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CAMILA MARIA BELARMINO DOS SANTOS

IMUNOEXPRESSÃO DE EFRINA-A1, EPH-A1 E EPH-A2 EM ADENOMA PLEOMÓRFICO E CARCINOMA MUCOEPIDERMOIDE GLANDULARES SALIVARES

CAMPINA GRANDE/PB
2024

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Estadual da Paraíba, como parte das exigências para obtenção do título de Mestre em odontologia.

Área de concentração: Clínica odontológica.

Orientador: Manuel Antonio Gordón-Núñez

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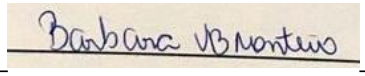

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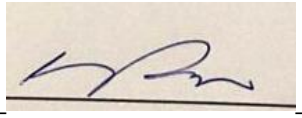

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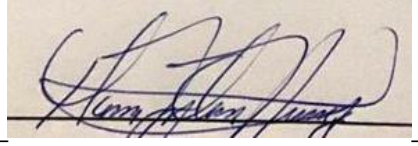

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RESUMO

Efrinas e seus receptores se tornam alvo de pesquisas relacionadas à etiopatogênese tumoral, visto que participam de sinalizações intracelulares bidirecionais que modulam fenômenos celulares e tumorais diversos, porém pouco se conhece sobre ação em neoplasias glandulares salivares. Foi avaliada a imunoexpressão de Efrina-A1, Eph-A1 e Eph-A2 em uma amostra de 8 adenomas pleomórficos (AP) e 8 carcinomas mucoepidermóide (CME) glandulares salivares em relação aos seus parâmetros histomorfológicos (critérios de Soares *et al.,* 2009 e de Brandwein *et al.,* 2009). Foram estabelecidos percentuais imunoexpressão citoplasmática e nuclear em cinco campos de maior imunorreatividade (400x) aos anticorpos monoclonais anti-Efrina-A1 e policlonais anti-Eph-A1 e anti-Eph-A2. Os dados foram analisados através dos testes de Mann-Whitney e de correlação de Spearman ($p < 0,05$). Observou-se altos percentuais de imunoexpressão citoplasmática de Efrina-A1, com discreta maior expressão em CME e baixos percentuais nucleares dessa efrina, principalmente em AP, com diferença significativa na sua expressão nuclear em relação grau de malignidade dos CME ($p <0,05$). Eph-A1 teve alta expressão citoplasmática na amostra, discretamente maior em AP e expressão nuclear em todos os CME e em 87,5% dos AP, sem diferença significativa da expressão citoplasmática e nuclear em relação aos parâmetros histomorfológicos da amostra. O Eph-A2 exibiu alta expressão citoplasmática, com tendência maior em CME e alta expressão nuclear na amostra, principalmente nos AP, sem diferenças significativas na expressão de Eph-A2 em citoplasma e núcleo em relação aos parâmetros histomorfológicos da amostra. Nos AP, houve correlação negativa entre as expressões citoplasmática de Efrina-A1 e nuclear de Eph-A1 ($r=-0,762; p=0,028$). Nos CME, houve correlação negativa entre as expressões citoplasmática de Eph-A1 e nuclear de Efrina-A1 ($r=-0,807; p=0,015$). Em conclusão, a maior expressão citoplasmática de Efrina-A1 em AP e CME, sugere sua ação na tumorigênese glandular salivar. A expressão nuclear de Eph-A1 sugere sua ação em vias de sinalização reversas, provavelmente regulando eventos do desenvolvimento de AP e CME. A maior expressão de Eph-A2 em CME sugere que esse perfil de expressão na tumorigênese maligna. A alta expressão citoplasmática das proteínas em relação aos parâmetros histomorfológicos da amostra, principalmente em CME de maior grau de malignidade sugere-se que essas podem exercer algum papel na regulação da patogenia dessas lesões, porém a baixa expressão nuclear, principalmente da Efrina-A1, sugere esse perfil de expressão das proteínas seja pouco envolvido na tumorigênese de AP e CME.

Palavras chaves: adenoma pleomórfico; carcinoma mucoepidermoide; efrina -A1; eph-A1; eph-A2.

ABSTRACT

Ephrins and their receptors have become the target of research related to tumor etiopathogenesis, as they participate in bidirectional intracellular signaling that modulate various cellular and tumor phenomena, but little is known about their action in salivary glandular neoplasms. The immunoexpression of Ephrin-A1, Eph-A1 and Eph-A2 was evaluated in a sample of 8 pleomorphic adenomas (PA) and 8 salivary glandular mucoepidermoid carcinomas (CME) in relation to their histomorphological parameters (criteria from Soares *et al*., 2009 and Brandwein *et al*., 20091). Cytoplasmic and nuclear immunoexpression percentages were established in five fields of greatest immunoreactivity (400x) to anti-Ephrin-A1 monoclonal and anti-Eph-A1 and anti-Eph-A2 polyclonal antibodies. Data were analyzed using the Mann-Whitney and Spearman correlation tests ($p < 0.05$). High percentages of cytoplasmic immunoexpression of Ephrin-A1 were observed, with a slight higher expression in CME and low nuclear percentages of this ephrin, mainly in AP, with a significant difference in its nuclear expression in relation to the degree of malignancy of the CME ($p <0.05$). Eph-A1 had high cytoplasmic expression in the sample, slightly higher in AP and nuclear expression in all CME and in 87.5% of AP, with no significant difference in cytoplasmic and nuclear expression in relation to the histomorphological parameters of the sample. Eph-A2 exhibited high cytoplasmic expression, with a higher tendency in CME and high nuclear expression in the sample, mainly in AP, without significant differences in the expression of Eph-A2 in cytoplasm and nucleus in relation to the histomorphological parameters of the sample. In AP, there was a negative correlation between cytoplasmic Ephrin-A1 and nuclear Eph-A1 expressions ($r=-0.762$; $p=0.028$). In CME, there was a negative correlation between cytoplasmic Eph-A1 and nuclear Ephrin-A1 expressions ($r=-0.807$; $p=0.015$). In conclusion, the greater cytoplasmic expression of Ephrin-A1 in AP and CME suggests its action in salivary gland tumorigenesis. The nuclear expression of Eph-A1 suggests its action in reverse signaling pathways, probably regulating events in the development of AP and CME. The higher expression of Eph-A2 in CME suggests this expression profile in malignant tumorigenesis. The high cytoplasmic expression of proteins in relation to the histomorphological parameters of the sample, especially in CME of higher degree of malignancy, suggests that they may play a role in regulating the pathogenesis of these lesions, however the low nuclear expression, mainly of Ephrin-A1, suggests that this protein expression profile is little involved in the tumorigenesis of AP and CME.

Keywords: pleomorphic adenoma; mucoepidermoid carcinoma; ephrin-A1; eph-A1; eph-A2.

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AP:	Adenoma pleomórfico.
CEP:	Comitê de ética em pesquisa.
CME:	Carcinoma mucoepidermoide.
CTNNB1:	Do inglês *catenin* (*cadherin associated protein*) *beta 1*, refere-se ao gene *CTNNB1*.
EPH:	Do inglês *erythopoietin-producing human hepatocellular receptor*, refere-se ao receptor de efrina.
ºGL:	Grau *Gay Lussac***,** representa a porcentagem de álcool puro presente em uma mistura.
HCl:	Ácido clorídrico.
GPI:	Glicosilfosfatidilinositol.
IBM:	Do inglês *International Business Machines Corporation*, traduzido como Corporação Internacional de Máquinas de Negócios.
SAM:	Do inglês *sterile α motif,* traduzido com motivo α estéril
SPSS:	Do inglês *Statistical package for social sciences*, traduzido como programa estatístico para ciências sociais.
UEPB:	Universidade Estadual da Paraíba.
µm:	Micrômetro.

SUMÁRIO

1 CONSIDERAÇÕES INICIAIS

Entre as neoplasias das glândulas salivares, pela sua maior frequência e comportamentos biológicos, destacam-se o Adenoma Pleomórfico (AP) e Carcinoma Mucoepidermoide (CME) (Galib *et al.,* 2023).

O AP ou tumor misto benigno trata-se de um tumor comum das glândulas salivares (Abdelhamid *et al*., 2022). Apesar de ser classificado como benigno, torna-se motivo de preocupação uma vez que há relatos na literatura quanto à taxa de recorrência e transformação maligna (Singh *et al*., 2017; Khanal, 2019; Baskaradass; Upreti, 2023). Sua etiopatogenia pode estar relacionada ao uso de radioterapia ou alterações genômicas que podem ser encontradas em aproximadamente 70% dos casos, como a fusão PLAG1 (8q12), fusão HMGA2 (12q14-15), além do CTNNB1, que pode superexpressar a oncoproteína PLAG1 (Asahina *et al*., 2019; Toper; Sarioglu, 2021)

Encontra-se com maior frequência em mulheres, principalmente na faixa etária de 40 a 60 anos. Além disso, o AP representa cerca de 60% dos tumores benignos na glândula parótida (Zoccali *et al.*, 2023). Clinicamente apresenta-se como uma massa indolor, de crescimento lento, com limites bem definidos, consistência borrachoide e endurecida (Abdelhamid *et al*., 2022).

Quanto à histomorfologia, frequentemente observa-se uma lesão encapsulada com componentes ductais (epiteliais), mioepiteliais e mesenquimais. O epitélio pode estar permeado por um estroma fibroso frouxo do tipo mixoide, condroide ou condromixoide (Almeslet, 2020; Arumugam *et al*., 2019; Khanal, 2019; Yousra; Saliha, 2021). O AP apresenta disposição variada quanto aos padrões morfológicos que são determinados quanto ao arranjo das células epiteliais e o tipo de estroma presente no tumor (Hellquist *et al*., 2019).

A classificação histomorfológica do AP, baseia-se no proposto por Seifert *et al*. (1976) em Tipo I, Clássico, quando o estroma representa de 30% a 50% do tumor; Tipo II, Estromal, quando o estroma caracteriza mais de 80%; Tipo III, representado pelos casos com predomínio de células neoplásicas (70%); Tipo IV, Monomórfico, caracterizado por lesões celularizadas com arranjo predominante monomórfico das células. Soares *et al.* (2009) apresentaram uma classificação dicotômica do AP baseando-se na composição celular e estromal: ricos em células e pobres em células. O uso dessa classificação tem como objetivo diminuir a subjetividade da classificação de Seifert *et al.,* (1976) uma vez que evita maior estratificação de uma amostra pequena e fortalece o poder dos dados estatísticos.

O CME, lesão maligna mais comum em glândulas salivares (Sama; Komiya; Guddati, 2022), tem origem no componente epitelial ou no parênquima glandular salivar e possui três graus de histodiferenciação, diretamente relacionados ao comportamento biológico da lesão e prognóstico para o paciente (Peraza *et al*., 2020). A lesão tem predileção pelo sexo feminino e terceira a sexta década de vida. A glândula parótida, palato, região retromolar e mucosa jugal são as regiões mais afetadas (Gill *et al.*, 2018).

Quanto a sua origem, relata-se possível etiopatogenia relacionada aos efeitos de radiação ionizante e translocação genética específica t(11;19)(q14-21; p12-13) com fusão CRTC1 (MECT1)-MAML2 (Chen *et al*., 2014). As questões genéticas são fatores importantes a serem analisados, uma vez que estão relacionadas à evolução dessa lesão de baixo grau para alto grau como também se relaciona com o prognóstico do paciente (Sama; Jomiya; Guddati, 2022). Clinicamente o paciente apresenta um aumento de volume local e não relata sintomatologia, porém pode associar-se a leve pressão e desconforto, a depender da localização e desenvolvimento da lesão (Devaraju *et al*., 2014).

Histologicamente, observa-se a presença de células mucosas, intermediárias e epidermoides, podendo ser colunares, claras ou oncocitoides. A lesão é classificada em 3 graus histopatológicos de malignidade: baixo, intermediário e alto. O baixo grau apresenta grandes espaços císticos preenchidos por mucina e revestidos por células secretoras de muco, intermediárias e epidermoides. O grau intermediário representa tumores mais sólidos e menos circunscritos. Os casos de alto grau possuem predominância de células epiteliais, com poucas células mucosas (Devaraju *et al*., 2014; Gill et al., 2018).

Quanto à classificação do grau histopatológico, existem algumas referências clássicas reconhecidas na literatura. Nesse contexto a classificação proposta por Brandwein *et al.* (2001), avalia os seguintes critérios: Componente intracístico <25%, fronte tumoral e sua invasão em pequenos ninhos e ilhas, atipia nuclear pronunciada, invasão linfática ou vascular, invasão óssea, mais de quatro mitoses por 10 campos em grande aumento, disseminação perineural e necrose. Assim, de acordo com o escore de pontos obtido por cada item, tem-se a classificação em Graus I, II, III. A respeito do tratamento de escolha, a maioria baseia-se apenas em ressecção cirúrgica completa, parcial e radioterapia (Peraza *et al*., 2020).

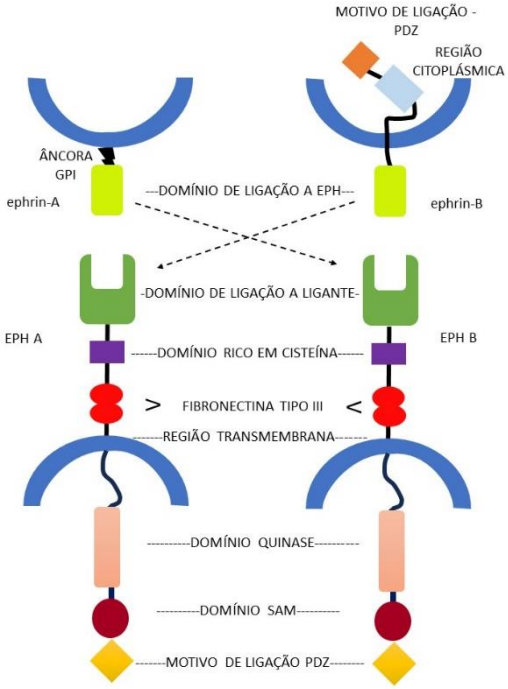
Visando entender os complexos processos etiopatogênicos das neoplasias glandulares salivares, diversos estudos têm analisado uma série de proteínas

(*cyclooxygenase-2, cyclin D1,* Par-4, Survivin, Muc-1, PHLDA1, FAZ, Ki-67, *caspase*-3, SPARC, VEGFC, VEGF-D, OCT4, CD44, ATG7, LC3A, LC3B, p-mTOR, p62) e seu papel no desenvolvimento dessas lesões (Tenório *et al.*, 2018; Silva *et al.*, 2019; Barroso *et al.*, 2020; Moura *et al.*, 2021; Pires *et al.*, 2023). Efrinas (*Ephrins*) e seus receptores (Eph), embora seja um assunto estudado em outras doenças, ainda constitui um tema pouco abordado em relação a neoplasias glandulares salivares (Ieguchi; Maru 2019; Xiao *et al*., 2020).

