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ORIGINAL PAPER

PD-L1 AND HLA-E EXPRESSION PATTERNS IN ADVANCED AND METASTATIC HEAD AND NECK SQUAMOUS CELL CARCINOMAS

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Abstract

Introduction. In head and neck squamous cell carcinoma (HNSCC), immune escape mechanisms, particularly the expression of programmed death ligand 1 (PD-L1) and human leucocyte antigen-alpha chain E (HLA-E), play a crucial role in tumour progression and therapeutic resistance.
The objective of this study was to evaluate the relationship between the expression of PD-L1 and HLA-E and their association with tumour grade and disease stage, highlighting their potential prognostic and therapeutic relevance.
Material and methods. The study was conducted retrospectively and analyzed archival tissue samples from patients with advanced or metastatic HNSCC. PD-L1

Résumé

Profils d'expression de PD-L1 et HLA-E dans les carcinomes épidermoïdes avancés et métastatiques de la tête et du cou

Introduction. Dans le carcinome épidermoïde de la tête et du cou (CECC), les mécanismes d'échappement immunitaire, notamment l'expression de PD-L1 et d'HLA-E, jouent un rôle crucial dans la progression tumorale et la résistance aux traitements.
L'objectif de cette étude était d'évaluer la relation entre l'expression de PD-L1 et de HLA-E et leur association avec le grade tumoral et le stade de la maladie, en soulignant leur intérêt pronostique et thérapeutique potentiel.

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and HLA-E expression were immunohistochemically assessed. Quantification of HLA-E intensity was performed using the HistoQuest image analysis software. Statistical analysis was also performed.

Results. A weak, statistically insignificant, negative correlation was found between PD-L1 and HLA-E expression (r= –0.0913). The average PD-L1 expression increased from grade G1 to G3, but ANOVA analysis showed no statistical significance ($F = 0.94$, $p > 0.05$). Both PD-L1 and HLA-E showed no significant differences relative to tumour stage ($p > 0.05$).

Conclusions. In this cohort of HNSCC patients, the expression of PD-L1 and HLA-E showed no significant correlation with each other and no statistically significant association with tumour grade or clinical stage. These findings underscore the pronounced heterogeneity of immune checkpoint expression in HNSCC and suggest that PD-L1 and HLA-E reflect independently regulated immune evasion mechanisms that cannot be reliably inferred from conventional clinicopathological parameters.

Keywords: HistoQuest, HLA-E, PD-L1, squamous cell carcinoma, head and neck.

List of abbreviations:
HNSCC – head and neck squamous cell carcinoma
HPV – human papillomavirus
PD-L1 – programmed death ligand 1
HLA-E – class I histocompatibility antigen, human leucocyte antigen-alpha chain E
NKG2 - natural killer cells (NK cells) genes 2

Matériel et méthodes. Cette étude rétrospective a analysé des échantillons tissulaires archivés provenant de patients atteints de CECC avancé ou métastatique. L'expression de PD-L1 et d'HLA-E a été évaluée par immunohistochimie. La quantification de l'intensité d'HLA-E a été réalisée à l'aide du logiciel d'analyse d'images HistoQuest. Une analyse statistique a également été effectuée.

Résultats. Une faible corrélation négative, statistiquement non-significative, a été observée entre l'expression de PD-L1 et celle de HLA-E (r = -0,0913). L'expression moyenne de PD-L1 augmentait du grade G1 au grade G3, mais l'analyse de variance (ANOVA) n'a montré aucune signification statistique ($F = 0,94$; $p > 0,05$). PD-L1 et HLA-E ne présentaient pas de différences significatives en fonction du stade tumoral ($p > 0,05$).

Conclusions. Dans cette cohorte de carcinomes épidermoïdes de la tête et du cou, l'expression de PD-L1 et de HLA-E ne présentait ni corrélation significative entre elles, ni association statistiquement significative avec le grade tumoral ou le stade clinique. Ces résultats soulignent l'hétérogénéité marquée de l'expression des points de contrôle immunitaires dans les HNSCC et suggèrent que PD-L1 et HLA-E reflètent des mécanismes d'échappement immunitaire régulés de manière indépendante, qui ne peuvent pas être déduits de façon fiable à partir des paramètres clinicopathologiques conventionnels.

