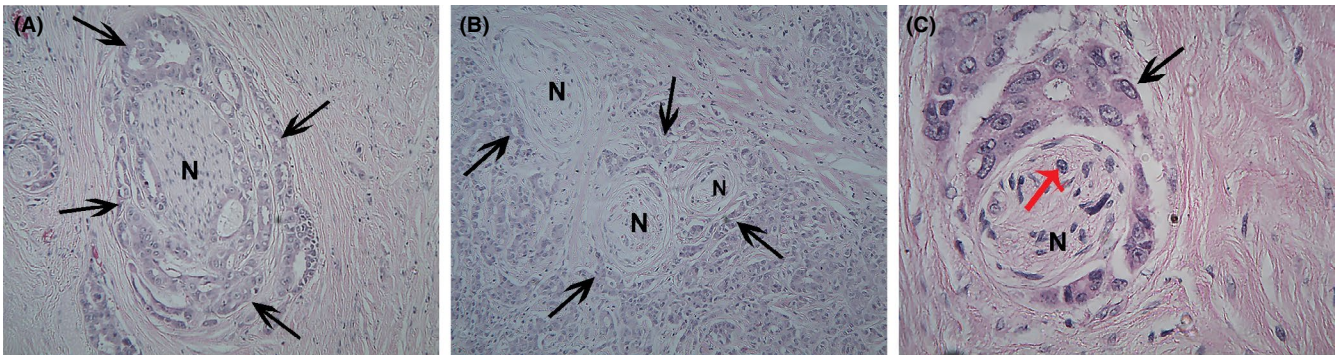

FIGURE 1 Haematoxylin and eosin stained sections of of PNI in OSCC. Complete encirclement of cancer cells around the nerve (A) (x100 magnification). Incomplete encirclement of cancer cells around the nerves (B) (x100 magnification). Intraneural invasion of cancer cells within and around the nerve (C) (x200 magnification). N indicates the nerve, black arrows point to cancer cells around the nerve, red arrow point to tumour inside the nerve

rehydration process, antigens were unmasked by heating the sections in 10mM sodium citrate buffer at 95°C (pH 6.0) using a water bath, and sections were allowed to cool in the buffer for 20 minutes before washing in deionised H_2O. The sections were incubated in 1% H_2O_2 for 5 minutes to bock endogenous peroxidase activity, followed by washing twice in PBS. Sections were blocked with 5% normal goat serum (NGS) in PBS for 1 hour at room temperature. This was followed by incubation with primary antibodies against NGF (1:50) and TrkA (1:50) diluted in 5% NGS/PBS in a humidified chamber overnight at 4°C. After equilibration, the sections were washed three times with PBS to remove the unbound antibody followed by incubation with biotinylated secondary antibody 1µg/ml diluted in 5% NGS/PBS for 45 minutes in a humidified chamber, the sections were incubated for 30 minutes with the ABC reagent and the excess ABC reagent was removed from the slides by washing with PBS. Visualisation was achieved by incubation in peroxidase substrate and chromogen mixture (3, 3′-diaminobenzidine (DAB) and hydrogen peroxide prepared in house for 5 minutes. The sections were counterstained with Mayer's haematoxylin (Sigma). The dehydration and mounting processes were followed as per manufactures instructions (Santa Cruz biotechnology). Positive controls used for NGF and TrkA were ductal epithelial cells in salivary gland tissues.[16] The NGF and TrkA antibodies were blocked using the respective blocking peptide (#365944P and #7268P, respectively, Santa Cruz biotechnology) by adding twice the volume of peptide as the volume of antibody used. These tissues were used as negative control.

2.3 | Immunohistochemical score

Assessment of the staining was carried out according to the scoring system reported previously in the literature with some modification.[16] Stained sections were scanned using a light microscope at low power. Tumour cells exhibiting a brown cytoplasmic, nuclear and/or surface membrane staining were counted as positive.