As efrinas são ligantes de receptores de efrinas (Groppa *et al*., 2018; Grandi *et al.*, 2019). Em humanos, são reconhecidas 9 efrinas, divididas nas classes A (Efrina-A1-5), B (Efrina-B1, Efrina-B2, Efrina-B3 e Efrina-B4) (Pasquale, 2008, LaCombre *et al*., 2022). Sua composição estrutural apresenta diferenças quando se compara as duas classes. As Efrinas-A estão fixadas à membrana celular por meio de uma âncora de glicosilfosfatatidilinositol (GPI). Por outro lado, as Efrinas-B são proteínas transmembranares que, na região intracelular, possuem um domínio PDZ (Xi, 2012; Kania; Klen, 2016).

Os Eph's são proteínas ligadas à membrana que formam a maior família de receptores de tirosina quinase e são classificados em duas classes: Eph-A (Eph-A1-8 e EphA10) e B (Eph-B1-4 e EphB6) (LaCombe *et al*., 2022). Eph's apresentam em sua estrutura componentes localizados na região extracelular e intracelular. Na região extracelular, possui um domínio de ligação ao ligante, que se liga ao receptor, um domínio rico em cisteína, duplo domínio de fibronectina tipo III. Transpassando a região membranar, apresenta um domínio tirosina quinase, domínio motivo α estéril (SAM - sterile α motif) e por fim, domínio PDZ (Xi, 2012; Kania; Klen, 2016).

Figura 1 – Estrutura de Efrinas e Eph’s



Fonte: Adaptada de Xi, 2012.

A interação das efrinas e eph’s constitui complexos responsáveis pela formação de vias de sinalização intracelulares bidirecionais que geram ou controlam mecanismos moleculares sobre a célula que expressa a efrina e a que expressa o Eph (Pasquale, 2008; Ieguchi; Maru, 2019; Jensen, 2000). Tais complexos formam-se pelo grau de afinidade dos seus domínios de ligação, sendo assim as Efrinas-A usualmente interagem com os Eph-A e as Efrinas-B com seus receptores cognatos Eph-B (Pasquale, 2005).

Uma das exceções a essa usual forma de interação é o caso da Efrina-A5, a qual, quando em altas concentrações, pode interagir com o Eph-B2 (Himanen *et al*., 2004). Além disso, as Efrina-B2 e Efrina-B3 podem formar complexos de interação com o Eph-A4 (Gale *et al*., 1996). Somado ao antes exposto, em cada subgrupo de efrinas/Eph podem ocorrer interações promíscuas com graus de afinidades variáveis (Pasquale, 2004; Lackmann; Boyd, 2008).

As sinalizações promovidas pelos complexos Efrina/Eph regulam uma ampla variedade de eventos celulares, incluindo morfologia, proliferação, migração e sobrevivência celular. Além de estar envolvidos no desenvolvimento neuronal, padronização vascular e inflamação durante a lesão tecidual, também participam de processos adesivos celulares à matriz extracelular (MEC) (Kania; Klein, 2016; Kou; Kandpal, 2018).

A regulação e a expressão aberrante das efrinas e/ou seus receptores e, consequentemente, das vias de sinalização nas quais participam, podem ser associadas a tumorigênese, incluindo alterações no potencial proliferativo, migratório e invasivo de uma variedade de cânceres humanos, fato que tornou tais proteínas o foco de estudos e possíveis alvos em terapêuticas antineoplásicas (Kou; Kandpal, 2018; Liang *et al*., 2019).

Um número limitado de pesquisas relacionadas à análise de efrinas e seus receptores em neoplasias glandulares salivares é disponível na literatura (Pubmed DataBase, acesso em 08/06/2024), porém sugere-se que essas proteínas desempenham funções complexas em diversos eventos etiopatogênicos de glândulas salivares, incluindo angiogênese, adesão, migração celular, invasão vascular, invasão perineural e progressão tumoral (Shao *et al*., 2013; Fukai *et al*., 2014).

Nesse contexto, Shao *et al.* (2013) avaliaram a expressão de Eph-A2 e efrina-A1 através de imunistoquímica, Westernblot e RTPCR em tempo real em 49 CAC primários e 10 amostras de tecidos de glândulas salivares normais, observando maior expressão de Eph-A2 e efrina-A1 em CAC em comparação com tecidos não neoplásicos e positivamente correlacionada com aumento de microdensidade vascular (MVD). Verificaram ainda que a superexpressão de Eph-A2 e efrina-A1, juntamente com MVD elevada, foi associada ao estágio TNM tumoral e à presença de invasão perineural e perivascular. Os CAC sólidos, que são acompanhados por piores prognósticos, exibiram maior expressão das proteínas avaliadas e da MVD em comparação com os subtipos cribriforme e tubular. Como conclusão, o estudo mostrou alta expressão de Eph-A2 e Efrina-A1 no CAC.

Por sua vez, Fukai *et al.* (2014) relataram o caso de um paciente masculino de 29 anos de idade com CAC que apresentou disseminação perineural do tumor ao longo do nervo mandibular. As células tumorais apresentaram características de transição epitelial-mesenquimal (EMT) e uma alta imunoexpressão de Eph-A2, sugerindo uma possível ligação entre o receptor e fenótipos tumorais agressivos.

A relação entre Efrina A1 e os receptores A1 e A2 apresenta-se em atividades fisiológicas já explicadas na literatura, porém essa comunicação se torna alvo de investigação uma vez que a ligação se faz presente em alguns processos patológicos através da angiogênese, migração celular e desenvolvimento de patologias. A expressão exacerbada da Efrina-A1 relaciona-se com a malignidade tumoral e mau prognóstico devido ao desenvolvimento de metástase (Ieguchi; Maru, 2019).

Em condições patológicas, a interação de EphA1 e EphA2 com a efrina-A1 em eventos como a angiogênese, fenômeno que fornece nutrientes e suprimento sanguíneo, favorece o crescimento tumoral, agravando o prognóstico da lesão, como tem sido relatado no câncer de mama (Ieguchi; Maru, 2019).

Ao analisar as funções de Eph-A1 em uma variedade de tumores, Wu *et al*. (2023) identificaram a expressão anormal em células tumorais de câncer gástrico, nasofaríngeo e de ovário. Eph-A1 mostrou-se diretamente relacionada com progressão do câncer e angiogênese tumoral. Apesar dessas considerações, o mecanismo envolvido ainda se encontra não elucidado. A ausência de informações corrobora a necessidade de mais pesquisas sobre o tema.

Evidências mostram que Eph-A2, por exemplo, em cânceres de origem epitelial e mesenquimal regula alguns fatores importantes no desenvolvimento tumoral, como iniciação, neoangiogênese e metástase (Dunne *et al*., 2016). Tais fatores relacionam-se diretamente com mau prognóstico, alto potencial de metástase e diminuição da sobrevida do paciente com tumor, tornando-se um participante importante na progressão maligna (Xiao *et al*., 2020).

Sugere-se que o Eph-A2 pode estar associada à promoção de invasão neoplásica dependente de quinase de adesão focal, devido sua ação na estimulação da expressão da metaloproteinase de matriz (MMP-2), a qual exerceria a ação de degradação da MEC, facilitando assim os eventos de invasão celular (Duxbury *et al*., 2004). Por sua vez, a alta expressão do Eph-B4 tem sido relatada em aproximadamente 58% dos cânceres de mama (Brantley-Sieders, 2011; Kaenel; Mosimann; Andres, 2012), porém mesmo com a superexpressão desse receptor nessa neoplasia, a sinalização direta da efrina-B2/Eph-B4 parece ativar a via anti-oncogênica ABL-CRK e regular negativamente a MMP-2 (Noren *et al*., 2006).

Escassos estudos relatam a análise em glândulas salivares. Dentre eles, o Carcinoma Adenoide Cístico e a interação com Efrinas da classe A1 com Eph A1/A2 (Shao *et al*., 2013; Fukai *et al*., 2014; Yan; Wang, 2022). Desta forma, o objetivo do presente trabalho foi avaliar a expressão da Efrina-A1 e os receptores Eph-A1 e Eph-A2 em AP e CME, visando obter maiores informações sobre a participação dessas proteínas nos mecanismos etiopatogênicos dessas neoplasias de glândulas salivares.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar a imunoexpressão da Efrina-A1, Eph-A1 e Eph-A2 em relação a parâmetros histomorfológicos de uma série de AP e CME glandulares salivares.

2.2 Objetivos específicos

- Analisar o perfil clínico (sexo, idade e localização anatômica das lesões) de uma amostra de AP e CME.
- Caracterizar o perfil histomorfológicos da amostra de AP e CME.
- Determinar os percentuais de células imunopositivas para Efrina -A1, Eph-A1 e Eph-A2 em células de AP e CME.
- Estabelecer correlações entre as imunoexpressões da Efrina -A1 e dos receptores Eph-A1, Eph-A2 em relação aos parâmetros histomorfológicos dos AP e dos CME.

3 METODOLOGIA

3.1 Caracterização do estudo

Estudo transversal com caráter descritivo correlacional da análise qualitativa e semiquantitativa da expressão imunoistoquímica de Efrina-A1 e dos receptores de efrinas Eph-A1, Eph-A2 em relação aos parâmetros histomorfológicos de uma série de AP e CME glandulares salivares.

3.2 Aspectos éticos

A presente pesquisa foi cadastrada na Base de Registros de Pesquisas envolvendo Seres Humanos (Plataforma Brasil) e submetida à análise de seu conteúdo pelo Comitê de Ética em Pesquisa da Universidade Estadual da Paraíba (UEPB) obtendo parecer aprovado (nº 6.798.135), respeitando a resolução 466/2012 do Conselho Nacional de Saúde/ Ministério da Saúde.

3.3 População

A população do estudo constituiu-se por todos os casos de AP e CME glandulares salivares diagnosticados e arquivados no Laboratório de Histopatologia Oral do Departamento de Odontologia da UEPB, no período de 2011 a 2024.

3.4 Amostra

O estudo foi realizado com uma amostra não probabilística por conveniência, constituída por 16 espécimes fixados em formol a 10% e incluídos em blocos de parafina, correspondendo a 8 casos de cada lesão.

3.4.1 Critérios inclusão e exclusão

Foram incluídos na amostra, espécimes provenientes de biópsia excisional e incisional de glândulas salivares maiores e menores que apresentaram material biológico suficiente para realização dos estudos histomorfológicos e imunoistoquímicos, cujos blocos de parafina apresentavam bom estado de conservação. Para o CME foram excluídos espécimes de pacientes submetidos a tratamento antineoplásico prévio.

3.5 Variáveis

O Quadro 1 exibe o elenco das variáveis independentes e dependentes avaliadas no presente estudo.

Quadro 1 – Elenco de variáveis que foram analisadas no estudo.

VARIÁVEIS DEPENDENTES		
VARIÁVEL	**DEFINIÇÃO**	**CATEGORIZAÇÃO**
Efrina-A1	Percentual de células com positividade.	• Células neoplásicas: citoplasma e núcleo
Eph-A1	Percentual de células com positividade.	• Células neoplásicas: citoplasma e núcleo.
Eph-A2	Percentual de células com positividade.	• Células neoplásicas: citoplasma e núcleo.
VARIÁVEIS INDEPENDENTES		
Tipo de lesão	Tumores glandulares salivares classificados de acordo com as características histopatológicas	• Adenoma pleomórfico • Carcinoma mucoepidermoide
Classificação histomorfológica dos AP de acordo com a quantidade e composição celular do estroma (Soares *et al.*, 2009)	Classificação dos AP baseada na composição celular e estromal	• Ricos em célula • Pobres em célula
Grau de malignidade dos CME	Classificação dos CME em relação aos critérios histológicos de malignidade (Brandwein *et al.*, 2001)	• Baixo grau de malignidade • Grau intermediário de malignidade • Alto grau de malignidade

Fonte: Elaborado pela autora, 2024.

3.6 Coleta de dados epidemiológicos

Baseou-se nas informações das fichas de registro e laudos de exame histopatológico, dos quais idade e sexo dos pacientes, além da localização anatômica e diagnóstico histopatológico das lesões corresponderam às variáveis estudadas (APÊNDICE A).

3.7 Estudo histomorfológico

A partir do material biológico incluído em parafina, foram obtidos cortes histológicos com 5μm de espessura, os quais foram estendidos em lâminas de vidro e submetidos à coloração de rotina da hematoxilina e eosina. Posteriormente, sob microscopia de luz (*Leica DM 500, Leica Microsystems Vertrieb GmbH, Wetzlar, DE*), um patologista sem conhecimento dos dados clínicos relacionados aos casos, realizou a análise histomorfológica dos tumores glandulares salivares segundo a classificação mais recente da OMS (Lépine, 2024).

A análise histomorfológica e classificação dos AP foi realizada de acordo com os critérios de quantidade e composição celular do estroma, onde foram classificados em: ricos em células (predominância de células epiteliais) e pobre em células (predominância de áreas condroides e mixoides) (Soares *et al.*, 2009).

Com base nos critérios de Brandwein *et al*. (2001), os CMEs foram classificados em baixo (Grau I) quando células mucosas eram predominantes, células intermediárias e formações císticas eram proeminentes e padrão de crescimento circunscrito, intermediário (Grau II), quando as células intermediárias eram predominantes sobre as células mucosas, com a maior parte solidamente arranjada, ou alto grau de malignidade (Grau III) quando havia predominância de células escamosas, mas com células intermediárias e mucosas também presentes. Tal classificação baseia-se diante de características histopatológicas descritas abaixo.

Quadro 2. Características histopatológicas para gradação do CME, adaptado de Brandwein *et al.* (2001)

Características	**Pontos**
Componente intracístico <25%	2
Front de invasão	2
Atipia nuclear	2
Invasão linfática e vascular	3

Invasão óssea	3
>4 mitoses por 10 campos em maior aumento	3
Invasão perineural	3
Necrose	3
Grau I Grau II Grau III	0 2-3 4 ou mais

Fonte: Brandwein *et al.* (2001).

3.8 Estudo imunoistoquímico

3.8.1 Método imunoistoquímico

A amostra selecionada, fixada em formol a 10% e incluída em parafina, foi submetida a cortes com 3µm de espessura, os quais foram estendidos em lâminas de vidro preparadas com adesivo à base de organosilano (*3-aminopropiltrietoxisilano, Sigma-Aldrich, St. Louis, MO, USA*). Posteriormente, o material foi submetido ao método da imunoperoxidase pela técnica baseada em polímeros de dextrano (*EnVision^{TM} Flex+, Dako North America Inc., Carpinteria, CA, USA*), utilizando anticorpos monoclonais anti-Efrina-A1 e anticorpos policlonais anti-Eph-A1 e anti-Eph-A2 (Quadro 3).