Mots-clés: HistoQuest, HLA-E, PD-L1, carcinome épidermoïde, tête et cou.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous group of malignancies and ranks as the seventh most common cancer worldwide, with about 890,000 new cases and 450,000 deaths annually, accounting for 4.5% of the global cancer burden[1]. It shows a marked male predominance (male-to-female ratio 2.5/ 3.1)[2]. Despite therapeutic advances, the 5-year survival for advanced stage disease remains below 50%, highlighting the need for better prognostic biomarkers and targeted therapies[3].

HNSCC is characterized by complex tumour-immune interactions that enable immune evasion and tumour progression[4,5]. Among these mechanisms, the upregulation of immune checkpoint molecules such as programmed death-ligand 1 (PD-L1) and non-classical major histocompatibility complex class I molecules, such as class I histocompatibility antigen, human leucocyte antigen-alpha chain E (HLA-E), is extremely important. PD-L1 binds on PD-1 on T-cells, leading to functional exhaustion and suppression of cytotoxic activity. HLA-E interacts with the NKG2A receptor on NK and CD8+ T-cells, providing an additional immune inhibitory signal[6].

The role of PD-L1 and HLA-E has received increasing attention. PD-L1 plays a central role as both a predictive biomarker and as targeted therapy in HNSCC. Simultaneously, HLA-E expression has been involved in immune evasion and poor prognosis in several tumour types, including HNSCC[7,8]. Furthermore, the interaction between HLA-E and the inhibitory receptor NKG2A has been proposed as a promising therapeutic target[9].

The objective of the study was to analyze the relationship between PD-L1, HLA-E, tumour differentiation and clinical stage in a cohort of patients with advanced or metastatic HNSCC.

Materials and methods

Study design

Sample selection – the study included 60 archival tissue samples from patients with advanced

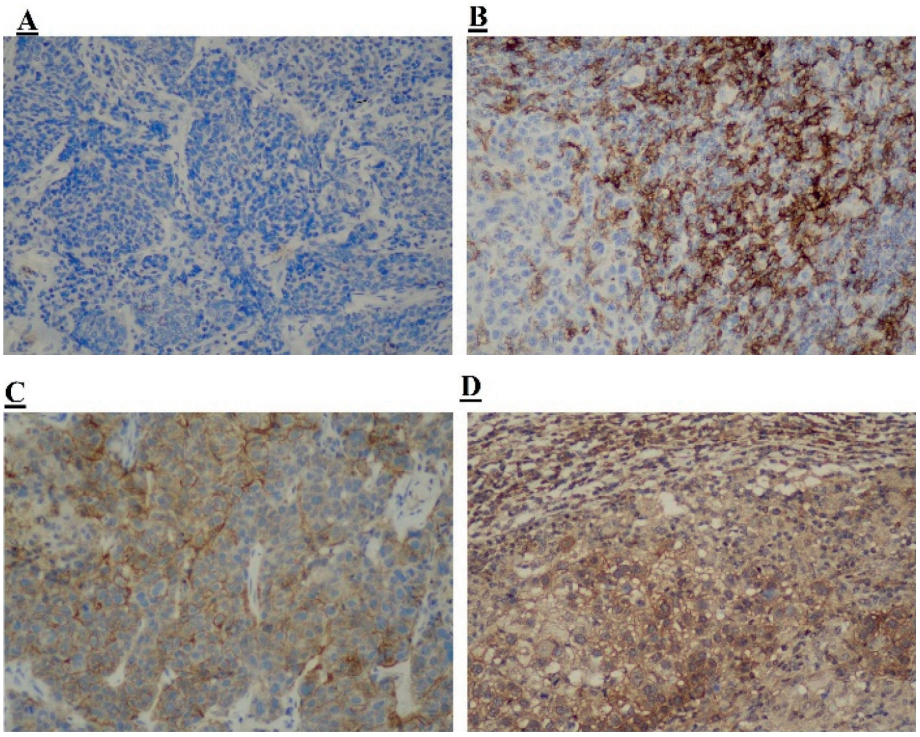


Figure 1. Representative immunohistochemical staining for PD-L1 expression in HNSCC, stratified by Combined Positive Score (CPS). (A) CPS =0 (17% of the patients); (B) CPS 1-19 (30%); (C) CPS 20-49 (33%); (D) CPS ≥50 (20%). The four panels illustrate the range of PD-L1 expression categories observed in the cohort. Magnification: x20

or metastatic HNSCC, diagnosed between 2021-2024, in the Department of Clinical Pathology at the University Hospital "St. George", Plovdiv, Bulgaria. The patients were predominantly male (75%) with a mean age of 69.4±9.1 years; most (73%) were older than 60 years old. The study was approved by the ethics committee of the Medical University Plovdiv, approval number R-KNE-20/02.02.2026.

