NATÁLIA MEDEIROS DIAS LOPES

AVALIAÇÃO DE PARÂMETROS DE ESTRESSE OXIDATIVO E MARCADORES INFLAMATÓRIOS SISTÊMICOS DE PACIENTES COM CARCINOMA PAPILÍFERO DE TIREOIDE E TIREOIDITE DE HASHIMOTO RESIDENTES NO NORTE DO PARANÁ

Londrina
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Tese apresentada ao Programa de Pós-graduação em Patologia Experimental da Universidade Estadual de Londrina - UEL, como requisito parcial para a obtenção do título de Doutor em Patologia Experimental.

Orientador: Profª. Drª. Alessandra Lourenço Cecchini Armani.

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LOPES, Natália Medeiros Dias Lopes. **Avaliação dos parâmetros de estresse oxidativo e marcadores inflamatórios sistêmicos de pacientes com Carcinoma Papilífero de tireoide e tireoidite de Hashimoto residentes no Norte do Paraná.** 2021. 80 f. Tese (Doutorado em Patologia Experimental) – Universidade Estadual de Londrina, Londrina, 2021.

RESUMO

O câncer de tireoide (CT) é a neoplasia maligna endócrina mais incidente, com maior acometimento entre as mulheres. É dividido em diferentes subtipos, sendo o carcinoma papilífero da tireoide (CPT) o mais frequente. Outras alterações podem se desenvolver na tireoide, como as doenças autoimunes, cuja principal representante é a tireoidite de Hashimoto (TH). Desde 1955, quando foi descrito pela primeira vez uma possível relação entre TH e CT, vários estudos têm sido elaborados na tentativa de elucidar esta correlação, entretanto, ainda não foram totalmente esclarecedores. Sabendo-se que o estresse oxidativo (EO) está relacionado com o desenvolvimento de diversos tipos de câncer, bem como a participação de marcadores inflamatórios, o objetivo deste trabalho foi determinar a participação do EO e dos marcadores inflamatórios sistêmicos em pacientes com CPT e TH residentes no norte do Paraná. Após aprovação ética (CEP-UEL nº 2.793.785), foram coletadas amostras de 115 pacientes, divididos entre os grupos: BENIGNO (n=63), CPT (n=27), TH (n=15) e CPT+TH (n=10). Um questionário para avaliar as características clínico patológicas e uma amostra de tecido também foram coletados. Sessenta e três indivíduos saudáveis foram utilizados como controle. Quanto às análises de EO, foram avaliados nos eritrócitos os parâmetros de defesa antioxidante Superóxido dismutase (SOD), Catalase (CAT) e Glutationa reduzida (GSH), além dos relacionados a lipoperoxidação, como a quimiluminescência induzida por terc-butil hidroperóxido e Malondialdeído (MDA), dosado no plasma. Os marcadores inflamatórios, interleucina-10 (IL-10), TGF-β1 e TNF-α também foram analisados no plasma. No microambiente tumoral avaliamos marcadores de estresse oxidativo (3-nitrotirosina e 4-hidroxinonenal), além de ki-67 e VEGF. Os resultados da CAT e SOD demonstraram valor maior nos grupos com alteração da tireoide em relação ao grupo controle. Quanto a GSH, os grupos BENIGNO e CPT apresentaram valores reduzidos em relação ao controle. Quando apenas os grupos CPT e CPT+TH foram comparados, nenhuma diferença significativa foi encontrada quanto aos parâmetros de EO, bem como quanto aos resultados dos marcadores inflamatórios. A capacidade de conter a lipoperoxidação induzida foi menor e um alto nível de MDA plasmático foi observado no grupo CPT. Para a análise entre CPT e CPT+TH, a participação do EO foi mais pronunciada no grupo CPT. Na imunohistoquímica, foi encontrado maior marcação no grupo CPT quanto aos parâmetros analisados, quando comparado ao CPT+TH. Pacientes com CPT sem TH apresentam maiores níveis de EO e marcadores de proliferação e angiogênese, assim, apresentam cenário mais favorável a metástases, e possível pior prognóstico, do que pacientes com associação entre CPT e TH.

Palavras-chave: carcinoma papilífero de tireoide; tireoidite de hashimoto; estresse oxidativo; marcadores inflamatórios.

LOPES, Natália Medeiros Dias. **Evaluation of oxidative stress parameters and systemic inflammatory markers of patients with Papillary thyroid carcinoma and Hashimoto's thyroiditis residing in North of Paraná.** 2021. 80 p. Thesis (Doctoral in Experimental Pathology) – Universidade Estadual of Londrina, Londrina, 2021.

ABSTRACT

Thyroid cancer (TC) is the most common endocrine malignancy, with greater involvement among women. It is divided into different subtypes, with papillary thyroid carcinoma (PTC) being the most frequent. Other changes can develop in the thyroid, such as autoimmune diseases, whose main representative is Hashimoto's thyroiditis (HT). Since 1955, when a possible relationship between HT and TC was first described, several studies have been carried out to elucidate this correlation, however, have not yet been fully clarifying. Knowing that oxidative stress (OE) is related to the development of several types of cancer, as well as the participation of inflammatory markers, the objective of this work was to determine the participation of OE and systemic inflammatory markers in patients with PTC and HT residents in North of Paraná. After ethical approval (CEP-UEL nº 2.793.785), samples were collected from 115 patients, divided between the groups: BENIGN (n= 63), PTC (n= 27), HT (n= 15) and PTC+HT (n= 10). A questionnaire to assess clinical pathological characteristics and a tissue sample were also collected. Sixty-three healthy individuals were used as controls. As for OE analyzes, the antioxidant defense parameters Superoxide dismutase (SOD), Catalase (CAT) and reduced Glutathione (GSH) were evaluated in the erythrocytes, in addition to those related to lipid peroxidation, such as chemiluminescence induced by tert-butyl hydroperoxide and Malondialdehyde (MDA), dosed in plasma. Inflammatory markers, interleukin-10 (IL-10), TGF-β1 and TNF-α were also analyzed in plasma. In the tumor microenvironment, we evaluated OE markers (3-nitrotyrosine and 4-hydroxynonenal), in addition to ki-67 and VEGF. The CAT and SOD results showed a higher value in the groups with thyroid disorders compared to the control group. As for GSH, the BENIGN and PTC groups showed reduced values in relation to the control. When only the PTC and PTC+HT groups were compared, no significant difference was found among the parameters of OE, as well as the results of the inflammatory markers. The ability to contain the induced lipid peroxidation was lower and a high level of plasma MDA was observed in the PTC group. For the analysis among PTC and PTC+HT, the participation of OE was more pronounced in the PTC group. In immunohistochemistry, greater marking was found in the PTC group for the parameters analyzed, when compared to PTC+HT. Patients with PTC without HT have higher levels of OE, proliferation and angiogenesis markers, thus, they have a more favorable scenario for metastases, and possibly a worse prognosis, than patients with an association among PTC and HT.

Keywords: papillary thyroid carcinoma; hashimoto's thyroiditis; oxidative stress; inflammatory markers.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

Anti-Tg	Anti-Tireoglobulina
Anti-TPO	Anti-Tireoperoxidade
Anti-TRAb	Anti-Receptor de TSH
ATP	Adenosina trifosfato
BRAF	Gene da Quinase RAF do tipo B
CAT	Catalase
CDT	Carcinoma diferenciado da tireoide
CFT	Carcinoma folicular da tireoide
CPT	Carcinoma papilífero da tireoide
CT	Câncer de tireoide
Cu/ZnSOD	Cobre Zinco Superóxido dismutase 1
DAIT	Doença autoimune da tireoide
DG	Doença de Graves
DNA	*Deoxyribonucleic acid* - Ácido Desoxirribonucleico
DUOX	*Dual oxidase* - Oxidase dupla
EO	Estresse oxidativo
ERO	Espécies reativas de oxigênio
GSH	Glutationa reduzida
GSSG	Glutationa oxidada
GR	Glutationa redutase
GPx	Glutationa peroxidase
H_2O_2	Peróxido de hidrogênio
IFN-γ	Interferon-gama
IL-2	Interleucina-2
IL-4	Interleucina-4

IL-10	Interleucina-10
IL-13	Interleucina-13
IL-17	Interleucina-17
INCA	Instituto Nacional do Câncer
L•	Radical lipídico
LOO•	Radical peroxila
MDA	Malondialdeído
MHC	Complexo principal de histocompatibilidade
MnSOD	Manganês Superóxido dismutase 2
NADPH	Nicotinamida adenina dinucleotídeo fosfato
NOX	Nicotinamida adenina dinucleotídeo fosfato oxidase
$O_2^{\bullet -}$	Ânion superóxido
•OH	Radical hidroxila
OMS	Organização Mundial da Saúde
RAS	Oncogene RAS
RL	Radical livre
RET	*Rearranged During transfection* – Proto-oncogene RET
SOD	Superóxido dismutase
T3	Triiodotironina
T4	Tiroxina
TH	Tireoidite de Hashimoto
TPO	Tireoperoxidase
TSH	Hormônio tireoestimulante
4-HNE	4-hidroxinonenal

SUMÁRIO

1 INTRODUÇÃO

O câncer de tireoide é considerado a neoplasia maligna endócrina mais comum, apresentando uma crescente taxa de incidência na população, com maior acometimento entre as mulheres. Apresenta uma classificação específica, de acordo com as características moleculares apresentadas e a origem tecidual de desenvolvimento, sendo o Carcinoma Papilífero da tireoide o subtipo mais frequente. Além do câncer, outras alterações podem acometer a glândula tireoide, como as doenças autoimunes. Dentre elas, a mais incidente é a tireoidite de Hashimoto. Descrita em 1912 por Hakaru Hashimoto, é considerada uma tireoidite linfocítica crônica, relacionada com a ativação anormal de linfócitos T e a destruição de células epiteliais da tireoide, com produção de autoanticorpos e resposta inflamatória crônica persistente, sendo uma das principais causas de desenvolvimento de hipotireoidismo no mundo.

A correlação entre doenças inflamatórias crônicas e câncer está bem estabelecida em vários tecidos e órgãos, porém, na tireoide esta associação ainda é controversa. Desde 1955, quando Dailey descreveu pela primeira vez uma possível relação entre a tireoidite de Hashimoto e o câncer de tireoide, diversos estudos têm sido elaborados na tentativa de elucidar a correlação entre estas doenças, entretanto, ainda não foram totalmente esclarecedores.

Apesar de ser apontada como um fator de risco para o desencadeamento do processo carcinogênico na tireoide, a tireoidite de Hashimoto parece estar associada a um menor índice de metástases linfonodais e melhor prognóstico aos pacientes que a possuem em associação com o carcinoma papilífero, além de menor recorrência e mortalidade.

Sabe-se que o estresse oxidativo, estabelecido pelo desequilíbrio entre o sistema de defesa antioxidante e agentes pró-oxidantes, como as espécies reativas de oxigênio, está relacionado com o desenvolvimento de diversas doenças, como o câncer. Quanto ao câncer de tireoide, a participação do estresse oxidativo não está totalmente estabelecida. O mesmo ocorre quanto ao processo inflamatório e o câncer de tireoide, em que carece de maiores informações.

Para adicionar conhecimento a esses achados clínicos e tentar esclarecer essa relação controversa entre carcinoma papilífero da tireoide e a tireoidite de Hashimoto é necessário conhecer os aspectos biológicos do microambiente

tireoidiano, bem como a resposta sistêmica do paciente. Portanto, compreender a participação do estresse oxidativo e de marcadores inflamatórios sistêmico, bem como o microambiente tumoral apresentado pelos pacientes com carcinoma papilífero de tireoide e tireoidite de Hashimoto se faz de grande importância.

2 REVISÃO DE LITERATURA

2.1 TIREOIDE

A tireoide, localizada abaixo da laringe e ocupando as regiões laterais e anterior da traqueia, é uma importante glândula do sistema endócrino, dividida em dois lobos laterais, ligados por um istmo. É composta por folículos, considerados as unidades funcionais da tireoide. Os folículos são formados por uma monocamada de células epiteliais, chamadas de tireócitos, que circundam o lúmen central, e é delimitado pela superfície apical dos tireócitos e preenchido por tireoglobulina, também chamada de 'coloide', uma glicoproteína precursora dos hormônios tireoidianos (KUMAR et al., 2016; CARVALHO; DUPUY, 2017).

O hormônio tireoestimulante (TSH) produzido pela hipófise, estimula a produção dos hormônios da tireoide. Cerca de 93% dos hormônios produzidos pela tireoide correspondem a tiroxina (T4) e 7% à triiodotironina (T3), que são essenciais para o crescimento normal e o metabolismo energético do organismo. Além destes, a calcitonina também é um hormônio produzido na tireoide, pelas células parafoliculares, cuja função está relacionada ao metabolismo do cálcio (KUMAR et al., 2016; ROSEN; SAPRA, 2020).

Alterações na produção dos hormônios da tireoide podem causar o desenvolvimento de doenças tireoidianas, como o hipotireoidismo e o hipertireoidismo, que são condições comuns e que afetam a população no mundo todo, com consequências graves para a saúde, se não tratadas. O hipertireoidismo ocorre quando há uma produção excessiva de hormônios e tem como principal representante a Doença de Graves (DG). Já o hipotireoidismo, é caracterizado quando a produção hormonal é deficiente (KUMAR et al., 2016; TAYLOR et al., 2018).

Das alterações que podem acometer a tireoide, as doenças autoimunes são as mais frequentes. São as principais desencadeadoras do hipotireoidismo, pois resultam de uma interrupção da tolerância aos autoantígenos tireoidianos, levando a um ataque imunológico à tireoide, com intensa infiltração de células, principalmente linfócitos, desencadeando destruição dos tireócitos, e consequentemente, menor produção hormonal. Dentre as doenças autoimunes da tireoide (DAIT), a principal representante é a tireoidite de Hashimoto (TH) (ANTONELLI et al., 2015; KUMAR et al., 2016).

2.2 TIREOIDITE DE HASHIMOTO

No Japão, em 1912, Hakaru Hashimoto descreveu pela primeira vez as principais características da tireoidite autoimune, ao relatar a histologia tireoidiana de quatro pacientes, que apresentavam infiltração linfocítica difusa, fibrose e atrofia parenquimatosa, as quais denominou de '*struma lymphomatosa'*. Desde então, esta alteração ficou conhecida como tireoidite de Hashimoto (AKAMIZU; AMINO, 2000; RAGUSA et al., 2019).

A TH é a DAIT mais frequente, sendo conhecida também como tireoidite linfocítica crônica. A quebra da autolerância e indução da autoimunidade na tireoide, com a presença de um intenso infiltrado inflamatório, composto principalmente por células T $CD4^+$ e $CD8^+$, que promovem a morte celular por liberação de citocinas ou por citotoxicidade, respectivamente, além da presença de anticorpos antitireoidianos, resulta em destruição progressiva dos tireócitos com consequente redução da produção hormonal (KUMAR et al., 2016; RAYMAN, 2019).

Os mecanismos que desencadeiam o ataque autoimune à tireoide e os fatores de risco ainda estão sob investigação, sem uma definição concreta até o momento, porém, a interação entre a suscetibilidade genética e fatores ambientais, incluindo fatores nutricionais, como a ingestão excessiva de iodo (RAYMAN, 2019), têm sido apontados como os principais gatilhos para a quebra da autotolerância imunológica na TH (ANTONELLI et al., 2015; RAGUSA et al., 2019). Além destes, o consumo abusivo de álcool, estresse e tabagismo também são considerados fatores que podem desencadear a TH (LIONTIRIS; MAZOKOPAKIS, 2017).

A incidência mundial anual de tireoidite de Hashimoto é estimada em 3,5-5 casos por 1000 indivíduos. Apresenta maior incidência em mulheres, com um risco maior com o aumento da idade, e um pico de ocorrência estimado entre 45 e 55 anos (ANTONELLI et al., 2015; RAGUSA et al., 2019). Morfologicamente, a TH apresenta atrofia gradual do tecido tireoidiano após a invasão pelos linfócitos, seguido de atrofia folicular e metaplasia oncocítica das células foliculares (PYZIK et al., 2015). Microscopicamente, há excessiva infiltração do parênquima por infiltrado inflamatório, composto principalmente por linfócitos (KUMAR et al., 2016).

O diagnóstico da TH é feito pelo quadro clínico dos pacientes, alterações hormonais, análise anatomopatológica e principalmente pela presença de autoanticorpos (RAGUSA et al., 2019). Os anticorpos antitireoidianos, anti-

tireoperoxidase (anti-TPO), anti-tireoglobulina (anti-Tg) e anti-receptor de TSH (anti-TRAb) apresentam correlação positiva com o aumento da reação inflamatória na tireoide e com o desenvolvimento de TH (PYZIK et al., 2015). Ehlers et al., (2016) observaram que pacientes com doença autoimune da tireoide e com dosagem elevada de anti-TPO (>500UI/mL) apresentavam risco moderadamente maior de desenvolver hipotireoidismo (EHLERS et al., 2016).

O anti-TPO é considerado o melhor marcador sorológico para estabelecer o diagnóstico de TH, pois são encontrados em aproximadamente 95% dos pacientes com TH e raramente em indivíduos saudáveis. Quanto ao anticorpo anti-Tg, apresenta menor sensibilidade e especificidade, pois apresenta positividade para cerca de 60-80% dos pacientes com TH e está presente em uma fração maior de indivíduos saudáveis, respectivamente, quando comparado ao anti-TPO (CATUREGLI et al., 2014; ANTONELLI et al., 2015).

Pacientes com TH estão mais propensos a desencadear outras doenças autoimunes, tanto endócrinas, como diabetes mellitus tipo 1, quanto não-endócrinas, como artrite reumatoide e lúpus eritematoso sistêmico (KUMAR et al., 2016). Podem apresentar também um risco maior de desenvolver câncer de tireoide (CT) (ANTONELLI et al., 2015; GOBARU et al., 2019).

2.3 Câncer de Tireoide

Em 2020 foram registrados cerca de 586 mil novos casos de câncer de tireoide no mundo, e mais de 43 mil mortes, segundo o *Global Cancer Observatory*, ocupando o nono lugar em incidência mundial, sendo, portanto, a neoplasia maligna endócrina mais comum (GCO, 2020). No Brasil, para cada ano do triênio 2020-2022, estima-se que haverá 1.830 casos novos em homens e 11.950 em mulheres, ocupando a quinta posição entre os tipos de câncer mais incidentes na população feminina brasileira, segundo o Instituto Nacional do Câncer (INCA, 2020). É também um dos tipos de câncer mais incidente em adultos jovens (NCCN, 2020).

Desde a década de 1980, tem-se registrado o aumento da incidência de CT, que se deve principalmente à introdução de novas técnicas de diagnóstico, associado ao maior acesso da população aos serviços de saúde (VACCARELLA et al., 2016), o que contribui para a detecção precoce e diagnósticos mais precisos, com redução da morbidade (HAN; KIM, 2018). Embora tenha uma alta incidência, a taxa

de mortalidade em decorrência do CT é relativamente baixa, variando entre 0,28 e 0,51 a cada 100 mil homens e mulheres, respectivamente (INCA, 2020).

Apesar do avanço no desenvolvimento de exames diagnósticos contribuir para a detecção precoce de diversas doenças, inclusive o CT, o uso da tecnologia radiológica em exames de raio-x e tomografia computadorizada, por exemplo, aumenta consideravelmente a exposição da população à radiação ionizante (ZHANG et al., 2015; HAN; KIM, 2018). A glândula tireoide está entre os órgãos mais radio sensíveis do corpo. As doses de radiação recebidas em cada exame, apesar de serem baixas, são relatadas como um possível e importante fator de risco para o desencadeamento do CT (ZHANG et al., 2015; HAN; KIM, 2018). Assim como a radiação recebida na radioterapia, principalmente para o tratamento de regiões do corpo próximas à tireoide (SUZUKI et al., 2019).

Apesar da radiação ionizante ser um dos fatores de risco mais bem estabelecidos até o momento (ZHANG et al., 2015; SUZUKI et al., 2019), outros fatores também podem estar relacionados com o desenvolvimento de CT. As espécies reativas de oxigênio (ERO) quando presentes em níveis elevados, podem induzir o estresse oxidativo, além de serem consideradas potentes agentes lesivos ao DNA (AMEZIANE-EL-HASSANI et al., 2019). Além destes, obesidade, tabagismo, desequilíbrio na ingestão de iodo (THUN et al., 2017), assim como histórico familiar de CT e a presença de doenças tireoidianas pré-existentes, como a TH, são considerados importantes fatores desencadeantes de CT (LIANG et al., 2017; MAROTTA et al., 2017; MÓLNAR et al., 2019).

A classificação do CT sofreu várias alterações ao longo do tempo. Em 1953, foi publicado pelo Instituto de Patologia das Forças Armadas dos Estados Unidos, a primeira classificação, que subdividia o CT em tumores benignos e malignos, com apenas duas variantes, papilar e folicular, sendo distinguidos com base na arquitetura tecidual (WARREN; MEISSNER, 1953). Com o passar do tempo, novas classificações foram estabelecidas, e de acordo com a mais recente, publicada pela Organização Mundial da Saúde em 2017 (BYCHKOV, 2020), o CT é classificado de acordo com o perfil molecular apresentado e quanto ao epitélio de origem (FIG. 1), sendo o carcinoma diferenciado da tireoide (CDT) o mais comum, representando cerca de 95% dos casos de CT. O CDT é proveniente do epitélio folicular e apresenta arquitetura tecidual semelhante ao epitélio tireoidiano normal (CAMESELLE-TEIJEIRO; SOBRINHO-SIMÕES, 2018; ASA, 2019).

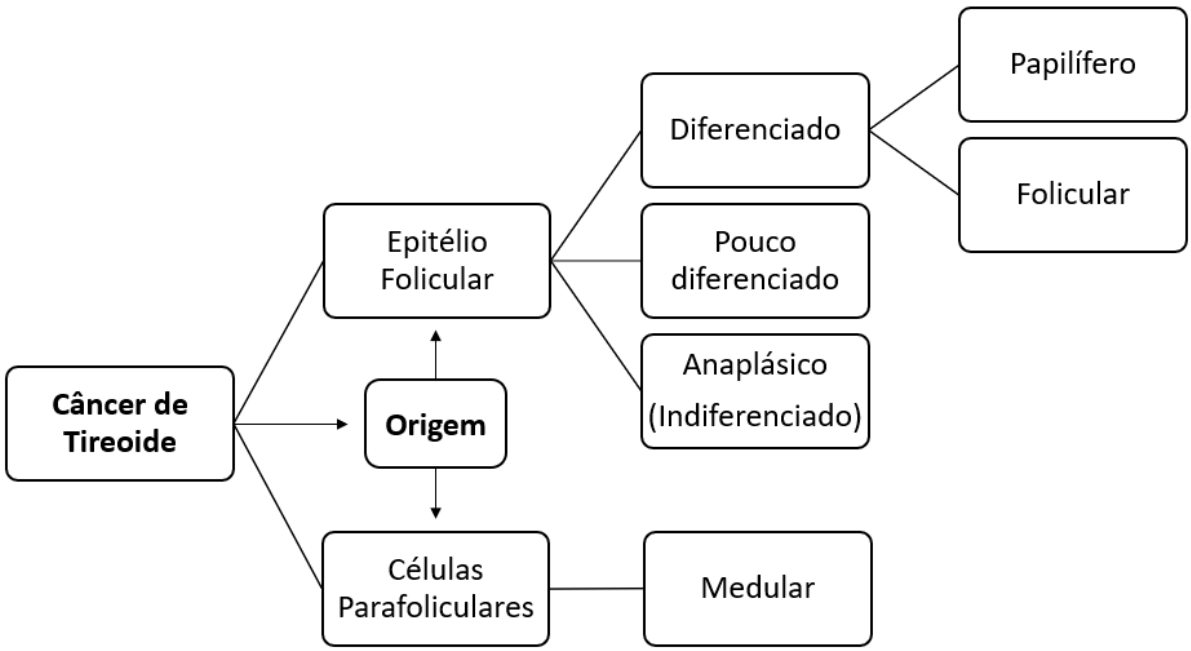

Figura 1. Classificação dos principais tipos de câncer de tireoide de acordo com a origem tecidual de seu desenvolvimento (Fonte: Próprio autor).