Quadro 3. Especificidade, referência, fabricante, diluição, recuperação antigênica e incubação dos anticorpos primários utilizados no estudo.

Especificidade	**Referência**	**Fabricante**	**Diluição**	**Recuperação Antigênica**	**Incubação**
Efrina-A1	MA5-29231	Invitrogen	1:600	Citrato, pH 6,0 *Steamer*, 90ºC, 30 min	*Overnight*
Eph-A1	PA1-30291	Invitrogen	1:6000	Citrato, pH 6,0 *Steamer*, 90ºC, 30 min	*Overnight*
Eph-A2	PA5-14574	Invitrogen	1:1500	Citrato, pH 6,0 *Steamer*, 90ºC, 30 min	*Overnight*

Fonte: Elaborado pela autora, 2024.

O protocolo imunoistoquímico utilizado encontra-se descrito abaixo:

⇒ Desparafinização: 2 banhos em xilol aquecido (15 minutos cada);

⇒ Reidratação em cadeia descendente de etanóis:

- Álcool etílico absoluto I (5 minutos);
- Álcool etílico absoluto II (5 minutos);
- Álcool etílico absoluto III (5 minutos);
- Álcool etílico 95°GL (5 minutos);
- Álcool etílico 80°GL (5 minutos);

⇒ Remoção de pigmentos formólicos com hidróxido de amônia a 10% em etanol 95°, à temperatura ambiente (10 minutos);

⇒ Lavagem em água corrente (10 minutos)

⇒ Duas passagens em água destilada (5 minutos cada);

⇒ Recuperação antigênica;

⇒ Lavagem em água corrente (10 minutos);

⇒ Duas passagens em água destilada (5 minutos cada);

⇒ Duas incubações dos cortes em solução de peróxido de hidrogênio 3% 10 volumes, em proporção de 1/1, para o bloqueio da peroxidase endógena tecidual (10 minutos cada);

⇒ Lavagem em água corrente (10 minutos);

⇒ Duas passagens em água destilada (5 minutos cada);

⇒ Duas passagens em solução de TRIS-HCl Tween pH 7,4 (5 minutos cada);

⇒ Incubação dos cortes com anticorpo primário, em solução diluente (*EnVision™ Flex antibody diluent*, Dako North America Inc., Carpinteria, CA, USA), a 4ºC em câmara úmida

⇒ Duas passagens em solução de TRIS-HCl Tween pH 7,4 (5 minutos cada);

⇒ Incubação com anticorpo secundário polimerizado à peroxidase (*EnVision™ Flex/ HRP*, Dako North America Inc., Carpinteria, CA, USA), a 4ºC em câmara úmida (30 minutos);

⇒ Duas passagens em solução de TRIS-HCl Tween pH 7,4 (5 minutos cada);

⇒ Revelação da reação com solução cromógena de 3,3-diaminobenzidina (*EnVision™ Flex DAB+*, Dako North America Inc., Carpinteria, CA, USA) (10 minutos);

⇒ Lavagem em água corrente (10 minutos);

⇒ Passagens rápidas em água destilada (2 trocas);

⇒ Contracoloração com hematoxilina de Harris, à temperatura ambiente (2,5 minutos);

⇒ Lavagem em água corrente (10 minutos);

⇒ Desidratação em álcool absoluto:

- Álcool etílico absoluto I (5 minutos);
- Álcool etílico absoluto II (5 minutos);
- Álcool etílico absoluto III (5 minutos);

⇒ Três passagens em xilol (2 minutos cada);

⇒ Montagem em polímero de acrilatos (*Entellan® new*, Merck KGaA, Darmstadt, DE).

3.8.2 Análise imunoistoquímica

Após o processamento dos cortes histológicos e tratamento imunoistoquímico, cada lâmina foi analisada sob microscopia de luz (*Leica DM 500, Leica Microsystems Vertrieb GmbH, Wetzlar, DE*), por dois examinadores previamente treinados. As lâminas foram escaneadas em imagens de alta resolução por meio de sistema digital (*MoticEasyScan Pro 6, Motic Inc.*, Richmond, BC, CAN) e, posteriormente, visualizadas no programa *DSAssistant* (*Motic Inc.*, Richmond, BC, CAN). Um patologista experiente realizou as análises imunoistoquímicas sem conhecimento dos dados clinicopatológicos relacionados aos casos. A análise da expressão das proteínas Efrina-A1, Eph-A1 e Eph-A2 foi realizada em células dos tumores selecionados, considerando a expressão citoplasmática/membranar e nuclear de cada proteína.

Para as proteínas citadas foram avaliadas a imunorreatividade em membrana/citoplasma e em núcleo, adaptando-se a metodologia proposta no estudo de Shao *et al.* (2013), na qual, sob aumento de 100x (*DSAssistant, Motic Inc., Richmond, BC, CAN*), identificou-se 5 campos de maior imunorreatividade em cada caso. Em seguida, esses campos foram capturados sob aumento de 400x (*DSAssistant, Motic Inc.*, Richmond, BC, CAN). Com o auxílio do programa *ImageJ®* (*Image Processing and Analysis in Java, National Institute of Health, Bethesda, MD, USA*), as células imunopositivas e negativas foram contadas em cada um dos campos microscópicos capturados. Por fim, estabeleceu-se os percentuais de células imunopositivas em relação ao total de células quantificadas.

3.9 Análise estatística

Os resultados obtidos com as análises clínicas, morfológicas e imunoistoquímicas foram organizados em um banco de dados informatizado com o

auxílio do programa *Microsoft Excel*® e em seguida transferidos ao *software IBM SPSS Statistics 20*® (*IBM SPPS Inc., Armonk, NY, USA*). Estatística descritiva foi utilizada para caracterização da amostra. Os dados obtidos com a avaliação dos percentuais de imunopositividade foram submetidos à análise de distribuição, por meio do teste de Shapiro-Wilk. Os dados não apresentaram distribuição normal. As comparações das medianas dos percentuais de imunopositividade para as proteínas foram realizadas pelo teste Mann-Whitney. Possíveis correlações entre os percentuais de imunopositividade para as proteínas analisadas foram avaliadas por meio do teste de correlação de Spearman. Para todos os testes, considerou-se um nível de significância de 5% ($p < 0,05$).

4 ARTIGO

4.1 Apresentação

O projeto de pesquisa foi desenvolvido e aprovado em qualificação pelo Programa de Pós-Graduação em Odontologia da UEPB. Mediante execução desse projeto, um artigo é apresentado nesta dissertação: **"Imunoexpressão de Efrina-A1, Eph-A1 e Eph-A2 em adenoma pleomórfico e carcinoma mucoepidermoide glandulares salivares".**

O artigo será submetido ao periódico *Head and Neck Pathology* (ISSN: 1936-0568; Fator de impacto: 2,6; Qualis Odontologia A1), cujas normas para submissão de trabalhos são apresentadas no Anexo B.

4.2 Artigo a ser submetido

IMUNOEXPRESSÃO DE EFRINA-A1, EPH-A1 E EPH-A2 EM ADENOMA PLEOMÓRFICO E CARCINOMA MUCOEPIDERMOIDE GLANDULARES SALIVARES

Título breve: **Imunoexpressão de Efrina-A1, Eph-A1-A2 em neoplasias de glândula salivar**

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Conformidade com os padrões éticos

Disponibilidade de dados

Não se aplica.

Disponibilidade de códigos

Não se aplica.

Conflito de interesses

Os autores declaram não haver conflito de interesses.

Aprovação ética

Todos os procedimentos realizados em estudos envolvendo participantes humanos estavam de acordo com as normas éticas do comitê de pesquisa institucional e/ou nacional e com o Declaração de Helsinque de 1964 e suas emendas posteriores ou padrões éticos comparáveis. O estudo foi aprovado pelo Comitê de Ética em Pesquisa (CEP) da Universidade Estadual da Paraíba, Campina Grande, Paraíba, Brasil (Aprovação nº 6.798.135).

Consentimento informado

Este estudo obteve a aprovação do CEP da Universidade Estadual da Paraíba, Campina Grande, Paraíba, Brasil (Aprovação nº 6.798.135) e a necessidade de consentimento informado foi dispensada.

ABSTRACT

Background: The Eph-Ephrin interaction is related to bidirectional intracellular signaling that regulates cellular and tumor phenomena. The immunoexpression of Ephrin-A1, Eph-A1 and Eph-A2 was evaluated in relation to histomorphological parameters of pleomorphic adenomas (PA) and mucoepidermoid carcinomas (CME) of salivary glands.

Methods: The immunoperoxidase method based on the dextran polymer technique and anti-Ephrin-A1 monoclonal antibodies / anti-Eph-A1 and anti-Eph-A2 polyclonal antibodies was used, the cytoplasmic/membranous and nuclear expression of proteins was analyzed . Percentages of immunopositive cells were established in relation to the total cells quantified and five tumor fields. The data obtained were statistically analyzed using the Mann-Whitney and Spearman correlation tests ($p < 0.05$).

Results: There was cytoplasmic immunoexpression of Ephrin-A1 in the sample, with a slight tendency towards higher expression in CME and low nuclear percentages of this ephrin, mainly in AP. There was a significant difference in the nuclear expression of this protein ($p < 0.05$). Eph-A1 showed high percentages of cytoplasmic immunoexpression in the sample, with a slightly higher median in AP and nuclear expression in all CME and in 87.5% of AP, without significant differences in its expression in the cytoplasm and nucleus of AP and CME, nor in relation to the histomorphological parameters of the sample.

Cytoplasmic immunoexpression of Eph-A2 was observed, with a slightly higher median in CME and nuclear immunoexpression in the sample, mainly in AP. There were no significant differences in the immunoexpression of Eph-A2 in the cytoplasm and nucleus of the sample, nor in relation to the histomorphological parameters of AP and CME. In AP, there was a negative correlation between the cytoplasmic expression of Ephrin-A1 and the nuclear expression of Eph-A1 ($r=-0.762; p=0.028$). In CME, there was a negative correlation between cytoplasmic Eph-A1 and nuclear Ephrin-A1 expressions ($r=-0.807; p=0.015$).

Conclusions: The immunoexpression profiles of Ephrin-A1, Eph-A1 and Eph-A2 in the sample, with their usual localization in membrane/cytoplasm and nuclear translocation, may indicate that these proteins appear to exert some or several cellular and tumor regulatory functions, influencing the etiopathogenesis and biological behavior of these lesions.

Keywords: Pleomorphic adenoma; Mucoepidermoid carcinoma; Ephrin-A1; Eph-A1; Eph-A2.

Introdução

Tumores que acometem as glândulas salivares são incomuns e representam 5% de todos os cânceres que acometem cabeça e pescoço. A Organização Mundial da Saúde (OMS) atualmente reconhece 33 tumores diferentes. Tais lesões apresentam variações quanto às características clínicas, componentes histológicos e comportamento biológico [1,2].

O adenoma pleomórfico (AP) é um tumor comum das glândulas salivares [3]. Apesar de benigno, torna-se motivo de preocupação pela taxa de recorrência e transformação maligna [4-6]. O carcinoma mucoepidermoide (CME), lesão maligna mais comum em glândulas salivares [7], possui três graus de histodiferenciação, diretamente relacionados ao comportamento biológico da lesão e prognóstico para o paciente [8].

Nessa circunstância, diversos estudos têm analisado uma série de proteínas e seu papel no desenvolvimento dessas lesões. [9-13]. Efrina e seus receptores (Eph), embora seja um assunto estudado em outras doenças, ainda são pouco estudadas em relação a neoplasias glandulares salivares [14,15].

As sinalizações promovidas pelos complexos Efrina/Eph regulam uma ampla variedade de eventos celulares, incluindo morfologia, proliferação, migração e sobrevivência celulares. A regulação e a expressão aberrante das efrinas e/ou Eph e, consequentemente, das vias de sinalização das quais participam, podem ser associadas a tumorigênese, incluindo alterações no potencial proliferativo, migratório e invasivo de células neoplásicas em uma variedade de cânceres humanos, fato que tornou tais proteínas o foco de estudos e possíveis alvos em terapêuticas antineoplásicas [16,17].

Face ao exposto, o objetivo do presente trabalho foi avaliar a expressão da Efrina-A1 e os receptores Eph-A1 e Eph-A2 em AP e CME, visando obter maiores informações sobre a participação dessas proteínas nos mecanismos etiopatogênicos dessas neoplasias de glândulas salivares.

Materiais e métodos

Amostra

A amostra foi composta por 8 casos de AP e 8 casos de CME. Os casos foram provenientes dos arquivos do Laboratório de Histopatologia Oral do Departamento de Odontologia da Universidade Estadual da Paraíba (UEPB). Foram incluídos na amostra, apenas casos com material biológico suficiente para realização dos estudos histomorfológicos e imunoistoquímicos. Foram excluídos os CME com histórico de tratamento antineoplásico prévio à obtenção do material biopsiado. O estudo foi aprovado pelo Comitê de Ética em Pesquisa da UEPB (Parecer nº 6.798.135).

Análise morfológica

A partir do material emblocado em parafina, cortes histológicos de 5 μm foram obtidos, em seguida estendidos em lâminas de vidro e seguiram para coloração através da técnica da Hematoxilina e Eosina. Um patologista oral experiente analisou morfologicamente cada caso, sob microscopia de luz (*Leica DM 500, Leica Microsystems Vertrieb GmbH, Wetzlar, DE*).

A classificação dos AP baseou-se na quantidade celular e composição estromal, compondo dois grupos: tumores ricos em células (predomínio de células epiteliais) e tumores pobres em células (predomínio de áreas mixoides e condroides) [7]. A classificação dos CME baseou-se nos parâmetros propostos por Brandwein *et al.* (2001), considerando o grau histopatológico de malignidade, em: grau I, II e III.

Imunoistoquímica

As amostras selecionadas, fixadas em formol a 10% e incluídas em parafina, foram submetidas a cortes com 3μm de espessura, os quais foram estendidos em lâminas de vidro silanizadas (*3-aminopropiltrietoxisilano, Sigma Aldrich Co., St. Louis, MO, USA*).