Regarding tumour differentiation, most cases (77%) were moderately differentiated, followed by poorly differentiated tumours (16%) and well differentiated tumours (7%). The most common tumour site was the larynx (38%), followed by oral cavity (30%), hypopharynx (12%), nasal cavity/ nasopharynx (10%), oropharynx (7%) and ear (3%).

PD-L1 expression (evaluated by CPS score) was 0 in 17% of patients, between 1-19% in 30%, between 20-49 in 33% and ≥50 in 20% of cases (Figure 1).

The HLA-E intensity values had a calculated mean intensity of 73.26± 8.24 and were grouped into 3 categories: (A) Low (61.60 – 75.90), (B) Medium (75.90- 90.16) and (C) High (90.16 –104.42) (Figure 2).

Immunohistochemistry

IHC staining was performed using a polyclonal antibody for HLA-E and a monoclonal antibody for PD-L1. Detection was carried out with the DAKO visualization system according to the protocols in line. Internal and external positive and negative controls were included and PD-L1 was assessed by two pathologists. Digital imaging was performed using

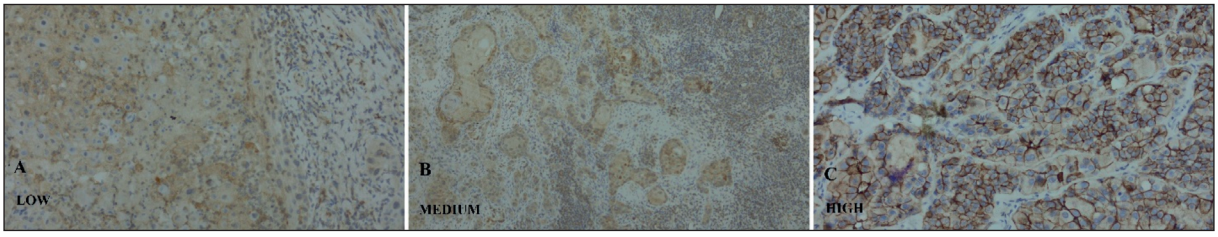


Figure 2. Representative immunohistochemical staining for HLA-E expression in HNSCC, stratified by mean intensity values. (A) Low expression; (B) Medium expression; (C) High expression. Expression categories were defined by quantitative image analysis using HistoQuest software. Magnification: x20