O carcinoma papilífero da tireoide (CPT) é o subtipo de CDT mais incidente, seguido pelo carcinoma folicular (CFT), observado em menor frequência (ABDULLAH et al., 2019; ASA, 2019). Quanto ao carcinoma pouco diferenciado, caracteriza-se por apresentar limitada diferenciação celular, sendo morfologicamente intermediária entre a apresentada pelos CDT e o carcinoma anaplásico, ou indiferenciado, sendo este último subtipo considerado raro, porém, com alta taxa de mortalidade. Além destes, há o carcinoma medular, derivado de células parafoliculares (FIG. 1) (CAMESELLE-TEIJEIRO; SOBRINHO-SIMÕES, 2018; MÓLNAR et al., 2019).

2.3.1 Carcinoma Papilífero da Tireoide

O carcinoma papilífero da tireoide é o mais frequente, representando cerca de 75-80% dos casos de CDT, com maior acometimento entre as mulheres. Seu desenvolvimento é lento e pode ocorrer em qualquer idade, porém, a incidência aumenta com o passar dos anos, sendo mais comum entre a terceira e a quinta década de vida dos pacientes, com pico médio aos 40 anos (ABDULLAH et al., 2019).

O CPT é um tumor epitelial com evidências de diferenciação folicular, com

características nucleares específicas (FIG. 2), como presença de núcleos em forma de ‘vidro fosco’ (núcleos claros), fenda na membrana nuclear (‘grãos de café’), pseudoinclusões intranucleares e corpos psamomatosos, que são corpúsculos calcificados, decisivos para o diagnostico deste tipo de câncer, visto que é muito raro em outros tipos de CT, além de arquitetura tecidual com presença de papilas e/ou invasão capsular (ASA, 2019; ABDULLAH et al., 2019).

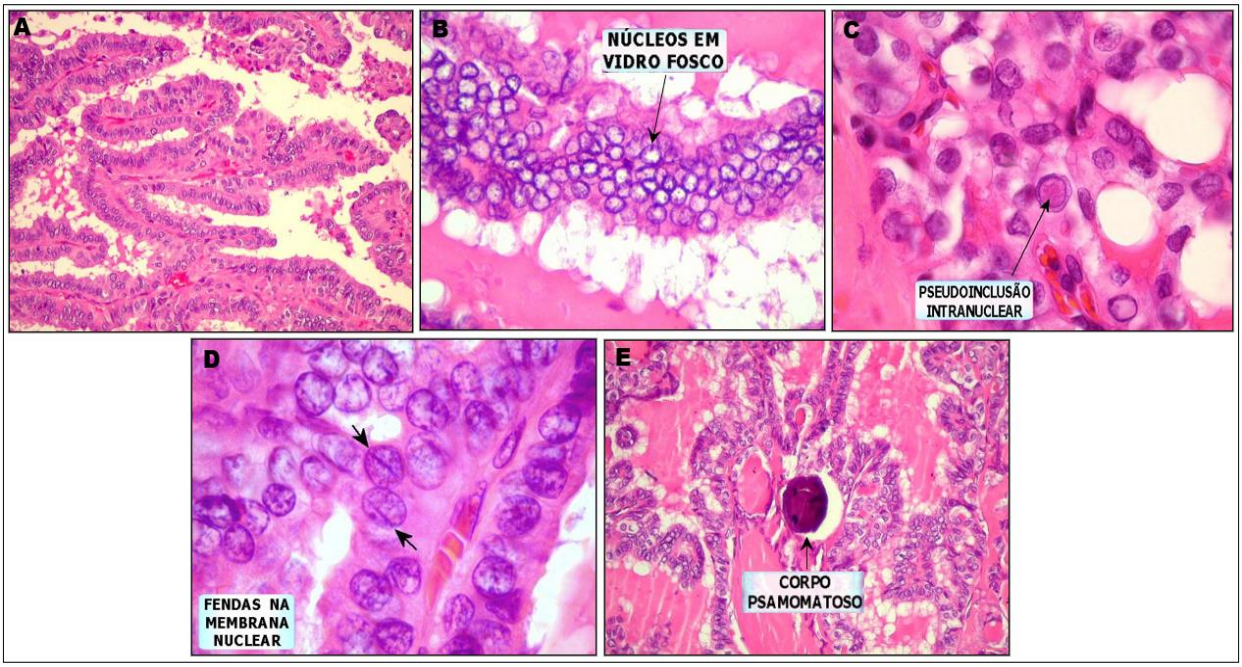

Figura 2. Características nucleares específicas do carcinoma papilífero de tireoide. (a) arquitetura tecidual em forma de papilas; (b) núcleos em ‘vidro fosco’; (c) pseudoinclusões intranucleares; (d) fendas na membrana nuclear e (e) presença de corpo psamomatoso. (Fonte: Adaptado do Atlas de Anatomia Patológica – UNICAMP).

Apesar da etiologia do CPT ser controversa, fato este que se assemelha aos outros tipos de CT, o histórico de exposição à radiação ionizante é considerado um fator de risco bem estabelecido. Quanto às alterações genéticas, mutações nos oncogenes BRAF, RAS ou RET podem ser encontradas em cerca de 70% dos casos. Estes eventos genéticos podem desencadear formas variantes de CPT, apresentando maior agressividade e diferentes características histopatológicas (ABDULLAH et al., 2019; GRACEFFA et al., 2019). Além destes, o histórico familiar de CPT e doença benigna da tireoide preexistente, como a TH, também podem ser considerados como fatores de risco, visto que na literatura, estudos apontam para uma possível correlação entre pacientes com TH e CPT (MÓLNAR et al., 2019; SULAIEVA et al., 2020). Diferentes estudos sugerem que não há, ou que é pouco frequente, a correlação entre TH e os outros subtipos de câncer de tireoide, incluindo variantes foliculares,

medulares ou anaplásicas (DE PAIVA et al., 2017; SULAIEVA et al., 2020).

2.4 ASSOCIAÇÃO ENTRE TIREOIDITE DE HASHIMOTO E CÂNCER DE TIREOIDE

Em 1863, Rudolf Virchow descreve pela primeira vez uma possível correlação entre inflamação e câncer (VIRCHOW, 1863). Desde então, várias evidências mostram que vários tipos de câncer podem ser desencadeados a partir de uma doença inflamatória crônica (LANDSKRON et al., 2014; KORNILUK et al., 2017; MURATA, 2018). Apesar de ser uma resposta benéfica, a fim de restaurar a homeostasia do organismo frente a uma lesão ou agente patológico, a resposta inflamatória ao se tornar crônica, pode compartilhar vias de sinalização com o processo carcinogênico, como sinais de sobrevivência celular para evitar apoptose, fatores pró-angiogênicos, quimiocinas e citocinas (LANDSKRON et al., 2014; KORNILUK et al., 2017).

A possibilidade do processo inflamatório desencadeado pela TH estar relacionado com o desenvolvimento do CT foi descrita pela primeira vez por Dailey et al., (1955), e posteriormente, vários estudos têm sido realizados no intuito de investigar esta associação (MAZOKOPAKIS et al., 2010; JANKOVIC et al., 2013; ZHANG et al., 2014; PAGANO et al., 2018; MÓLNAR et al., 2019; SULAIEVA et al., 2020). Em uma meta-análise realizada por Lai et al., (2017), observaram que a presença de CPT foi mais frequentemente encontrado em pacientes com TH, sugerindo que a resposta inflamatória presente na TH pode criar um cenário favorável para desencadear o processo carcinogênico, caracterizando-a, portanto, como um possível fator de risco para o CPT (LAI et al., 2017).

Em um estudo retrospectivo realizado por Uhliarova e Hajtman (2018), ao analisarem os dados clínico histopatológicos de pacientes submetidos à tireoidectomia, parcial ou total, observaram que a TH foi significativamente mais frequentemente associada ao CT e ao microcarcinoma de tireoide em comparação com outras alterações benignas, sendo que, entre os casos de CT, o tipo histológico mais encontrado foi o CPT (62%) (UHLIAROVA; HAJTMAN, 2018). Dados semelhantes foram obtidos por Jackson et al., (2020), que observaram aumento de 1,5 vezes no risco de desenvolver CT em pacientes que tinham TH, quando comparado aos pacientes que não apresentavam (JACKSON et al., 2020).

Apesar da possibilidade de ser um fator de risco para o desenvolvimento de CT, a associação entre TH e CT pode ser benéfica. Molnár et al., (2019)

observaram que, a multifocalidade tumoral pode ser encontrada com maior frequência em pacientes que apresentam CT e TH concomitante, mas, por outro lado, estes pacientes apresentavam taxas de metástases linfáticas significativamente menores (MOLNÁR et al., 2019), o que corrobora com outros estudos, que sugerem que a coexistência de CT e TH acarreta em um melhor prognóstico aos pacientes e menor agressividade do tumor (DVORKIN et al., 2013; DOBRINJA et al., 2016; SONG et al., 2018).

A destruição celular decorrente dos distúrbios autoimunes da tireoide, como o que ocorre na TH, pode estar relacionada à destruição de células tumorais via mecanismos imunes humorais e mediados por células T citotóxicas, contribuindo assim, para um efeito favorável da associação entre TH e CT (JEONG et al., 2012; ZENG et al., 2018). Apesar de indicar a necessidade de mais estudos para verificar esta afirmação, Graceffa et al., (2019) também sugerem que o possível vínculo favorável entre TH e CPT pode ser devido à presença da resposta autoimune que se desenvolve junto com uma resposta imune antitumoral (GRACEFFA et al., 2019). Ao analisar as possíveis ligações imunológicas, Sulaieva et al., (2020) observaram que pacientes com CPT e TH possuíam número maior de células T $CD8^{+}$, comparado aos pacientes que apresentam apenas CPT, refletindo assim a capacidade do sistema imunológico de gerar e recrutar células T citotóxicas na área do tumor, o que poderia explicar o efeito protetor da TH na progressão do CPT (SULAIEVA et al., 2020).

Respostas imunes do tipo Th17 levam à produção de Interleucina-17 (IL-17), que sabidamente participa de processos autoimunes, como na TH (FIGUEROA-VEJA et al., 2010). Zeng et al., (2018) verificaram uma correlação negativa entre IL-17 com metástases linfonodais em pacientes com TH e CPT, indicando que possivelmente as células Th17 podem apresentar função antitumoral (ZENG et al., 2018). O perfil de citocinas apresentado em pacientes com CPT e TH pode ser alterado. Zivancevic-Simonovic et al., (2015) observaram que além do padrão de citocinas previamente esperado, Th17, por se tratar de uma doença autoimune, houve uma produção expressiva de outras citocinas, relacionadas com o perfil Th1 e Th2, como IFN-γ e IL-4, respectivamente. Porém, indicam a necessidade de maiores estudos para confirmar e correlacionar os resultados obtidos (ZIVANCEVIC-SIMONOVIC et al., 2015).

Lu et al., (2020) analisaram a participação da interleucina-10 (IL-10), considerada uma interleucina anti-inflamatória, em pacientes com CPT e TH em

associação, visto que há relatos de sua elevada expressão em pacientes nestas condições (STANCIU et al., 2015). Ao investigar sua função na imunidade tumoral, observaram que a presença da IL-10 estava relacionada à maior expressão do MHC classe I, quando comparado à pacientes com CPT isolado, e que resultaria em melhora da imunidade antitumoral, visto que a maior expressão de MHC está relacionada com menores índices de evasão da morte pelas células neoplásicas (STANCIU et al., 2015; LU et al., 2020). Além da IL-10, a interleucina-2 (IL-2) parece desempenhar papel semelhante, aumentando também a expressão do MHC classe I. Sugerindo, portanto, que a presença destas interleucinas estaria relacionada à um prognostico favorável apresentado por pacientes com CPT e TH associados (HU et al., 2020; LU et al., 2020).

Apesar dos relatos na literatura sugerindo a correlação entre TH e CT, esta afirmação ainda é controversa. Alguns autores afirmam que não há evidências claras para apoiar esta associação, visto que não encontraram correlação estatisticamente significante entre elas (JANKOVIC et al., 2013; DEL RIO et al., 2019). Assim, esta relação parece estar longe de ser esclarecida, do que diz respeito aos mecanismos fisiopatológicos e clínicos que interligam essas duas alterações tireoidianas.

2.5 Estresse oxidativo e Câncer de tireoide

As espécies reativas de oxigênio (ERO), podem ser divididas em dois grupos: os radicais livres (RL), que são moléculas que apresentam um elétron desemparelhado na sua camada de valência, como por exemplo, o ânion superóxido ($O_2^{\bullet -}$) e o radical hidroxila (•OH); e moléculas não radicalares, como o peróxido de hidrogênio (H_2O_2) (LUKOSZ et al., 2010). A produção destes compostos ocorre de forma contínua e fisiológica no organismo, através de diversas reações bioquímicas importantes, como na geração de ATP (adenosina trifosfato), por meio da cadeia transportadora de elétrons na mitocôndria. As ERO são neutralizadas pelo sistema antioxidante celular, que tem a função de inibir e/ou reduzir os danos causados pelas ERO. Este sistema é constituído por antioxidantes enzimáticos, como a Superóxido Dismutase (SOD), Catalase (CAT) e Glutationa Peroxidase (GPx), e pelos não-enzimáticos, como a glutationa e as vitaminas E e C, por exemplo (VEAL et al., 2007; SIES, 2015).

A SOD é a primeira e uma das mais importantes enzimas na linha de defesa

do sistema antioxidante, estando presente essencialmente em todas as células do corpo. Se apresenta sob 2 principais isoformas: a citoplasmática, dependente dos cofatores cobre e zinco (Cu/ZnSOD ou SOD1) e a mitocondrial, dependente de manganês (MnSOD ou SOD2). Atua através de um mecanismo de ação comum à todas isoformas, no qual catalisa a dismutação do ânion superóxido, resultando na geração de H_2O_2 (FIG. 3), sendo este, menos lesivo, quando comparado aos danos causados pelo ânion superóxido (LUKOSZ et al., 2010; WANG et al., 2018).

O peróxido de hidrogênio apresenta ação antimicrobiana, pois através da oxidação lipídica, a membrana plasmática dos microrganismos é degradada, ocasionando sua morte. Porém, em excesso o H_2O_2 é muito prejudicial às células, pois na presença dos metais ferro e cobre, pela Reação de Fenton, pode ser convertido em radical hidroxila (•OH) (FIG. 3). Para conter o excesso de H_2O_2, há enzimas, como a catalase, que promovem a conversão de peróxido de hidrogênio à água e oxigênio molecular (FIG. 3). A CAT é expressa principalmente nos rins, fígado e hemácias, e está localizada principalmente nos peroxissomos, mas também na mitocôndria e no núcleo (LUKOSZ et al., 2010; GLORIEUX et al., 2015).

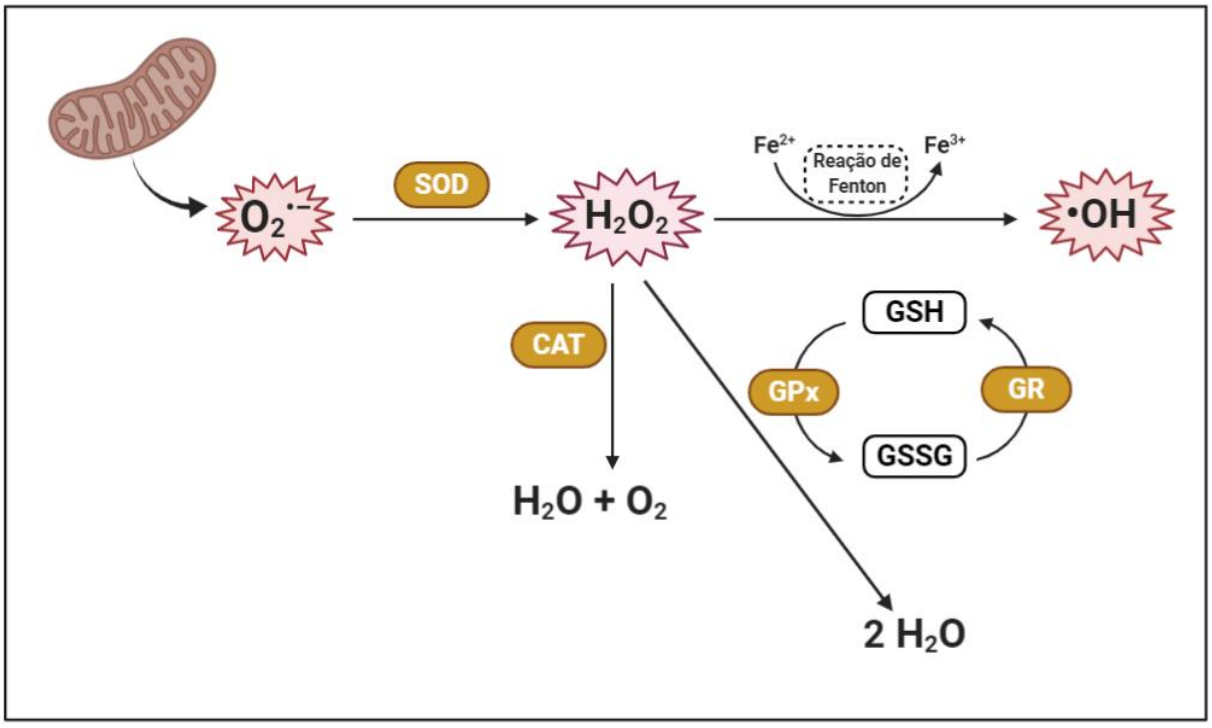

Figura 3. Ação das enzimas antioxidantes na neutralização de radicais livres. O ânion superóxido gerado pela cadeia transportadora de elétrons mitocondrial é convertido em peróxido de hidrogênio pela enzima superóxido dismutase. Após sua geração, o peróxido de hidrogênio pode ser convertido em água e oxigênio pela enzima Catalase ou pela Glutationa peroxidase, no entanto, para que este processo ocorra, é necessário a conversão da

glutationa reduzida em glutationa oxidada, que posteriormente pode retornar à sua forma reduzida pela ação da Glutationa redutase. Além disso, pode ocorrer a interação do peróxido de hidrogênio com íons ferro (ou cobre), que através da Reação de Fenton, resulta na formação de radical hidroxila. CAT: catalase; Fe: ferro; GSH: glutationa reduzida; GSSG: glutationa oxidada; GPx: glutationa peroxidase; GR: glutationa redutase; H_2O_2: Peróxido de hidrogênio; H_2O: Água; $O_2^{\bullet-}$: Ânion superóxido; O_2: Oxigênio; •OH: Radical hidroxila; SOD: superóxido dismutase (Fonte: Próprio autor).

Além da CAT, outro sistema capaz de converter o H_2O_2 em água e oxigênio é o da Glutationa, que é considerada o grupamento tiol (-SH) mais abundante nas células, principalmente nas hemácias e nos hepatócitos. A glutationa se apresenta em sua maior parte, cerca de 98%, como glutationa reduzida (GSH) e o restante na sua forma oxidada (GSSG). Durante períodos de EO, a GSH é convertida em GSSG pela GPx, e pode ser revertida para GSH pela glutationa redutase (GR) (FIG. 3). Assim, a razão GSH/GSSG pode ser utilizada para avaliar o estado redox dos sistemas biológicos, crucial para manutenção da homeostase intracelular (LU, 2013; SIES, 2015).

Quando ocorre um desequilíbrio entre a geração de ERO e sua neutralização pelo sistema antioxidante, configura o processo chamado de estresse oxidativo (EO) (HOLMSTRÖM; FINKEL, 2014; SIES, 2015). O EO pode causar danos significativos na estrutura e nas funções celulares, além de danos no DNA, que podem induzir mutações somáticas e transformação neoplásica, desencadeando os processos de iniciação e progressão da carcinogênese, sendo, portanto, considerado um importante fator de risco para vários tipos de câncer, dentre eles, o CT (REUTER et al., 2010; MOLONEY; COTTER, 2018).

Além da cadeia transportadora de elétrons da mitocôndria, outra fonte de ERO celular é representada pela NADPH oxidase (NOX), que é um complexo enzimático ligado à membrana plasmática, e que age como doador de elétrons para redução do oxigênio e formação de ânion superóxido (RABÊLO et al., 2010). A família NOX é composta por 7 isoformas (NOX 1, NOX 2, NOX3, NOX4, NOX5, DUOX1 e DUOX2), envolvidos em diferentes funções biológicas (PANDAY et al., 2015). Na tireoide, 3 tipos já foram descritos: DUOX1, DUOX 2 e mais recentemente, NOX4. DUOX1 e 2, estão localizados na membrana apical dos tireócitos e agem em conjunto com os fatores de maturação DUOXA1 e 2, na presença de cálcio. São responsáveis pela geração de H_2O_2, utilizado pela enzima tireoperoxidase (TPO) na produção dos hormônios T3 e T4. Quanto ao NOX4, pode estar localizado no núcleo, retículo

endoplasmático e mitocôndrias, e é responsável pela produção intracelular de H_2O_2 (WEYEMI et al., 2010; MUZZA et al., 2016; AMEZIANE-EL-HASSANI et al., 2019).

Durante a produção hormonal, ERO são geradas na tireoide como parte do processo normal, no entanto, são neutralizadas pelas enzimas antioxidantes, limitando, portanto, os danos oxidativos. Entretanto, algumas condições como, proliferação de células tumorais e inflamação da glândula tireoide podem alterar o equilíbrio entre as ERO e os níveis de antioxidantes, gerando um ambiente de EO e consequentemente, resultando em danos no DNA, que podem contribuir para a carcinogênese (RAMLI et al., 2017).

A síntese dos hormônios tireoidianos requer a iodação da tirosina, aminoácido presente na tireoglobulina, processo este que é catalisado pela TPO e que necessita de uma quantidade específica de H_2O_2 para uma produção adequada. Neste cenário, o DUOX2 é a principal fonte de peróxido de hidrogênio no folículo tireoidiano (SZANTO et al., 2019). Grandes quantidades de H_2O_2 produzidas durante a síntese hormonal pode ser uma possível explicação aos danos oxidativos do DNA e a alta taxa de mutação espontânea observada na glândula tireoide, em comparação com outros órgãos. Em contraste com a produção fisiológica de ERO desempenhada pelo DUOX2, a participação da NOX4 tem sido relacionada à geração de níveis patologicamente elevados de H_2O_2, culminando em danos ao DNA e instabilidade genômica (AMEZIANE-EL-HASSANI et al., 2016). Processos estes, que estão relacionados ao início da carcinogênese na tireoide, sendo, portanto, a NOX4 identificada como uma potencial fonte prejudicial de ERO (FIG. 4) (AMEZIANE-EL-HASSANI et al., 2016; AZOUZI et al., 2017; SZANTO et al., 2019).

Weyemi et al., (2010) observaram que a NOX4 pode estar expressa em altos níveis nos tireócitos e que produz H_2O_2 continuamente. Além disso, NOX4 estaria regulada positivamente no CT, particularmente no CPT, sugerindo que esse sistema gerador de H_2O_2 poderia estar envolvido no mecanismo de sinalização das células tumorais da tireoide (WEYEMI et al., 2010).

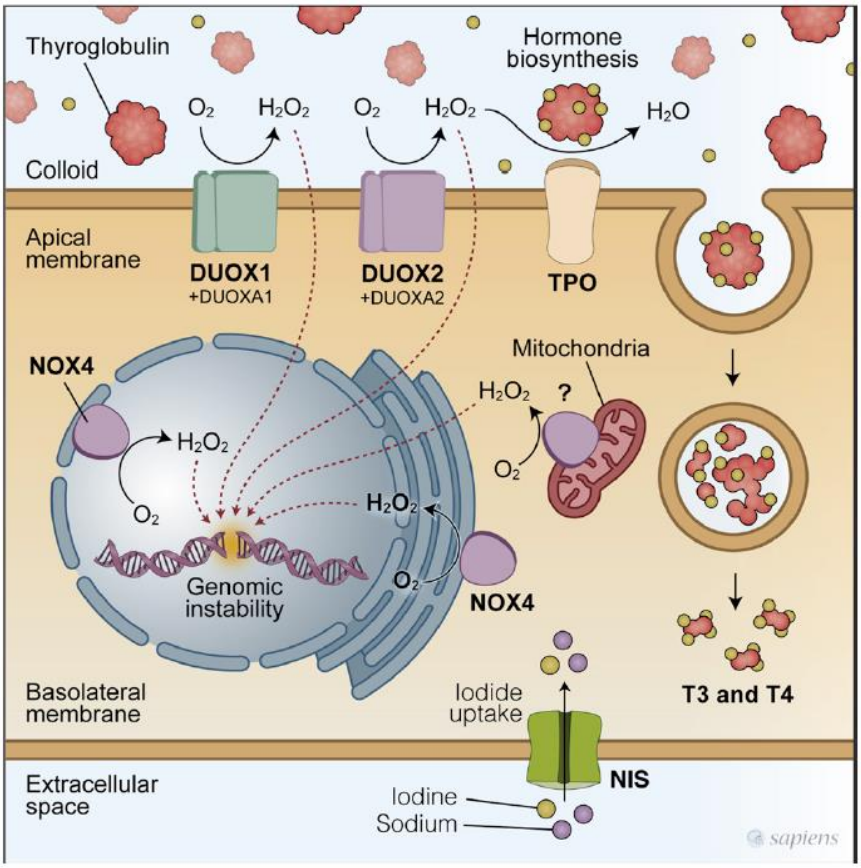

Figura 4. Papel das NADPH oxidases geradoras de H_2O_2 no dano ao DNA na tireoide. A tireoperoxidase utiliza o peróxido de hidrogênio, produzido pelo DUOX2, na síntese dos hormônios T3 e T4. Em um contexto patológico, o H_2O_2 produzido pelo DUOX2 pode difundir através da membrana apical do tireócito e atingir o núcleo, de forma direta. Outra via, seria através da produção de H_2O_2 pela NOX4, que pode ser encontrada no núcleo e retículo endoplasmático. A presença do H_2O_2 resulta em aumento do estresse oxidativo nuclear, promovendo danos ao DNA e instabilidade genômica. DUOX: oxidase dupla; H_2O_2: peróxido de hidrogênio; NOX4: NADPH oxidase 4; TPO: tireoperoxidase; T3: triiodotironina; T4: tiroxina (Fonte: Adaptado de Ameziane-El-Hassani et al., 2019).