Os cortes teciduais foram desparafinizados, reidratados e submetidos à recuperação antigênica (**Tabela 1**). Para o bloqueio da peroxidase endógena tecidual, os cortes foram imersos em peróxido de hidrogênio a 3% e posteriormente incubados com anticorpos primários (**Tabela 1**), lavados com tampão Citrato pH 6 e tratados com complexo baseado em polímeros de dextrano (*EnVision™ Flex+, Dako North America Inc.*, Carpinteria, *CA, USA*). A atividade da peroxidase foi visualizada por meio da imersão dos cortes em diaminobenzidina (*EnVision™ Flex DAB+, Dako North America*

Inc., Carpinteria, CA, USA), resultando em um produto de coloração acastanhada. Por fim, os cortes teciduais foram contracorados com hematoxilina de Harris, desidratados e montados com lamínula. Tecido glandular salivar normal foi utilizado para controle positivo. Para controle negativo, houve a omissão dos anticorpos primários.

Análise imunoistoquímica

A imunoexpressão de Efrina-A1, Eph-A1 e Eph-A2 foi avaliada de forma quantitativa, adaptando-se a metodologia utilizada no estudo de Shao *et al.* [21]. Para os anticorpos, considerou-se como positividade a coloração acastanhada de células tumorais, tanto citoplasmática quanto nuclear.

As lâminas foram escaneadas em imagens digitais de alta resolução (*MoticEasyScan Pro 6, Motic Inc., Richmond, BC, CAN*) e, subsequentemente, visualizadas no programa *DSAssistant* (*Motic Inc., Richmond, BC, CAN*). Uma examinadora previamente treinada realizou as análises imunoistoquímicas, sem conhecimento dos dados clinicopatológicos relacionados aos casos. Sob aumento de 100× (*DSAssistant, Motic Inc., Richmond, BC, CAN*), foram elencadas áreas de maior imunorreatividade aos anticorpos e sob aumento de 400× (*DSAssistant, Motic Inc., Richmond, BC, CAN*), foram fotomicrografados cinco campos nessas áreas de maior imunorreatividade. O programa *ImageJ® (Image Processing and Analysis in Java, National Institute of Health, Bethesda, MD, USA*) foi utilizado para a contagem de células imunomarcadas e negativas em cada campo da amostra. Os valores obtidos nos campos foram somados, estabelecendo-se o percentual de células imunopositivas em AP e CME em relação ao total de células contadas.

Análise estatística

Os dados coletados foram analisados com o auxílio do programa *IBM SPSS Statistics* (versão 20.0; *IBM Corp., Armonk, NY, USA).* O teste de Shapiro-Wilk revelou distribuição não normal dos dados, sendo o teste não paramétrico de Mann-Whitney utilizado para comparar as medianas dos percentuais de células imunopositivas para Efrina-A1, Eph-A1 e Eph-A2 em relação aos grupos estudados. Para analisar possíveis correlações entre as imunoexpressões dessas proteínas, aplicou-se o teste de correlação de *Spearman*. Para todos os testes estatísticos utilizados no presente estudo, foi considerado o nível de significância de 5% ($p < 0,05$).

Resultados

Parâmetros clinicopatológicos

A análise dos dados clínicos dos AP revelou 75% da amostra era do sexo feminino, com idades variando de 22 a 86 anos e média de 46,43 anos, além disso, 87,5% dos AP eram de glândulas salivares menores (**Tabela 2**). Nos CMEs, 50% dos casos eram no sexo feminino, com idades variando de 19 a 83 anos e média de 43,63 anos. A maioria (75%) ocorreu em glândulas salivares menores (**Tabela 2**).

Análise histomorfológica

A análise das características histopatológicas dos AP revelou 4 casos do subtipo rico em células e 4 do subtipo pobre em células. No grupo dos CME, a avaliação do grau histopatológico de malignidade demonstrou três casos com grau I, três casos com grau II e dois casos com grau III.

Imunoexpressão de Efrina-A1

Expressão citoplasmática de Efrina-A1 foi observada em todos os casos analisados, com altos percentuais de positividade em todos os grupos e mediana discretamente maior em CME **(Figura 4).** A análise da expressão nuclear revelou positividade em todos os grupos de lesões, com baixos percentuais de células imunopositivas para Efrina-A1, com mediana discretamente maior em núcleos de AP **(Figura 2-A).** Não foram observadas diferenças estatisticamente significativas na expressão de Efrina-A1 em citoplasma e núcleo entre AP e CME (**Tabela 3; Figura 4 e 5**).

Não foram observadas diferenças estatisticamente significativas na imunoexpressão citoplasmática e nuclear de Efrina-A1 em relação ao subtipo histológico dos AP **(Figura 6 e 7)**. Considerando o grau histopatológico dos CMEs, observou-se diferença estatisticamente significativa na expressão nuclear de Efrina-A1 entre grau I e graus II/III (p = 0,024*)* (**Figura 9**).

Imunoexpressão de Eph-A1

Expressão citoplasmática de Eph-A1 foi identificada em todos os casos de AP e CME, com altos percentuais de positividade em todos os grupos, com mediana discretamente maior em AP (**Figura 3-E**). A expressão nuclear foi positiva para todos os casos de CME e 87,5% de AP analisados, com mediana discretamente maior em AP

(**Figura 2-B**). Não foram observadas diferenças estatisticamente significativas na expressão de Eph-A1 em citoplasma e núcleo de AP e CME (**Tabela 3; Figura 4 e 5**).

Não foram constatadas diferenças estatisticamente significativas na imunoexpressão citoplasmática e nuclear de Eph-A1 em relação aos parâmetros histomorfológicos da amostra (**Figuras 6-9**).

Imunoexpressão de Eph-A2

Expressão citoplasmática de Eph-A2 foi observada em todos os casos analisados, com altos percentuais de positividade em todos os grupos e mediana discretamente maior em CME (**Figura 3-F)**. A análise da expressão nuclear revelou positividade em todos os casos de AP e 87,5% dos CME analisados, com baixos percentuais de células imunopositivas, com mediana discretamente maior em núcleos de AP (**Figura 2-C**). Não foram observadas diferenças estatisticamente significativas na expressão de Eph-A2 em citoplasma e núcleo de AP e CME (**Figuras 4 e 5**).

Não foram observadas diferenças estatisticamente significativas na imunoexpressão citoplasmática e nuclear de Eph-A2 em relação aos parâmetros histomorfológicos de AP e CME (**Figuras 6-9**).

Correlações entre as imunoexpressões de Efrina-A1, Eph-A1 e Eph-A2

Nos AP, observou-se correlação negativa entre as expressões citoplasmática de Efrina-A1 e nuclear de Eph-A1 (*r=-0,762; p*=0,028*)*. Nos CME, identificou-se correlação negativa entre as expressões citoplasmática de Eph-A1 e nuclear de Efrina-A1 (*r=-0,807; p=0,015*) (**Tabela 4**).

Discussão

A interação de Efrina e Eph participa da geração de vias de sinalizações intracelulares bidirecionais que podem produzir ou regular mecanismos sobre a célula que expressa Efrina e a que expressa Eph [14,18,19]. Essa interação relaciona-se com processos celulares normais, como morfologia, proliferação, migração e sobrevivência celular [20,21].

No entanto, de forma patológica, a expressão de Efrina-A1, Eph-A1 e Eph-A2 têm sido relacionadas com a iniciação e progressão de tumores, uma vez que parecem ser responsáveis por promover angiogênese. Evidências citam o Eph-A2 como

regulador de iniciação tumoral, neoangiogênese e metástase em cânceres de origem epitelial e mesenquimal [14, 22, 23].

No presente estudo a imunoexpressão citoplasmática e nuclear da Efrina-A1, Eph-A1 e Eph-A2 em AP e CME glandulares salivares foi marcante em geral, na maioria da amostra, porém com destaque para maiores percentuais de imunopositividade citoplasmática da Efrina-A1 e baixos percentuais de imunopositividade nuclear, sugerindo que a imunoexpressão dessas proteínas pode estar envolvida na patogenia de AP e CME.

O perfil de imunoexpressão citoplasmática da Efrina-A1 na amostra deste estudo assemelha-se com achados anteriores [24] onde foi avaliada a imunoexpressão de Efrina-A1 e Eph-A2 em Carcinoma Adenoide Cístico (CAC). Os achados desta pesquisa corroboram os de Shao *et al.* [24], ao observarem alta imunoexpressão membranar/citoplasmática da Efrina-A1, sugerindo que essa expressão se relacionava com a atividade angiogênica, uma vez que a imunoexpressão de Efrina-A1 se correlacionou significativamente com a MDV ($p<0,01$).

Nesse contexto, embora não tenha sido feita análise das possíveis atividades celulares/tumorais na amostra deste estudo, pode-se inferir que essa proteína esteja envolvida na patogenia de AP e CME, uma vez que, segundo Rud *et al*. [25] é importante relatar que a expressão citoplasmática e ou membranar de Efrina-A1 sugerem papeis importantes na progressão tumoral, porém as funções dependem de fatores como o tipo celular que a expressa e/ou o receptor com o qual está interagindo, quer seja, o seu receptor cognato, o Eph-A1 ou alternativo, Eph-A2.

Considerando a expressão nuclear de Efrina-A1, os baixos percentuais observados neste estudo e a ausência de relatos de expressão dessa proteína na literatura, podem levar a inferir a pouca ou nenhuma participação desse padrão de expressão nas neoplasias analisadas.

É conhecido que os Eph são usualmente expressos na superfície celular onde tipicamente exercem seu papel recebendo e/ou transmitindo sinais vindos do microambiente externo para o meio intracelular [26-28]. Eles formam a maior família de receptores tirosina quinase (RTKs), considerados reguladores da morfogênese da ramificação de glândulas mamárias [29].

Zhou *et al.* [30] avaliaram a imunoexpressão de Eph-A2 em células de câncer de mama, nas quais observaram altos níveis de expressão citoplasmática desse receptor, sugerindo que Eph-A2 esteja envolvido na etiopatogenia do câncer de mama. Diante do

antes exposto e considerando que a mama constitui um tecido glandular e APLICANDO esses fatos aos tecidos glandulares salivares, pode-se sugerir que, a superexpressão dessa proteína possa participar de eventos moleculares relacionados à tumorigênese dessas lesões.

Nesse contexto, é importante destacar que a imunoexpressão de Eph-A2 já foi relatada em tecido glandular salivar normal com baixa imunoexpressão dessa proteína em citoplasma [24], pelo qual podemos inferir que nesses tecidos o Eph-A2 está expresso de forma constitutiva, passando a ter alta imunoexpressão em processos neoplásicos [24,31].

Os dados do nosso estudo, com a maioria da amostra exibindo altos percentuais de expressão citoplasmática/membranar, principalmente nos AP, corroboram os achados da literatura [32], podendo inferir-se que a expressão citoplasmática/membranar de Eph-A1 na amostra deste estudo sugere seu envolvimento em eventos relacionados à etiopatogenia dos AP e CME, provavelmente na histodiferenciação e sobrevivência celulares no AP e nas características etiopatiogênicas tumorais do CME, incluindo desenvolvimento e propagação do fenótipo malignos e outras atividades celulares e do microambiente tumoral.

Quanto à imunoexpressão nuclear dos Eph, relata-se que em alguns contextos celulares (particularmente em células transformadas), esses receptores podem ser expressos no núcleo celular, através da translocação nuclear [26]. Considerando essa informação, salienta-se que, embora o Eph-A1 tenha sido expresso nas duas lesões analisadas, houve destaque para a expressão nuclear em todos os CME e em 87,5% dos AP, sugerindo a ocorrência de translocação nuclear desse receptor, achados que corroboram as informações da literatura [26].

Considerando esse fato, relata-se que a localização nuclear é aberrante para os Eph, podendo ser associada à transformação neoplásica, influenciando no aparecimento e progressão do fenótipo transformado mediante o aumento da ocorrência de eventos pro-tumorigênicos como incremento da taxa de proliferação celular, atividade angiogênica considerável, invasão e potencial metastático celulares, fatores que repercutem no comportamento biológico mais agressivo de algumas neoplasias malignas [26].

Além disso, LaCombe *et al*. [26] sugerem que a localização do receptor Eph-A1 pode sofrer modificações na topografia celular, quer seja em membrana/citoplasma ou núcleo celular e que essas localizações diferentes podem depender da fase do ciclo

celular em que a célula se encontra durante seu crescimento e proliferação. Face ao exposto, pode-se inferir que o perfil de imunoexpressão desse receptor na amostra avaliada, em membrana/citoplasma e núcleo, pode estar relacionado às diferentes fases do ciclo celular das células neoplásicas, influenciando nas atividades necessárias para que a célula tenha os requerimentos necessários para sua proliferação e, consequentemente, no comportamento biológico das lesões.

Importante destacar que estudos sugerem que a translocação nuclear do Eph-A1, possa ocorrer na condição integralmente completa desse receptor, ou na sua forma clivada, tendo ações opostas, quer seja pro-tumoral ou antitumoral, respectivamente [30], porém ainda se desconhece através de quais mecanismos essa translocação ocorre e quais seriam os eventos específicos que regulam na atividade pró e antitumoral.

Quanto à imunoexpressão de Eph-A2, Fukai *et al.* [31] ao avaliarem a imunoexpressão desse receptor e da Efrina-A1, visando informações sobre seus possíveis envolvimentos com a transição epitelial-mesenquimal (EMT) em CAC, encontraram resultados semelhantes ao presente estudo, com imunoexpressão citoplasmática desse receptor. Considerando a imunoexpressão de Eph-A2, os autores sugeriram que sua expressão independe da expressão da Efrina-A1, a qual foi negativa no seu estudo, sugerindo que a expressão de Eph-A2 pode evidenciar sua participação em eventos sinalizadores que ocorrem na EMT e, portanto, exercendo algum papel no potencial invasivo e metastático do CAC.

O antes exposto, e os altos percentuais de imunoexpressão citoplasmática de Eph-A2 em toda a amostra, sem diferença estatisticamente significativa entre as lesões, porém, descritivamente, com maiores medianas de expressão citoplasmática desse receptor em CME, poderiam indicar que o Eph-A2 pode exercer alguns papeis importantes na patogenia dos AP e CME, principalmente nessa última lesão, com provável envolvimento em eventos que promovem a instalação e/ou progressão do fenótipo maligno, como angiogênese ou crescimento tumoral.

A expressão nuclear de Eph-A2, principalmente em AP, sugere a ocorrência de translocação nuclear desse receptor e sua participação na regulação de mecanismos patológicos relacionados, provavelmente, à proliferação celular e manutenção da atividade proliferativa celular e suas interações com outras células epiteliais e do microambiente tumoral, visando o crescimento e/ou expansão dos AP.

Por outro lado, embora toda a amostra de CME não tenha apresentado imunoexpressão nuclear para Eph-A2, sugere a translocação nuclear desse receptor e

provavelmente, seu envolvimento na regulação de eventos celulares diferentes daqueles que esse receptor regula na sua localização habitual na superfície celular.