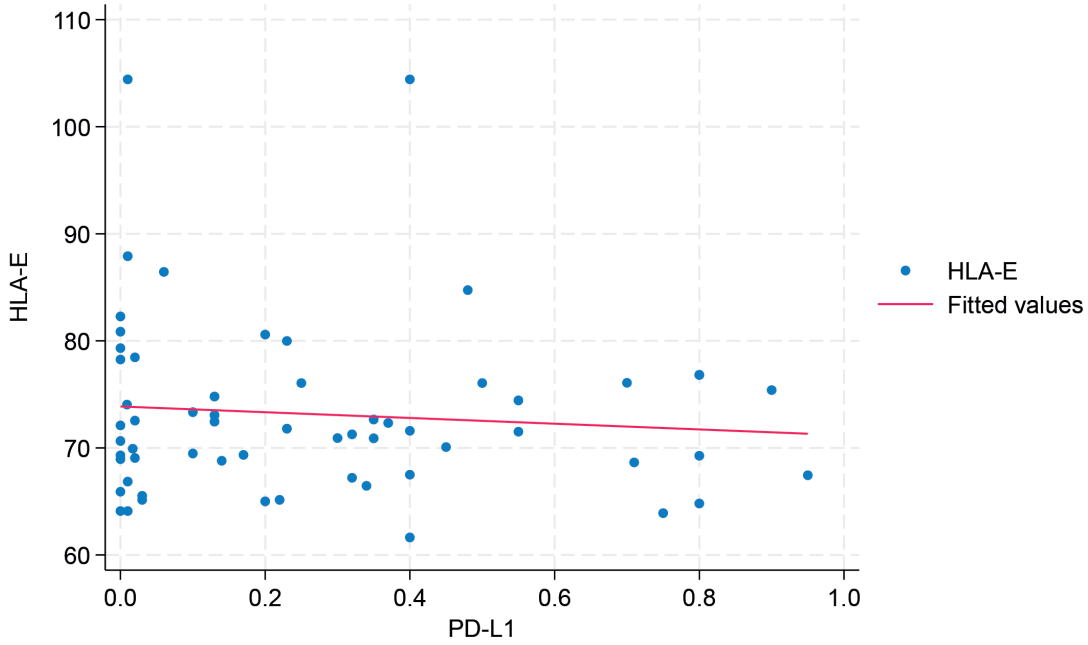


Figure 3. Scatter plot of the correlation between PD-L1 and HLA-E expression.

Leica DM6 B Fully Automated Upright Microscope System for Life Science Research. All specimens were captured at three different magnifications (x4, x10, x20), with final image analysis conducted at x20 magnification. Quantification Using HistoQuest Software: tumour cell expression levels of HLA-E were quantified automatically by the software, minimizing interobserver variability and providing objective measurements.

Statistical Analysis

The statistical analysis was conducted to explore associations and differences in PD-L1 and HLA-E expression across different variables. Data are expressed as mean±standard deviation unless otherwise indicated. To evaluate the linear relationship between PD-L1 and HLA-E, Pearson's correlation coefficient was calculated. A linear regression model was also constructed to assess the predictive value of PD-L1 on HLA-E expression. The association between PD-L1 expression and tumour differentiation (grades G1, G2, G3) was assessed using one-way analysis of variance (ANOVA). To verify the homogeneity of variances across groups, Bartlett's and Levene's tests were applied. In addition, the Spearman's rank correlation coefficient was calculated to identify the strength and direction of association between PD-L1 expression and histological grade as a ranked variable. To assess the relationship between marker expression and clinical tumour stage, the non-parametric Kruskal – Wallis test was employed. All statistical analyses were conducted using Stata 18.5 BE with a level of significance 0.05.

Results

The expression patterns of PD-L1 and HLA-E were analyzed across multiple pathological parameters, using both parametric and non-parametric statistical methods. Correlation between PD-L1 and HLA-E: the Pearson correlation coefficient between those two markers was –0.0913, indicating a very weak, statistically insignificant negative correlation. A linear regression model yields an OLS-estimated $\beta = 2.69$ (p-value > 0.05) and an $R^2 = 0.008$. The observed scatter plot showed wide dispersion of data points around the regression line, confirming the lack of a meaningful linear relationship between the markers (Figure 3).

PD-L1 expression and tumour differentiation: PD-L1 expression increased with loss of tumour differentiation: G1 tumours had the lowest mean PD-L1 expression (0.1425±0.1729), G2 tumours showed intermediate levels (0.2611±0.2793), and G3 tumours had the highest mean expression (0.3687±0.3109). However, one-way ANOVA did not reveal a statistically significant difference among group means ($F = 0.94$, $p > 0.05$). Both Bartlett's and Levene's tests confirm the equality of variances across groups ($p > 0.05$), supporting the validity of the ANOVA assumptions. Figure 4 presents the means and standard deviations for the grade groups of PD-L1.

Additionally, Spearman's rank correlation analysis was conducted. The estimated ρ is close to 0, indicating a very low correlation with the grade variable and PD-L1 values. The variables appear to be independent ($\rho = 0.1901$, $p > 0.05$).