A utilização de H_2O_2 para a síntese de hormônios, faz com que a tireoide seja facilmente exposta ao EO em situações em que há alteração na produção hormonal. O TSH, hormônio produzido pela hipófise, exerce papel estimulante sobre a síntese dos hormônios tireoidianos. Níveis elevados de TSH tem sido observado em casos de CT, sugerindo que a estimulação excessiva do TSH pode contribuir para o desencadeamento de EO na tireoide, e consequentemente, favorecendo o processo de carcinogênese local (STANLEY et al., 2016; ROWE et al., 2017).

Ao analisar a atividade das enzimas antioxidantes, Ramli et al., (2017) observaram que pacientes com doenças na tireoide apresentavam um sistema de defesa antioxidante mais baixo do que os controles, potencialmente predispondo-os

ao EO. Além disso, as diferentes condições patológicas da tireoide podem influenciar quanto aos níveis de antioxidante no soro e nas hemácias desses pacientes, implicando na capacidade variável de prevenção ao EO (RAMLI et al., 2017).

Devido à natureza oxidativa da síntese hormonal, a tireoide pode ser um dos órgãos mais vulneráveis aos efeitos deletérios do estresse oxidativo, portanto, um equilíbrio entre a produção e a eliminação de ERO é de extrema importância. Neste contexto, as enzimas antioxidantes apresentam relevante função, visto que a proteção dos tireócitos contra danos oxidativos é essencial para o seu bom funcionamento. A atividade dos antioxidantes pode se apresentar com padrões diferentes, visto que as células cancerígenas exibem alta variabilidade na modulação das atividades das enzimas antioxidantes, quando comparadas com as células normais (SZANTO et al., 2019). Stanley et al., (2016), observaram que os níveis das enzimas glutationa peroxidase e SOD estavam aumentadas em pacientes com CPT, enquanto a catalase permaneceu inalterada, quando comparadas a outras condições, como bócio e a TH (STANLEY et al., 2016).

A lipoperoxidação é um processo que consiste no ataque dos radicais livres sobre os ácidos graxos poli-insaturados presentes principalmente na membrana plasmática das células. Como consequência, pode ocorrer alteração nos processos de troca iônica, rompimento e liberação do conteúdo celular, culminando na morte da célula (SU et al., 2019). A lipoperoxidação é uma reação em cadeia, que consiste em 3 etapas: iniciação, propagação e terminação (FIG. 5). Inicialmente, o radical hidroxila (•OH) sequestra um átomo de hidrogênio do ácido graxo poli-insaturado da membrana, formando o radical lipídico (L•), que irá reagir com o oxigênio, na primeira etapa da propagação, resultando na formação do radical peroxila (LOO•), que por sua vez sequestra um novo átomo de hidrogênio de outro ácido graxo poli-insaturado, formando novamente o L•, na segunda etapa da propagação. A última etapa, terminação, corre quando os radicais L• e LOO• produzidos nas etapas anteriores, reagem entre si formando produtos não radicalares (FIG. 5) (AYALA et al., 2014; GASCHLER; STOCKWELL, 2017; SU et al., 2019).

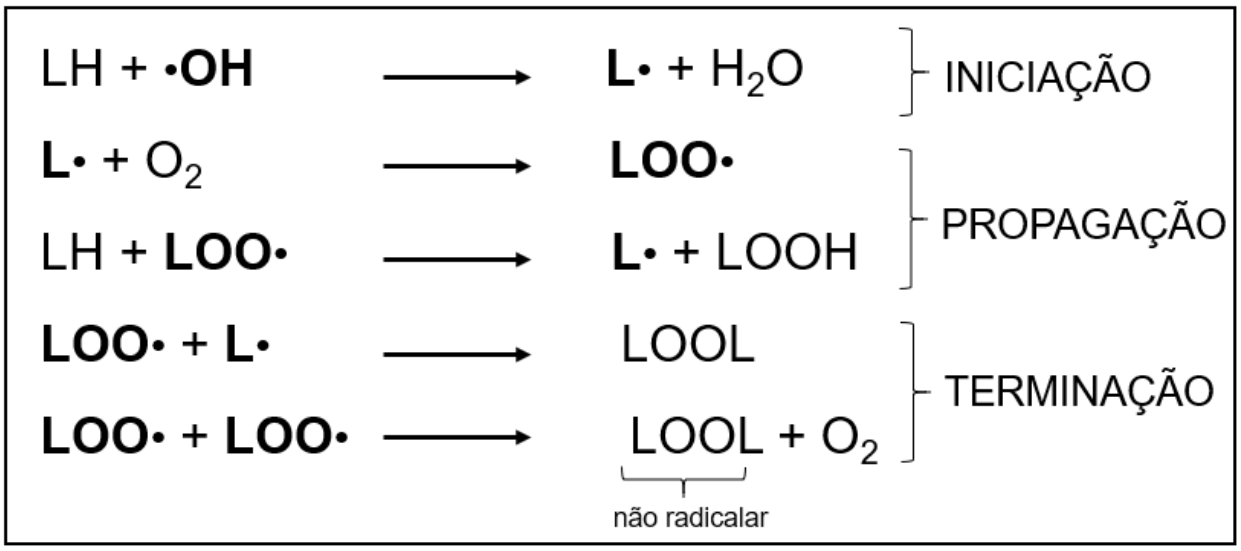

Figura 5. Etapas do processo de lipoperoxidação. (Fonte: próprio autor).

Como resultado da lipoperoxidação, há formação de aldeídos, como o Malondialdeído (MDA) e o 4-hidroxinonenal (4-HNE), considerados produtos finais da lipoperoxidação, amplamente utilizados, portanto, como biomarcadores de estresse oxidativo (AYALA et al., 2014; TSIKAS, 2017).

Portanto, analisar o comportamento das enzimas antioxidantes, bem como de biomarcadores de lipoperoxidação, para avaliar o estresse oxidativo em pacientes com CT é de grande importância.

3 OBJETIVO

Determinar os parâmetros de estresse oxidativo e marcadores inflamatórios sistêmicos de pacientes com carcinoma papilífero de tireoide e tireoidite de Hashimoto residentes no norte do Paraná.

3.1 OBJETIVOS ESPECÍFICOS

Determinar as características clínico patológicas e demográficas de pacientes com alterações benignas, TH, CPT e CPT+TH residentes no norte do Paraná.

Avaliar os parâmetros bioquímicos e imunológicos de pacientes com alterações benignas, TH, CPT e CPT+TH.

Avaliar e comparar os parâmetros de estresse oxidativo sistêmico de pacientes com alterações benignas, TH, CPT e CPT+TH, com indivíduos controles.

Mensurar parâmetros de estresse oxidativo no microambiente tumoral dos pacientes com as diferentes alterações na tireoide, especialmente dos pacientes com CPT e TH.

4 ARTIGO PARA PUBLICAÇÃO

O presente trabalho originou um artigo científico que será incluído nesta tese como Apêndice A. Todas as análises experimentais contidas foram realizadas na Universidade Estadual de Londrina, nos laboratórios de Patologia Molecular e no laboratório de Fisiopatologia e Radicais Livres.

O trabalho será submetido para publicação em revista científica e intitula-se “Role of papillary thyroid carcinoma patients with Hashimoto thyroiditis: evaluation of oxidative stress and inflammatory markers”. Além deste, um artigo de revisão foi publicado em 2020 na revista científica *Pathology - Research and Practice* com o título “Thyroid cancer and thyroid autoimmune disease: A review of molecular aspects and clinical outcomes”, e foi incluído nesta tese como Apêndice B.

5 CONSIDERAÇÕES FINAIS

Com os resultados obtidos neste trabalho, observamos que o estresse oxidativo, tanto a nível sistêmico quanto no microambiente tumoral, está mais evidente no grupo que apresenta apenas carcinoma papilífero de tireoide, quando comparado aos pacientes que possuem associação à tireoidite de Hashimoto. Além disso, os pacientes com CPT tendem a apresentar uma pior evolução da doença, visto que apresentam também marcação mais expressiva de marcadores de proliferação celular e angiogênese, o que pode favorecer ao desencadeamento de metástases, quando comparados aos pacientes com associação à TH.

REFERÊNCIAS

ABDULLAH, M. I., JUNIT, S. M., NG, K. L., JAYAPALAN, J. J., KARIKALAN, B., HASHIM, O. H. Papillary Thyroid Cancer: Genetic Alterations and Molecular Biomarker Investigations. **International journal of medical sciences**, v.16(3), p.450–460, 2019.

AKAMIZU, T., AMINO, N. Hashimoto's Thyroiditis. In K. R. Feingold (Eds.) et. al., **Endotext. MDText.com**, 2000.

AMEZIANE-EL-HASSANI, R., BUFFET, C., LEBOULLEUX, S., DUPUY, C. Oxidative stress in thyroid carcinomas: biological and clinical significance. **Endocrine-related cancer**, v.26(3), p.R131–R143, 2019.

AMEZIANE-EL-HASSANI, R., SCHLUMBERGER, M., DUPUY, C. NADPH oxidases: new actors in thyroid cancer?. Nature reviews. **Endocrinology**, v.12(8), p.485–494, 2016.

ANTONELLI, A., FERRARI, S. M., CORRADO, A., Di DOMENICANTONIO, A., FALLAHI, P. Autoimmune thyroid disorders. **Autoimmunity reviews**, v.14(2), p.174–180, 2015.

ASA, S. L. The Current Histologic Classification of Thyroid Cancer. **Endocrinol Metab Clin N Am,** v.48, p.1–22, 2019.

AYALA, A., MUÑOZ, M. F., ARGÜELLES, S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. **Oxidative medicine and cellular longevity**, 2014, 360438, 2014.

AZOUZI, N., CAILLOUX, J., CAZARIN, J. M., KNAUF, J. A., CRACCHIOLO, J., AL GHUZLAN, A., HARTL, D., POLAK, M., CARRÉ, A., El MZIBRI, M., FILALI-MALTOUF, A., AL BOUZIDI, A., SCHLUMBERGER, M., FAGIN, J. A., AMEZIANE-EL-HASSANI, R., DUPUY, C. NADPH Oxidase NOX4 Is a Critical Mediator of BRAFV600E-Induced Downregulation of the Sodium/Iodide Symporter in Papillary Thyroid Carcinomas. **Antioxidants & redox signaling**, v.26(15), p.864–877, 2017.

BRASIL. Ministério da Saúde - **INCA**, Rio de Janeiro; 2020.

BYCHKOV, A. World Health Organization (WHO) classification. PathologyOutlines.com website. http://www.pathologyoutlines.com/topic/thyroidwho.html. Acesso em: 01 abr. 2020.

CAMESELLE-TEIJEIRO, J.M., SOBRINHO-SIMÕES, M. Nueva clasificación de la OMS de los tumores tiroideos: una categorización pragmática de las neoplasias de la glándula tiroides. **Endocrinol Diabetes Nutr,** v.65(3), p.133-135, 2018.

CARVALHO, Denise P., DUPUY, Corinne. Thyroid hormone biosynthesis and release. **Molecular and cellular endocrinology**, v.458, p.6–15, 2017.

CATUREGLI, P., DE REMIGIS, A., ROSE, N.R. Hashimoto thyroiditis: clinical and

diagnostic criteria. **Autoimmunity Reviews**, v.13, p.391-397, 2014.

DAILEY, M.E., LINDSAY, S., SKAHEN, R. Relation of thyroid neoplasms to Hashimoto disease of the thyroid gland. **AMA Arch Surg**, v.70, p. 291–297, 1955.

DE PAIVA, R.C., GRØNHØJ, C., FELDT-RASMUSSEN, U., VON BUCHWALD, C. Association between Hashimoto's Thyroiditis and Thyroid Cancer in 64,628 Patients. **Frontiers in oncology**, v.7, p.53, 2017.

DEL RIO, P., MONTANA, C., COZZANI, F., ROSSINI, M., LODERER, T., DALL'AGLIO, E., CATALDO, S., MARINA, M., GRAZIANO, C. Is there a correlation between thyroiditis and thyroid cancer?. **Endocrine**, v.66(3), p.538–541, 2019.

DOBRINJA, C., MAKOVAC, P., PASTORICCHIO, M., CIPOLAT MIS, T., BERNARDI, S., FABRIS, B., PISCOPELLO, L., DE MANZINI, N. Coexistence of chronic lymphocytic thyroiditis and papillary thyroid carcinoma. Impact on presentation, management, and outcome. **International journal of surgery (London, England),** v.28 Suppl 1, p.S70–S74, 2016.

DVORKIN, S., ROBENSHTOK, E., HIRSCH, D., STRENOV, Y., SHIMON, I., BENBASSAT, C.A. Differentiated thyroid cancer is associated with less aggressive disease and better outcome in patients with coexisting Hashimotos thyroiditis. **J Clin Endocrinol Metab**. v.98(6), p.2409–2414, 2013.

EHLERS, M., JORDAN, A. L., FELDKAMP, J., FRITZEN, R., QUADBECK, B., HAASE, M., ALLELEIN, S., SCHMID, C., SCHOTT, M. Anti-Thyroperoxidase Antibody Levels >500 IU/ml Indicate a Moderately Increased Risk for Developing Hypothyroidism in Autoimmune Thyroiditis. **Hormone and metabolic research.** 48(10), 623–629, 2016.

FIGUEROA-VEJA, N., ALFONSO-PEREZ, M., BENEDICTO, I., SANCHEZ-MADRID, F., GONZALEZ-AMARO, R., MARAZUELA, M. Increased circulating pro-inflammatory cytokines and Th17 lymphocytes in Hashimoto's thyroiditis. J **Clin Endocrinol Metab**. v.95, p. 953-962, 2010.

GASCHLER, M. M., STOCKWELL, B. R. Lipid peroxidation in cell death. **Biochemical and biophysical research communications**. 482(3), 419–425, 2017.

GLOBAL CANCER OBSERVATORY. **Globocan 2020**. 2020.

GOBARU, M., ASHIDA, K., YOSHINOBU, S., NAGAYAMA, A., KABASHIMA, M., IWATA, S., HASUZAWA, N., TSURUTA, M., WADA, N., NAKAYAMA, H., MOTOMURA, S., TAJIRI, Y., NOMURA, M. Human Leukocyte Antigen (HLA) Subtype-Dependent Development of Myasthenia Gravis, Type-1 Diabetes Mellitus, and Hashimoto Disease: A Case Report of Autoimmune Polyendocrine Syndrome Type 3. **The American journal of case reports**, v. 20, p.1709–1714, 2019.

GLORIEUX, C., ZAMOCKY, M., SANDOVAL, J. M., VERRAX, J., CALDERON, P. B. Regulation of catalase expression in healthy and cancerous cells. **Free radical biology & medicine**, v.87, p.84–97, 2015.

GRACEFFA, G., PATRONE, R., VIENI, S., CAMPANELLA, S., CALAMIA, S., LAISE, I., CONZO, G., LATTERI, M., CIPOLLA, C. Association between Hashimoto's thyroiditis and papillary thyroid carcinoma: a retrospective analysis of 305 patients. **BMC endocrine disorders**, v.19(Suppl 1), p.26, 2019.

HAN, M. A., KIM, J. H. Diagnostic X-Ray Exposure and Thyroid Cancer Risk: Systematic Review and Meta-Analysis. **Thyroid:official journal of the American Thyroid Association**, v.28(2), p.220–228, 2018.

HOLMSTRÖM, K.M., FINKEL, T. 2014 Cellular mechanisms and physiological consequences of redox-dependent signalling. **Nature Reviews: Molecular Cell Biology,** v.15, p.411–421, 2014.

HU, J. Q., LEI, B. W., WEN, D., MA, B., ZHANG, T. T., LU, Z. W., WEI, W. J., WANG, Y. L., WANG, Y., LI, D. S., JI, Q. H., LIAO, T. IL-2 enhanced MHC class I expression in papillary thyroid cancer with Hashimoto's thyroiditis overcomes immune escape *in vitro*. **Journal of Cancer**, v.11(14), p.4250–4260, 2020.

JACKSON, D., HANDELSMAN, R. S., FARRÁ, J. C., LEW, J. I. Increased Incidental Thyroid Cancer in Patients With Subclinical Chronic Lymphocytic Thyroiditis. **The Journal of surgical research**, v.245, p.115–118, 2020.

JANKOVIC, B., Le, K. T., HERSHMAN, J. M. Clinical Review: Hashimoto's thyroiditis and papillary thyroid carcinoma: is there a correlation?. **The Journal of clinical endocrinology and metabolism**, v.*98*(2), p.474–482, 2013.

JEONG, J. S., KIM, H. K., LEE, C. R., PARK, S., PARK, J. H., Kang, S. W., JEONG, J. J., NAM, K. H., CHUNG, W. Y., & PARK, C. S. Coexistence of chronic lymphocytic thyroiditis with papillary thyroid carcinoma: clinical manifestation and prognostic outcome. **Journal of Korean medical science**, v.27(8), p.883–889, 2012.

KORNILUK, A., KOPER, O., KEMONA, H., & DYMICKA-PIEKARSKA, V. From inflammation to cancer. **Irish journal of medical science**, v.186(1), p.57–62, 2017.

KUMAR, V., ABBAS, A. K., FAUSTO, N., ASTER, J. C. **Robbins e Cotran: patologia - bases patológicas das doenças**. 9. ed. Rio de Janeiro, RJ: Elsevier, 2016.

LAI, X., XIA, Y., ZHANG, B., LI, J., & JIANG, Y. A meta-analysis of Hashimoto's thyroiditis and papillary thyroid carcinoma risk. **Oncotarget**, v.*8*(37), p.62414–62424, 2017.

LANDSKRON, G., DE LA FUENTE, M., THUWAJIT, P., THUWAJIT, C., HERMOSO, M. A. Chronic inflammation and cytokines in the tumor microenvironment. **Journal of immunology research**, v.2014, p.149185, 2014.

LIANG, J., ZENG, W., FANG, F., YU, T., ZHAO, Y., FAN, X., GUO, N., GAO, X. 2017. Clinical analysis of Hashimoto thyroiditis coexistent with papillary thyroid cancer in 1392 patients. **Acta otorhinolaryngologica itálica**. v.37, p.393-400, 2017.

LIONTIRIS, M. I., & MAZOKOPAKIS, E. E. A concise review of Hashimoto thyroiditis

(HT) and the importance of iodine, selenium, vitamin D and gluten on the autoimmunity and dietary management of HT patients.Points that need more investigation. **Hellenic journal of nuclear medicine**, v.20(1), p.51–56, 2017.

LU, S.C. Glutathione synthesis. **Biochim Biophys Acta**. v.1830(5), p.3143-3153, 2013.

LU, Z. W., HU, J. Q., LIU, W. L., WEN, D., WEI, W. J., WANG, Y. L., WANG, Y., LIAO, T., & JI, Q. H. IL-10 restores MHC class Ⅰ expression and interferes immunity in papillary thyroid cancer with Hashimoto's thyroiditis. **Endocrinology**, bqaa062. Advance online publication, 2020.

LUKOSZ, M., JAKOB, S., BUCHNER, N., ZSCHAUER, T.C., ALTSCHMIED, J., HAENDELER, J. 2010 Nuclear redox signaling. **Antioxidants and Redox Signaling** v.12, p.713–742, 2010.

MAROTTA, V., SCIAMMARELLA, C., CHIOFALO, M.G., GAMBARDELLA, C., BELLEVICINE, C., GRASSO, M., CONZO, G., DOCIMO, G., BOTTI, G., LOSITO, S., TRONCONE, G., DE PALMA, M., GIACOMELLI, L., PEZZULLO, L., COLAO, A., FAGGIANO, A. Hashimoto's thyroiditis predicts outcome in intrathyroidal papillary thyroid cancer. **Endocr Relat Cancer**. v.24(9), p.485-493, 2017.

MAZOKOPAKIS, E. E., TZORTZINIS, A. A., DALIERAKI-OTT, E. I., TSARTSALIS, A. N., SYROS, P. K., KAREFILAKIS, C. M., PAPADOMANOLAKI, M. G., STARAKIS, I. K. Coexistence of Hashimoto's thyroiditis with papillary thyroid carcinoma. A retrospective study. **Hormones**, v.9(4), p.312–317, 2010.

MOLNÁR, C., MOLNÁR, S., BEDEKOVICS, J., MOKÁNSZKI, A., GYŐRY, F., NAGY, E., MÉHES, G. Thyroid Carcinoma Coexisting with Hashimoto’s Thyreoiditis: Clinicopathological and Molecular Characteristics Clue up Pathogenesis. **Pathology & Oncology Research**, v. 25, p.1191–1197, 2019.

MOLONEY, J. N., COTTER, T. G. ROS signalling in the biology of cancer. **Seminars in cell & developmental biology**, v.80, p.50–64, 2018.

MURATA, M. Inflammation and câncer. **Environmental Health and Preventive Medicine**, v.23, p.50, 2018.

MUZZA, M., COLOMBO, C., CIRELLO, V., PERRINO, M., VICENTINI, L., & FUGAZZOLA, L. Oxidative stress and the subcellular localization of the telomerase reverse transcriptase (TERT) in papillary **thyroid cancer. Molecular and Cellular Endocrinology**, v.431, p.54–61, 2016.

NCCN Guidelines for Patients: **Thyroid Cancer**, 2020.

PAGANO, L., MELE, C., SAMA, M. T., ZAVATTARO, M., CAPUTO, M., DE MARCHI, L., PAGGI, S., PRODAM, F., AIMARETTI, G., MARZULLO, P. Thyroid cancer phenotypes in relation to inflammation and autoimmunity. **Frontiers in bioscience (Landmark edition),** v.23, p.2267–2282, 2018.

PANDAY, A., SAHOO, M. K., OSORIO, D., BATRA, S. NADPH oxidases: an overview from structure to innate immunity-associated pathologies. **Cellular & molecular immunology**, v.12(1), p.5–23, 2015.

PYZIK, A., GRYWALSKA, E., MATYJASZEK-MATUSZEK, B., & ROLIŃSKI, J. Immune disorders in Hashimoto's thyroiditis: what do we know so far?.**Journal of immunology research**, v.2015, p.1-8, 2015.

RABÊLO, L.A., SOUZA, V.N., FONSECA, L.J.S., SAMPAIO, W.O. Desbalanço redox: NADPH oxidase como um alvo terapêutico no manejo cardiovascular. **Arquivos Brasileiros de Cardiologia**, v.94(5), p.684-693, 2010.

RAGUSA, F., FALLAHI, P., ELIA, G., GONNELLA, D., PAPARO, S. R., GIUSTI, C., CHURILOV, L. P., FERRARI, S. M., ANTONELLI, A. Hashimotos' thyroiditis: Epidemiology, pathogenesis, clinic and therapy. **Best practice & research. Clinical endocrinology & metabolism**, v.33(6), p.101367, 2019.

RAMLI, N., MAT JUNIT, S., LEONG, N. K., RAZALI, N., JAYAPALAN, J. J., ABDUL AZIZ, A. Analyses of antioxidant status and nucleotide alterations in genes encoding antioxidant enzymes in patients with benign and malignant thyroid disorders. **PeerJ**, v.5, p.e3365, 2017.