Analyses of intra-class correlation by the main observer twice and inter-class correlation by three independent observers were carried out to test the scoring results consistency gave a Cronbach's alpha of more than 0.8. NGF and TrkA staining scoring was performed as follows: the percentage of tissue staining was designated as 1 when 0%-25% of tumour cells were stained, 2 when 25%-50% of tumour cells were stained and 3 when >50% of tumour cells were stained. The intensity of tissue staining was categorised as 1 for weak staining, 2 for moderate staining and 3 for strong staining. The final scoring was determined according to the product of staining intensity and the percentage of tissue staining ranging from 0-6 categorised as low expression (IHC = 0-2) and high expression (IHC = 3-6).

2.4 | Statistical analysis

Data were analysed using SPSS package (IBM statistics version 22). Data related to categorical variables were described in terms of numbers with percentage and as mean with standard deviation (±) for continuous variables. Descriptive statistics were computed for each variable according to PNI status with a 95% confidence interval. The correlation between PNI and NGF/TrkA expression status with clinical/histopathological characteristics was analysed by chi-square or Fisher's exact test where appropriate. Spearman's correlation (r_s) was used to assess the significance of relationships between the expression level of NGF and TrkA. Five-year overall survival (OS), disease-specific survival (DSS) and disease-free survival (DFS)[24] were calculated using the Kaplan-Meier method, the difference in survival rate was assessed by log-rank test and a *P*-value of <.05 was considered statistically significant.

3 | RESULTS

3.1 | Analysis of the clinical data

A total of 430 OSCC patients' clinical data were analysed in this study, 242 (56%) were male and 188 (44%) female with an age range of 29 to 105 years (mean age 72.5 ± 12.2). The commonest site for OSCC was the tongue and floor of mouth for both PNI-positive (69.9%) and PNI-negative OSCC (68%). For those cases where data were available, more than 50% of the patient population were smokers and 82% had a history of alcohol consumption. The demographical and clinical characteristics of 430 OSCC patients according to the presence and absence of PNI are summarised in Table 1.

3.2 | Correlation between PNI and clinicopathological parameters in OSCC cohort

PNI was seen in 75/430 (17.4%) of patients with OSCC. Thirty-five (46%) patients presented with a history of pain at the time of diagnosis in the PNI-positive group compared to 43 (12%) patients with PNI-negative group ($P < .05$). In the PNI-positive group, a significant proportion of patients (24%) had an advanced pathological T stage (T4), nodal involvement (N2) (21%), extranodal extension (26%), increased DOI (>4 mm) (61%), discohesive invasive front (50%), poor tumour differentiation (38%) and lymphovascular invasion (34%), when compared to patients without PNI, and these results are highly significant, with $P < .05$. A third of the patients with PNI had stage IV disease (33%) (UICC staging) in comparison with patients without PNI the majority of whom presented with stage I (27%) ($P < .05$). It is important to note here that histopathological parameters of TNM stage, invasive front, grade of differentiation and DOI had a significant proportion of missing data.

At the time of the analysis, 56 (74%) of 75 patients with PNI were deceased in comparison with 247 (69%) of 355 patients without PNI. Out of these 56 patients with PNI, 19 (34%) patients were reported dead due to OSCC. Descriptive statistics of the clinicopathological characteristics of 430 OSCC patients according to the presence and absence of PNI are shown in Table 2.

TABLE 1 Demographical and clinical characteristics of OSCC cohort according to the presence and absence of PNI