No entanto, a ausência de diferenças significativas da imunoexpressão nuclear de Eph-A2, em relação aos parâmetros histomorfológicos das lesões analisadas, evidencia-se que algum papel esse receptor execute no microambiente tumoral, que possa repercutir no comportamento biológico de AP e CME, no entanto, o tamanho reduzido da amostra, pode ter influenciado os resultados estatísticos, podem não representar a realidade da correlação desse receptor em relação aos parâmetros histopatológicos da amostra.

Considerando que uma análise imunoistoquímica limita as condições de obter maiores informações que possa auxiliar no entendimento dos mecanismos associados à expressão das proteínas, os achados deste estudo tornam-se relevantes, por ser o primeiro estudo em relatar o perfil de expressão membrana/citoplasmática e nuclear de Efrina-a1, Eph-A1 e Eph-A2 em AP e CME, indicando que essas proteínas parecem exercer alguma ou algumas funções na etiopatogenia dessas lesões.

Porém, maiores estudos, principalmente de biologia molecular precisam ser planejados e executados, ampliando a amostra, no sentido de obter maiores e mais robustas evidências científicas sobre as ações que estariam associadas ao perfil de imunoexpressão dessas proteínas, e quais os mecanismos envolvidos no desenvolvimento dessas ações.

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Fig. 2 Imunoexpressão citoplasmática e nuclear de (**A**) Efrina-A1, (**B**) Eph-A1 e (**C**) Eph-A2 em AP.

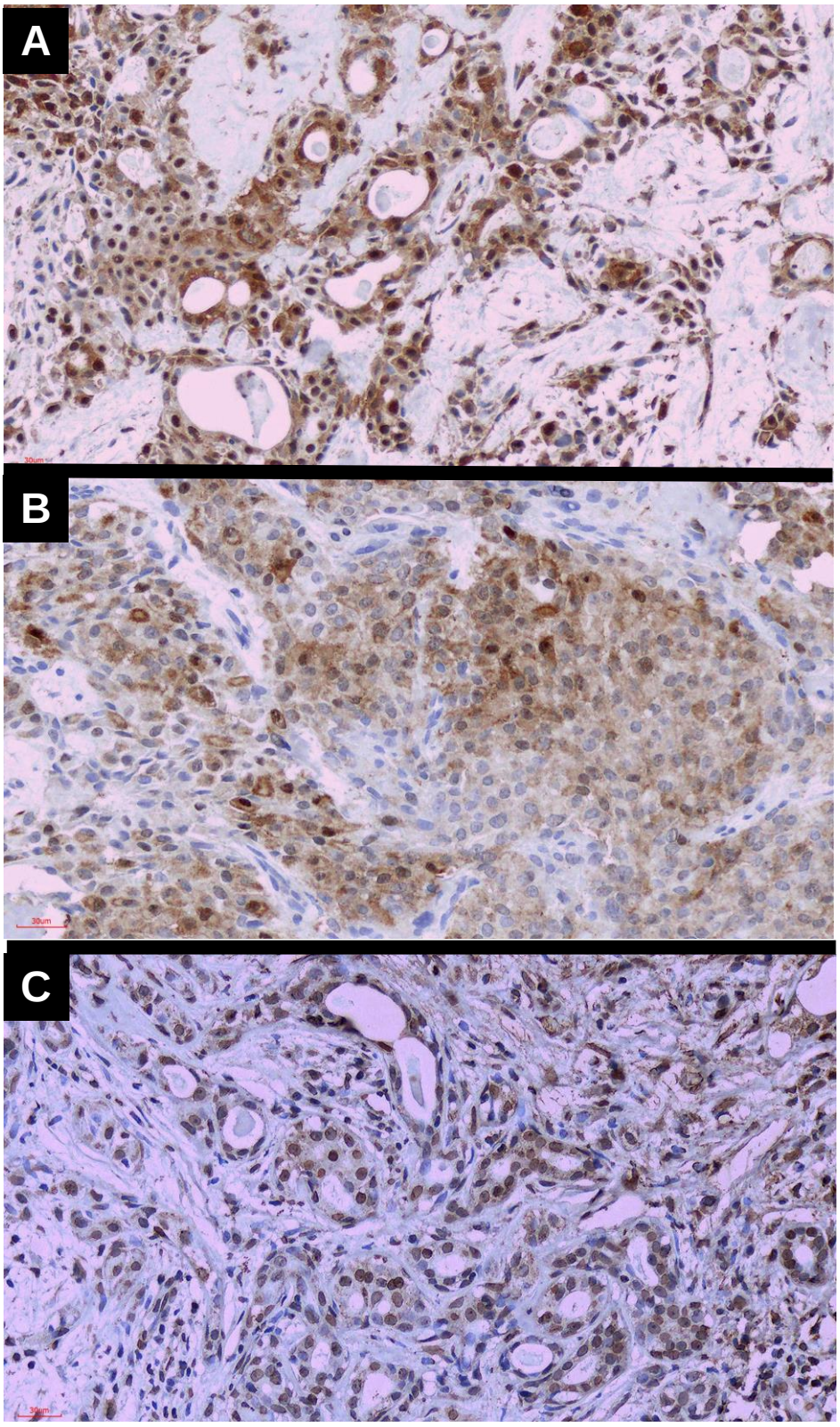


Fig. 3 Imunoexpressão citoplasmática e nuclear de (**D**) Efrina-A1, (**E**) Eph-A1 e (**F**) Eph-A2 em CME.

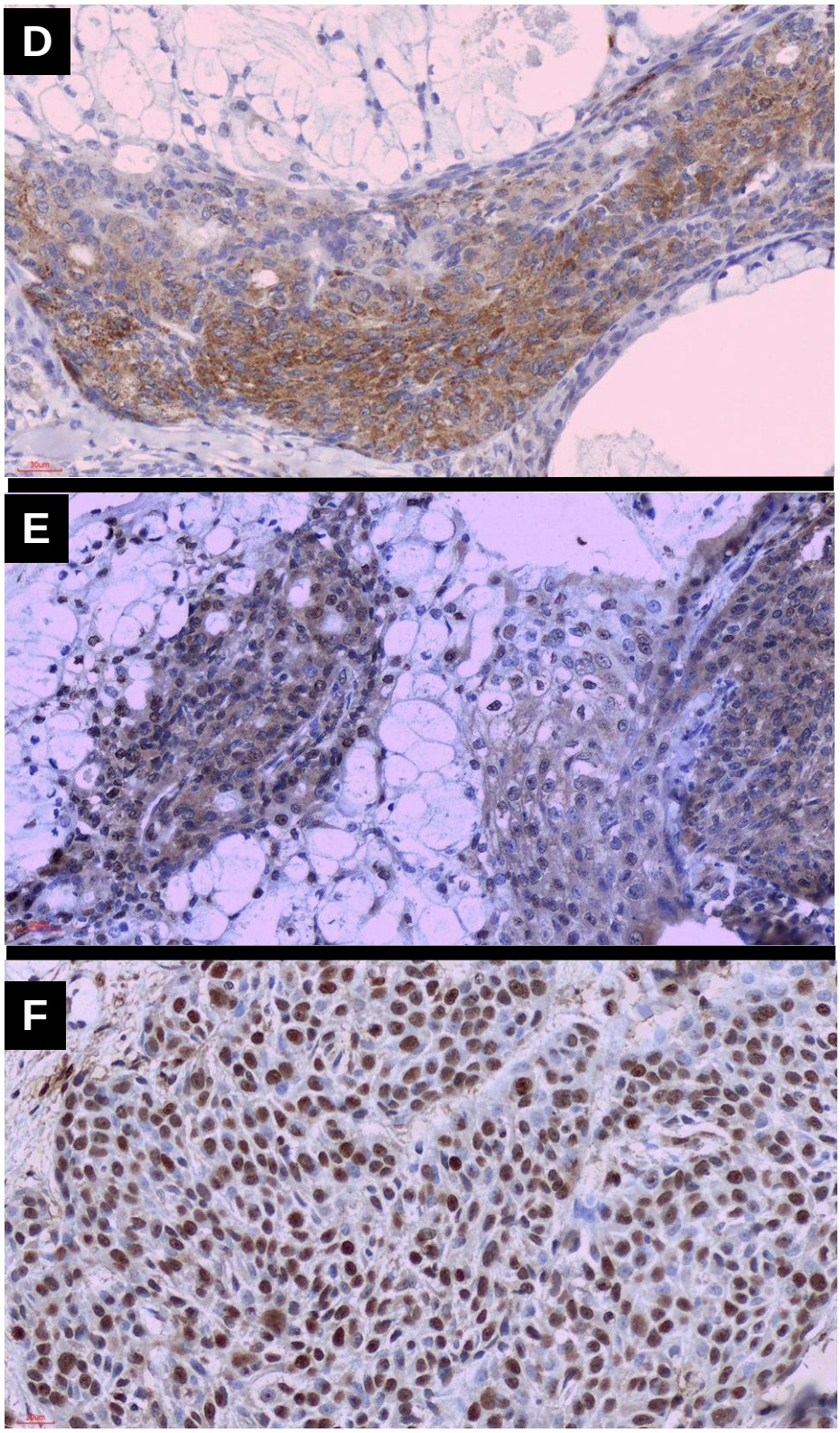


Fig. 4 Box plot ilustrando os percentuais de positividade citoplasmática para Efrina-A1, Eph-A1 e Eph-A2 em AP e CME

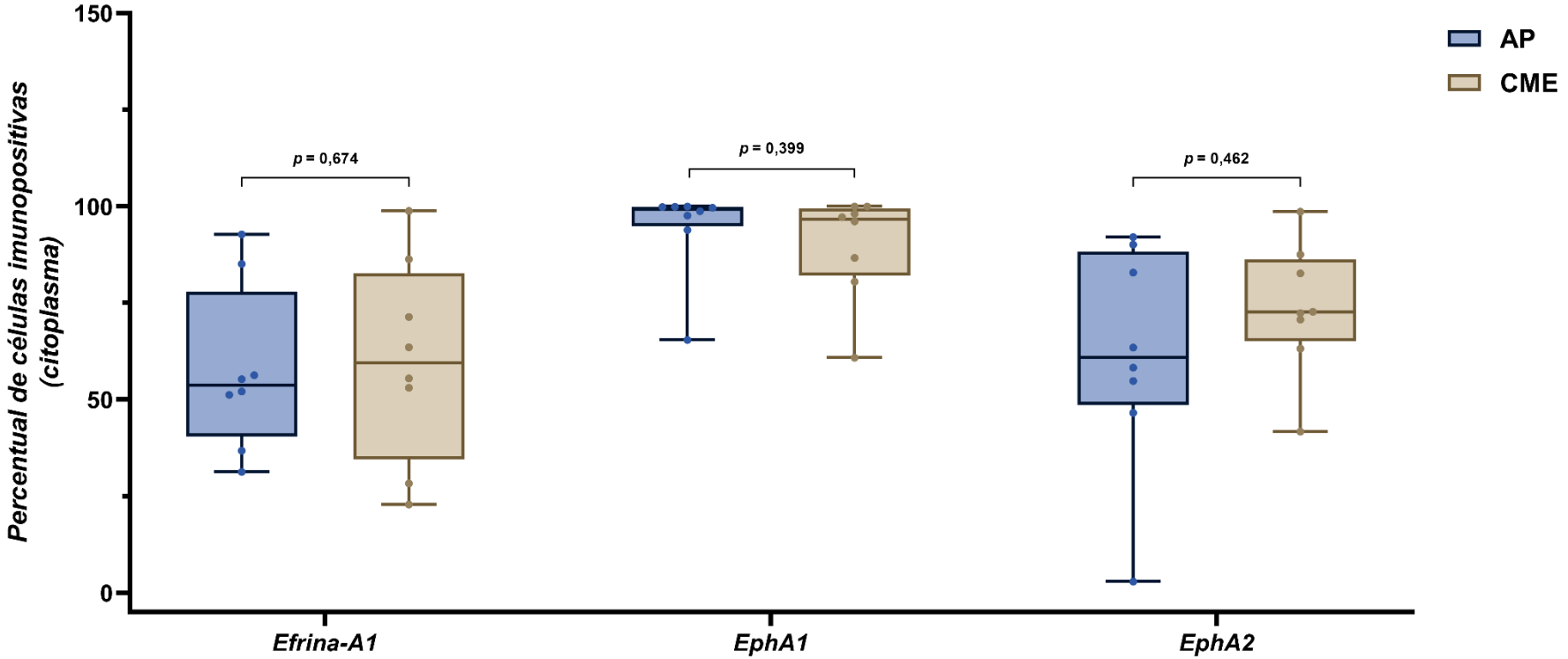


Fig. 5 Box plot ilustrando os percentuais de positividade nuclear para Efrina-A1, Eph-A1 e Eph-A2 em AP e CME.

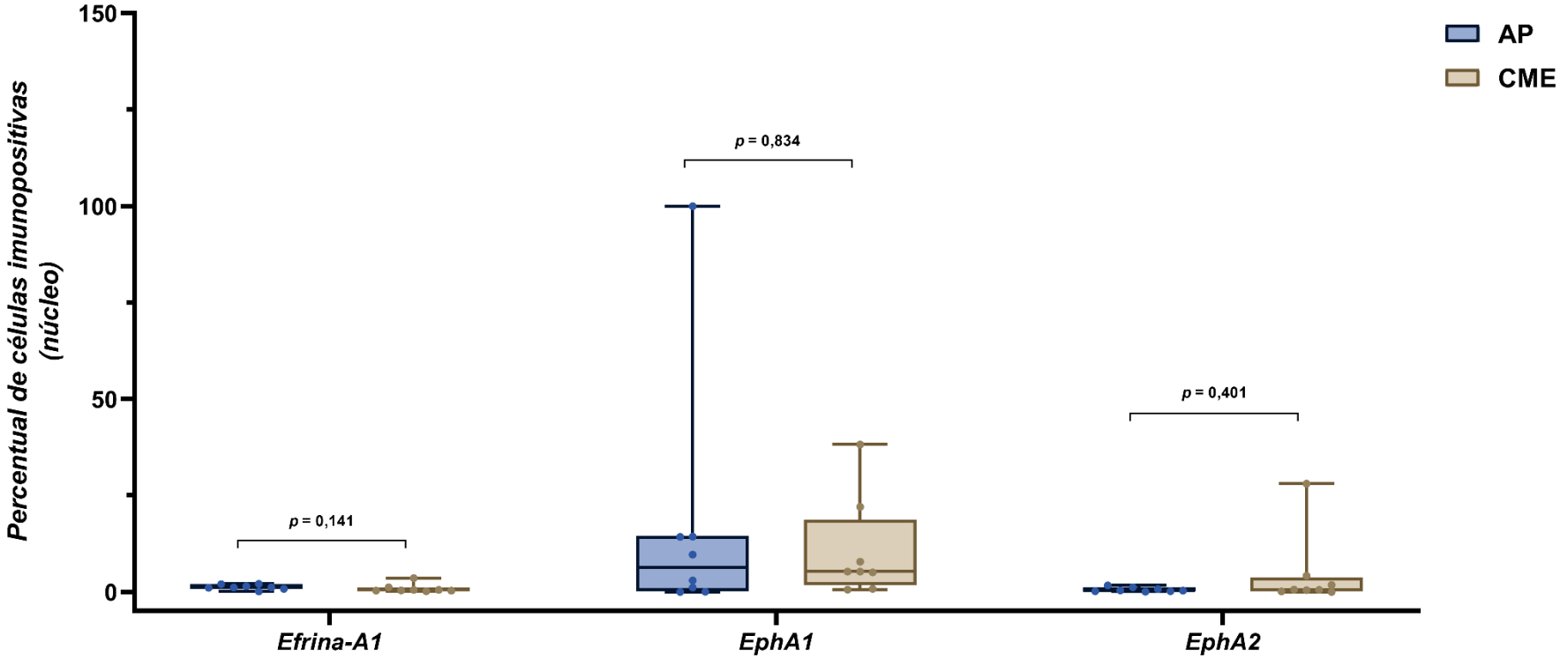


Fig. 6 Box plot ilustrando os percentuais de positividade citoplasmatica para Efrina-A1, Eph-A1 e Eph-A2 em AP, segundo classificação histomorfológica.