RAYMAN, M.P. Multiple nutritional factors and thyroid disease, with particular reference to autoimmune thyroid disease. **Proc Nutr Soc**. v.78(1), p.34-44, 2019.

REUTER, S., GUPTA, S. C., CHATURVEDI, M. M., & AGGARWAL, B. B. Oxidative stress, inflammation, and cancer: how are they linked?. **Free radical biology & medicine**, v.49(11), p.1603–1616, 2010.

ROSEN, R.D., SAPRA, A. **Embryology, Thyroid**. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Disponível em: https://www.ncbi.nlm.nih.gov/books/NBK551611/

ROWE, M. E., OSORIO, M., LIKHTEROV, I., URKEN, M. L. Evaluation of ultrasound reporting for thyroid cancer diagnosis and surveillance. **Head & neck**, v.39(9), p.1756–1760, 2017.

SIES, H. Oxidative stress: a concept in redox biology and medicine. **Redox biology,** v.4, p.180–183, 2015.

SONG, E., JEON, M. J., PARK, S., KIM, M., OH, H. S., SONG, D. E., Kim, W. G., KIM, W. B., SHONG, Y. K., KIM, T. Y. Influence of coexistent Hashimoto's thyroiditis on the extent of cervical lymph node dissection and prognosis in papillary thyroid carcinoma. **Clinical endocrinology**, v.88(1), p.123–128, 2018.

STANCIU, A.E., SERDAREVIC, N., HURDUC, A.E., STANCIU, M.M. IL-4, IL-10 and high sensitivity-CRP as potential serum biomarkers of persistent/recurrent disease in papillary thyroid carcinoma with/without Hashimoto's thyroiditis. **Scandinavian journal of clinical and laboratory investigation**. v.75(7), p.539-48, 2015.

STANLEY, J. A., NEELAMOHAN, R., SUTHAGAR, E., VENGATESH, G., JAYAKUMAR, J., CHANDRASEKARAN, M., BANU, S. K., ARULDHAS, M. M. Lipid peroxidation and antioxidants status in human malignant and non-malignant thyroid tumours. **Human & experimental toxicology**, v.35(6), p.585–597, 2016.

SU, L. J., ZHANG, J. H., GOMEZ, H., MURUGAN, R., HONG, X., XU, D., JIANG, F., PENG, Z. Y. Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. **Oxidative medicine and cellular longevity**. 5080843, 2019.

SULAIEVA, O., SELEZNIOV, O., SHAPOCHKA, D., BELEMETS, N., NECHAY, O., CHERESHNEVA, Y., TSOMARTOVA, D., IVANOVA, M. Hashimoto's thyroiditis attenuates progression of papillary thyroid carcinoma: deciphering immunological links. **Heliyon**, v.6(1), p.e03077, 2020.

SUZUKI, K., SAENKO, V., YAMASHITA, S., MITSUTAKE, N. Radiation-Induced Thyroid Cancers: Overview of Molecular Signatures. **Cancers**, v.11(9), p. 1290, 2019.

SZANTO, I., PUSZTASZERI, M., MAVROMATI, M. H2O2 Metabolism in Normal Thyroid Cells and in Thyroid Tumorigenesis: Focus on NADPH Oxidases. **Antioxidants (Basel, Switzerland),** v.8(5), p.126, 2019.

TAYLOR, P. N., ALBRECHT, D., SCHOLZ, A., GUTIERREZ-BUEY, G., LAZARUS, J. H., DAYAN, C. M., OKOSIEME, O. E. Global epidemiology of hyperthyroidism and hypothyroidism. **Nature reviews. Endocrinology**, v.14(5), p.301–316, 2018.

THUN, M. J., LINET, M.S., CERHAN, J.R., HAIMAN, C.A., SCHOTTENFELD, D. **Cancer epidemiology and prevention**. 4th ed. New York: Oxford University Press, 2017.

TSIKAS, D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. **Analytical biochemistry**, 524, 13–30, 2017.

UHLIAROVA, B., HAJTMAN, A. Hashimoto's thyroiditis - an independent risk factor for papillary carcinoma. **Braz J Otorhinolaryngol.** v.84(6), p.729–735, 2018.

VACCARELLA, S., FRANCESCHI, S., BRAY, F., WILD, C. P., PLUMMER, M., DAL MASO, L. Worldwide Thyroid-Cancer Epidemic? The Increasing Impact of Overdiagnosis. **The New England journal of medicine**, v.375(7), p.614–617, 2016.

VEAL, E.A., DAY, A.M., MORGAN, B.A. Hydrogen peroxide sensing and signaling. **Molecular Cell** v.26, p.1–14, 2007.

Virchow, R. **Cellular pathology as based upon physiological and pathological histology**. J. B. Lippincott, Philadelphia, 1863.

WANG, Y., BRANICKY, R., NOË, A., HEKIMI, S. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. **The Journal of cell biology**, v.217(6), p.1915–1928, 2018.

WARREN, S., MEISSNER, W.A., **Tumors of the thyroid gland. Atlas of tumor pathology,** Series 1, Fascicle 14. Washington, DC: Armed Forces Institute of Pathology; 1953.

WEYEMI, U., CAILLOU, B., TALBOT, M., AMEZIANE-EL-HASSANI, R., LACROIX, L., LAGENT-CHEVALLIER, O., AL GHUZLAN, A., ROOS, D., BIDART, J. M., VIRION, A., SCHLUMBERGER, M., DUPUY, C. Intracellular expression of reactive oxygen species-generating NADPH oxidase NOX4 in normal and cancer thyroid tissues. **Endocrine-related cancer**, v.17(1), p.27–37, 2010.

ZENG, R., LYU, Y., ZHANG, G., SHOU, T., WANG, K., NIU, H., YAN, X. Positive effect of RORγt on the prognosis of thyroid papillary carcinoma patients combined with Hashimoto's thyroiditis. **Am J Transl Res.** v.10(10), p.3011-3024, 2018.

ZHANG, Y., CHEN, Y., HUANG, H., SANDLER, J., DAI, M., MA, S., UDELSMAN, R. Diagnostic radiography exposure increases the risk for thyroid microcarcinoma: a population-based case-control study. **European journal of cancer prevention: the official journal of the European Cancer Prevention Organisation (ECP)**, v.24(5), p.439–446, 2015.

ZHANG, Y., DAI, J., WU, T., YANG, N., YIN, Z. The study of the coexistence of Hashimoto's thyroiditis with papillary thyroid carcinoma. **J Cancer Res Clin Oncol,** v.140(6), p.1021–1026, 2014.

ZIVANCEVIC-SIMONOVIC, S., MIHALJEVICIVANA, O., MAJSTOROVIC, I., POPOVIC, S., MARKOVIC, S., MILOSEVIC-DJORDJEVIC, O., JOVANOVIC, Z., MIJATOVIC-TEODOROVIC, L., MIHAJLOVIC, D., COLIC, M. Cytokine production in patients with papillary thyroid cancer and associated autoimmune Hashimoto thyroiditis. **Cancer Immunology, Immunotherapy**. v.64(8), p.1011–1019, 2015.

APÊNDICES

APÊNDICE A – Role of papillary thyroid carcinoma patients with Hashimoto thyroiditis: Evaluation of oxidative stress and inflammatory markers.

Role of papillary thyroid carcinoma patients with Hashimoto thyroiditis: Evaluation of oxidative stress and inflammatory markers.

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Abstract

Introduction and objective: Thyroid cancer is the most common endocrine malignancy. Among the subtypes, papillary thyroid carcinoma (PTC) is the most frequent. Hashimoto's thyroiditis (HT), an autoimmune disease, can affect the thyroid gland. Despite several research aimed to understand the relationship between HT and PTC, the precise involved mechanisms are under debate yet. Oxidative stress (OE) and inflammatory environment participate in the development of several types of cancer, thus the objective of this work was to determine the participation of OE and systemic inflammatory markers in patients with PTC and HT. **Methods:** Samples were collected from 115 patients, divided into four groups with thyroid changes: BENIGN (n= 63), PTC (n= 27), HT (n= 15) and PTC+HT (n= 10). Sixty-three healthy individuals participate as control. To quantify the EO, the antioxidant enzymes activity of Superoxide dismutase (SOD), Catalase (CAT) and the reduced form of Glutathione (GSH) were evaluated; markers of lipid peroxidation, Malondialdehyde (MDA) and lipid hydroperoxide were also measured. The inflammatory markers IL-10, TGF-β1 and TNF-α were analyzed. For the tumor microenvironment, immunohistochemistry was performed to mark indicators of OE (3-nitrotyrosine and 4-hydroxynonenal), cell proliferation (Ki-67) and angiogenesis (VEGF). **Results:** CAT and SOD activities were higher in the groups with thyroid disorders compared to the control group. The GSH concentration was decreased in the BENIGN and PTC groups, compared to control group. When only the PTC and PTC+HT groups were analyzed, no significant differences were found in the antioxidant defense. Regarding inflammatory markers, no significant differences were found among the PTC and PTC+HT groups. The ability to contain the induced lipid peroxidation was lower and a high level of plasma MDA was observed in the PTC group. All the immunohistochemistry markers had higher scores in PTC group, compared to PTC+HT. **Conclusion:** Therefore, it was possible to observe the more pronounced presence of OE and the greatest activity of the cell proliferation and angiogenesis markers, factors that contribute to tumor progression and metastasis in the PTC group than PTC+HT.

Keywords: Papillary thyroid carcinoma. Hashimoto's thyroiditis. Oxidative stress.

Introduction

Thyroid cancer is the most common endocrine malignancy in the population, accounting for 3.4% of all cancers diagnosed annually. In Brazil, approximately 12 thousand new cases/year of the 2020-2022 are expected [1]. Among the differentiated subtypes of thyroid cancer, papillary thyroid carcinoma (PTC) is the most frequent, corresponding to 80% of the total cases [2,3]. In addition to malignant neoplasms, other changes can develop in the thyroid, such as autoimmune diseases. Discovered by Hakaru Hashimoto in 1912, Hashimoto's thyroiditis (HT) is the main autoimmune disease that affects the thyroid gland, being responsible for triggering an intense inflammatory infiltrate and cellular destruction, resulting in reduced production of thyroid hormones, with consequent hypothyroidism [4,5]. HT is more prevalent in women and its worldwide annual incidence is estimated to be 3,5-5 cases per 1000 individuals [4].

The association between thyroid cancer and HT has been addressed when Dailey et al., (1955) first suggested this possible correlation [6]. Since then, several studies have been carried out to unraveling this issue, and to elucidate the main mechanisms that make this association possible [3,7,8].

Although it can be considered as a risk factor for the triggering of thyroid carcinogenesis [9], HT has also been related to a better prognosis for patients who have it in association with thyroid cancer, especially PTC. Reports in the literature show that patients with PTC with HT have significantly reduced rates of lymphatic metastases and less tumor aggressiveness [3,10,11]. However, the exact mechanism behind it is still not precisely known.

During the production of thyroid hormones, the use of hydrogen peroxide is necessary, therefore, the generation of reactive oxygen species (ROS) is part of the

normal functioning of the gland; this event would make the thyroid a vulnerable organ and frequently exposed to the damage caused by oxidative stress (OE) [12,13]. Under normal conditions, ROS are neutralized by the antioxidant defense system. In situations where there is an imbalance among the generation of ROS and its neutralization, the OE can damage the gland [14].

Although the molecular mechanism remains unclear, the participation of OE, as well as the inflammatory markers are also still unknown. Therefore, the objective of this work was to evaluate the parameters related to OE and systemic inflammatory markers of patients with PTC combined HT and identify the possible correlation between these diseases.

Methods and Materials

Study population and design

Ethical approval for this study was obtained by the Research Ethics Committee of the *Universidade Estadual of Londrina* (REC-UEL process n. 2.793.785). Consents were obtained from all the patients and controls before proceeding with the collection of samples.

A total of 115 patients with thyroid alteration were recruited from *Hospital Universitario of the Universidade Estadual of Londrina (HU-UEL), Hospital do Coração of Londrina*, *Irmandade Santa Casa of Londrina* and *Hospital Norte Paranaense of Arapongas*, from August 2018 to December 2020. Patients were classified into four groups: Benign thyroid disorders, such as nodular colloid goiter and follicular adenoma (BENIGN), Papillary thyroid carcinoma (PTC), Hashimoto's thyroiditis (HT) and patients with PTC and HT in association (PTC+HT). Patients with hepatic, cardiac or renal dysfunction and using antioxidant therapy, were excluded from the study. The

blood samples were taken prior to surgery, for OE parameters and for inflammatory markers analysis. A sample of thyroid tissue was collected after thyroidectomy, for immunohistochemical analysis. A questionnaire was applied to obtain the pathological clinical characteristics of the patients. In addition, a total of 63 healthy individuals from a database in our laboratory served as a control group. All the samples from patients and healthy individuals were processed and analyzed at the Molecular Pathology Laboratory of the *Universidade Estadual of Londrina*.

Blood samples

Blood was drawn by venipuncture from all the participants. Blood samples were collected in heparin tubes, to obtain plasma and erythrocytes, and in a dry tube to obtain serum. The whole blood samples were centrifuged at 3500rpm for 5 minutes, separated in serum and plasma, and stored at -20ºC. The erythrocytes were washed and centrifuged three times with 1ml of 0.9% sodium chloride solution for 5 minutes at 4°C before storage in Alsever buffer at 4°C, for a maximum of two weeks after collection.

The biochemical (TSH and free T4) and immunological (TPOAb and TGAb) parameters were obtained from results of preoperative exams of the patients, carried out by accredited laboratories.

Systemic oxidative stress parameters

Lipid peroxidation was evaluated by chemiluminescence (CL) induced via tert-butyl hydroperoxide [15]. Erythrocyte were diluted in iced 10mM phosphate monobasic buffer, pH 7.4 (0.9% NaCl). The chemiluminescent reaction was initiated by the

addition of tert-butyl and curves were obtained in a Lumat^3 LB9508 luminometer (Berthold Technologies GmbH & Co.KG).

The amount of malondialdehyde (MDA) was determined in blood plasma by high performance liquid chromatography, readings are taken at 535nm during an 11-minute run, and the results are expressed in nM of MDA, as previously described [16].

Catalase (CAT) activity was determined in erythrocyte as described previously [17]. The enzymatic kinetics was reading at a wavelength of 240nm. Superoxide dismutase (SOD) activity in erythrocytes was analyzed based on the autoxidation of pyrogallol method, as previously described [18], with kinetic reading at 420nm, at 37º Celsius. Reduced glutathione (GSH) levels were determined in erythrocytes and was read at 412nm, as previously described [19]. These methods were normalized against total protein concentrations identified, as described before [20,21].

Inflammatory markers analysis

The systemic inflammatory markers were analyzed in the plasma samples and was measured using an Enzyme-Linked Immunosorbent Assay (ELISA) kit, for Interleukin-10 (IL-10) (eBioscience Ref: 88-7106-88), Tumor Necrosis Factor Alpha (TNF-α) (eBioscience Ref: 88-7346-88) and Transforming Growth Factor Beta (TGF-β1) (eBioscience Ref: 88-50390-88), according to the manufacturer's instructions.

Immunohistochemistry

Immunohistochemical analysis was performed using nodular tissue samples from all groups of patients, collected after total or partial thyroidectomy. A normal tissue sample, located distant from the nodule, was also taken, and used as control. The paraffin-embedded thyroid samples were submitted to immunohistochemical analysis.

Briefly, 5µm-thick sections mounted on silane-coated slides (Sigma Aldrich St. Louis, MO, USA) were deparaffinized, rehydrated, immersed in 10mmol x L^{-1} citrate buffer, pH 6.0, and submitted to heat-induced epitope retrieval using a vapor lock for 20 minutes. The slides were rinsed with phosphate-buffered saline (PBS) and immersed in 3% hydrogen peroxide for 30 minutes to block endogenous peroxidase. Non-specific protein binding was blocked with bovine serum albumin (BSA) for 30min. Sections were incubated with primary antibodies specific for 3-nitrotyrosine (3-NT) (Santa Cruz Biotechnology, Santa Cruz, CA, USA, dilution 1:100), 4-Hydroxynonenal (4-HNE) (Bioss USA Antibodies, dilution 1:100), vascular endothelial growth factor (VEGF) (Abcam, USA, dilution 1:200) and Ki-67 (Novus Biologicals, USA, dilution 1:100) for 2 hours at 37°C in a humid chamber. Following washes in PBS, an HRP-conjugated secondary antibody (#7074S, Cell Signaling Technology) were incubated for 1 hour. The substrate 3, 3′ diaminobenzidine tetrahydrochloride (DAB) was used to produce a dark brown/black precipitate. The slides were counterstained with Harris hematoxylin, dehydrated, and mounted with balm. As a negative control, the cuts were incubated with PBS. For statistical purposes, the samples were scored for total area (3-NT, 4-HNE and VEGF) and percentage of marked nuclei (Ki-67) as follows: 0, negative (0%); 1, weak staining (10-25% per field); 2, moderate staining (26-50% per field); 3, strong staining (> 50% per field). A blinded analysis was performed by two observers.

Statistical analysis

The statistical analysis was performed using the software STATA (version 13; Stata Corp., Texas, USA) and Prism 8.4 (GraphPad Software, San Diego, CA, USA). Continuous variables were described as means and their respective standard deviations (SD). Categorical variables are shown as number and percentage and

compared by Fisher's exact test. The Shapiro-Wilk test verified the normality of the data. Parametric data were analyzed by Student's t-test for two groups (PTC vs PTC+HT), or one-way ANOVA followed by Tukey's post-hoc test for analyzes of more than two groups. Non-parametric data were analyzed using the Kruskal-Wallis test and Dunn's post-hoc test and expressed by violin plot. Differences were considered statistically significant when $p<0.05$.

Results

According to the result of the histopathological analysis, patients were divided into four groups: 63 (54.8%) BENIGN, 27 (23.5%) with PTC; 15 (13.0%) patients with HT and 10 (8.7%) patients with PTC+HT. Although more female (87.3%) than male patients were included in our study, no significant differences were observed in the sex ratio. Some demographic data and clinical characteristics such as age, body mass index, smoking, type 2 diabetes, hypertension, family history of thyroid disease, family history of cancer, personal history of cancer and the presence of other concomitant diseases were analyzed, and did not show statistically significant difference between groups, as shown in table 1.

Data more specifically related to thyroid diseases, such as tumor size, presence of single or multiple nodules, primary lesion and presence of metastases were evaluated between the groups. As it can be seen in table 2, no statistically significant results were found. Regarding biochemical and immunological parameters, the dosage of thyroid-stimulating hormone (TSH), free thyroxine (fT4), the anti-thyroid antibodies thyroglobulin (TGAb) and thyroid peroxidase antibody (TPOAb) was evaluated. Circulating TGAb levels were significantly normal in patients (p=0.010). As for the other parameters, no differences were observed (table 3).

Antioxidant response and oxidative stress parameters

The results of the antioxidants systemic are shown in figure 1. The catalase activity (Fig.1a) is significantly higher in the PTC ($p=0.023$) and PTC+HT ($p<0.001$) groups, when compared to the control. The activity of SOD enzyme (Fig. 1b) was significantly higher in all groups, compared to the control group ($p<0.0001$). The levels of the non-enzymatic antioxidant GSH (Fig. 1c) were reduced in the BENIGN ($p=0.001$) and PTC ($p=0.004$) groups compared to the control group. To observe the possible interference of HT in patients with thyroid cancer, we made a comparative analysis among PTC vs PTC+HT, for all analyzed parameters, as will be presented below. We also analyzed the isolated participation of HT, but no relevant results were observed (data not shown). No significant differences were observed in the activity of the enzyme catalase (Fig. 1d), SOD (Fig. 1e), and in the concentration of GSH (Fig. 1f) among PTC and PTC+HT groups.

Lipid peroxidation induced by tert-butyl allows an analysis of the damage induced by ROS in cells, more specifically in their membranes. The speed of rising of the curve to the maximum emission peak was higher in the BENIGN and PTC group, compared to the control group ($p=0.0351$ and $p=0.0008$, respectively) (Fig. 2a). The levels of MDA (Fig. 2b) were significantly increased in the BENIGN ($p<0.0001$), PTC ($p<0.0001$), HT ($p<0.0001$) and PTC+HT ($p=0.016$) groups, compared to control group. When the speed of rising of the curve was analyzed emphasizing the PTC and PTC+HT group, it was significantly higher in the PTC group ($p=0.043$) (Fig. 2c). As for MDA levels (Fig. 2d), the PTC group obtained a higher value than the PTC+HT group ($p=0.010$).

Systemic inflammatory markers

The results of the systemic inflammatory markers were analyzed (Fig. 3), and the results of IL-10 are shown (Fig. 3a). No statistically significant differences were found among groups. As to the results of TGF-β1 (Fig. 3b), it is possible to observe that the BENIGN ($p<0.0004$), PTC ($p=0.034$) and PTC+HT ($p=0.001$) groups showed significantly lower results compared to control group. About the proinflammatory cytokine TNF-α (Fig. 3c), no statistically significant differences were found among groups. When the cytokines IL-10 (Fig. 3d), TGF-β1 (Fig. 3e) and TNF-α (Fig. 3f) were analyzed, no significant difference was observed among PTC and PTC+HT groups.

Immunohistochemistry analysis

A representative figure from the immunohistochemistry analysis was added (Fig. 4) and the results obtained from the analyzes are shown in figures 5 and 6. About the results of OE markers (Fig. 5), the 3-NT score is higher in the groups PTC ($p<0.0001$), HT ($p<0.0001$), and PTC+HT ($p<0.0001$), when compared to normal tissue (Fig. 5a). As for 4-HNE, the score of PTC and PTC+HT groups were higher than normal tissue ($p<0.0001$ and $p<0.0001$, respectively) (Fig. 5b). When we analyzed only PTC and PTC+HT groups, we observed higher score in PTC compared to the PTC+HT group for 3-NT ($p=0.012$) (Fig. 5c) and for 4-HNE ($p=0.043$) (Fig. 5d). About the markers of cell proliferation and angiogenesis (Fig. 6), the ki-67 score was higher in PTC and PTC+HT groups, compared to normal tissue ($p<0.0001$ and $p=0.001$, respectively) (Fig. 6a). For VEGF, the normal tissue score was lower than all other groups (Fig. 6b). The analysis restricted to PTC and PTC+HT groups, PTC group had a higher score than the PTC+HT group for ki-67 ($p=0.001$) (Fig. 6c) and VEGF ($p=0.036$) (Fig. 6d).

Discussion

Studies to better understand the contribution of HT to PTC and its relationship are still scarce, as well as the participation of OE [14,22,23]. It has also been shown that different populations may present different patterns of behavior of thyroid hormones, according to the region in which they live, eating habits, especially related to iodine intake, among other factors [24,25,26].

In this study, we evaluated the antioxidant defense and lipid peroxidation markers in the blood and OE markers in the tumor microenvironment. Also, systemic inflammatory markers, in the blood of patients with alterations in the thyroid gland, recruited from hospitals in the Northern region of Paraná, Brazil.

Reactive oxygen species are produced continuously in our body during metabolic processes, such as in the mitochondrial respiratory chain [27,28]. Among the main ROS, we can mention the superoxide anion and hydrogen peroxide, which are metabolized by the enzymes SOD and CAT, respectively. These enzymes are part of our antioxidant defense system, to minimize the reactivity of ROS [29,30]. The increase in CAT and SOD activities, as we observed, maybe due to the positive regulation in the synthesis of these enzymes, as a protective response to oxidative stress. Ramli et al., (2017), suggested that the increase in serum SOD and CAT activities in some groups of thyroid disorders indicates an increase in the production of superoxide anion and hydrogen peroxide, respectively [14]. Stanley et al., (2016) observed that SOD, GPx, and CAT levels were consistently elevated in all malignant thyroid tumor tissues, although they did not show any variation in multi-nodular goiter tissues [22].

Disorders in GSH homeostasis are involved in the etiology and progression of many human diseases, including cancer [31,32]. GSH is being consumed due to OE

in patients with PTC, consequently decreasing its systemic level, corroborating with a previous study [24]. GSH is one of the most important intracellular antioxidants and this reduction can lead to increased susceptibility to OE, implicated in the progression of thyroid cancer, as we can find in the literature, that reduced GSH levels can be found in certain types of cancer, favoring its progression [32,33].