Variables	Patients without PNI, n = 355 (82.6%)	Patients with PNI, n = 75 (17.4%)
Gender		
Male	202 (65.9)	40 (53.3)
Female	153 (43.1)	35 (46.7)
Age (y)		
Mean ± (Range)	73.3 ± 12.1 (34-105)	68.8 ± 12 (29-95)
Smoking		
Yes	105 (29.6)	23 (30.7)
No	36 (10.1)	15 (20)
Ex-smoker	31 (8.7)	8 (10.7)
Data missing	183 (51.5)	29 (38.7)
Alcohol		
Yes	97 (27.3)	32 (42.7)
No	21 (5.9)	6 (8)
Data missing	237 (66.8)	37 (49.3)
Site		
Tongue	133 (37.5)	35 (46.7)
Floor of the mouth	79 (22.4)	16 (21.3)
Tongue & floor of the mouth	12 (3.4)	5 (6.7)
Retromolar trigone	21 (5.9)	5 (6.7)
Alveolus	24 (6.8)	5 (6.7)
Buccal mucosa	41 (11.6)	3 (4)
Lower lip	40 (11.3)	3 (4)
Hard palate	5 (1.4)	3 (4)
Treatment		
Surgery	220 (62)	57 (76)
Surgery + radiotherapy with/without chemotherapy	15 (4.2)	7 (9.3)
No surgery (Chemoradiotherapy)	120 (33.8)	11 (14.7)

Note: ± standard deviation.

Abbreviation: OSCC, oral squamous cell carcinoma; PNI, perineural invasion.

3.3 | NGF and TrkA expression in OSCC samples

High expression of NGF was seen in 111 of 132 (84%) OSCC as an intense staining in the cytoplasm and nucleus of cancer cells in a diffuse pattern. One hundred and twenty-two (92%) OSCC had a high expression of TrkA that was present diffusely in the surface membrane and cytoplasm of tumour cells. Immunoreactivity of NGF and TrkA was weakly present in 21% and 8% of the OSCC, respectively (Figure 2). Neural tissues showed moderate to strong intensity of NGF and Trk immunoreactivity. A strong positive correlation was seen between NGF immunoreactivity and TrkA immunoreactivity in OSCC samples (r_s = 0.65). r_s = Spearman's correlation coefficient.

3.4 | Relationship between NGF and TrkA expression with clinicopathological parameters in OSCC samples

PNI-positive OSCC expressed NGF and TrkA with a greater frequency than PNI-negative OSCC ($P < .5$). There were significant differences between NGF/TrkA expression levels, DOI > 4 mm and lymphovascular invasion ($P < .05$). Furthermore, patients who presented with pain had a significantly higher NGF expression in their tumour compared with patients with low NGF expression who presented with pain ($P < .5$). Correlation between NGF and TrkA expression levels and clinicopathological parameters including the presence and absence of PNI of 132 OSCC samples is shown in Table 3.

3.5 | Survival analysis

The presence of PNI was associated with poor DSS in OSCC ($P < .05$). The trend for OS and DFS was similar but not significant. The overall 5-year survival rate was worse for patients with OSCC with high expression of NGF and TrkA compared to OSCC patients with low NGF and TrkA expression (34% vs 48%, 34% vs 60%, respectively). However, Kaplan-Meier survival analysis revealed no significant association of NGF and TrkA expression levels with OS, DSS and DFS ($P > .05$) (Figure 3).

4 | DISCUSSION

PNI is observed when cancer cells invade and grow along nerve tracts away from the primary tumour and is recognised as one of the most important prognostic factors in OSCC.[9] Our study found that the incidence of PNI was 17.4% in OSCC, and this is consistent with the trend of overall frequency of PNI that ranges from 2.5% to 71% in OSCC.[6] The vast majority of PNI-positive OSCC were tumours arising in the tongue and floor of the mouth. These results are consistent with other studies where heterogeneous anatomical sites were employed.[6] Some studies have shown that infiltration of the perineural space of the nerves by tumour cells correlates with tumour size, depth and pattern of invasion, the degree of tumour differentiation, presence of nodal metastasis and presence of extranodal extension in OSCC.[10,11,25] Our study demonstrates a significant correlation between the presence of PNI and these histological features in OSCC.