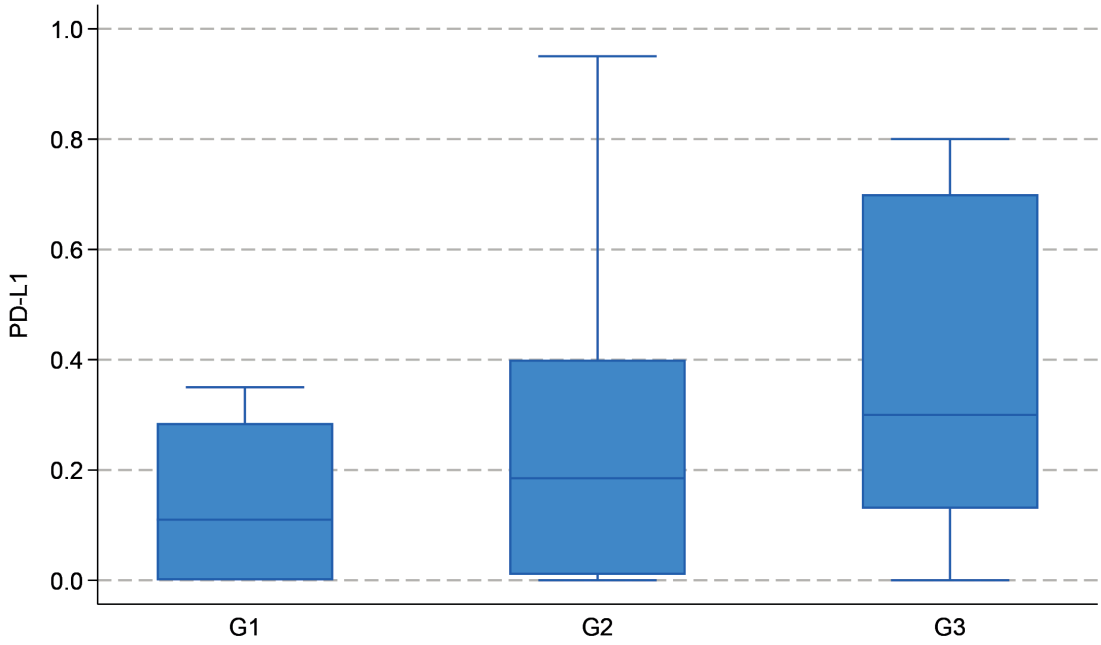


Figure 4. Means and dispersion of PD-L1 expression by histological grade (G1, G2, G3).

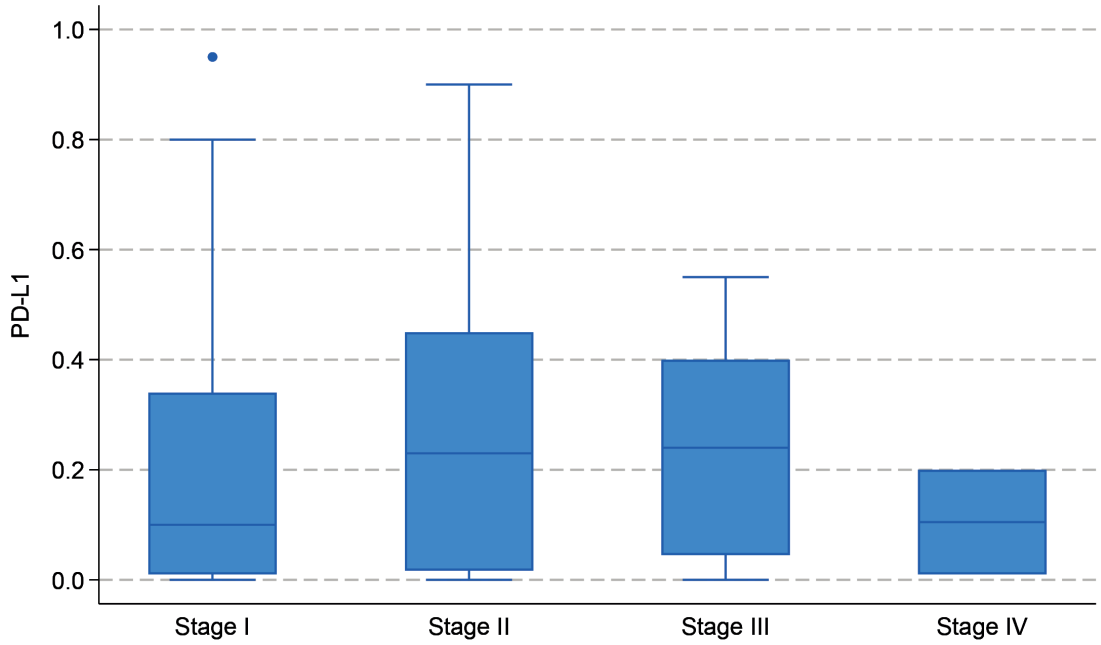


Figure 5. Boxplot of PD-L1 expression by tumour stage.