The action of free radicals on phospholipid membranes characterizes the lipid peroxidation process, with consequent destruction of the membrane structure and loss of metabolic exchanges, which can culminate in cell death [35]. As we have shown, patients with PTC have a lower ability to contain induced lipid peroxidation, potentially predisposing them to OE. As also observed [14], the antioxidant capacity of thyroid cancer patients is more limited. Considering that the smaller number of antioxidants available in the sample, less will be the ability to contain the beginning of lipid peroxidation, consequently, higher elevation speed of the lipid peroxidation curve. Thus, we observed for the first time that specifically patients with PTC without HT have the less oxidizing capacity to contain OE, than patients with PTC+HT.

In our MDA analysis, which is a product formed at the end of the lipid peroxidation process [36], reveals that patients with thyroid changes have higher levels of lipid peroxidation, which proves the vulnerability to the toxic effects of ROS, as also observed by Erdamar et al., (2010) [34,37]. In addition, the formation of MDA is more intense in our patients with PTC without association with HT. The increase in lipid peroxidation may be due to the inability of peroxide-sequestering enzymes to keep up with the high rate of peroxides being produced [22].

Taking together the blood analysis of antioxidants and lipid peroxidation markers, we affirm that there is an imbalance between the antioxidant defense system and the pro-oxidants agents in patients with thyroid cancer and that patients with PTC

have more relevant systemic OE than patients with HT associated, this is an important point that we found in this study.

Inflammatory microenvironments play a key role in the pathogenesis of thyroid cancer [38], thus we conducted an analysis of blood pro and anti-inflammatory cytokines to better understand the inflammatory profile of patients with different thyroid alterations. Information about the influence of the anti-inflammatory cytokine IL-10 on thyroid disorders is scarce and controversial yet. In our study, we have not found conclusive results about its influence in patients with thyroid cancer. Cunha et al., (2015) observed a positive correlation between the positivity of IL-10 and the size of tumor, suggesting that the aggressiveness of the tumor may be associated with the expression of IL-10 [39]. In contrast, IL-10 successfully recovers MHC class I expression and increases tumor antigenicity in PTC with concomitant HT, as observed by Lu et al., (2020) [40]. Therefore, further studies are needed to better clarify the participation of IL-10 in thyroid cancer.

TGF-β1 has a dual function and pleiotropic effects and can act as a tumor suppressor or an oncogenic factor. In normal cells, TGF-β1 inhibits cell proliferation and stimulates cell differentiation. Conversely, in cancer cells, TGF-β1 promotes tumor progression and metastasis [41,42]. The relationship between PTC+HT and TGF-β1, especially at systemic levels, is briefly discussed. As our results, Bertol et al., (2020) also observed reduced plasma levels of TGF-β1, suggesting this to be a potential factor associated with susceptibility to the development of PTC [43]. In tissue, the higher expression of TGF-β1 is related to a worse prognosis for patients with PTC [44,45], and in cell culture, TGF-β1 can promote the invasion and migration of PTC cells [46]. Given the variety of inconclusive results found, therefore, further analysis is necessary to better understand this relationship.

TNF-α is a cell signaling protein involved in the systemic inflammatory response, produced mainly by activated macrophages. It can be secreted by $CD4^+$ lymphocytes, NK cells, neutrophils, mast cells and other cells [47]. Although we did not observe statistically significant results in the analysis of TNF-α, Zhang et al., (2019) observed its high expression in serum of patients with PTC+HT [48,49], and Bertol et al., (2020) correlated the systemic levels of TNF-α with a worse prognosis presented by the patients with PTC [43]. In cell culture, Coperchini et al., (2015) show that TNF-α does enhance the expression by thyroid cancer cells of CCR6, a chemokine receptor with a specific and well-established role in the development of cancer metastasis and that this resulting in more aggressive tumor behavior [50].

Together, our results show a higher incidence of OE, especially in PTC group when compared to the PTC associated with HT, corroborating with other studies [11,12,22] and indicates a possible worse prognosis in patients without the association with HT. However, to better understand this relationship, we analyzed the tumor microenvironment of these groups by immunohistochemistry.

The generation of 3-NT, the product of protein tyrosine nitration, is associated with OE conditions [51] and its high expression can be observed in cancer conditions [52], as it could be evidenced in our results. Patel et al., (2002) observed the highest expression of 3-NT in PTC, when analyzing thyroid tumors from children and adolescents [53]. But the correlation among 3-NT and thyroid cancer, little has been explored in the literature, specifically in relation to PTC associated with HT, highlighting the importance of our result and the need for further studies on this matter. As for 4-HNE, it is an aldehyde resulting from lipid peroxidation, widely used for the analysis of OE [54] and can be considered an important factor in carcinogenesis due to its ability to bind covalently to DNA [55]. About the correlation with thyroid cancer, few reports

are found, and when we observe its high expression in patients with PTC without HT, evidencing the participation of OE in these patients, make our results being the first to get this information.

Ki-67 is a well-known growth and cell proliferation marker, widely used to assess tumor progression [56], and its high expression is found in cancer [57,58]. Corroborating with our results, Zhou et al., (2015) observed a higher expression of Ki-67 in PTC, and correlates this to the major size of the tumor, invasion and metastasis of cervical lymph node. This suggests Ki-67 as an important marker to evaluate clinical progress and estimate the prognosis of these patients [59].

During tumor development and progression, pro-angiogenic factors, such as VEGF, are pathologically increased [60]. As we observed, patients with PTC without HT have a high activity of this marker. Previous studies also have shown that VEGF activity is higher in thyroid cancer and this overexpression has a direct link with the tumor prognosis [61,62], suggesting VEGF as an important factor for tumor growth and development, given the necessity for new vascularization for the growth of malignant tumors [60].

Conclusion

In summary, the results presented we can indicate the more pronounced presence of OE, both systemic and in the tumor microenvironment of patients with PTC without HT. In addition, these patients tend to have a worse disease progression, since there is a greater activity of OE markers, cell proliferation, and angiogenesis, which may be more favorable to the triggering of metastases.

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References

[1] BRASIL. Ministério da Saúde - INCA, Rio de Janeiro; 2020.

[2] Asa SL. The Current Histologic Classification of Thyroid Cancer. Endocrinol Metab Clin North Am. 2019 Mar;48(1):1-22. doi: 10.1016/j.ecl.2018.10.001. PMID: 30717895.

[3] Molnár C, Molnár S, Bedekovics J, Mokánszki A, Győry F, Nagy E, Méhes G. Thyroid Carcinoma Coexisting with Hashimoto's Thyreoiditis: Clinicopathological and Molecular Characteristics Clue up Pathogenesis. Pathol Oncol Res. 2019 Jul;25(3):1191-1197. doi: 10.1007/s12253-019-00580-w. Epub 2019 Jan 21. PMID: 30666518; PMCID: PMC6614143.

[4] Ragusa F, Fallahi P, Elia G, Gonnella D, Paparo SR, Giusti C, Churilov LP, Ferrari SM, Antonelli A. Hashimotos' thyroiditis: Epidemiology, pathogenesis, clinic and therapy. Best Pract Res Clin Endocrinol Metab. 2019 Dec;33(6):101367. doi: 10.1016/j.beem.2019.101367. Epub 2019 Nov 26. PMID: 31812326.

[5] Rayman, M.P. Multiple nutritional factors and thyroid disease, with particular reference to autoimmune thyroid disease. Proc Nutr Soc. v.78(1), p.34-44, 2019. doi:10.1017/S0029665118001192

[6] Dailey ME, Lindsay S, Skahen R. Relation of thyroid neoplasms to Hashimoto disease of the thyroid gland. AMA Arch Surg. 1955 Feb;70(2):291-7. doi: 10.1001/archsurg.1955.01270080137023. PMID: 13227748.

[7] Pagano L, Mele C, Sama MT, Zavattaro M, Caputo M, De Marchi L, Paggi S, Prodam F, Aimaretti G, Marzullo P. Thyroid cancer phenotypes in relation to inflammation and autoimmunity. Front Biosci (Landmark Ed). 2018 Jun 1;23:2267-2282. PMID: 29772561.

[8] Sulaieva O, Selezniov O, Shapochka D, Belemets N, Nechay O, Chereshneva Y, Tsomartova D, Ivanova M. Hashimoto's thyroiditis attenuates progression of papillary thyroid carcinoma: deciphering immunological links. Heliyon. 2020 Jan 8;6(1):e03077. doi: 10.1016/j.heliyon.2019.e03077. PMID: 31938743; PMCID: PMC6953714.

[9] Jackson D, Handelsman RS, Farrá JC, Lew JI. Increased Incidental Thyroid Cancer in Patients With Subclinical Chronic Lymphocytic Thyroiditis. J Surg Res. 2020 Jan;245:115-118. doi: 10.1016/j.jss.2019.07.025. Epub 2019 Aug 12. PMID: 31415932.

[10] Song E, Jeon MJ, Park S, Kim M, Oh HS, Song DE, Kim WG, Kim WB, Shong YK, Kim TY. Influence of coexistent Hashimoto's thyroiditis on the extent of cervical lymph node dissection and prognosis in papillary thyroid carcinoma. Clin Endocrinol (Oxf). 2018 Jan;88(1):123-128. doi: 10.1111/cen.13475. Epub 2017 Oct 4. PMID: 28906015.

[11] Moon S, Chung HS, Yu JM, Yoo HJ, Park JH, Kim DS, Park YJ. Associations between Hashimoto Thyroiditis and Clinical Outcomes of Papillary Thyroid Cancer: A Meta-Analysis of Observational Studies. Endocrinol Metab (Seoul). 2018 Dec;33(4):473-484. doi: 10.3803/EnM.2018.33.4.473. PMID: 30513562; PMCID:

PMC6279904.

[12] Ameziane-El-Hassani R, Schlumberger M, Dupuy C. NADPH oxidases: new actors in thyroid cancer? Nat Rev Endocrinol. 2016 Aug;12(8):485-94. doi: 10.1038/nrendo.2016.64. Epub 2016 May 13. PMID: 27174022.

[13] Szanto I, Pusztaszeri M, Mavromati M. H2O2 Metabolism in Normal Thyroid Cells and in Thyroid Tumorigenesis: Focus on NADPH Oxidases. Antioxidants (Basel). 2019 May 10;8(5):126. doi: 10.3390/antiox8050126. PMID: 31083324; PMCID: PMC6563055.

[14] Ramli NSF, Mat Junit S, Leong NK, Razali N, Jayapalan JJ, Abdul Aziz A. Analyses of antioxidant status and nucleotide alterations in genes encoding antioxidant enzymes in patients with benign and malignant thyroid disorders. PeerJ. 2017 Jun 1;5:e3365. doi: 10.7717/peerj.3365. PMID: 28584708; PMCID: PMC5457668.

[15] Gonzalez Flecha B, Llesuy S, Boveris A. Hydroperoxide-initiated chemiluminescence: an assay for oxidative stress in biopsies of heart, liver, and muscle. Free Radic Biol Med. 1991;10(2):93-100. doi: 10.1016/0891-5849(91)90002-k. PMID: 1849867.

[16] Victorino VJ, Panis C, Campos FC, Cayres RC, Colado-Simão AN, Oliveira SR, Herrera AC, Cecchini AL, Cecchini R. Decreased oxidant profile and increased antioxidant capacity in naturally postmenopausal women. Age (Dordr). 2013 Aug;35(4):1411-21. doi: 10.1007/s11357-012-9431-9. Epub 2012 May 28. PMID: 22645022; PMCID: PMC3705106.

[17] Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-6. doi: 10.1016/s0076-6879(84)05016-3. PMID: 6727660.

[18] Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974 Sep 16;47(3):469-74. doi: 10.1111/j.1432-1033.1974.tb03714.x. PMID: 4215654.

[19] Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem. 1969 Mar;27(3):502-22. doi: 10.1016/0003-2697(69)90064-5. PMID: 4388022.

[20] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951 Nov;193(1):265-75. PMID: 14907713.

[21] Miller, G.L. Protein determination for large numbers of samples. Analytical Chemistry 1959, 31, 964-964. doi: 10.1021/ac60149a611.

[22] Stanley JA, Neelamohan R, Suthagar E, Vengatesh G, Jayakumar J, Chandrasekaran M, Banu SK, Aruldhas MM. Lipid peroxidation and antioxidants status in human malignant and non-malignant thyroid tumours. Hum Exp Toxicol. 2016 Jun;35(6):585-97. doi: 10.1177/0960327115597982. Epub 2015 Aug 12. PMID:

26270564.

[23] Mseddi, M, Ben Mansour, R, Gouiia, N, Mnif, F, Bousselaa, R, Abid, M, Boudaouara, T, Attia, H, Lassoued, S. A comparative study of nuclear 8-hydroxyguanosine expression in Autoimmune Thyroid Diseases and Papillary Thyroid Carcinoma and its relationship with p53, Bcl-2 and Ki-67 cancer related proteins. Advances in medical sciences, 2017;62(1),45–51. https://doi.org/10.1016/j.advms.2016.06.003.

[24] Gerić M, Domijan AM, Gluščić V, Janušić R, Šarčević B, Garaj-Vrhovac V. Cytogenetic status and oxidative stress parameters in patients with thyroid diseases. Mutat Res. 2016 Nov 1;810:22-29. doi: 10.1016/j.mrgentox.2016.09.010. Epub 2016 Sep 29. PMID: 27776688.

[25] Teng X, Shan Z, Chen Y, Lai Y, Yu J, Shan L, Bai X, Li Y, Li N, Li Z, Wang S, Xing Q, Xue H, Zhu L, Hou X, Fan C, Teng W. More than adequate iodine intake may increase subclinical hypothyroidism and autoimmune thyroiditis: a cross-sectional study based on two Chinese communities with different iodine intake levels. Eur J Endocrinol. 2011 Jun;164(6):943-50. doi: 10.1530/EJE-10-1041. Epub 2011 Mar 28. PMID: 21444648.

[26] Katagiri R, Yuan X, Kobayashi S, Sasaki S. Effect of excess iodine intake on thyroid diseases in different populations: A systematic review and meta-analyses including observational studies. PLoS One. 2017 Mar 10;12(3):e0173722. doi: 10.1371/journal.pone.0173722. PMID: 28282437; PMCID: PMC5345857.

[27] Slimen IB, Najar T, Ghram A, Dabbebi H, Ben Mrad M, Abdrabbah M. Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review. Int J Hyperthermia. 2014 Nov;30(7):513-23. doi: 10.3109/02656736.2014.971446. PMID: 25354680.

[28] Larosa V, Remacle C. Insights into the respiratory chain and oxidative stress. Biosci Rep. 2018 Oct 2;38(5):BSR20171492. doi: 10.1042/BSR20171492. PMID: 30201689; PMCID: PMC6167499.

[29] He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. Cell Physiol Biochem. 2017;44(2):532-553. doi: 10.1159/000485089. Epub 2017 Nov 17. PMID: 29145191.

[30] Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem. 2015 Jun 5;97:55-74. doi: 10.1016/j.ejmech.2015.04.040. Epub 2015 Apr 22. PMID: 25942353.

[31] Lu SC. Glutathione synthesis. Biochim Biophys Acta. 2013 May;1830(5):3143-53. doi: 10.1016/j.bbagen.2012.09.008. Epub 2012 Sep 17. PMID: 22995213; PMCID: PMC3549305.

[32] Traverso N, Ricciarelli R, Nitti M, Marengo B, Furfaro AL, Pronzato MA, Marinari UM, Domenicotti C. Role of glutathione in cancer progression and chemoresistance. Oxid Med Cell Longev. 2013;2013:972913. doi: 10.1155/2013/972913. Epub 2013

May 20. PMID: 23766865; PMCID: PMC3673338.

[33] Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL. Glutathione dysregulation and the etiology and progression of human diseases. Biol Chem. 2009 Mar;390(3):191-214. doi: 10.1515/BC.2009.033. PMID: 19166318; PMCID: PMC2756154.

[34] Lassoued S, Mseddi M, Mnif F, Abid M, Guermazi F, Masmoudi H, El Feki A, Attia H. A comparative study of the oxidative profile in Graves' disease, Hashimoto's thyroiditis, and papillary thyroid cancer. Biol Trace Elem Res. 2010 Dec;138(1-3):107-15. doi: 10.1007/s12011-010-8625-1. Epub 2010 Mar 5. PMID: 20204550.

[35] Gaschler MM, Stockwell BR. Lipid peroxidation in cell death. Biochem Biophys Res Commun. 2017 Jan 15;482(3):419-425. doi: 10.1016/j.bbrc.2016.10.086. Epub 2017 Feb 3. PMID: 28212725; PMCID: PMC5319403.

[36] Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014:360438. doi: 10.1155/2014/360438. Epub 2014 May 8. PMID: 24999379; PMCID: PMC4066722.

[37] Erdamar H, Cimen B, Gülcemal H, Saraymen R, Yerer B, Demirci H. Increased lipid peroxidation and impaired enzymatic antioxidant defense mechanism in thyroid tissue with multinodular goiter and papillary carcinoma. Clin Biochem. 2010 May;43(7-8):650-4. doi: 10.1016/j.clinbiochem.2010.02.005. Epub 2010 Feb 18. PMID: 20171198.

[38] Guarino V, Castellone MD, Avilla E, Melillo RM. Thyroid cancer and inflammation. Mol Cell Endocrinol. 2010 May 28;321(1):94-102. doi: 10.1016/j.mce.2009.10.003. Epub 2009 Oct 14. PMID: 19835928.

[39] Cunha LL, Morari EC, Nonogaki S, Marcello MA, Soares FA, Vassallo J, Ward LS. Interleukin 10 expression is related to aggressiveness and poor prognosis of patients with thyroid cancer. Cancer Immunol Immunother. 2017 Feb;66(2):141-148. doi: 10.1007/s00262-016-1924-4. Epub 2016 Nov 17. PMID: 27858102.

[40] Lu ZW, Hu JQ, Liu WL, Wen D, Wei WJ, Wang YL, Wang Y, Liao T, Ji QH. IL-10 Restores MHC Class I Expression and Interferes With Immunity in Papillary Thyroid Cancer With Hashimoto Thyroiditis. Endocrinology. 2020 Oct 1;161(10):bqaa062. doi: 10.1210/endocr/bqaa062. PMID: 32348468; PMCID: PMC7469947.

[41] Colak S, Ten Dijke P. Targeting TGF-β Signaling in Cancer. Trends Cancer. 2017 Jan;3(1):56-71. doi: 10.1016/j.trecan.2016.11.008. Epub 2017 Jan 3. PMID: 28718426.

[42] Gu S, Feng XH. TGF-β signaling in cancer. Acta Biochim Biophys Sin (Shanghai). 2018 Oct 1;50(10):941-949. doi: 10.1093/abbs/gmy092. PMID: 30165534.

[43] Bertol BC, de Araújo JNG, Sadissou IA, et al. Plasma levels of soluble HLA-G and cytokines in papillary thyroid carcinoma before and after thyroidectomy. Int J Clin Pract.

2020;74(10):e13585. doi:10.1111/ijcp.13585.

[44] Matoba H, Sugano S, Yamaguchi N, Miyachi Y. Expression of transforming growth factor-beta1 and transforming growth factor-beta Type-II receptor mRNA in papillary thyroid carcinoma. Horm Metab Res. 1998 Oct;30(10):624-8. doi: 10.1055/s-2007-978946. PMID: 9851670.

[45] Wang N, Jiang R, Yang JY, Tang C, Yang L, Xu M, Jiang QF, Liu ZM. Expression of TGF-β1, SNAI1 and MMP-9 is associated with lymph node metastasis in papillary thyroid carcinoma. J Mol Histol. 2014 Aug;45(4):391-9. doi: 10.1007/s10735-013-9557-9. Epub 2013 Nov 26. PMID: 24276590.

[46] Xie J, Liu Y, Du X, Wu Y. TGF-β1 promotes the invasion and migration of papillary thyroid carcinoma cells by inhibiting the expression of lncRNA-NEF. Oncol Lett. 2019;17(3):3125-3132. doi:10.3892/ol.2019.9947.

[47] Balkwill F. TNF-alpha in promotion and progression of cancer. Cancer Metastasis Rev. 2006 Sep;25(3):409-16. doi: 10.1007/s10555-006-9005-3. PMID: 16951987.

[48] Zhang N, Wang Q, Tian Y, Xiong S, Li G, Xu L. Expressions of IL-17 and TNF-α in patients with Hashimoto's disease combined with thyroid cancer before and after surgery and their relationship with prognosis. Clin Transl Oncol. 2020 Aug;22(8):1280-1287. doi: 10.1007/s12094-019-02253-1. Epub 2019 Dec 23. PMID: 31873916.

[49] Kobawala TP, Trivedi TI, Gajjar KK, Patel DH, Patel GH, Ghosh NR. Significance of TNF-α and the Adhesion Molecules: L-Selectin and VCAM-1 in Papillary Thyroid Carcinoma. J Thyroid Res. 2016;2016:8143695. doi: 10.1155/2016/8143695. Epub 2016 Jan 11. PMID: 26881177; PMCID: PMC4737049.

[50] Coperchini F, Pignatti P, Carbone A, Bongianino R, Di Buduo CA, Leporati P, Croce L, Magri F, Balduini A, Chiovato L, Rotondi M. TNF-α increases the membrane expression of the chemokine receptor CCR6 in thyroid tumor cells, but not in normal thyrocytes: potential role in the metastatic spread of thyroid cancer. Tumour Biol. 2016 Apr;37(4):5569-75. doi: 10.1007/s13277-015-4418-7. Epub 2015 Nov 17. PMID: 26577851.

[51] Batthyány C, Bartesaghi S, Mastrogiovanni M, Lima A, Demicheli V, Radi R. Tyrosine-Nitrated Proteins: Proteomic and Bioanalytical Aspects. Antioxid Redox Signal. 2017;26(7):313-328. doi:10.1089/ars.2016.6787.

[52] Djikic D, Markovic D, Bogdanovic A, Mitrovic-Ajtic O, Suboticki T, Diklic M, Beleslin-Cokic B, Bjelica S, Kovacic M, P Cokic V. Oxidative and nitrosative stress in myeloproliferative neoplasms: the impact on the AKT / mTOR signaling pathway. J BUON. 2018 Sep-Oct;23(5):1481-1491. PMID: 30570876.

[53] Patel A, Fenton C, Terrell R, Powers PA, Dinauer C, Tuttle RM, Francis GL. Nitrotyrosine, inducible nitric oxide synthase (iNOS), and endothelial nitric oxide synthase (eNOS) are increased in thyroid tumors from children and adolescents. J Endocrinol Invest. 2002 Sep;25(8):675-83. doi: 10.1007/BF03345100. PMID: 12240898.

[54] Perluigi M, Coccia R, Butterfield DA. 4-Hydroxy-2-nonenal, a reactive product of lipid peroxidation, and neurodegenerative diseases: a toxic combination illuminated by redox proteomics studies. Antioxid Redox Signal. 2012;17(11):1590-1609. doi:10.1089/ars.2011.4406.

[55] Gasparovic AC, Milkovic L, Sunjic SB, Zarkovic N. Cancer growth regulation by 4-hydroxynonenal. Free Radic Biol Med. 2017 Oct;111:226-234. doi: 10.1016/j.freeradbiomed.2017.01.030. Epub 2017 Jan 25. PMID: 28131901.

[56] Yang C, Zhang J, Ding M, et al. Ki67 targeted strategies for cancer therapy. Clin Transl Oncol. 2018;20(5):570-575. doi:10.1007/s12094-017-1774-3.

[57] Arshad H, Ahmad Z, Hasan SH. Gliomas: correlation of histologic grade, Ki67 and p53 expression with patient survival. Asian Pac J Cancer Prev. 2010;11(6):1637-40. PMID: 21338209.

[58] Choudhury M, Singh S, Agarwal S. Diagnostic utility of Ki67 and p53 immunostaining on solitary thyroid nodule--a cytohistological and radionuclide scintigraphic study. Indian J Pathol Microbiol. 2011 Jul-Sep;54(3):472-5. doi: 10.4103/0377-4929.85077. PMID: 21934205.

[59] Zhou Y, Jiang HG, Lu N, Lu BH, Chen ZH. Expression of ki67 in papillary thyroid microcarcinoma and its clinical significance. Asian Pac J Cancer Prev. 2015;16(4):1605-8. doi: 10.7314/apjcp.2015.16.4.1605. PMID: 25743839.

[60] Siveen KS, Prabhu K, Krishnankutty R, et al. Vascular Endothelial Growth Factor (VEGF) Signaling in Tumour Vascularization: Potential and Challenges. Curr Vasc Pharmacol. 2017;15(4):339-351. doi:10.2174/1570161115666170105124038.