The process of PNI requires activation of some neurotrophic growth factors such as NGF and its TrkA receptor that may play a role in the mechanism of PNI.[26] Previous studies have shown a significant association of NGF and TrkA overexpression with PNI in

TABLE 2 Descriptive statistics of the clinicopathological factors in the OSCC cohort according to the presence or absence of PNI

Variables	Patients without PNI, n = 355 (82.6%)	Patients with PNI, n = 75 (17.4%)	*P*-value
p-T Stage			
T1	109 (30.7)	21 (28)	.001*
T2	72 (20.3)	23 (30.7)	
T3	19 (5.4)	1 (1.3)	
T4	26 (7.3)	18 (24)	
Data missing	129 (36.3)	12 (16)	
p-N Stage			
N0	181 (51)	38 (50.7)	.004*
N1	14 (3.9)	9 (12)	
N2	30 (8.5)	16 (21.30	
N3	0	0	
Data missing	130 (36.6)	12 (16)	
p-Extracapsular Extension			
Yes	33 (9.3)	20 (26.7)	.0001*
No	322 (90.7)	55 (73.3)	
p-DOI (mm)			
<4 mm	89 (25.1)	10 (13.3)	.0001*
>4 mm	112 (31.5)	46 (61.3)	
Data missing	154 (43.3)	19 (25.3)	
p-Histological grade			
Well	57 (16.1)	3 (4)	.001*
Moderate	207 (58.3)	38 (50.7)	
Poor	82 (23.1)	29 (38.7)	
Data missing	9 (2.5)	5 (6.7)	
p-Invasive front			
Cohesive	101 (28.5)	18 (24)	.002*
Discohesive	78 (22)	38 (50.7)	
Data missing	17 (49.6)	19 (25.3)	
p-Lymphovascular Invasion			
Yes	34 (9.6)	26 (34.7)	.0001*
No	32 (90.4)	49 (65.3)	
p-Bone invasion			
Yes	22 (6.2)	8 (10.7)	.1
No	333 (93.8)	67 (89.3)	NS
TNM Staging			
I	99 (27.9)	16 (21.3)	.001*
II	50 (14.1)	18 (24)	
III	32 (9)	4 (5.3)	
IV	44 (12.4)	25 (33.3)	
Data missing	130 (36.6)	12 (16)	
Pain			
Yes	43 (12.1)	35 (46.7)	.0001*
No	312 (87.9)	40 (53.3)	
Local recurrence			
Yes	86 (24.2)	16 (21.3)	.5
No	269 (75.8)	59 (78.7)	NS
Survival status			
Alive	108 (30.4)	19 (25.3)	.2
Deceased	247 (69.6)	56 (74.7)	NS
Deceased form OSCC	38 (15)	19 (34)	

Note: Comparison between two groups by Pearson's chi-square or Fisher's exact test, where appropriate.

Abbreviations: NS, not significant; OSCC, oral squamous cell carcinoma; PNI, perineural invasion.

*Significant value.

OSCC.[16-20] In the present study, we demonstrated NGF and TrkA expression in OSCC; interestingly, the expression levels were significantly higher in PNI-positive compared with PNI-negative OSCC. Moreover, there was a positive relationship between the expression of NGF and TrkA in OSCC in agreement with another study on OSCC.[16] Besides the cancer cells, immunoreactivity of NGF and TrkA was remarkably present in nerve tissues in OSCC in this study. Therefore, this may suggest that there is a close interaction between the cancer cells and neural tissue, where NGF is expressed by cancer cells causing tumour cells and nerves to grow together and enhance their physical contact, and therefore induce PNI. This hypothesis is supported by a previous study showed the influence of NGF/TrkA interactions between cancer cells and neural tissues in OSCC that may facilitate the development of PNI.[16]

The clinical features of PNI have been studied in different types of cancer, and it is well known that PNI is often clinically silent.[27] However, in this study, PNI is notably accompanied by pain in OSCC patients with PNI. Another study also reported that pain was a symptom in the majority of patients with PNI-positive OSCC at the time of presentation.[28] NGF and its receptor TrkA are believed to be major mediators of pain in pancreatic and prostate cancer.[13,29] To our knowledge, this is the first report to show that overexpression of NGF might influence pain generation in patients with OSCC.