Marker expression and disease stage: to explore the relationship between immune marker expression and tumour stage, Kruskal-Wallis tests were used due to the non-normal distribution of data. PD-L1 expression showed insignificant variation across clinical stages (χ^2 = 1.143, p > 0.05). (Figure 5). The mean values and standard deviation measures of PD-L1 marker in stage groups are as follows: stage I (0.253±0.34); stage II (0.293±0.288); stage III (0.25±0.207); stage IV (0.105±0.134).

Similarly, HLA-E expression varied insignificantly by disease stage (χ^2 = 1.947, p > 0.05) (Figure 6). The mean values and standard deviation measures of HLA-E marker in stage groups are as follows: stage I (70.38 ± 4.56); stage II (74.51 ± 9.47); stage III (71.87 ± 6.16); stage IV (76.46 ± 16.192).

These results indicate that both PD-L1 and HLA-E expressions are not driven by tumour grade or stage and do not correlate with each other, underscoring the biological heterogeneity of immune evasion

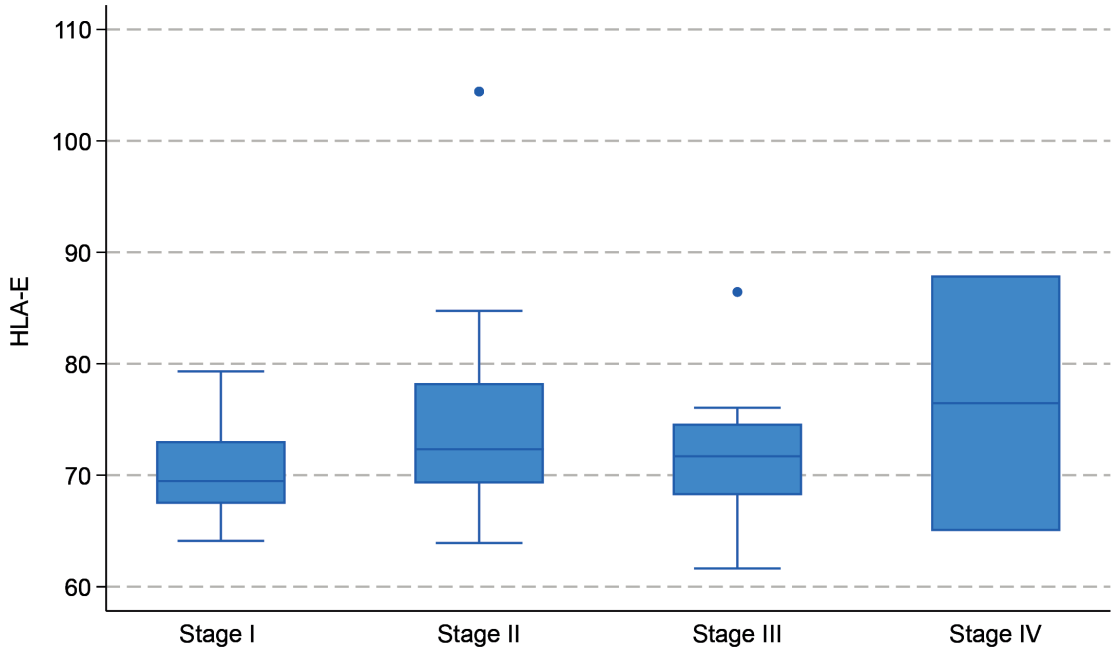


Figure 6. Boxplot of HLA-E expression by tumour stage.

pathways in cancer. This lack of association highlights the importance of evaluating immune markers individually, especially in a clinical setting.

Discussion

The present study provides evaluation of PD-L1 and HLA-E expression patterns in HNSCC, with a particular focus in their relationship to tumour differentiation, disease stage and potential marker associations.

Our analysis demonstrated a very weak and statistically insignificant correlation between PD-L1 and HLA-E expression, as evidenced by both Pearson correlation and linear regression analysis. The low correlation coefficient and minimal explained variance indicate that these two immune markers are largely independently regulated within the tumour microenvironment. These findings suggest that combined targeting of adaptive and innate immune inhibitory mechanisms could be required to overcome tumour immune resistance.