[61] Stuchi LP, Castanhole-Nunes MMU, Maniezzo-Stuchi N, Biselli-Chicote PM, Henrique T, Padovani Neto JA, de-Santi Neto D, Girol AP, Pavarino EC, Goloni-Bertollo EM. VEGFA and NFE2L2 Gene Expression and Regulation by MicroRNAs in Thyroid Papillary Cancer and Colloid Goiter. Genes (Basel). 2020 Aug 19;11(9):954. doi: 10.3390/genes11090954. PMID: 32824922; PMCID: PMC7563674.

[62] Salajegheh A, Pakneshan S, Rahman A, Dolan-Evans E, Zhang S, Kwong E, Gopalan V, Lo CY, Smith RA, Lam AK. Co-regulatory potential of vascular endothelial growth factor-A and vascular endothelial growth factor-C in thyroid carcinoma. Hum Pathol. 2013 Oct;44(10):2204-12. doi: 10.1016/j.humpath.2013.04.014. Epub 2013 Jul 8. PMID: 23845470.

Table 1. Clinical pathological and demographic characteristics of patients with thyroid disorders (n=115).

Characteristics	BENIGN (n= 63)	HT (n= 15)	PTC (n= 27)	PTC+HT (n= 10)	p value
Sex					0.937
Male (%)	7 (50.0)	2 (14.3)	4 (28.6)	1 (7.1)	
Female (%)	56 (55.5)	13 (12.8)	23 (22.7)	9 (8.9)	
Age (years) (Mean ± SD)	51.1 ± 16.8	55.1 ± 13.2	44.0 ± 13.1	43.4 ± 11.1	0.066
BMI					0.762
Normal (%)	21 (51.2)	5 (12.2)	10 (24.4)	5 (12.2)	
Overweight (%)	23 (67.6)	3 (8.8)	6 (18.6)	2 (5.8)	
Obesity (%)	14 (46.6)	6 (20.0)	8 (26.7)	2 (6.7)	
Smoking					0.922
No (%)	46 (54.1)	9 (10.6)	22 (25.8)	8 (9.4)	
Yes (%)	4 (66.7)	1 (16.7)	1 (16.7)	0 (0.0)	
Ex-smoker (%)	9 (64.3)	2 (14.3)	3 (21.4)	0 (0.0)	
Type 2 Diabetes					0.214
No (%)	46 (50.6)	13 (14.3)	23 (25.3)	9 (11.1)	
Yes (%)	15(75.0)	1 (5.0)	4 (20.0)	0 (0.0)	
Hypertension					0.786
No (%)	39 (54.2)	8 (11.1)	18 (25.0)	7 (9.7)	
Yes (%)	23 (57.5)	6 (15.0)	9 (22.5)	2 (5.0)	
Family history of thyroid disease					0.115
No (%)	26 (50.9)	4 (7.8)	14 (27.4)	7 (13.7)	
Yes (%)	35 (58.3)	10 (16.6)	13 (21.6)	2 (3.3)	
Family history of cancer					1.000
No (%)	24 (54.5)	6 (13.6)	11 (25.0)	3 (6.8)	
Yes (%)	37 (55.2)	8 (11.9)	16 (23.8)	6 (8.9)	
History of cancer					0.481
No (%)	58 (56.8)	13 (12.7)	23 (22.5)	8 (7.8)	
Yes (%)	3 (37.5)	1 (12.5)	3 (37.5)	1 (12.5)	
Other diseases					0.962
No (%)	49 (54.4)	14 (15.5)	18 (20.0)	9 (10.0)	
Yes (%)	7 (58.3)	1 (8.3)	3 (25.0)	1 (8.3)	

BENIGN: Benign thyroid disorders; BMI: Body mass index; HT: Hashimoto's thyroiditis; PTC: Papillary thyroid carcinoma; PTC+HT: Papillary thyroid carcinoma associated with Hashimoto's thyroiditis. Categorical data are presented as number and percentage, and continuous as mean ± standard deviation. $p < 0.05$ was considered statistically significant.

Table 2. Description of the changes presented by patients with different thyroid alterations (n=115).

	BENIGN (n= 63)	HT (n= 15)	PTC (n= 27)	PTC+HT (n= 10)	p value
Primary Injury					0.074
No (%)	2 (22.2)	1 (11.1)	5 (55.5)	1 (11.1)	
Yes (%)	60 (58.2)	13 (12.6)	22 (21.3)	8 (7.7)	
Tumor size					0.113
< 1cm	9 (36.0)	4 (16.0)	9 (36.0)	3 (12.0)	
≥ 1 cm	53 (61.6)	9 (10.4)	17 (19.7)	7 (8.1)	
Nodules					0.164
Multiple (%)	49 (62.0)	9 (11.3)	16 (20.2)	5 (6.3)	
Single (%)	14 (41.1)	5 (14.7)	10 (29.4)	5 (14.7)	
Metastasis					0.526
No (%)	NA	NA	15 (75.0)	5 (25.0)	
Yes (%)	NA	NA	12 (70.6)	5 (29.4)	

BENIGN: Benign thyroid disorders; HT: Hashimoto's thyroiditis; NA: Not applicable; PTC: Papillary thyroid carcinoma; PTC+HT: Papillary thyroid carcinoma associated with Hashimoto's thyroiditis. Categorical data are presented as number and percentage. $p < 0.05$ was considered statistically significant.

Table 3. Biochemical and immunological parameters presented by patients with thyroid disorders before surgery.

	BENIGN	HT	PTC	PTC+HT	p value
TSH (µlU/mL)	1.44 (±1.34)	4.08 (±5.61)	3.88 (±8.91)	2.37 (±0.63)	0.149
fT4 (ng/dL)	1.13 (±0.54)	1.17 (±0.32)	0.99 (±0.24)	1.25 (±0.08)	0.358
TPOAb (IU/mL)					0.216
Normal (%)	79.2	33.3	75.0	50.0	
Increased (%)	20.8	66.7	25.0	50.0	
TGAb (IU/mL)					0.010*
Normal (%)	93.3	33.3	100.0	50.0	
Increased (%)	6.7	66.7	0.0	50.0	

BENIGN: Benign thyroid disorders; fT4: Free thyroxine (reference value: 0.70 to 1.80ng/dL); HT: Hashimoto's thyroiditis; PTC: Papillary thyroid carcinoma; PTC+HT: Papillary thyroid carcinoma associated with Hashimoto's thyroiditis; TGAb: Thyroglobulin antibody; TPOAb: Thyroid peroxidase antibody; TSH: Thyroid-stimulating hormone (reference value: 0,45 to 4,5µIU/L). Categorical data are presented as percentage, and continuous as mean ± standard deviation. *p <0.05 was considered statistically significant.

Figure 1

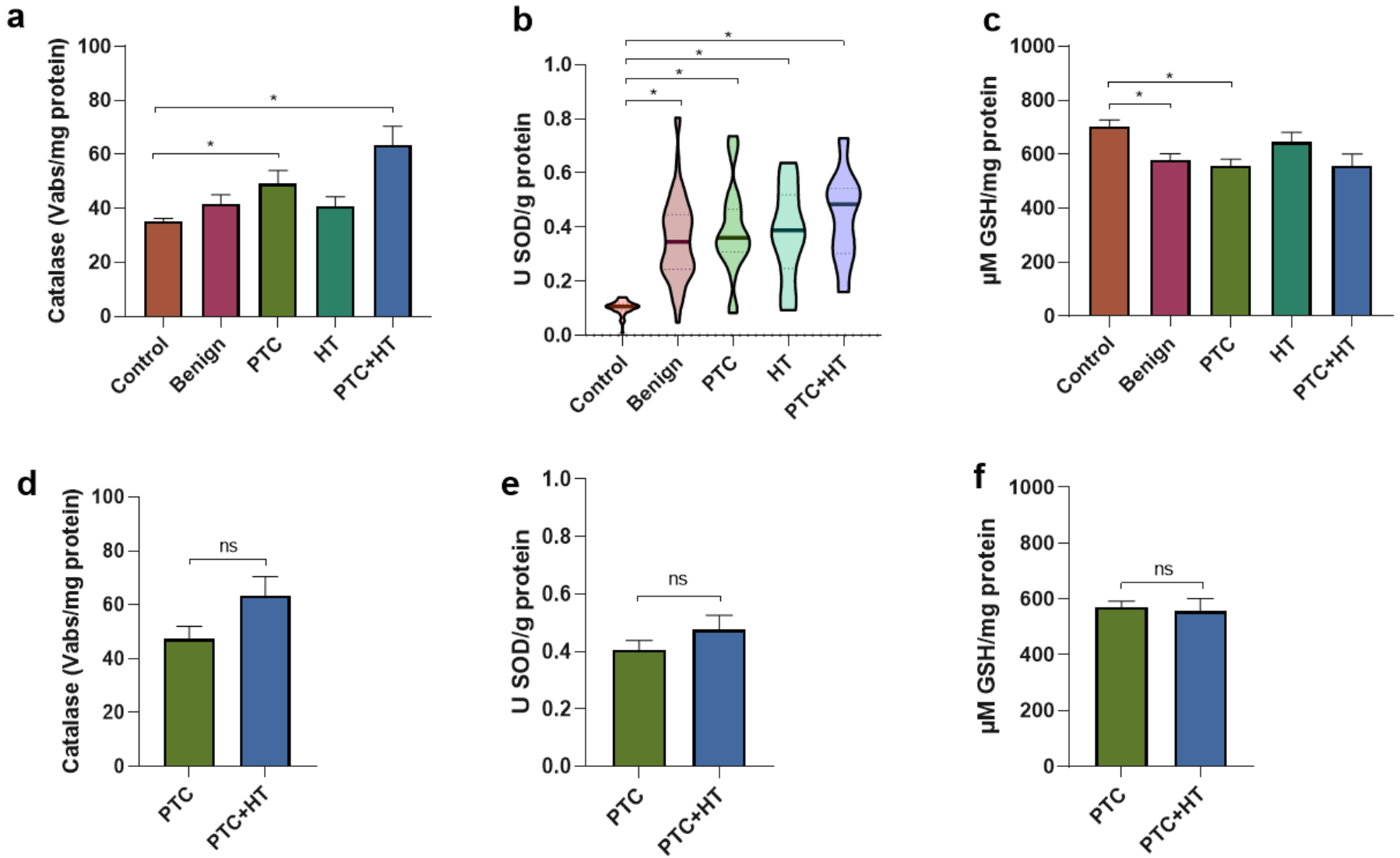

Figure 1. Systemic antioxidant parameters in erythrocyte. (a) catalase activity; (b) superoxide dismutase (SOD) activity; (c) Levels of reduced glutathione (GSH); comparison of the activity of (d) catalase, (e) SOD and (f) GSH, only among the PTC vs PTC+HT groups. Nonparametric data were expressed as violin plots. HT: Hashimoto's thyroiditis; PTC: Papillary thyroid carcinoma; PTC+HT: Papillary thyroid carcinoma associated with Hashimoto's thyroiditis; ns: not significant; * indicates significant difference ($p < 0.05$).

Figure 2

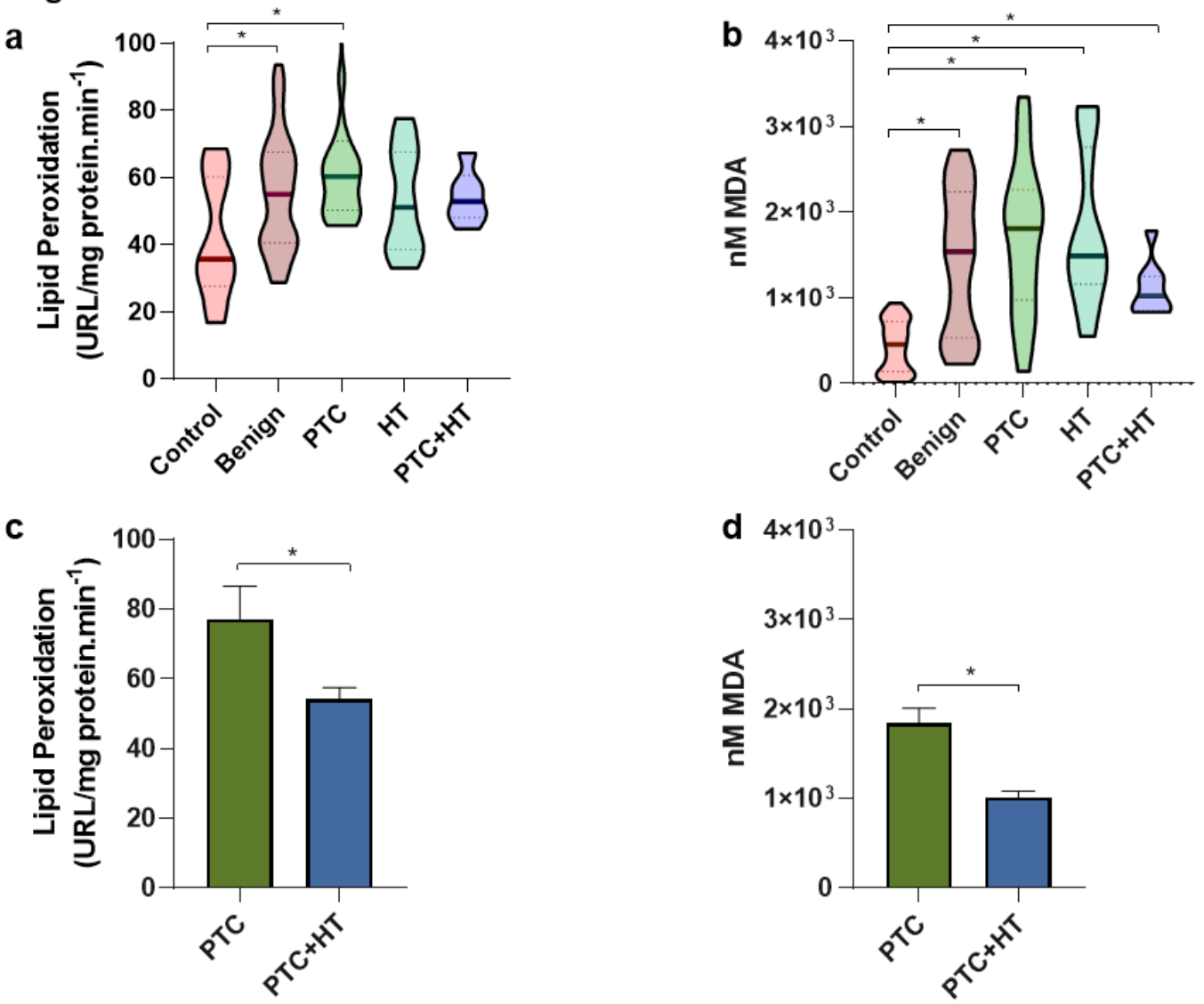

Figure 2. Lipid peroxidation markers. (a) speed of ascent of the curve of lipid peroxidation in erythrocytes; (b) malondialdehyde (MDA) in plasma; comparison of (c) speed of ascent of the curve of lipid peroxidation and (d) MDA levels, only among the PTC vs PTC+HT groups. Results of nonparametric data are expressed as violin plots. HT: Hashimoto's thyroiditis; PTC: Papillary thyroid carcinoma; PTC+HT: Papillary thyroid carcinoma associated with Hashimoto's thyroiditis; * indicates significant difference ($p < 0.05$).

Figure 3

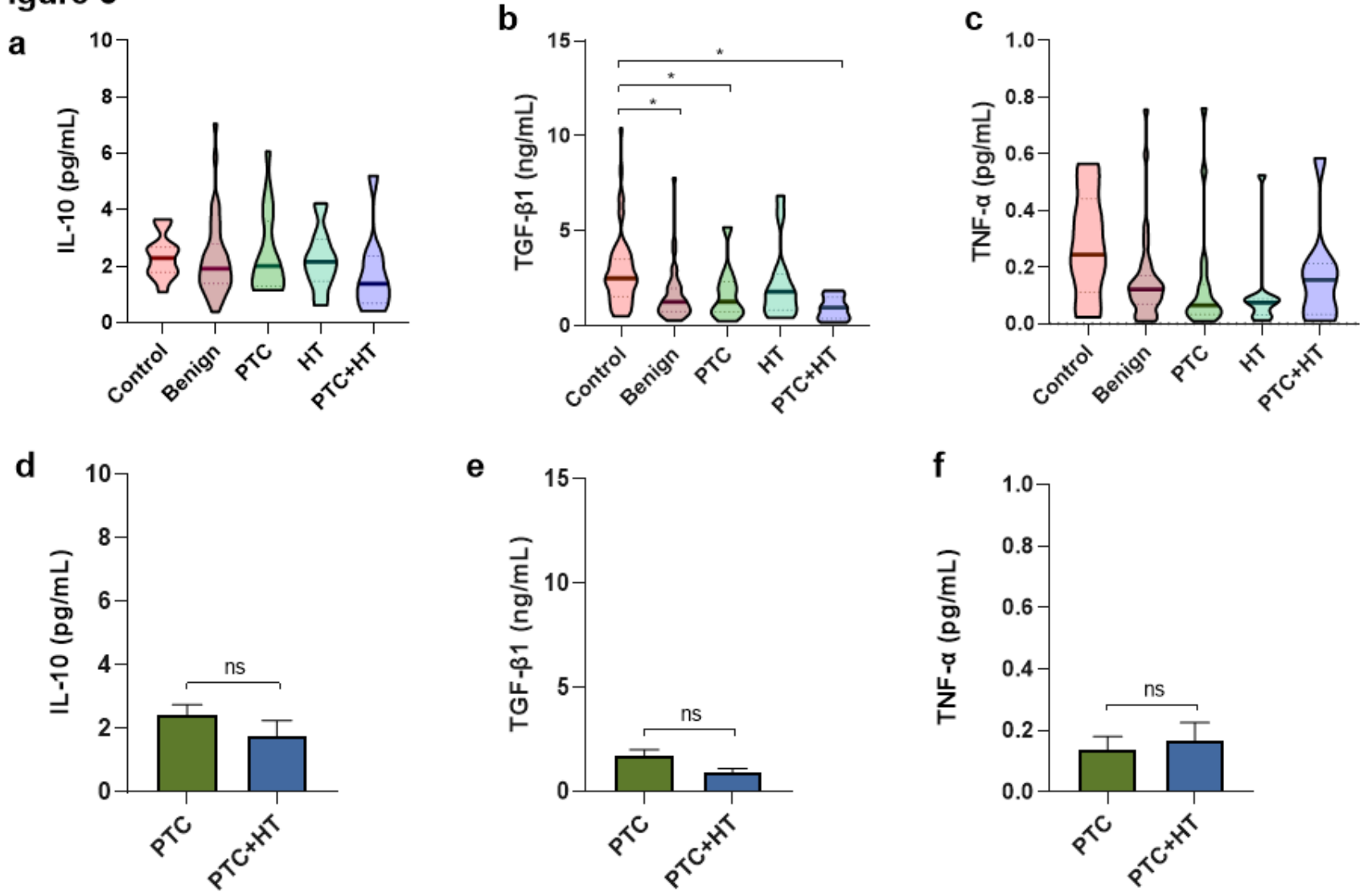

Figure 3. Systemic inflammatory markers. (a) Levels of IL-10; (b) TGF-β1; (c) TNF-α; comparison of (d) IL-10, (e) TGF-β1and (f) TNF-α, only among the PTC vs PTC+HT groups. All cytokines were analyzed in plasma. Nonparametric data were expressed as violin plots. HT: Hashimoto's thyroiditis; PTC: Papillary thyroid carcinoma; PTC+HT: Papillary thyroid carcinoma associated with Hashimoto's thyroiditis; ns: not significant; * indicates significant difference ($p < 0.05$).

Figure 4

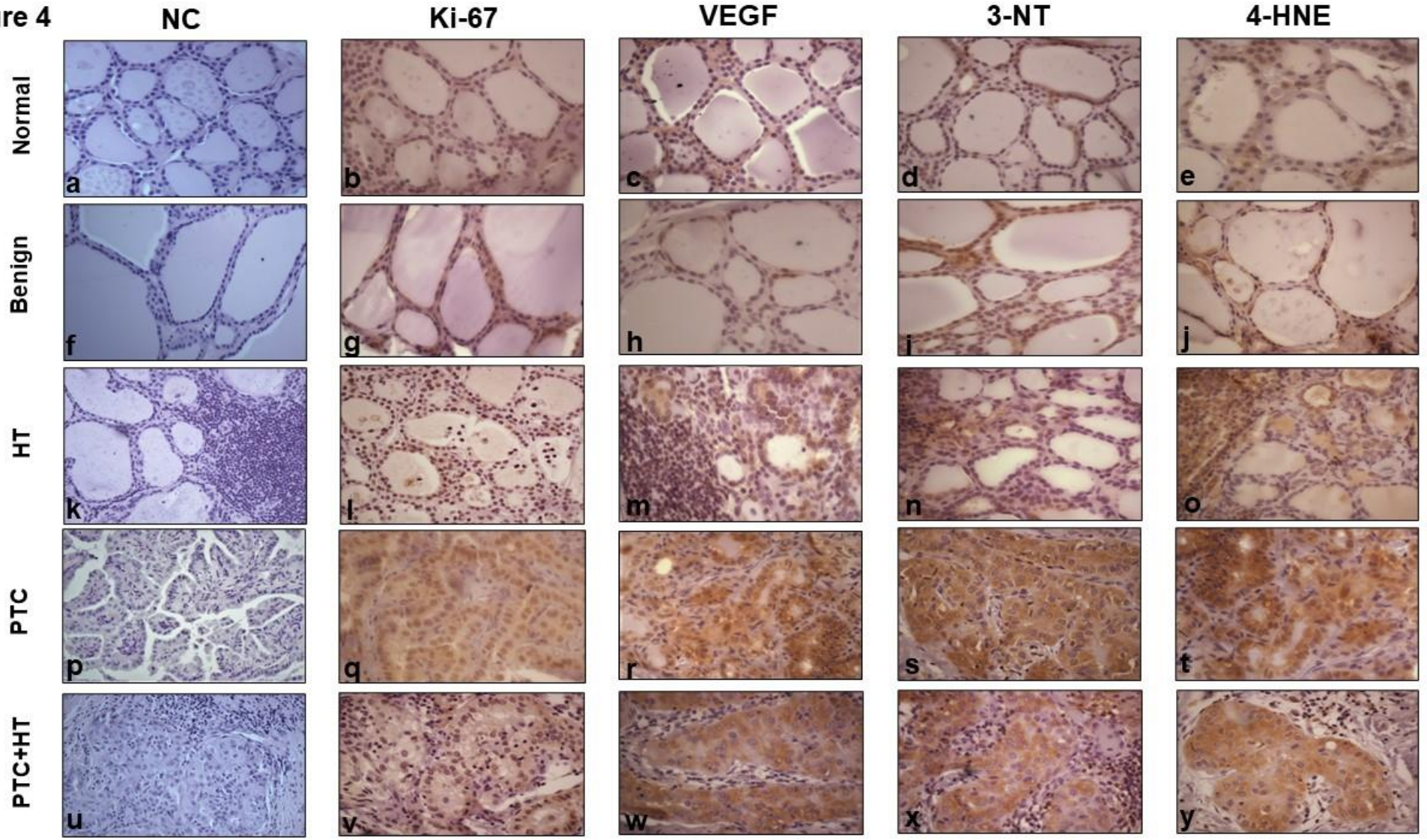

Figure 4. Representative image of immunohistochemistry. (a, f, k, p and u) negative control; (b, c, d and e) Ki-67, VEGF, 3-NT and 4-HNE immunolabeling in normal tissue; (g, h, i, and j) Benign alterations; (l, m, n and o) HT; (q, r, s and t) PTC; and (v, w, x and y) PTC+HT; (immunohistochemistry, x40; NC, x20). HT: Hashimoto's thyroiditis; NC: negative control; PTC: Papillary thyroid carcinoma; PTC+HT: Papillary thyroid carcinoma associated with Hashimoto's thyroiditis.