A previous study has shown an association of NGF overexpression with some pathological parameters in OSCC; however, TrkA immunoreactivity was not associated with any pathological factors in the same study.[19] Interestingly, our findings demonstrated a significant correlation of NGF and TrkA overexpression with lymphovascular invasion and increased DOI (>4mm) OSCC which might indicate that NGF and its receptor TrkA can predict aggressive behaviour of OSCC. This may contribute to the theory that NGF/TrkA may induce lymphovascularisation around nerves in cancers with an increase

DOI, thus supplying the nutrients for tumour and nerve growth which may enhance PNI.[30]

Some studies found a significant relationship between PNI and 5-year survival rate in OSCC.[7-9] In the current study, PNI was significantly associated with a poorer 5-year DSS in OSCC patients. Few studies have evaluated the impact of NGF expression on patient's survival in OSCC, with conflicting reports of higher NGF expression being associated with a significantly worse overall survival[19] and no association between NGF expression and survival.[18] Our study found no significant association of NGF and TrkA expression levels with OS, DSS and DFS in OSCC. The variation in the prognostic significance of NGF and TrkA expression in OSCC in the literature and our study could have been due to the unequal distribution of parameters between the PNI-positive and PNI-negative group that might reduce the predictive power of NGF and TrkA as biomarkers, and the retrospective nature of this study that needs to be further validated in prospective studies. However, one of the strengths of this study is that it includes a large, well-characterised cohort of 430 cases of OSCC from a geographically defined population diagnosed and treated in NHS Tayside reporting the incidence and prognostic impact of PNI as a pathologic entity on patients with OSCC in Scotland. It also highlights the potential of NGF and TrkA as biomarkers of PNI-related OSCC. Further understanding