Regarding tumour differentiation, PD-L1 expression showed a descriptive increase from well-differentiated (G1) to poorly differentiated (G3) tumours. However, this pattern did not reach statistical significance in either parametric (ANOVA) or non-parametric (Spearman) analysis. The absence of significance, despite a visible trend, may reflect limited subgroup sizes, particularly in the G1 subcategory and highlights the importance of cautious interpretation. These findings indicate that while PD-L1 expressions may vary across histological grades, tumour differentiation alone is insufficient to reliably predict PD-L1 expression levels.

Similarly, the analysis of immune marker expression across clinical stages revealed no statistically significant difference for either PD-L1 or HLA-E. Although the mean values showed variability among stage groups, particularly for HLA-E, the wide dispersion within groups suggests substantial inter-tumour heterogeneity rather than a stage dependent expression pattern. These results imply that neither PD-L1 nor HLA-E expression is driven by tumour stage, reinforcing the concept that immune escape mechanisms in HNSCC are complex and not strictly linked to conventional pathological parameters.

In line with the findings of Wen et al.[10], who demonstrated an association between HLA-E expression and local recurrence in advanced laryngeal carcinoma, our study shows uniform HLA-E expression across all recurrent carcinoma samples. Although our cohort lacks a non-recurrent comparison group, the consistent presence of HLA-E in recurrent tumours supports the hypothesis that HLA-E mediated immune escape may contribute to tumour persistence and recurrence.

Consistent with the work of Andre et al.[11], which demonstrated that HLA-E enrichment suppresses antitumour immunity through CD94/NKG2A signaling on exhausted CD8+ T cells, our findings suggest that increased HLA-E expression is associated with adverse local tumour behavior. Together, these data support the clinical relevance of the HLA-E /NKG2A immune checkpoint in HNSCC.

Importantly, the absence of correlation between these markers further supports the notion that evaluating a single immune checkpoint may provide an incomplete picture of tumour immune evasion.

Consistent with the findings of Ono et al.[12], our results support the concept that immune-related biomarkers in HNSCC reflect functional immune context rather than conventional pathological parameters. Both studies show that PD-L1-related pathways do not correlate independently with tumour stage or differentiation, underscoring that immune checkpoint expression must be interpreted within the broader immune microenvironment. Together, these data reinforce the importance of integrated immune profiling to understand the immune regulation of these tumours.

While the present study does not establish prognostic or predictive value for PD-L1 or HLA-E, it provides a basis for future investigations incorporating larger cohorts, survival outcomes and response to treatment to better define their potential clinical relevance. Additionally, combined assessment of adaptive and innate immune checkpoint pathways may offer a more comprehensive understanding of the escape mechanisms in HNSCC.

Conclusions

Our findings are further corroborated in the context by the evolving landscape of head and neck cancer treatment. With the emergence of immune checkpoint inhibitors targeting PD-1/PD-L1 and newer agents blocking the NKG2-HLA-E axis, dual marker evaluation could bring information for further therapeutic stratification[13,14]. Studies have already shown that patients with high PD-L1 expressions may benefit more from the targeted therapies, and combining these with agents targeting HLA-E related pathways could enhance efficacy in poor or non-responders[15].

In summary, while the present study does not establish prognostic or predictive value for PD-L1 or HLA-E, it provides a basis for future investigations incorporating larger cohorts, survival outcomes and response to treatment to better define their potential clinical relevance. Additionally, combined assessment of adaptive and innate immune checkpoint pathways may offer a more comprehensive understanding of the escape mechanisms in HNSCC.

Authors' contributions

M.S. provided original draft preparation, review & editing, and visualization; S.N.G. provided diagnosis, conceptualization, methodology, software of picturs, IHH analysis of PD-L1, writing –supervision; B.I.V. provided validation, immunohistochemical analysis of HLA-E, visualization; A.R.C. provided validation, writing –supervision; E.B.I. provided conceptualization, methodology, software analysis, draft text; T.G.I. provided conceptualization, methodology, software analysis; N.G.A. provided statistical analysis; A.V.B. provided conceptualization, methodology, software analysis. All authors have read and agreed to the published version of the manuscript. All authors have contributed to the manuscript in its entirety and have approved the submitted version.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article".

"The authors declare that all the procedures of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from the patients regarding the conducted studies, scientific research, and future publications. "

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