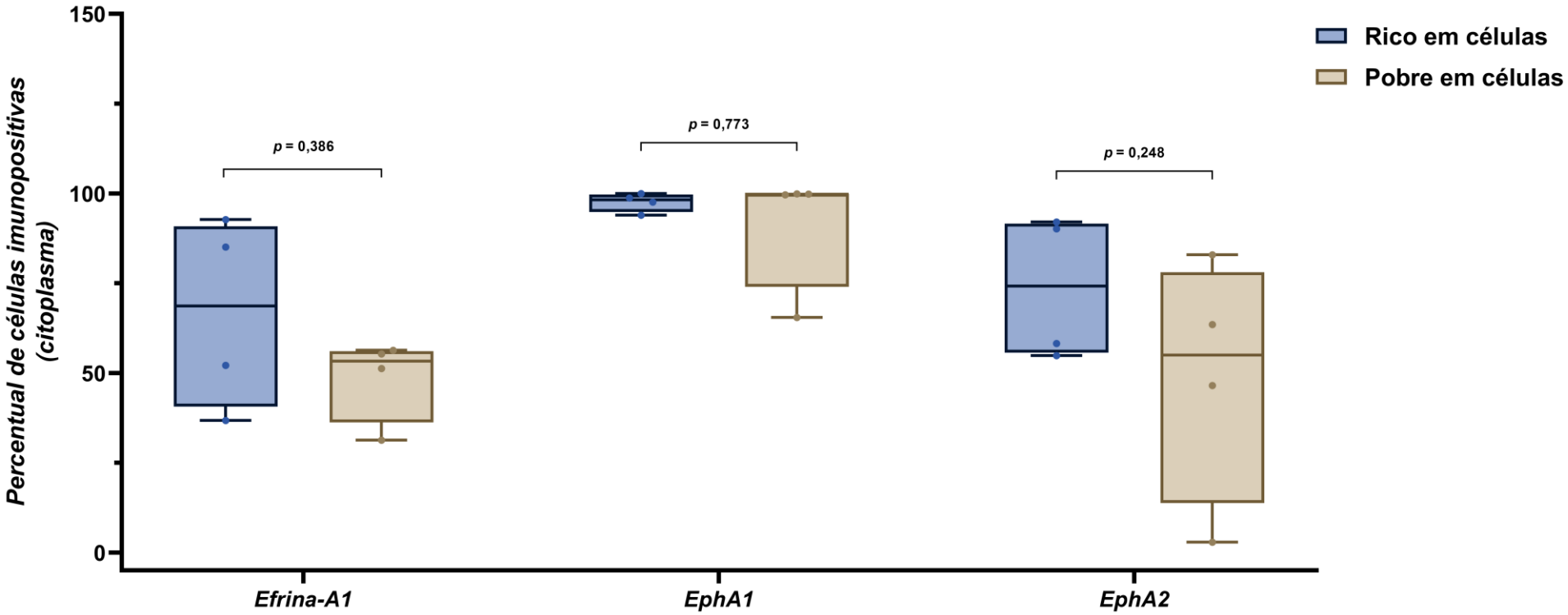


Fig. 7 Box plot ilustrando os percentuais de positividade nuclear para Efrina-A1, Eph-A1 e Eph-A2 em AP, segundo classificação histomorfológica.

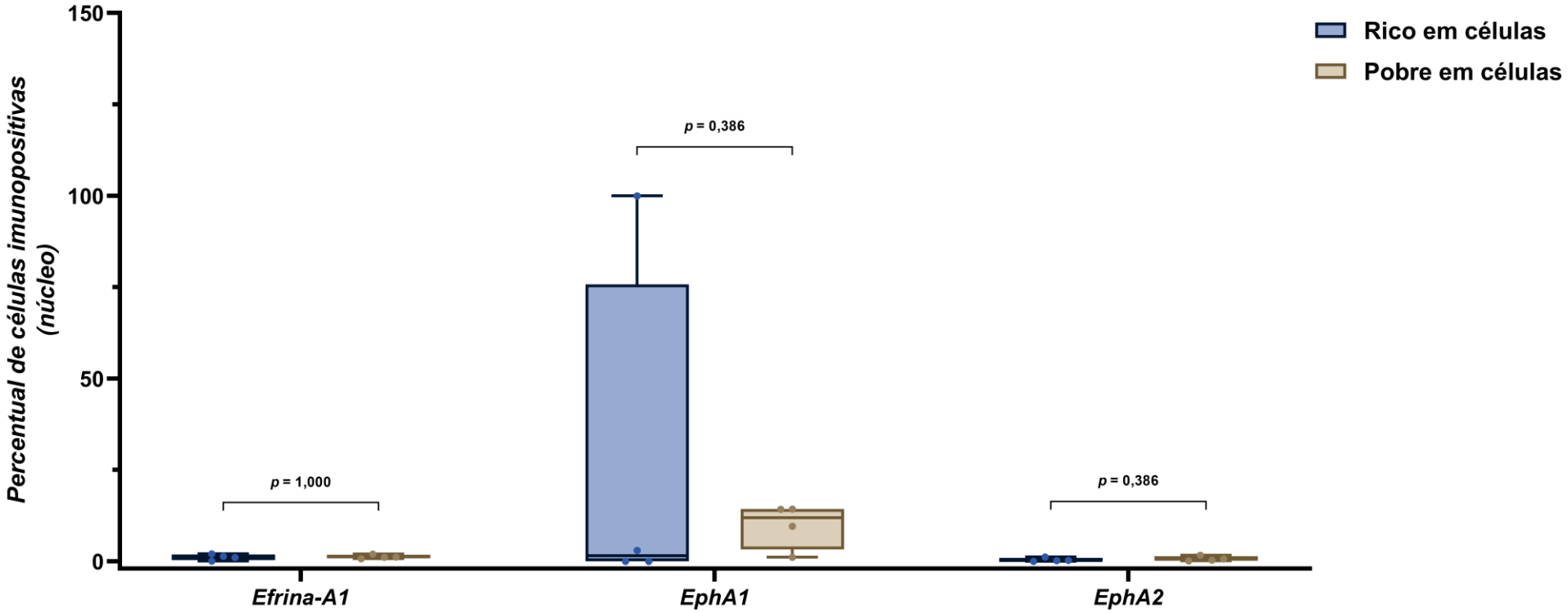


Fig. 8 Box plot ilustrando os percentuais de positividade citoplasmática para Efrina-A1, Eph-A1 e Eph-A2 em CME, segundo classificação histomorfológica.

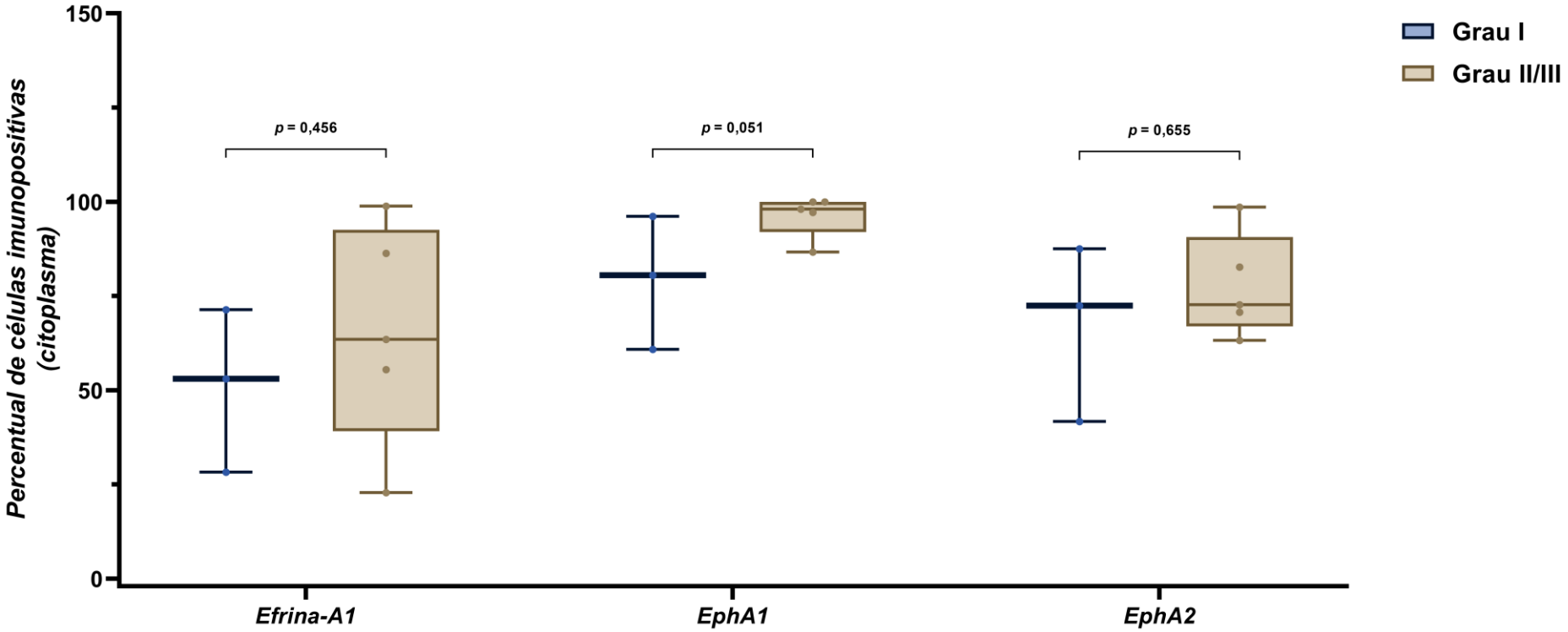
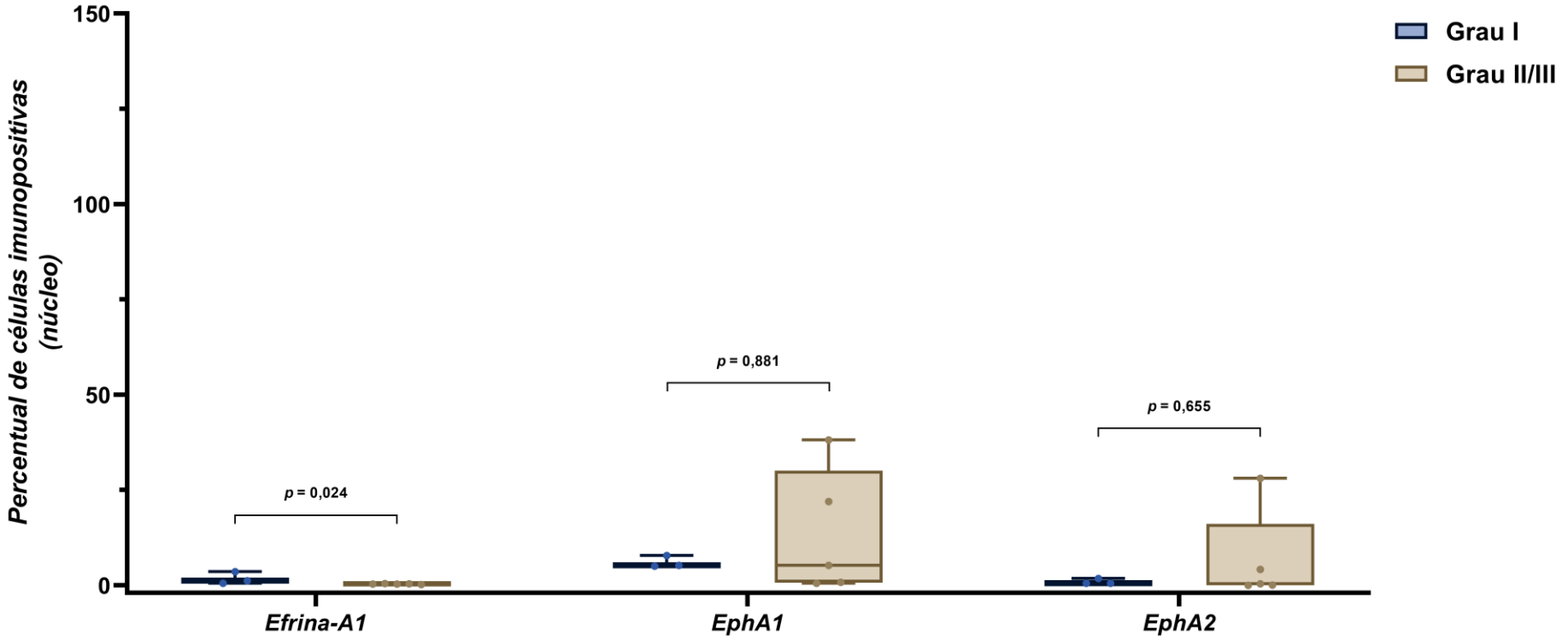


Fig. 9 Box plot ilustrando os percentuais de positividade nuclear para Efrina-A1, Eph-A1 e Eph-A2 em CME, segundo classificação histomorfológica.



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Tabelas

Tabela 1. Especificidade, referência, fabricante, diluição, recuperação antigênica e incubação dos anticorpos primários utilizados no estudo.