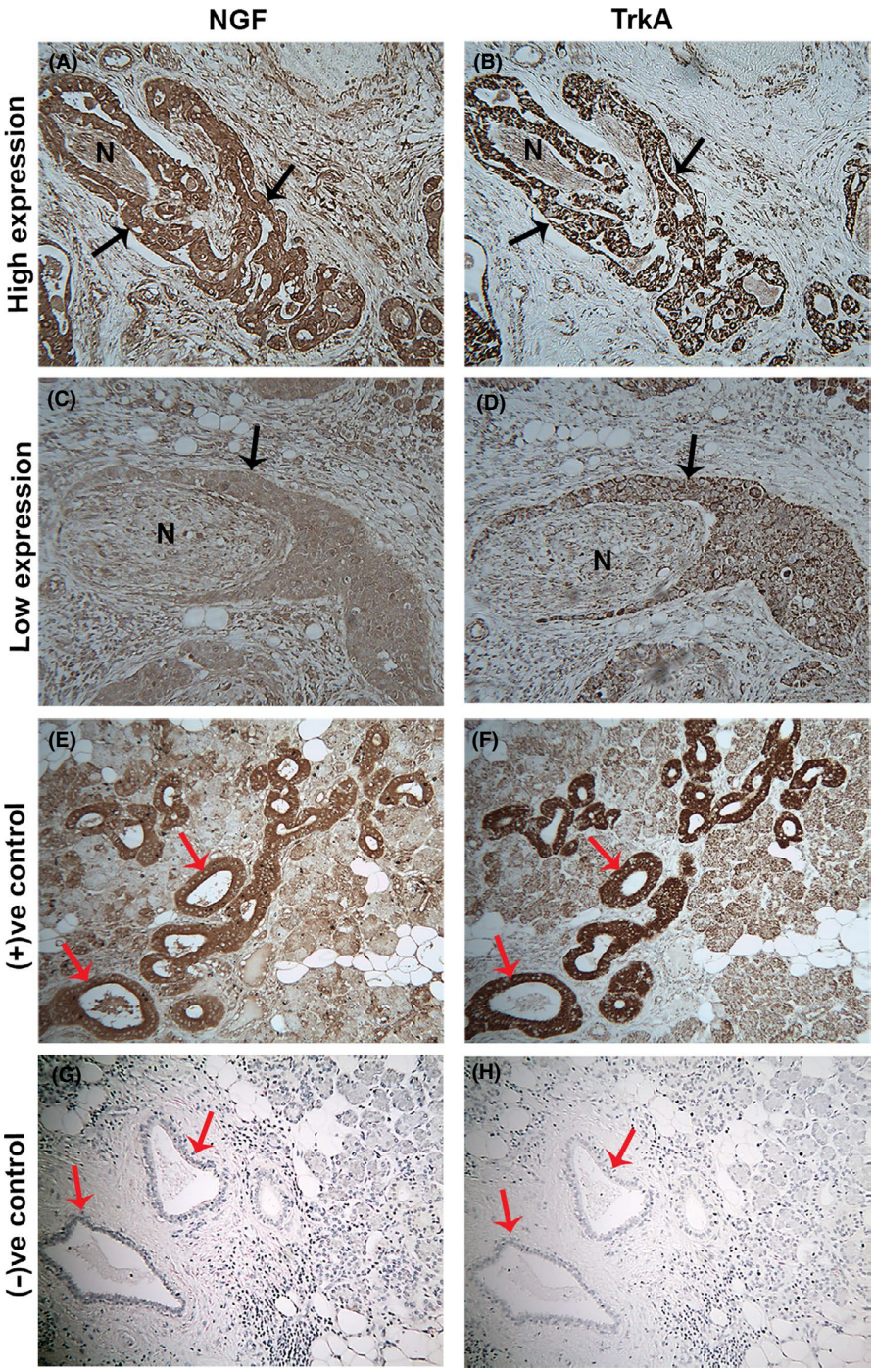

FIGURE 2 OSCC tissues stained with NGF and TrkA antibodies. Representative of high expression of NGF (A) and TrkA (B) in PNI-positive OSCC exhibiting intensive cytoplasmic/nuclear NGF and cytoplasmic/membrane TrkA immunoreactivity in cancer cells invading the nerve. Representative of low expression of NGF (C) and TrkA (D) in PNI-positive OSCC showing weak cytoplasmic/nuclear NGF and cytoplasmic/membrane TrkA staining in cancer cells invading the nerve. Ductal epithelial cells in salivary gland tissues act as a strong positive control for NGF (E) and TrkA (F). Complete blocking of NGF and TrkA antibodies after incubation with blocking peptides and no immunoreactivity for NGF (G) and Trk (H) was detected in stained sections. N indicates the nerve, black arrows point to cancer cells, and a red arrow points to ductal epithelial cells in salivary gland tissue. Images were captured at x100 magnification

TABLE 3 Correlation between NGF and TrkA expression level and clinicopathological parameters in OSCC samples