Figure 5

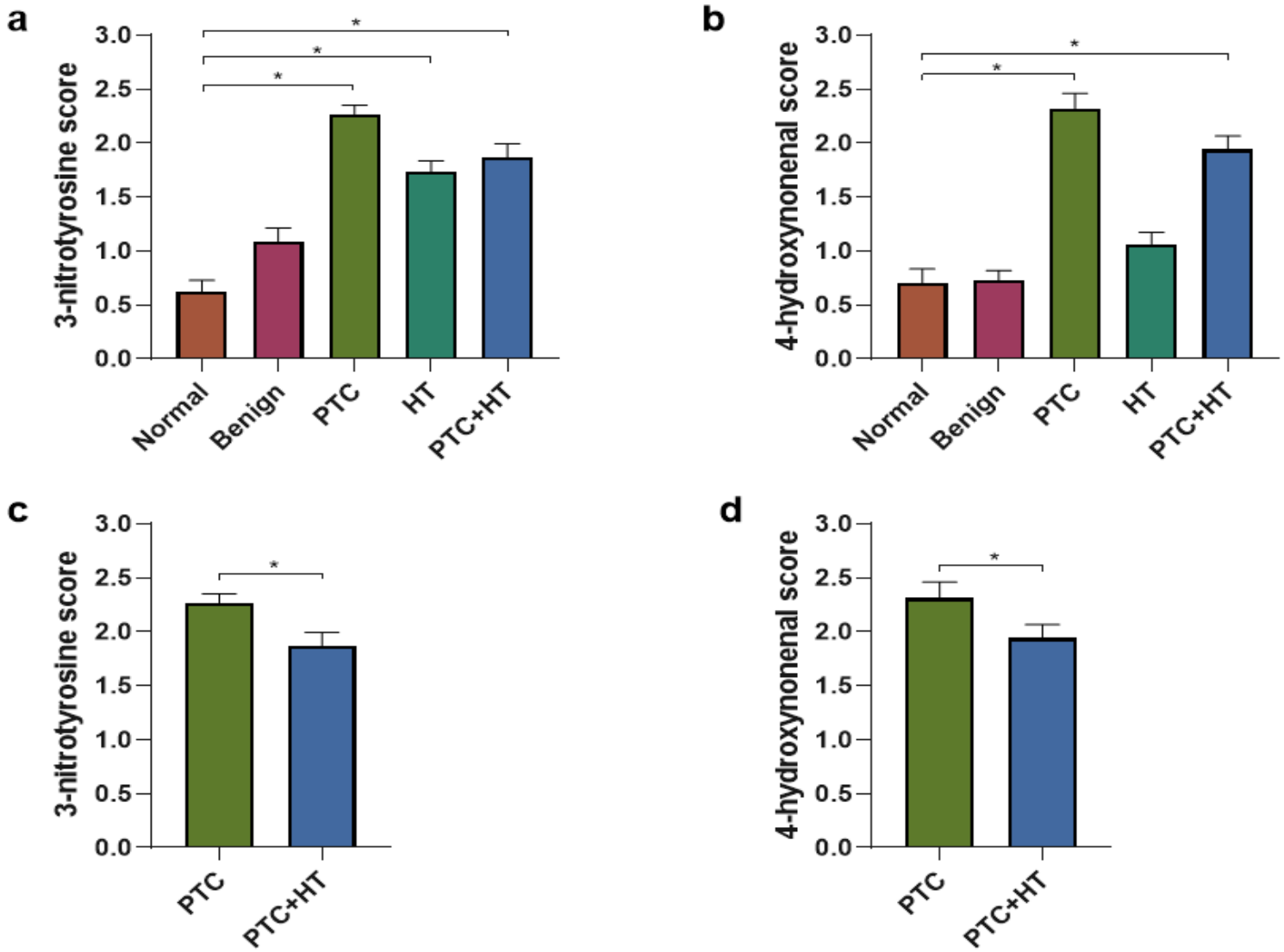

Figure 5. Analysis of oxidative stress markers by immunohistochemistry. (a) 3-nitrotyrosine score; (b) 4-hydroxynonenal score; comparison of (c) 3-nitrotyrosine score and (d) 4-hydroxynonenal score, only among the PTC vs PTC+HT groups. HT: Hashimoto's thyroiditis; PTC: Papillary thyroid carcinoma; PTC+HT: Papillary thyroid carcinoma associated with Hashimoto's thyroiditis; * indicates significant difference ($p < 0.05$).

Figure 6

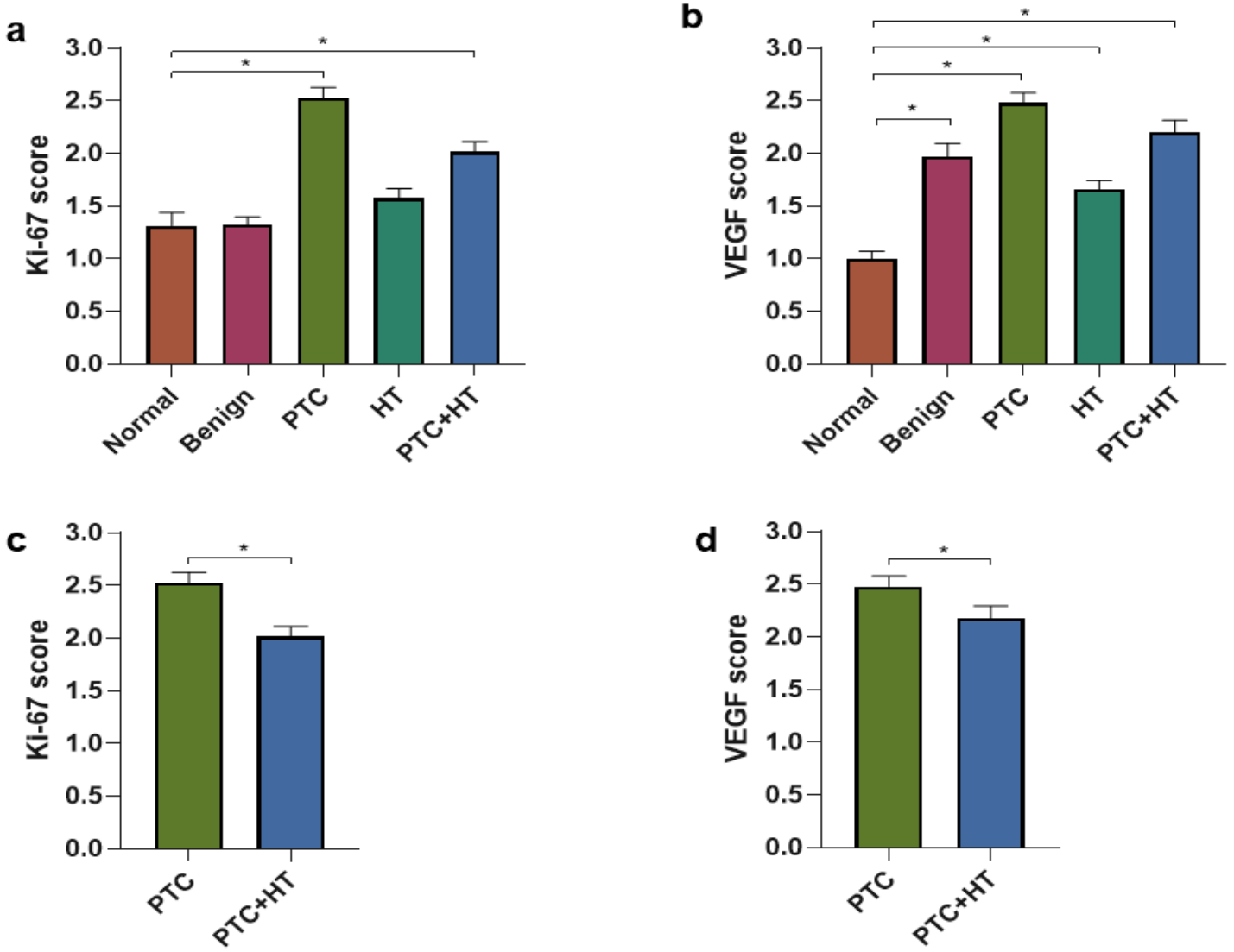

Figure 6. Proliferation and angiogenesis markers by immunohistochemistry. (a) ki-67 score; (b) VEGF score; comparison of (c) ki-67 score and (d) VEGF score, only among the PTC vs PTC+HT groups. HT: Hashimoto's thyroiditis; PTC: Papillary thyroid carcinoma; PTC+HT: Papillary thyroid carcinoma associated with Hashimoto's thyroiditis; * indicates significant difference ($p < 0.05$).

APÊNDICE B - Thyroid cancer and thyroid autoimmune disease: A review of molecular aspects and clinical outcomes.

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Thyroid cancer and thyroid autoimmune disease: A review of molecular aspects and clinical outcomes

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ABSTRACT

Thyroid cancer (TC) is the most prevalent malignant neoplasm that affects the endocrine system. Hashimoto's thyroiditis (HT), also known as chronic lymphocytic thyroiditis, is the most common autoimmune thyroid disease (AITD) that, together with Graves' disease (GD), represent the main autoimmune diseases that affect the thyroid gland. Some studies suggest a greater risk of AITD and the development of TC, while others, investigate its relationship with TC progression and patient prognosis. In this review, we have analyzed published data on the molecular aspects related to the association between AITD and TC, addressing their influence on TC progression, diagnosis, and prognosis of the patients. MEDLINE database (PubMed) platform was used as a search engine and the original articles related to the topic were selected using the keywords combination "thyroid cancer and Hashimoto thyroiditis" or "thyroid carcinoma and thyroid autoimmune disease". After the selection, we categorized the main findings of the papers into four topics: antitumor immunity, tumor progression, diagnosis, and prognosis. Although most of the studies have pointed out the presence of AITD as a factor that increases the risk of TC, few molecular mechanisms to support this conclusion have been described. Additionally, little information is available to explain, pathophysiologically, the effects of autoimmunity in TC diagnosis, progression, and prognosis.

1. Introduction

Thyroid cancer (TC) is the most common endocrine malignant neoplasm worldwide, presenting an increasing record number of new cases every year, predominantly in females [1,2]. According to the World Health Organization, among the subtypes, papillary thyroid carcinoma (PTC) is the most common in the population [3], representing about 75–80 % of the thyroid cancer cases [4], followed by follicular carcinoma, medullary carcinoma, and anaplastic carcinoma, that occur less frequently [5]. Despite the increasing incidence, mortality rates have been steadily decreasing, mainly because of the advances in diagnosis and treatment [6].

Hashimoto's thyroiditis (HT), also called chronic lymphocytic thyroiditis, is the most common autoimmune thyroid disease (AITD). It is characterized by the presence of chronic inflammatory infiltrates, which, together with Graves' disease (GD), represent the main autoimmune diseases that affect the thyroid gland [2,7,8].

Chronic inflammation triggered by HT may be related to the increased risk of developing thyroid cancer, however, despite some studies addressing this issue, no conclusion has been drawn concerning this relationship [2,5,9,10]. Regarding prognosis, some researchers state that the evolution of TC, when associated with HT, is more favorable [11,12]. On the other hand, there are authors proposing that this association is related to an unfavorable prognosis [13–15].

Considering that there is no consensus on the relationship between TC and HT, the aim of this review was to analyze the current published

Abbreviations: Anti-Tg, anti-thyroglobulin; Anti-TPO, anti-thyroperoxidase; AITD, autoimmune thyroid disease; CK19, cytokeratin 19; COX-2, inducible isoform of cyclooxygenase; CRP, C-reactive protein; DTC, differentiated thyroid carcinoma; FNAB, fine-needle aspiration biopsy; Foxp3, forkhead box P3; GD, Graves' disease; HT, Hashimoto's thyroiditis; IgG4, immunoglobulin G4; IL-4, Interleukin-4; IL-10, interleukin-10; IL-17, interleukin-17; IFN-γ, interferon-gamma; miRNAs, MicroRNAs; PTC, papillary thyroid carcinoma; PD-L1, programmed death ligand 1; TC, thyroid cancer; WHO, World Health Organization; 8-OHdG, 8-hydroxy-2-deoxyguanosine

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Fig. 1. Flowchart of inclusion of articles used in the review.
This diagram shows how the selection of articles used to write this review was made, based on MEDLINE database (PubMed).

studies that report the possible molecular mechanisms related to the association between AITD and thyroid cancer, addressing their influence on the diagnosis and prognosis of the patients.

2. Methods

The bibliographic search was performed in the MEDLINE database (PubMed) using the keywords "thyroid cancer and Hashimoto thyroiditis" or "thyroid carcinoma and thyroid autoimmune disease". The papers (published in the last 10 years) were selected according to the scheme shown in Fig. 1.

3. Molecular markers associated with antitumor immunity

The immune response triggered against TC and AITD is different. In TC, the immune response is more tolerant and allows tumor growth. In autoimmune diseases, the response is more aggressive, triggering cell destruction and reduction of the normal function of the gland [16]. The role of HT in TC seems to be ambiguous. HC is considered a risk factor for TC development, but its presence can be associated with a better prognosis for patients [17] due to the immune response resulting from the chronic lymphocytic infiltration, which can control tumor aggressiveness [17,18]. Another explanation is the pattern of cytokine production presented by patients with HT associated with PTC, which indicates increased production of Th2 cytokines, with the maintenance of IFN-γ and IL-17 production [19]. In Table 1, we show the relevance of the different biomarkers in relation to the prognosis for patients with PTC and for those with PTC associated with AITD.

There is evidence that Th17 cells, in addition to their well-established function in autoimmune disease, would be involved in antitumor immunity [20]. It was observed [17] that IL-17-specific nuclear marker (RORγt) was expressed in tumor tissue of patients with and without HT-PTC. In patients with high expression of this marker, the lymph node metastasis rate was lower, indicating that IL-17 can inhibit this process and exert antitumor activity in the tumor microenvironment of the patients possessing PTC with or without HT [17].

In Graves' disease, the presence of a strong humoral immune response appears to be protective against TC. When analyzing the inflammatory infiltrate of patients with TC and GD, a high concentration of activated NK cells and a higher ratio of M1 / M2 macrophages were observed, which might provide to the patients a more effective form of tumor immunity and low aggressiveness of TC [16].

Foxp3 is known to be related to the development of autoimmune diseases and there is growing evidence of its suppressive role in the antitumor response [21]. Regarding thyroid diseases, its role has not yet been well established [22], but some authors have shown that higher expression of Foxp3 is correlated with greater tumor aggressiveness in patients with PTC [23,24].

Besides being markers of AITD, anti-thyroperoxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies appear to be related to PTC as a possible risk factor; however, the presence of these antibodies would be associated with a better prognosis for these patients [25,26]. Regarding the triggering of metastasis, patients with anti-TPO and anti-Tg have lower distant metastasis rates than patients without these antibodies, possibly indicating a protective factor [27,28].

Table 1
Interference of different biomarkers in the prognosis of papillary thyroid carcinoma (PTC) and their association with autoimmune thyroid diseases (AITD).

Biomarker	PTC	PTC and AITD
IL-17	Favorable	Favorable
Anti-TPO	Favorable	Unfavorable
NK cells and macrophages M1/M2	Favorable	Indifferent
Foxp3	Unfavorable	Indifferent
PD-L1	Unfavorable	Indifferent
Osteopontin	Unfavorable	Favorable
IL-10 and IL-4	Unfavorable	Unfavorable
IgG4	Indifferent	Unfavorable

IL-17: interleukin-17; Anti-TPO: anti-thyroperoxidase; PD-L1: programmed death ligand 1; IL-10: interleukin-10; IL-4: interleukin-4.

4. Molecular markers associated with tumor progression

TC has a good prognosis and survival rate of about 97 % when detected early [29], but the patients who develop lymph node metastasis tend to have disease recurrence, decreasing the survival rate [30]. The identification of biomarkers related to tumor aggressiveness can help the diagnosis and the prediction of tumor progression, thereby improving the treatment directions [29]. The expression of immunomodulatory proteins, such as programmed death-ligand 1 (PD-L1), in neoplastic cells has been associated with more aggressive tumor characteristics and poor prognosis in different types of cancer [31], including TC [29]. Lubin and collaborators [30] analyzed PD-L1 expression in different cases of PTC development and found that PD-L1 expression in primary PTC strongly correlates with PD-L1 expression in local lymph node metastasis, and its expression correlates with greater tumor aggressiveness and worse prognosis [29,30]. Ahn and collaborators [32] observed that PD-L1 was more expressed in advanced thyroid cancer subtypes, such as the anaplastic thyroid carcinoma and the thyroid follicular carcinoma.

Used as a biomarker of oxidative DNA damage, 8-hydroxy-2-deoxyguanosine (8-OHdG) may be associated with the development of some diseases, such as cancer. Regarding TC, a higher expression of this biomarker was observed in the patients with PTC when compared to the patients with AITD [33]. Although slightly less evident, patients with HT and GD also show expression of this biomarker due to the oxidative stress caused by AITD and GD, which may possibly favor the development of TC [34].

Osteopontin, a secreted glycoprotein, is associated with several physiological and pathological processes, such as wound healing, tumorigenesis, inflammation, among others [35]. Tumor cells with high metastatic potential express higher levels of osteopontin, which is

related to poor patient prognosis in some cancers [36]. Regarding TC, some studies have been demonstrated that osteopontin expression is positively correlated with the presence of lymph node metastasis [37,38]. When analyzed osteopontin expression in the patients with HT-associated PTC, its expression was significantly lower than when compared to the patients without PTC, suggesting that the concomitant presence of HT may decrease PTC aggressiveness, negatively regulating the expression of osteopontin [39].

GD is the major cause of hyperthyroidism [40–42]. The presence of GD increases the risk of developing differentiated thyroid carcinoma (DTC) when compared to the patients without AITD [43]. The incidence of DTC in children has been increasing, being one of the most incident thyroid alterations in this population [44]. When analyzing children and young adults, MacFarland and collaborators [45] observed that patients with DTC associated with GD have common disease characteristics from those patients with isolated DTC. This result was maintained over the long term, suggesting that the presence of GD does not alter the course of DTC in this population.

The expression of inflammation mediators and the development of thyroid neoplasms are still not well understood. The expression of COX-2 (inducible isoform of cyclooxygenase), the enzyme that catalyzes prostaglandin formation from arachidonic acid [46], is associated with pathological conditions such as stress and inflammation, and its increased activity is associated with the occurrence of some kinds of neoplasia [47], including the thyroid ones [48]. Krawczyk-Rusiecka and collaborators [49] observed that the level of COX-2 gene expression was significantly higher in patients with PTC than in samples from the patients with benign nodules and HT. Furthermore, the patients with HT had a similar level of COX-2 expression than the patients with benign nodules, indicating that COX-2 does not participate in the mechanisms involved in the molecular association of HT and PTC [49].

5. Molecular markers with diagnostic value

Fine-needle aspiration biopsy (FNAB) is the most important tool in the distinction of malignant from benign nodules, but this technique has a relatively high rate of false-negative results, that range from 0.7 to 21 %, requiring its association with the ultrasound aspects. Clinical and biochemical findings, coupled with specific markers, tend to be more efficient, thus reducing the need for repetitive FNAB [50].

Many markers are used for the diagnosis of PTC, both in tissue and cytological samples, but they did not present high sensitivity and specificity [51]. Cytokeratin 19 (CK19) is a protein that is part of the epithelial cell cytoskeleton and is highly expressed in PTC and, therefore, it is a marker of this type of TC [52]. P63, which is a member of the p53 tumor suppressor gene family, is also considered a marker of PTC [53]. When analyzing the expression of these markers, Divani and collaborators [54] observed that all samples were positive for CK19. For P63, approximately 66 % of the samples were positive, suggesting that, although specific, P63 presents lower sensitivity for PTC than CK19 [54].

MicroRNAs (miRNAs) are non-coding RNAs acting as negative gene regulators and are involved in several cellular functions, such as proliferation, differentiation, apoptosis, and others; so, the dysregulation of these miRNAs is involved in tumorigenesis [55]. The analysis of the expression of some PTC-related miRNAs showed that the patients with GD presented intermediate levels of expression for miRNAs 146b, 221, and 222 when compared with the normal and PTC tissues, indicating that these miRNAs may have an influence in the PTC development in patients with GD [56].

Multifocal PTC is related to higher recurrence rates, distant metastasis, and worse prognosis than the unifocal [57]. According to Dong and collaborators [58], the detection of high levels of anti-TPO is indicative of multifocal PTC in the patients with HT, which suggests the utilization of this marker as a parameter for choosing the surgical treatment, with total thyroidectomy being the best option [58]. Anti-TPO and anti-Tg are the most common autoantibodies found in the patients with AITD and may also be found in low concentrations in the patients with nontoxic goiter without thyroid abnormalities [59]. The analysis of the pattern of epitopes presented in different groups of patients with thyroid abnormalities showed that the pattern of anti-Tg recognition in the patients with HT-associated PTC was more similar to that found in the patients with HT than in the patients that presented only PTC. The authors concluded that the pattern of epitopes varies according to the presence or absence of lymphocyte infiltrate in those patients [60].

The IgG4-related disease is a newly identified syndrome characterized by high levels of IgG4 in some conditions, such as in HT [61]. It represents an immune-mediated fibroinflammatory condition, in which the pathophysiology process is incompletely understood until now [62]. Patients with Warthin-type papillary carcinoma, a rare variation of PTC, presenting different clinical courses, and dense lymphocytic infiltration [63] demonstrated correlation with high levels of IgG4 when associated with HT, suggesting that the presence of IgG4 in these conditions may be indicative of this subtype of TC [64].

6. Prognosis

The thyroid cancer is the most incident endocrine tumor, affecting more often adults than children [65] with a rise in the number of cases in the last decades, for both adults and children [66]. The prognosis of thyroid carcinomas varies according to associated, concomitant, or intrinsic aspects, such as specific characteristics of the tumor cells, gender, and the presence of AITD.

According to Zirilli and collaborators [67] there is a growing trend of incidence of differentiated thyroid carcinomas as the population ages. Among the young population, especially those under 18 years old, there are differences regarding the morphology and behavior of the tumor [68]. The most prevalent phenotype in the juvenile population is the aggressive one, with areas of invasion in the adjacent tissue, pulmonary metastasis, lymphadenoma, and cervical mass growth [70]. Nonetheless, for this juvenile population, the long-term prognosis is independent of this aggressive condition, being able to respond better to the treatment than the adults [69]. The cause of this more favorable prognosis is not fully elucidated yet, but it is speculated that there are some connections with the high rate of association with AITD, by which this population is commonly affected [67].

A meta-analysis showed a relatively reproducible pattern of incidence of thyroid carcinoma with AITD, demonstrating a significant association between thyroiditis, thyroid cancer, and bilateral thyroid microcarcinoma [70]. This association presents a higher incidence in women [9,70–73] with a predominance of PTC [9,70]. The concomitant presence of AITD and TC is probably responsible for the early detection of hidden neoplastic changes in the gland, which can favor for a better prognosis [73]. Pellegriti and collaborators [75] showed that the expected prognosis of patients with DTC and GD is not favorable, presenting a high probability of malignancy and the necessity of more aggressive treatments since a relapse in these patients is higher when compared to patients that do not present GD associated-carcinoma [74–76]. The association between DTC and GD was characterized by a high mortality, with elevated rates of metastasis, and relapse in patients. In these cases, the unfavorable prognosis is independent of the tumor size, age or gender [74].

In patients with HT, the most common association with TC is related to PTC [77]. The concomitant presence of HT in patients with TC was also associated with a worse prognosis [75,78] due to the high levels of TSH related to HT, which can induce tumor growth and lymph node metastasis.

The first time when the association between TC and HT has emerged in the literature was in 1955 [79], and since then, the question regarding the presence of this association and its effects on the prognosis has become the focus of many disagreements. For Paparodis and

collaborators [72], the main reason for such contradiction involves different perspectives, one based on clinical patterns and the other on pathological findings. Spencer and Fatemi [80] point out that the disparity about the information related to the prognosis is because of the differences in the research designs, regarding the variation in the sample size, or in the methodologies, as well as in the quantification of anti-Tg, for example. The detection and measurement of serum thyroglobulin (Tg) are considered a method for monitoring DTC, even after total thyroidectomies or radioactive iodine treatment [81]. The positive detection of Tg is related to a less favorable prognosis, but the presence of anti-Tg is known to interfere with the quantification of serum Tg, so the presence of anti-Tg in the patients with AITD may influence prognostic assessment [82]. The specificity of anti-Tg epitopes recognized by these autoantibodies explains the persistence of carcinoma even after the total or partial thyroidectomy procedure [80], corroborating with Lupoli [81] that observed that the non-specificity of antibodies in some epitopes is associated with lower cancer recurrence rates, proving to be a more important tool for predicting effective treatments and more accurate prognostic assessment.

Anti-Tg strongly correlates with PTC associated with HT, as shown by Hwangbo and Park [73]. These authors also found that anti-Tg correlates with ultra-sensitive C-reactive protein (CRP), characterized as a marker of cancer relapse, inflammation, and worse prognosis [75,83,84]. They also pointed out that IL-10 and IL-4 are present in large quantities in the association of PTC and HT. This correlation of anti-Tg, CRP, IL-10, and IL-4 was identified in patients with relapsed HT-PTC, while Tg was not correlated with this scenario, showing that anti-Tg is an important prognostic factor that indicates a less favorable prognosis in patients with TC [75].