Especificidade	Referência	Fabricante	Diluição	Recuperação Antigênica	Incubação
Efrina-A1	MA5-29231	Invitrogen	1:600	Citrato, pH 6,0 *Steamer*, 90ºC, 30 min	*Overnight*
Eph-A1	PA1-30291	Invitrogen	1:6000	Citrato, pH 6,0 *Steamer*, 90ºC, 30 min	*Overnight*
Eph-A2	PA5-14574	Invitrogen	1:1500	Citrato, pH 6,0 *Steamer*, 90ºC, 30 min	*Overnight*

Tabela 2. Distribuição absoluta e relativa dos casos de adenomas pleomorficos e carcinomas mucoepidermoides de acordo com os parâmetros clinicopatológicos.

Parâmetros Clinicopatológicos	**AP (n=8)**	**CME (n=8)**
Sexo dos Pacientes		
Feminino	6 (75,0%)	4(50,0%)
Masculino	2(25,0%)	4(50,0%)
Idade dos pacientes (média em anos)*		
Média	46,43	43,63
Variação	22-86	19-83
Desvio Padrão	23,29	21,72
Localização da Lesão		
Glândula salivar menor	7(87,5%)	6(75,0%)
Glândula salivar maior	1(12,5%)	2(25,0%)
Subtipo histopatológico dos AP		
Rico em células	4(50,0%)	N/A
Pobre em células	4(50,0%)	N/A
Grau histopatológico dos CME		
Grau I	N/A	3(37,5%)
Grau II	N/A	3(37,5%)
Grau III	N/A	2(25,0%)

*Sem dados de idade para um caso
N/A= Não se aplica

Tabela 3. Tamanho da amostra, número de casos positivos, mediana, mínimo e máximo dos percentuais de células imunopositivas (citoplasma e núcleo) para Efrina-A1, Eph-A1 e Eph-A2 em relação aos grupos de lesões.

	n	***Casos positivos (%)***	***Mínimo***	***Máximo***	***Mediana***	***DP***	***Valor p***
Efrina A1 Citoplasma							
AP	8	8 (100)	31,28	92,76	53,72	21,37	0,674
CME	8	8 (100)	22,84	98,83	59,50	26,18	
Efrina A1 Núcleo							
AP	8	8 (100)	0,08	2,06	1,18	0,65	0,141
CME	8	8 (100)	0,19	3,58	0,45	1,13	
Efrina A1 - AP Citoplasma							
Pobre em células	4	4 (100)	-	-	-	-	0,386
Rico em células	4	4 (100)	-	-	-	-	
Efrina A1 - AP Núcleo			-	-	-	-	
Pobre em células	4	4 (100)	-	-	-	-	1,000
Rico em células	4	4 (100)					
Efrina A1 - CME Citoplasma			-	-	-	-	
			-	-	-	-	
Grau I	3	3 (100)					0,456
Grau II/III	5	5 (100)	-	-	-	-	
Efrina A1 - CME Núcleo			-	-	-	-	
Grau I	3	3 (100)	-	-	-	-	***0,024***
Grau II/III	5	5 (100)	-	-	-	-	
EpH -A1 Citoplasma							
AP	8	8 100)	65,43	100	99,20	11,88	0,399
CME	8	8 (100)	60,86	100	96,69	13,65	
EpH -A1 Núcleo							
AP	8	7 (87,5)	0,00	100	6,30	33,76	0,834
CME	8	8 (100)	0,56	38,18	5,23	13,01	
EpH A1 - AP Citoplasma							
Pobre em células	4	4 (100)	-	-	-	-	0,773
Rico em células	4	4 (100)	-	-	-	-	
EpH A1 - AP Núcleo							
Pobre em células	4	4 (100)	-	-	-	-	*0,386*
Rico em células	4	4 (100)	-	-	-	-	
EpH A1 - CME Citoplasma							
Grau I	3	3 (100)	-	-	-	-	***0,051***
Grau II/III	5	5 (100)	-	-	-	-	
EpH A1 - CME Núcleo							
Grau I	3	3 (100)	-	-	-	-	*0,881*
Grau II/III	5	5 (100)	-	-	-	-	

EpH A2 Citoplasma							
AP	8	8 (100)	2,85	92,08	60,86	29,11	*0,462*
CME	8	8 (100)	41,72	98,61	72,58	17,05	
EpH -A2 Núcleo							
AP	8	8 (100)	0,04	1,67	0,32	0,57	*0,401*
CME	8	7 (87,5)	0,00	28,08	0,54	9,64	
EpH A2 - AP Citoplasma							
Pobre em células	4	4(100)	-	-	-	-	*0,248*
Rico em células	4	4(100)	-	-	-	-	
EpH A2 - AP Núcleo							
Pobre em células	4	4(100)	-	-	-	-	*0,386*
Rico em células	4	4(100)	-	-	-	-	
EpH A2 - CME Citoplasma							
Grau I	3	3 (100)	-	-	-	-	*0,655*
Grau II/III	5	5 (100)	-	-	-	-	
EpH A2 - CME Núcleo							
Grau I	3	3 (100)	-	-	-	-	*0,655*
Grau II/III	5	5 (100)	-	-	-	-	

Tabela 4. Tamanho da amostra, coeficiente de correlação de Spearman (*r*) e significância estatística (*p*) para as imunoexpressões de Efrina-A1, Eph-A1 e Eph-A2.

Localização/Correlações	AP (n=8)		CME (n=8)	
	r	*p*	*r*	*p*
Citoplasma X Núcleo				
Efrina A1 (citoplasma) x Eph A1 (núcleo)	-0,762	**0,028**	-0,381	0,352
Eph A1 (citoplasma) x Efrina A1 (núcleo)	-0,643	0,086	-0,807	**0,015**

5 CONSIDERAÇÕES FINAIS

Embora a literatura seja escassa na imunoexpressão de efrinas e seus receptores em tecidos glandulares salivares, os achados deste estudo sobre a imunoexpressão de Efrina-A1, Eph-A1 e Eph-A2, sugerem potencial participação dessas proteínas na patogênese de AP e CME.

A maior imunoexpressão citoplasmática de Efrina-A1 em AP e CME, sugere um envolvimento dessa proteína na transmissão de sinais moduladores de eventos moleculares relacionados à tumorigênese glandular salivar.

A imunoexpressão citoplasmática e nuclear de Eph-A1, principalmente nessa última localização nas neoplasias avaliadas, parece corroborar a literatura ao sugerir que a expressão de nuclear sugere a participação desse receptor em vias de sinalização reversas intracelulares para o meio extracelular, provavelmente regulando mecanismos como a proliferação e morfologia celular, eventos adesivos célula-célula/célula-MEC e crescimento tumoral em AP e CME.

Embora não tenha sido observada diferença estatisticamente significativa da expressão citoplasmática e nuclear de Eph-A2 na amostra, seus maiores percentuais de imunopositividade em CME, corroboram a literatura, sugerindo sua maior expressão em neoplasias malignas.

Considerando a alta imunoexpressão citoplasmática das proteínas avaliadas em relação aos parâmetros histomorfológicos dos AP e CME, sugere-se que essas possam exercer algum papel nas vias de sinalização que regulam mecanismos relacionados à patogenia dessas lesões, com destaque para sua imunoexpressão em CME de maior grau de malignidade. Por sua vez, a pouca imunoexpressão nuclear dessas proteínas na amostra analisada, principalmente da Efrina-A1, sugere pouco envolvimento da expressão nuclear dessas proteínas na tumorigênese das neoplasias avaliadas.

Investigações mais amplas se fazem necessárias para melhor compreensão das vias de sinalização modulados por Efrina-A1, Eph-A1, Eph-A2 e outras proteínas envolvidas nessas vias, importantes para os mecanismos moleculares relacionados à tumorigênese glandular salivar, visando subsidiar o conhecimento para o desenvolvimento de alternativas terapêuticas para essas lesões tendo como alvo tais proteínas.

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APÊNDICE A - FICHA UTILIZADA PARA COLETA DE DADOS CLÍNICO-PATOLÓGICOS

CASO	IDADE	SEXO	LOCALIZAÇÃO	LESÃO	CLASSIFICAÇÃO HISTOPATOLÓGICA

Fonte: Elaborado pela autora, 2024.

ANEXO A - PARECER DO COMITÊ DE ÉTICA EM PESQUISA DA UEPB

UNIVERSIDADE ESTADUAL DA PARAÍBA - PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA - UEPB / PRPGP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: IMUNOEXPRESSÃO DE EPHRIN-A1 E EPH-A1, EPH-A2 EM ADENOMA PLEOMÓRFICO, CARCINOMA MUCOEPIDERMOIDE E CARCINOMA ADENOIDE CÍSTICO GLANDULARES SALIVARES.

Pesquisador: MANUEL ANTONIO GORDÓN NÚÑEZ

Área Temática:

Versão: 1

CAAE: 79213523.5.0000.5187

Instituição Proponente: Universidade Estadual da Paraíba - UEPB

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 6.798.135

Apresentação do Projeto:

Estudo transversal com caráter descritivo probabilístico correlacional da análise qualitativa e semiquantitativa da expressão imunoistoquímica Ephrin-A1 e dos receptores de efrinas Eph-A1, Eph-A2 em relação aos parâmetros histomorfológicos de uma série de adenomas pleomórficos, carcinomas mucoepidermoides e carcinomas adenoides císticos glandulares salivares e em tecido glandular salivar normal. O estudo será realizado com uma amostra não probabilística por conveniência, constituída por 80 espécimes fixados em formol a 10% e incluídos em blocos de parafina, correspondendo a 20 casos de cada lesão e 20 amostras de tecido glandular salivar normal.

Objetivo da Pesquisa:

Geral:

Avaliar a imunoexpressão da Ephrin-A1 e dos receptores Eph-A1 e Eph-A2 em relação a parâmetros histomorfológicos de uma série de AP, CME e CAC glandulares salivares.

Específicos:

Analisar o perfil clínico (sexo, idade e localização anatômica das lesões) de uma amostra de AP, CME e CAC;

Caracterizar o perfil histomorfológicos da amostra de AP, CME e CAC;

Endereço: Av. das Baraúnas, 351- Campus Universitário
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Telefone: (83)3315-3373 **Fax:** (83)3315-3373 **E-mail:** cep@setor.uepb.edu.br

Determinar os percentuais de células imunopositivas para Ephrin-A1 e dos receptores de efrinas Eph-A1, Eph-A2 no componente epitelial neoplásico e em células do componente estromal de AP, CME, CAC, além de no parênquima e mesênquima de tecido glandular normal;
Estabelecer correlações entre as imunoexpressões da Ephrin-A1 e dos receptores EphA1, Eph-A2 em relação aos parâmetros histomorfológicos dos AP (Segundo os critérios de Seifert et al. (1976) e Viana et al. (2013)), dos CME (Segundo os critérios de Brandwein et al. (2001)) e dos CAC (de acordo com os critérios de Ellis e Auclair (1996) e Weert et al. (2015).

Avaliação dos Riscos e Benefícios:

Por se tratar de uma pesquisa que envolve a coleta de dados em fichas clínicas e o uso de lâminas histológicas e espécimes biológicos mantidos em arquivo, os riscos potenciais implicados na participação neste estudo são mínimos. Nesse contexto, deve-se considerar a possibilidade de exposição dos dados pessoais dos pacientes. Esse risco será minimizado pelo uso de codificações que garantirão a privacidade e confidencialidade dos dados, assegurando que não sejam utilizadas quaisquer indicações que possam identificar os participantes da pesquisa. Os benefícios podem superar os possíveis riscos, ao contribuir para uma melhor compreensão dos mecanismos relacionados à patogenia de AP, CME, CAC.

Comentários e Considerações sobre a Pesquisa:

A proposta do projeto é relevante academicamente, uma vez que analisa o papel das Efrinas e seus receptores como mediadores importantes em processos patogênicos de lesões neoplásicas de diversas origens. Além disso, este esclarecimento contribui ao conhecimento da patogênese de tumores glandulares salivares.

Considerações sobre os Termos de apresentação obrigatória:

Folha de rosto: anexada;
Autorização Institucional: Anexada
Termo de Compromisso do Pesquisador Responsável: anexado
Termo de concordância com a pesquisa: anexado
TCLE: justificado a ausência.

Recomendações:

O projeto é relevante, apresenta importância acadêmica e social. A metodologia está clara e

adequada ao que se propõe. Todos os termos foram anexados.

Conclusões ou Pendências e Lista de Inadequações:

O projeto apresenta todos os documentos necessários, desta forma está aprovado salvo melhor entendimento.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_2259840.pdf	19/04/2024 22:53:36		Aceito
Outros	TERMO_AUT_INST_COLETA_DADOS_EM_ARQUIVOS.pdf	19/04/2024 22:52:38	MANUEL ANTONIO GORDÓN NÚÑEZ	Aceito
Declaração de Instituição e Infraestrutura	TERMO_DE_AUTORIZACAO_INSTITUCIONAL_TAI.pdf	19/04/2024 22:46:14	MANUEL ANTONIO GORDÓN NÚÑEZ	Aceito
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_2259840.pdf	05/12/2023 09:53:33		Aceito
Folha de Rosto	FOLHA_ROSTO_GORDONNUNEZ.pdf	05/12/2023 09:52:19	MANUEL ANTONIO GORDÓN NÚÑEZ	Aceito
Outros	TERMO_COMPROMISSO.pdf	05/12/2023 01:35:57	MANUEL ANTONIO GORDÓN NÚÑEZ	Aceito
Declaração de Pesquisadores	DECLARACAO_PESQUISADORES.pdf	05/12/2023 01:33:30	MANUEL ANTONIO GORDÓN NÚÑEZ	Aceito
Cronograma	CRONOGRAMA.pdf	05/12/2023 01:26:44	MANUEL ANTONIO GORDÓN NÚÑEZ	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	DISPENSA_TCLE.pdf	05/12/2023 01:25:12	MANUEL ANTONIO GORDÓN NÚÑEZ	Aceito
Declaração de concordância	DECLARACAO_CONCORDANCIA.pdf	05/12/2023 01:23:45	MANUEL ANTONIO GORDÓN NÚÑEZ	Aceito
Projeto Detalhado / Brochura Investigador	GORDONNUNEZ_EPHRIN_EPH_A1_A2_TUMORES_GLANDULARES.pdf	05/12/2023 01:22:41	MANUEL ANTONIO GORDÓN NÚÑEZ	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

UNIVERSIDADE ESTADUAL DA PARAÍBA - PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA - UEPB / PRPGP

Continuação do Parecer: 6.798.135

CAMPINA GRANDE, 30 de Abril de 2024

Assinado por:
Gabriela Maria Cavalcanti Costa
(Coordenador(a))

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ANEXO B – NORMAS PARA SUBMISSÃO DE ARTIGOS AO PERIÓDICO *HEAD AND NECK PATHOLOGY*

Instructions for Authors – Head and Neck Pathology

General Information

We only accept manuscript submission via our online manuscript submission system. Please follow the hyperlink "Submit manuscript" on this page and upload all of your manuscript files following the instructions given on screen.