OSCC samples	NGF Expression			TrkA expression		
	High n = 111 (84%)	Low n = 21 (16%)	*P*-value	High n = 122 (92%)	Low n = 10 (8%)	*P*-value
p-T stage						
T1	41 (39)	12 (57)		47 (40)	6 (60)	
T2	34 (32)	7 (33)	.2	37 (31)	4 (40)	.2
T3	2 (2)	0	NS	2 (2)	0	NS
T4	29 (27)	2 (10)		31 (26)	0	
Missing data	5	0		5	0	
p-N Stage						
N0	68 (64)	17 (81)	.3	76 (65)	9 (90)	.4
N1	16 (15)	1 (5)	NS	17 (15)	0	NS
N2	22 (21)	3 (14)		24 (20)	1 (10)	
N3	0	0		0	0	
Data missing	5	0		5	0	
p-Extracapsular Extension						
Yes	31 (28)	4 (19)	.3	34 (28)	1 (10)	.2
No	80 (72)	17 (81)	NS	88 (72)	9 (90)	NS
p-DOI (mm)						
<4 mm	21 (21)	11 (52)	**.003***	25 (23)	7 (70)	**.003***
>4 mm	79 (79)	10 (48)		86 (77)	3 (30)	
Data missing	11	0		11	0	
p-Histological grade						
Well	11 (10)	1 (5)	.3	11 (9)	1 (10)	.7
Moderate	53 (48)	14 (66)	NS	61 (50)	6 (60)	NS
Poor	47 (42)	6 (28)		50 (41)	3 (30)	
p-Invasive front						
Cohesive	39 (39)	12 (57)	.1	47 (43)	4 (40)	.8
Discohesive	60 (61)	9 (43)	NS	63 (57)	6 (60)	NS
Data missing	12	0		12	0	
Perineural Invasion						
Yes	61 (55)	0	**.0001***	61 (50)	0	**.002***
No	50 (45)	21 (100)		61 (50)	10 (100)	
p-Lymphovascular Invasion						
Yes	35 (32)	1 (5)	**.01***	36 (30)	0	**.04***
No	76 (68)	20 (95)		86 (70)	10 (100)	
p-Bone invasion						
Yes	14 (13)	2 (10)	.6	16 (13)	0	.6
No	97 (87)	10 (90)	NS	106 (87)	10 (100)	NS
TNM Staging						
I	33 (31)	10 (47)	.5	37 (32)	6 (60)	.1
II	25 (24)	5 (24)	NS	27 (23)	(30)	NS
III	10 (9)	1 (5)		11 (9)	0	
IV	38 (36)	5 (24)		42 (36)	1 (10)	
Data missing	5	0		5	0	
Pain						
Yes	41 (37)	3 (14)	**.04***	42 (34)	2 (20)	.4
No	70 (63)	18 (86)		80 (66)	8 (80)	NS

(Continues)

TABLE 3 (Continued)

OSCC samples	NGF Expression		*P*-value	TrkA expression		*p*-value
	High n = 111 (84%)	Low n = 21 (16%)		High n = 122 (92%)	Low n = 10 (8%)	
Recurrence						
Yes	28 (25)	7 (33)	.4	31 (25)	4 (40)	.4
No	83 (75)	14 (67)	NS	91 (75)	6 (60)	NS
Survival status						
Alive	38 (34)	10 (48)	.2	42 (34)	6 (60)	.1
Deceased	73 (66)	11 (52)	NS	80 (66)	4 (40)	NS
Deceased from disease	23 (32)	3 (27)		26 (33)	0	

Note: Comparison between two groups by Pearson's chi-square or Fisher's exact test, where appropriate.

Abbreviations: NGF, nerve growth factor; NS, not significant; OSCC, oral squamous cell carcinoma; TrkA, tyrosine kinase A.

*Significant value.