Submission of a manuscript implies:

- that the work described has not been published before;
- that it is not under consideration for publication anywhere else;
- that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out

The publisher will not be held legally responsible should there be any claims for compensation

All manuscripts are evaluated via iThenticate for signs of any potential plagiarism. For information on the concept of self-plagiarism, visit the following online http://www.ithenticate.com/resources/papers/ethics-of-self-plagiarism#

ORCID ID

This publication requires that the corresponding author provides his/her ORCiD ID before proceeding with submission. For more information about this journal's ORCiD policy, please visit the ORCID FAQ

Types of Papers

Research

This section is the major emphasis of the journal, with a focus on head and neck pathology topics, and devoted to scientific reporting of results of original clinicopathologic research.

Review

Reviews present, contrast, and evaluate information from previously published research to address a specific question or topic related to the aims and scope of the journal.

Correspondence

Correspondences provide a format for discussions of matters associated with the publication. Correspondences are published at the discretion of the Editor and those presenting original material are subject to peer review. Correspondences are written without subheadings and have a maximum length of 1,000 words and 5-10 pertinent references.

Case Report

In general, case reports are not encouraged and the editors strictly limit the number of case reports per issue. For the Editors to consider a case report it must contain all pertinent clinical, imaging, pathology, and ancillary information on a particularly novel or exceedingly rare entity, set within the context of a thorough and investigative review of the literature.

Submissions of single case reports or limited series of common entities will be rejected and returned to the author.

Comment

These can portray your view on a topical or controversial subject in the field, as well as anything of likely interest to the readers, such as policy debates and community announcements. Please note supplementary information will not be accepted as part of the submission.

No abstract required; Main body of text (excluding references, tables/figures, figure legends) not to exceed 1,500 words; Max 2 table or figure; Max 15 references.

Perspective

A scholarly overview and discussion of the primary research literature that does not meet the criteria for a review article - either because the scope is too narrow, or a primary purpose of the piece is to advocate a controversial position or a speculative hypothesis, or to discuss work primarily from one or a few research groups. They are intended to stimulate discussion and consideration of new approaches to investigation and understanding of a field.

Unstructured abstract max. 250 words; Max of 4 tables or figures; Max 25 references.

Image

Submissions to this section illustrate classic examples of common entities with clinical photographs, radiographic imaging, intraoperative photographs, gross pathology images, cytology, classic histology, and any pertinent supporting studies (histochemical, immunohistochemical, immunofluorescence, molecular, genetic and/or ultrastructural). The text includes a brief summary of clinical and histopathologic findings and potential differential diagnostic considerations clinically or pathologically. On occasion, we may accept a classic presentation of a very rare entity if these criteria are fulfilled.

The text should be approximately 250-500 words, appropriate legends for the figures shown and 3-5 pertinent references.

Meeting Report

The proceedings of the North American Society of Head and Neck Pathology (part of the United States and Canadian Academy of Pathology) annual meeting are published annually in the spring issue of the journal. These invited manuscripts must be submitted under the Meeting Report article type. The manuscript requires text to include an abstract of no more than 250 words, an introduction, discussion, and conclusion followed by tables, references, and figure legends. Up to 8 color illustrations may be included and no more than 30 pertinent and current references. Submission guidelines for images and tables are as noted below.

Instructions for complying with Ethical Standards

Please replace the first sentence with: Please include a separate section called “Compliance with Ethical Standards” within your manuscript.

Please always include each type of statement as well as the exact statement order and wording as described below for the five disclosure statements. Please always add all seven statements, even if one or more are not applicable; if any are not relevant to the content please state that this declaration is “not applicable”.

1. Funding (must be included): “This study was funded by X (grant number X)”

In this paragraph all sources of funding for the research reported should be declared. The role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared.

If not applicable, please add the following sentence:
"This study was not supported by any funding."

2. Conflict of Interest (must be included): "Author A has received research grants from Company A. Author B has received a speaker honorarium from Company X and owns stock in Company Y. Author C is a member of committee Z."
If not applicable, please add the following sentence:
"The authors declare that they have no conflict of interest."
In this paragraph all financial and non-financial competing interests must be declared in this section. If you are unsure whether you or any of your co-authors have a competing interest please contact the editorial office.

3. Ethical approval (must be included):
- **For studies involving patients**, please add the following sentence:
"All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."
- **For retrospective studies**, please add the following sentence:
"For this type of study formal consent is not required."
- **For studies with animals**, the following statement should be included in the text: "All applicable international, national, and/or institutional guidelines for the care and use of animals were followed."
If applicable (where such a committee exists): "All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted."
- **If articles do not contain studies with human participants or animals** by any of the authors, please select one of the following statements:
"This article does not contain any studies with human participants performed by any of the authors."
"This article does not contain any studies with animals performed by any of the authors."
"This article does not contain any studies with human participants or animals performed by any of the authors."
A statement on the **Institutional Review Board (IRB) approval** of your study must be also included in this section.

4. Consent to Participate (must be included): "Informed consent was obtained from all individual participants included in the study."
For all research involving human subjects, informed consent to participate in the study should be obtained from participants (or their parent or legal guardian in the case of children under 16).
If not applicable, please add one of the following sentences:
"For this type of study informed consent is not required."
"This study has obtained IRB approval from (indicate the relevant board) and the need for informed consent was waived."

5. Consent for publication (must be included): “Consent for publication was obtained for every individual person’s data included in the study.”
If your manuscript contains any individual person’s data in any form, consent to publish must be obtained from that person, or in the case of children, their parent or legal guardian. All presentations of case reports must have consent to publish.
If not applicable, please add the following sentence:
“For this type of study consent for publication is not required.”

6. Availability of data and materials (must be included):
This statement should explain how to access data supporting the results and analysis in the article, including links/citations to publicly archived datasets analysed or generated durinh the study.
If it is not possible to share research data publicly, for instance when individual privacy could be compromised, this statement should describe how data can be accessed and any conditions for reuse.
When creating a data availability statement, authors are encouraged to consider the minimal dataset that would be necessary to interpret, replicate and build upon the findings reported in the article.

7. Code Availability (must be included):
If any software application or custom code has been used in the preparation of your article, please describe this here.

Editorial procedure
Peer Review Policy
Peer review is the system used to assess the quality of a manuscript before it is published. Independent researchers in the relevant research area assess submitted manuscripts for originality, validity and significance to help Editors determine whether the manuscript should be published in their journal.

Head and Neck Pathology operates a single-blind peer-review system, where the reviewers are aware of the names and affiliations of the authors, but the reviewer reports provided to authors are anonymous. The benefit of single-blind peer review is that it is the traditional model of peer review that many reviewers are comfortable with, and it facilitates a dispassionate critique of a manuscript.

Submitted manuscripts will generally be reviewed by two to three experts who will be asked to evaluate whether the manuscript is scientifically sound and coherent, whether it duplicates already published work, and whether or not the manuscript is sufficiently clear for publication. Reviewers will also be asked to indicate how interesting and significant the research is. The Editors will reach a decision based on these reports and, where necessary, they will consult with members of the Editorial Board. Where an Editor is on the author list or has any other competing interest regarding a specific manuscript, another member of the Editorial Board will be assigned to assume responsibility for overseeing peer review.

Any commissioned content, or any content included in special issues/collections, undergoes the same peer review process as a standard submission. The papers will be assessed by the journal's editorial team and at least 2 external peer reviewers. If a Guest Editor is leading the special issue/collection they will be required to have at least 2 independent peer reviewers assess each submission and the final decision will remain with the Editors-in-Chief.

Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

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Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink "Submit manuscript" and upload all of your manuscript files following the instructions given on the screen.

Source Files

Please ensure you provide all relevant editable source files at every submission and revision. Failing to submit a complete set of editable source files will result in your article not being considered for review. For your manuscript text please always submit in common word processing formats such as .docx or LaTeX.

Submitting Declarations

Please note that Author Contribution information and Competing Interest information must be provided at submission interface. Only the information submitted via the interface will be used in the final published version. Please make sure that if you are an editorial board member and also a listed author that you also declare this information in the Competing Interest section of the interface.

Please see the relevant sections in the submission guidelines for further information on these statements as well as possible other mandatory statements.

The following materials are required to be included with the online submission, or the manuscript will be rejected and returned to the author:

- Cover letter that describes the significance and novelty of the work and includes the statements "All authors have read and approved the manuscript" and "This manuscript is not under consideration elsewhere," as well as any additional information that may impact the review process.
- Corresponding author's complete contact information to include address, phone number, and e-mail address.
- Structured Abstract of no more than 350 words (for Original, Review, Meetings Proceedings and Sine Qua Non articles).
- Original Papers and Case Reports should have an Abstract, Introduction, Materials and Methods, Results, and Discussion, with supplementary materials if necessary.
- All Tables submitted at the end of the paper, not embedded within it, listed in order cited in the manuscript.
- The anatomic site(s) best suited for the original article (part of Classifications section).

- Full names and affiliations of all authors, complete with first and middle names or initials, and e-mail addresses for each.
- Two reviewer suggestions that include names and e-mail addresses.
- Indication of whether or not the paper was invited.
- Please refer to the Instructions for Complying with Ethical Standards and the Compliance with Ethical Standards Sections for additional important requirements that must appear after the Title Page.

Stats Declaration
For all submissions, within the Cover Letter authors must include one or the other of the following statements:
Statistical methods employed are performed by an experienced person, with authorship (acknowledgement) on the manuscript, and the contributor(s) specified.
Or
No statistical analysis was performed in the preparation of this manuscript.

Title Page
Please make sure your title page contains the following information.

Title
The title should be concise and informative.

Author information
- The name(s) of the author(s)
- The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country
- A clear indication and an active e-mail address of the corresponding author
- If available, the 16-digit ORCID of the author(s)

If address information is provided with the affiliation(s) it will also be published.
For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Large Language Models (LLMs), such as ChatGPT, do not currently satisfy our authorship criteria. Notably an attribution of authorship carries with it accountability for the work, which cannot be effectively applied to LLMs. Use of an LLM should be properly documented in the Methods section (and if a Methods section is not available, in a suitable alternative part) of the manuscript.

Abstract
Please provide a structured abstract of 150 to 250 words which should be divided into the following sections:
- Purpose (stating the main purposes and research question)
- Methods
- Results
- Conclusions

For life science journals only (when applicable)
- Trial registration number and date of registration for prospectively registered trials
- Trial registration number and date of registration, followed by "retrospectively registered", for retrospectively registered trials

Keywords
Please provide 4 to 6 keywords which can be used for indexing purposes.

Statements and Declarations
The following statements should be included under the heading "Statements and Declarations" for inclusion in the published paper. Please note that submissions that do not include relevant declarations will be returned as incomplete.

- **Competing Interests:** Authors are required to disclose financial or non-financial interests that are directly or indirectly related to the work submitted for publication. Please refer to "Competing Interests and Funding" below for more information on how to complete this section.

Please see the relevant sections in the submission guidelines for further information as well as various examples of wording. Please revise/customize the sample statements according to your own needs.

Important information regarding Abstract
For Research articles and Reviews, please provide a structured abstract of up to 350 words organized under the following sections:

- Purpose (stating the main purposes and research question)
- Methods
- Results
- Conclusion

Abbreviations, footnotes, and references should not be used in the abstract, with the exception of standard, repetitive abbreviations.

Additional information regarding the Title Page
The title page should also contain the following information:

- Total number of each:
  1.text pages, including title page, references, and figure legends;
  2.tables; and
  3.figures.

Text

Text Formatting
Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX. We recommend using Springer Nature's LaTeX template.

Headings
Please use no more than three levels of displayed headings.

Abbreviations
Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes
Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.
Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.
Always use footnotes instead of endnotes.

Acknowledgments
Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Scientific style
Please always use internationally accepted signs and symbols for units (SI units).

References
Citation
Reference citations in the text should be identified by numbers in square brackets. Some examples:
1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3,7].

Reference list
The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.
The entries in the list should be numbered consecutively.
If available, please always include DOIs as full DOI links in your reference list (e.g. “https://doi.org/abc”).

- Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8.Ideally, the names of all authors should be provided, but the usage of “et al.” in long author lists will also be accepted: Smith J, Jones M Jr, Houghton L et al. (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

- Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see ISSN.org LTWA.

If you are unsure, please use the full journal title.

Authors preparing their manuscript in LaTeX can use the bibliography style file sn-basic.bst which is included in the Springer Nature Article Template.

Tables

- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and Illustrations Guidelines

Electronic Figure Submission

- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art

- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art

- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.
- Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.

- Combination artwork should have a minimum resolution of 600 dpi.

Color Art

- Color art is free of charge for online publication.
- If black and white will be shown in the print version, make surethat the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
- If the figures will be printed in black and white, do not refer to color in the captions.
- Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.

Figure Numbering

- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in text in consecutive numerical order.
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- If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,"A1, A2, A3, etc." Figures in online appendices [Supplementary Information (SI)] should, however, be numbered separately.

Figure Captions

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

- Figures should be submitted separately from the text, if possible.
- When preparing your figures, size figures to fit in the column width.
- For large-sized journals the figures should be 84 mm (for doublecolumn text areas), or 174 mm (for single-column text areas) wide and not higher than 234 mm.
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Accessibility
In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

- All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)
- Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)
- Any figure lettering has a contrast ratio of at least 4.5:1

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Please check Springer's policy on generative AI images and make sure your work adheres to the principles described therein.

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Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.
Before submitting research datasets as Supplementary Information, authors should read the journal's Research data policy. We encourage research data to be archived in data repositories wherever possible.
Submission

- Supply all supplementary material in standard file formats.
- Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
- To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.
- High resolution (streamable quality) videos can be submitted up to a maximum of 25GB; low resolution videos should not be larger than 5GB.

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- Aspect ratio: 16:9 or 4:3
- Maximum file size: 25 GB for high resolution files; 5 GB for low resolution files
- Minimum video duration: 1 sec
- Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Text and Presentations

- Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.

- A collection of figures may also be combined in a PDF file.

Spreadsheets

- Spreadsheets should be submitted as .csv or .xlsx files (MS Excel).

Specialized Formats

- Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

- It is possible to collect multiple files in a .zip or .gz file.

Numbering

- If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.
- Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.
- Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

- For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

- Supplementary Information (SI) will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

- The manuscript contains a descriptive caption for each supplementary material
- Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

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The participant has consented to the submission of the case report to the journal.

Patients signed informed consent regarding publishing their data and photographs.

Sample statements if identifying information about participants is available in the article:

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Sample statements for **"Consent to participate"**:

Informed consent was obtained from all individual participants included in the study.

Informed consent was obtained from legal guardians.

Written informed consent was obtained from the parents.

Verbal informed consent was obtained prior to the interview.

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