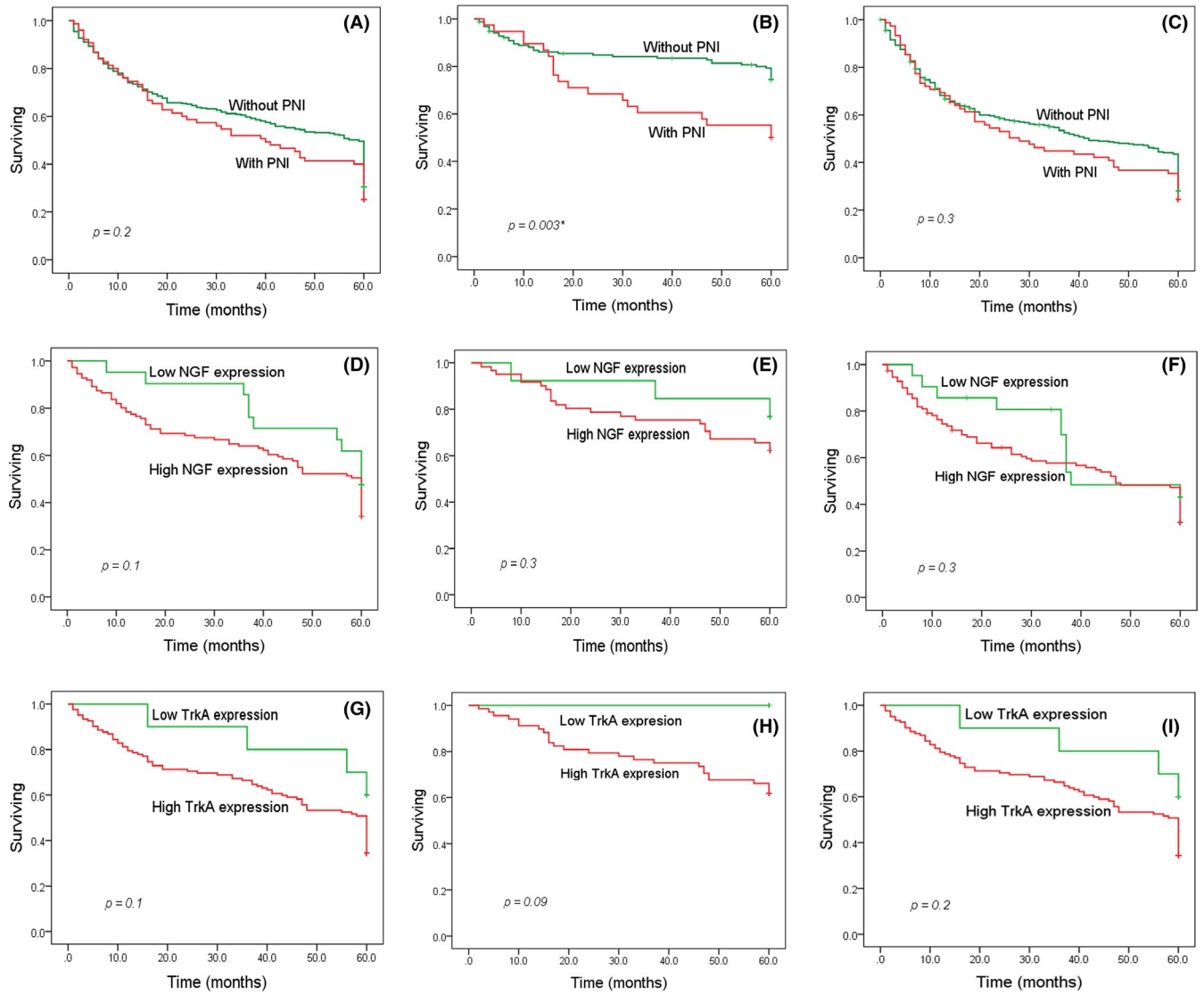

FIGURE 3 Kaplan-Meier survival analysis for PNI, NGF and TrkA expression in OSCC. Kaplan-Meier survival plots for overall survival (OS) (A), disease-specific survival (DSS) (B) and disease-free survival (DFS) (C) in a cohort of 430 patients with OSCC, PNI-positive OSCC represented by red line and PNI-negative OSCC represented in green. Kaplan-Meier survival plots showing the relation between NGF expression in 132 OSCC samples and OS (D), DSS (E) and DFS (F), high NGF expression represented by red line and low NGF expression represented by green line. Kaplan-Meier survival plots for TrkA expression in 132 OSCC for OS (G), DSS (H) and DFS (I), high TrkA expression represented by red line and low TrkA expression represented by green line. (p = Log-Rank test)

of the role that NGF/TrkA may play in OSCC would be necessary for the development of therapy modalities targeting the actions NGF/TrkA, and this may represent a potential route for the treatment of PNI and its related symptoms in OSCC in future.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

Huda Alkhadar: Data curation; Formal analysis; Investigation; Project administration; Writing-original draft; Writing-review & editing. **Michaelina Macluskey:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Methodology; Supervision; Writing-original draft; Writing-review & editing. **Sharon White:** Investigation; Supervision; Validation; Writing-original draft; Writing-review & editing. **Ian Ellis:** Conceptualization; Methodology; Project administration; Supervision; Validation; Writing-original draft; Writing-review & editing.

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