The high production of IL-4 and IL-10 was observed in both HT [75] and GD [74]. The presence of IL-4 in the neoplastic area was associated with some properties such as antiapoptotic capacity, the survival of the tumor cells, metastasis, and invasion in several *in vitro* cancer cell models [85,86]. For this reason, high levels of IL-4 in tumor tissue are associated with poor prognosis [87]. IL-4 receptor was considered a therapeutic target in the anaplastic thyroid carcinoma [87]. The presence of IL-10 protects the tumor from the immune system and can control T cell proliferation and differentiation by preventing the effector's mechanisms of immune cells, which favors the escape of immune recognition by loss of antigen expression [88]. Together, these cytokines support the growth and aggressiveness of the thyroid tumor, resulting in unfavorable prognosis. IL-4 and IL-10 are products of Th2 immune response, a different profile from that observed in HT, where a predominant Th1 response is found, with a raising production of proinflammatory cytokines. Stanciu and collaborators [75] point out that when HT is associated with PTC, there is a shift in the cytokine profile and a massive increase in the production of Th2 cytokines. However, it is important to highlight that Zivancevic-Simonovic and collaborators [19] showed that the increase in Th2 cytokines was accompanied by the maintenance of IFN-γ and IL-17 production [19].

Li and collaborators [89] rated HT as positive or negative for IgG4, histopathologically. The presence of IgG4 in HT-PTC patients is more common than its absence and it is related with worse prognostic parameters [90]. Yu and collaborators [76] cited two hypotheses about the concomitant occurrence of these two diseases: the first one addresses carcinoma as a facilitator of the development of autoimmunity, and the second one, more accepted by the group, postulates that lymphocytic infiltration and IgG4 production due to pre-existing autoimmune inflammation facilitates the occurrence of PTC. This facilitation is related to TGF-alpha1 production, presence of regulatory T cells (Treg), and Th2 immune response. The unfavorable prognosis is due to a higher number of relapses, infiltration into the adjacent tissues, lymph node metastasis, and increased tumor size [76].

7. Conclusion

The analysis of the information available in the literature allows us to conclude that most of the studies indicate that the presence of autoimmune thyroid disease is a factor that increases the risk of thyroid cancer. Nevertheless, this inference is mainly based on epidemiological studies, with little comprehension of the molecular mechanisms to support it. The main mechanisms described to explain this increased risk are related to the induction of oxidative DNA lesions by chronic inflammation and the shift of immune response to Th2 profile. The exact role of the different types of the immune response in the physiopathology of autoimmune thyroid diseases and thyroid cancer is poorly understood. There is no consensus about the effects of autoimmunity in the clinical outcomes and prognosis of patients with thyroid cancer. Although some studies point out that autoimmunity can decrease proteins related to tumor aggressiveness, others relate it to worse clinical outcomes by increasing proteins that favor tumor progression.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2020.153098.

References

[1] M.P. Vanderpump, The epidemiology of thyroid disease, Br. Med. Bull. 99 (2011) 39–51.
[2] N.S. Morais, J. Stuart, H. Guan, Z. Wang, E.S. Cibas, M.C. Frates, C.B. Benson, N.L. Cho, M.A. Nehs, C.A. Alexander, E. Marqusee, M.I. Kim, J.H. Lorch, J.A. Barletta, T.E. Angell, E.K. Alexander, The impact of hashimoto thyroiditis on thyroid nodule cytology and risk of thyroid cancer, J. Endocr. Soc. 3 (4) (2019) 791–800.
[3] J.J. Wiltshire, T.M. Drake, L. Uttley, S.P. Balasubramanian, Systematic review of trends in the incidence rates of thyroid cancer, Thyroid 26 (2016) 11.
[4] A. Bychkov. World Health Organization (WHO) classification. PathologyOutlines.com website. http://www.pathologyoutlines.com/topic/thyroidwho.html/ (Accessed July 25th, 2019).
[5] C. Molnár, S. Molnár, J. Bedekovics, A. Mokánszki, F. Győry, E. Nagy, G. Méhes, Thyroid carcinoma coexisting with Hashimoto's thyreoiditis: clinicopathological and molecular characteristics clue up pathogenesis, Pathol. Oncol. Res. 25 (2019) 1191–1197.
[6] C. La Vecchia, M. Malvezzi, C. Bosetti, W. Garavello, P. Bertuccio, F. Levi, E. Negri, Thyroid cancer mortality and incidence: a global overview, Int. J. Cancer 136 (9) (2015) 2187–2195.
[7] D.A.S. McLeod, D.S. Cooper, The incidence and prevalence of thyroid autoimmunity, Endocrine 42 (2012) 252–265.
[8] M. Borowczyk, A. Janicki, G. Dworacki, E. Szczepanek-Parulska, M. Danieluk, J. Barnett, M. Antonik, M. Kałużna, B. Bromińska, R. Czepczyński, M. Bączyk, K. Ziemnicka, M. Ruchała, Decreased staging of diferentiated thyroid cancer in patients with chronic lymphocytic thyroiditis, J. Endocrinol. Invest. 42 (2019) 45–52.
[9] J. Liang, W. Zeng, F. Fang, T. Yu, Y. Zhao, X. Fan, N. Guo, X. Gao, Clinical analysis of Hashimoto thyroiditis coexistent with papillary thyroid cancer in 1392 patients, Acta otorhinolaryngologica itálica 37 (2017) 393–400.
[10] T.M. Myshunina, B.D. Guda, M.Y. Bolgov, N.I. Mikhailenko, N.D. Tronko, Differentiated thyroid carcinomas associated with chronic thyroiditis: biological and clinical properties, Exp. Oncol. 40 (2) (2018) 128–131.
[11] J.S. Jeong, H.K. Kim, C. Lee, S. Park, J.H. Park, S. Kang, J.S. Jeong, K. Nam, W.Y. Chung, C.S. Park, Coexistence of chronic lymphocytic thyroiditis with papillary thyroid carcinoma: clinical manifestation and prognostic outcome, J. Korean Med. Sci. 27 (2012) 883–889.
[12] A. Selek, B. Cetinarslan, I. Tarkun, Z. Canturk, B. Ustuner, Z. Akyay, Thyroid autoimmunity: is really associated with papillary thyroid carcinoma? Eur. Arch. Otorhinolaryngol. 274 (3) (2016) 1677–1681.
[13] C.S.G. Anil, A. Gursoy, Hashimoto's thyroiditis is not associated with increased risk

of thyroid Cancer in patients with thyroid nodules: a single-center prospective study, Thyroid 20 (2010) 601–606.
[14] G. Grani, A. Calvanese, G. Carbotta, M. D'Alessandri, A. Nesca, M. Bianchini, M. Del Sordo, M. Vitale, A. Fumarola, Thyroid autoimmunity and risk of malignancy in thyroid nodules submitted to fine-needle aspiration cytology, Head Neck 37 (2) (2014) 260–264.
[15] V. Marotta, C. Sciammarella, M.G. Chiofalo, C. Gambardella, C. Bellevicine, M. Grasso, G. Conzo, G. Docimo, G. Botti, S. Losito, G. Troncone, M. De Palma, L. Giacomelli, L. Pezzullo, A. Colao, A. Faggiano, Hashimoto's thyroiditis predicts outcome in intrathyroidal papillary thyroid cancer, Endocr. Relat. Cancer 24 (9) (2017) 485–493.
[16] R. Zeng, Y. Lyu, G. Zhang, T. Shou, K. Wang, H. Niu, X. Yan, Positive effect of RORγt on the prognosis of thyroid papillary carcinoma patients combined with Hashimoto's thyroiditis, Am. J. Transl. Res. 10 (10) (2018) 3011–3024.
[17] E.Y. Kim, W.G. Kim, W.B. Kim, T.Y. Kim, J.M. Kim, J.S. Ryu, S.J. Hong, G. Gong, Y.K. Shong, Coexistence of chronic lymphocytic thyroiditis is associated with lower recurrence rates in patients with papillary thyroid carcinoma, Clin. Endocrinol. 71 (4) (2009) 581–586.
[18] S. Zivancevic-Simonovic, O. MihaljevicIvana, I. Majstorovic, S. Popovic, S. Markovic, O. Milosevic-Djordjevic, Z. Jovanovic, L. Mijatovic-Teodorovic, D. Mihajlovic, M. Colic, Cytokine production in patients with papillary thyroid cancer and associated autoimmune Hashimoto thyroiditis, Cancer Immunol. Immunother. 64 (8) (2015) 1011–1019.
[19] W. Zou, N.P. Restifo, TH17 cells in tumour immunity and immunotherapy, Nat. Rev. Immunol. 10 (4) (2010) 248–256.
[20] W. Zou, Regulatory T cells, tumour immunity and immunotherapy, Nat. Rev. – Immunol. 6 (2006) 295–307.
[21] M. Marazuela, M.A. García-López, N. Figueroa-Vega, H. Fuente, B. Alvarado-Sánchez, A. Monsiváis-Urenda, F. Sánchez-Madrid, R. González-Amaro, Regulatory T cells in human autoimmune thyroid disease, J. Clin. Endocrinol. Metab. 91 (9) (2006) 3639–3646.
[22] J.D. French, Z.J. Weber, D.L. Fretwell, S. Said, J.P. Klopper, B.R. Haugen, Tumor-associated lymphocytes and increased FoxP3+ regulatory T cell frequency correlate with more aggressive papillary thyroid Cancer, J. Clin. Endocrinol. Metab. 95 (5) (2010) 2325–2333.
[23] T. Pilli, P. Toti, R. Occhini, M.G. Castagna, S. Cantara, M. Caselli, S. Cardinale, L. Barbagli, F. Pacini, Chronic lymphocytic thyroiditis (CLT) has a positive prognostic value in papillary thyroid cancer (PTC) patients: the potential key role of Foxp3+ T lymphocytes, J. Endocrinol. Invest. 41 (2018) 703–709.
[24] I. Vasileiadis, G. Boutzios, G. Charitoudis, E. Koukoulioti, T. Karatzas, Thyroglobulin antibodies could be a potential predictive marker for papillary thyroid carcinoma, Ann. Surg. Oncol. 21 (8) (2014) 2725–2732.
[25] X. Wu, Y. Lun, H. Jiang, Q. Gang, S. Xin, Z. Duan, J. Zhang, Coexistence of thyroglobulin antibodies and thyroid peroxidase antibodies correlates with elevated thyroid-stimulating hormone level and advanced tumor stage of papillary thyroid cancer, Endocrine 46 (2014) 554–560.
[26] C.T. Shen, X.Y. Zhang, Z.L. Qiu, Z.K. Sun, W.J. Wei, H.J. Song, Q.Y. Luo, Thyroid autoimmune antibodies in patients with papillary thyroid carcinoma: a double-edged sword? Endocrine 58 (1) (2017) 176–183.
[27] F. Veit, D. Graf, S. Momberger, B. Helmich-Kapp, I. Ruschenburg, A. Peters, J. Kussmann, W. Saeger, K.W. Schmidt, M. Toetsch, K. Nestler, K. Mann, Papillary thyroid cancer and coexisting autoimmune thyroiditis, Horm. Metab. Res. 49 (2017) 869–872.
[28] S. Chowdhury, J. Veyhl, F. Jessa, O. Polyakova, A. Alenzi, C. MacMillan, R. Ralhan, P.G. Walfish, Programmed death-ligand 1 overexpression is a prognostic marker for aggressive papillary thyroid cancer and its variants, Oncotarget 7 (22) (2016) 32318–32328.
[29] D. Lubin, E. Baraban, A. Lisby, S. Jalali-Farahani, P. Zhang, V. Livolsi, Papillary thyroid carcinoma emerging from hashimoto thyroiditis demonstrates increased PD-L1 expression, which persists with metastasis, Endocr. Pathol. 29 (2018) 317–323.
[30] K.C. Ohaegbulam, A. Assal, E. Lazar-Molnar, Y. Yao, X. Zang, Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway, Trends Mol. Med. 21 (1) (2015) 24–33.
[31] S. Ahn, T.H. Kim, S.W. Kim, C.S. Ki, H.W. Jang, J.S. Kim, J.S.H. Kim, J.H. Choe, J.H. Shin, S.Y. Hahn, Y.L. Oh, J.H. Chung, Comprehensive screening for PD-L1 expression in thyroid cancer, Endocr. Relat. Cancer 24 (2) (2017) 97–106.
[32] A. Valavanidis, T. Vlachogianni, C. Fiotakis, 8-hydroxy-2' -deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis, J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 27 (2) (2009) 120–139.
[33] M. Mseddi, R.B. Mansour, N. Gouiia, F. Mnif, R. Bousselaa, M. Abid, T. Boudaouara, H. Attia, S. Lassoued, A comparative study of nuclear 8-hydroxyguanosine expression in autoimmune thyroid diseases and papillary thyroid carcinoma and its relationship with p53, Bcl-2 and Ki-67 cancer related proteins, Adv. Med. Sci. 62 (2017) 45–51.
[34] K.X. Wang, D.T. Denhardt, Osteopontin: role in immune regulation and stress responses, Cytokine Growth Factor Rev. 19 (2008) 333–345.
[35] N.S. Fedarko, A. Jain, A. Karadag, M.R. Van Eman, L.W. Fisher, Elevated serum bone sialoprotein and osteopontin in colon, breast, prostate, and lung cancer, Clin. Cancer Res. 7 (2001) 4060–4066.
[36] V. Guarino, P. Faviana, G. Salvatore, M.D. Castellone, A.M. Cirafici, V. De Falco, A. Celetti, R. Giannini, F. Basolo, R.M. Melillo, M. Santoro, Osteopontin is overexpressed in human papillary thyroid carcinomas and enhances thyroid carcinoma cell invasiveness, J. Clin. Endocrinol. Metab. 90 (9) (2005) 5270–5278.
[37] K.H. Kang, Osteopontin expression in papillary thyroid carcinoma and its relationship with the BRAF mutation and tumor characteristics, J. Korean Surg. Soc. 84 (2013) 9–17.
[38] S.H. Park, C.S. Park, Kim Young, S. Nam-Goong, Y.S. Kim, J.C. Lee, Choi Jl, J.W. Park, E.S. Kim, Osteopontin levels in patients with papillary thyroid cancer according to the presence of Hashimoto's thyroiditis, Asian Pacific J. Cancer Prev. 16 (6) (2015) 2447–2451.
[39] X. Shu, J. Ji, X. Li, J. Sundquist, K. Sundquist, K. Hemminki, Cancer risk in patients hospitalised for Graves' disease: a population-based cohort study in Sweden, Br. J. Cancer 102 (9) (2010) 1397–1399.
[40] M. Ehlers, M. Schott, S. Allelein, Graves' disease in clinical perspective, Front. Biosci. (Landmark Ed) 24 (2019) 35–47.
[41] S.M. Ferrari, I. Ruffilli, G. Elia, F. Ragusa, S.R. Paparo, A. Patrizio, V. Mazzi, A. Antonelli, P. Fallahi, Chemokines in hyperthyroidism, J. Clin. Transl. Endocrinol. 16 (2019) 100196.
[42] S. Wei, Z.W. Baloch, V.A. LiVolsi, Thyroid carcinoma in patients with Graves' disease: an institutional experience, Endocr. Pathol. 26 (1) (2015) 48–53.
[43] S.A. Rivkees, Pediatric Graves' disease: controversies in management, Horm. Res. Paediatr. 74 (5) (2010) 305–311.
[44] S.P. MacFarland, A.J. Bauer, N.S. Adzick, L.F. Surrey, J. Noyes, K. Kazahaya, S. Mostoufi-Moab, Disease burden and outcome in children and young adults with concurrent graves disease and differentiated thyroid carcinoma, J. Clin. Endocrinol. Metab. 103 (8) (2018) 2918–2925.
[45] F. Burdan, A. Chałas, J. Szumiło, Cyclooxygenase and prostanoids - biological implications, Postepy. Hig. Med. Dosw. (Online) 60 (2006) 129–141.
[46] J.L. Masferrer, K.M. Leahy, A.T. Koki, B.S. Zweifel, S.L. Settle, B.M. Woerner, D.A. Edwards, A.G. Flickinger, R.J. Moore, K. Seibert, Antiangiogenic and anti-tumor activities of cyclooxygenase-2 inhibitors, Cancer Res. 60 (2000) 1306–1311.
[47] Y. Ito, H. Yoshida, K. Nakano, Y. Takamura, A. Miya, K. Kobayashi, T. Yokozawa, F. Matsuzuka, N. Matsuura, K. Kuma, A. Miyauchi, Cyclooxygenase-2 expression in thyroid neoplasms, Histopathology 42 (2003) 492–497.
[48] K. Krawczyk-Rusiecka, K. Wojciechowska-Durczynska, A. Cyniak-Magierska, A. Zygmunt, A. Lewinski, Assessment of cyclooxygenase-1 and 2 gene expression levels in chronic autoimmune thyroiditis, papillary thyroid carcinoma and nontoxic nodular goitre, Thyroid Res. 7 (10) (2014) 1–8.
[49] S. Hwang, D.Y. Shin, E.K. Kim, W.I. Yang, J.W. Byun, S.J. Lee, G. Kim, S.J. Im, E.J. Lee, Focal lymphocytic thyroiditis nodules share the features of papillary thyroid cancer on ultrasound, Yonsei Med. J. 56 (5) (2015) 1338–1344.
[50] C.C. Cheung, S. Ezzat, J.L. Freeman, I.B. Rosen, S.L. Asa, Immunohistochemical diagnosis of papillary thyroid carcinoma, Mod. Pathol. 14 (2001) 338–342.
[51] S.J. Raphael, G. McKeown-Eyssen, S.L. Asa, High-molecular-weight cytokeratin 19 in the diagnosis of thyroid tumours, Mod. Pathol. 7 (1994) 295–300.
[52] M. Bonzanini, P.L. Amadori, C. Sagramoso, P.D. Palma, Expression of cytokeratin 19 and protein p63 in fine needle aspiration biopsy of papillary thyroid carcinoma, Acta Cytol. 52 (5) (2008) 541–548.
[53] S.N. Divani, G.P. Kalodimos, M.A. Lioupis, N.C. Syrmos, Hashimoto's thyroiditis and papillary thyroid carcinoma. Are cytokeratin 19 and P63 proteins of any diagnostic value? Hell. J. Nucl. Med. 19 (3) (2016) 250–253.
[54] J.B. Cowland, C. Hother, K. Gronbaek, MicroRNAs and cancer, APMIS 115 (2007) 1090–1106.
[55] M. Pohl, F. Grabellus, K. Worm, G. Arnold, M. Walz, K.W. Schmid, S.Y. Sheu-Grabellus, Intermediate microRNA expression profile in Graves' disease falls between that of normal thyroid tissue and papillary thyroid carcinoma, J. Clin. Pathol. 70 (2017) 33–39.
[56] S.F. Kuo, S.F. Lin, T.C. Chao, C. Hsueh, K.J. Lin, J.D. Lin, Prognosis of multifocal papillary thyroid carcinoma, Int. J. Endocrinol. 2013 (2013) 1–6.
[57] S. Dong, Q. Xia, Y.J. Wu, High TPOab levels (&1300 IU/ml) indicate multifocal PTC in Hashimoto's thyroiditis patients and support total thyroidectomy, Otolaryngol. Head Neck Surg. 153 (1) (2015) 20–26.
[58] C. Marcocci, M. Marino, Thyroid-directed antibodies, in: L.E. Braverman, R. Utiger (Eds.), Part II Laboratory Assessment of Thyroid Function, Lippincott Williams and Wilkins, Philadelphia, PA, 2005, pp. 360–372.
[59] F. Latrofa, D. Ricci, L. Grasso, P. Vitti, L. Masserini, F. Basolo, C. Ugolini, G. Mascia, A. Lucacchini, A. Pinchera, Characterization of Thyroglobulin Epitopes in Patients with Autoimmune and Non-autoimmune, (2008).
[60] Thyroid Diseases Using Recombinant Human Monoclonal Thyroglobulin Autoantibodies. J. Clin. Endocrinol. Metab. 93(2):591–596.
[61] F. Jokisch, I. Kleinlein, B. Haller, T. Seehaus, H. Fuerst, M. Kremer, A small subgroup of Hashimoto's thyroiditis is associated with IgG4-related disease, Virchows Arch. 468 (2016) 321–327.
[62] D. Lang, J. Zwerina, H. Pieringer, IgG4-related disease: current challenges and future prospects, Ther. Clin. Risk Manag. 12 (2016) 189–199.
[63] H.H. Jun, S.M. Kim, S.W. Hong, Y.S. Lee, H.S. Chang, C.S. Park, Warthin-like variant of papillary thyroid carcinoma: single institution experience, ANZ J. Surg. 86 (6) (2016) 492–494.
[64] M. Hirokawa, E. Nishihara, N. Takada, M. Higuchi, M. Kotakemori, T. Hayashi, A. Miyauchi, Warthin-like papillary thyroid carcinoma with immunoglobulin G4-positive plasma cells possibly related to Hashimoto's thyroiditis, Jpn. Endocr. Soc. 65 (2) (2017) 175–180.
[65] D.A. Siegel, J. King, E. Tai, N. Buchanan, U.A. Ajani, Cancer incidence rates and trends among children and adolescents in the United States, 2001–2009, Pediatrics 134 (2014) 945–955.
[66] M. Russo, P. Malandrino, M. Moleti, A. D'Angelo, M. Tavarelli, G. Sapuppo, Thyroid cancer in the pediatric age in Sicily: influence of the volcanic environment, Anticancer Res. 37 (2017) 1515–1522.
[67] G. Zirilli, L. Cannavò, F. Vermiglio, M.A. Violi, F. De Luca, M. Wasniewska, Differentiated thyroid carcinoma presentation may be more aggressive in children and adolescents than in young adults, Ital. J. Pediatr. 44 (1) (2018) 13.

[68] S.H. LaFranchi, Inaugural management guidelines for children with thyroid nodules and differentiated thyroid cancer: children are not small adults, Thyroid 25 (2015) 713–715.
[69] S. Markovina, P.W. Grigsby, J.K. Schwarz, T. DeWees, J.F. Moley, B.A. Siegel, Treatment approach, surveillance, and outcome of well-differentiated thyroid cancer in childhood and adolescence, Thyroid 24 (2014) 1121–1126.
[70] B. Uhliarova, A. Hajtman, Hashimoto's thyroiditis-an independent risk factor for papillary carcinoma, Braz. J. Otorhinolaryngol. 84 (6) (2018) 729–735.
[71] M. Ehlers, M. Schott, Hashimoto's thyroiditis and papillary thyroid cancer: are they immunologically linked? Trends Endocrinol. Metab. 25 (1) (2014) 656–664.
[72] R. Paparodis, S. Imam, K. Todorova-Koteva, A. Staii, J.C. Jaume, Hashimoto's thyroiditis pathology and risk for thyroid cancer, Thyroid 24 (7) (2014) 1107–1114.
[73] Y. Hwangbo, Y.J. Park, Genome-wide association studies of autoimmune thyroid diseases, thyroid function, and thyroid cancer, Endocrinol. Metab. 33 (2) (2018) 175–184.
[74] G. Pellegriti, C. Mannarino, M. Russo, R. Terranova, I. Marturano, R. Vigneri, A. Belfiore, Increased mortality in patients with differentiated thyroid cancer associated with Graves' disease, J. Clin. Endocrinol. Metab. 98 (3) (2013) 1014–1021.
[75] A.E. Stanciu, N. Serdarevic, A.E. Hurduc, M.M. Stanciu, IL-4, IL-10 and high sensitivity-CRP as potential serum biomarkers of persistent/recurrent disease in papillary thyroid carcinoma with/without Hashimoto's thyroiditis, Scand. J. Clin. Lab. Invest. 75 (7) (2015) 539–548.
[76] Y. Yu, J. Zhang, G. Lu, T. Li, Y. Zhang, N. Yu, X. Guo, Clinical relationship between IgG4-positive Hashimoto's thyroiditis and papillary thyroid carcinoma, J. Clin. Endocrinol. Metab. 101 (4) (2016) 1516–1524.
[77] S. Benvenga, On the association between Hashimoto' s thyroiditis and papillary thyroid carcinoma: looking 100 years back and, hopefully, fewer years ahead to sort out this association, Thyroid 23 (2013) 1180–1181.
[78] F. Boi, F. Pani, S. Mariotti, Thyroid autoimmunity and thyroid cancer: review focused on cytological studies, Eur. Thyroid J. 6 (2017) 178–186.
[79] M.E. Dailey, S. Lindsay, R. Skahen, Relation of thyroid neoplasms to Hashimoto disease of the thyroid gland, AMA Arch. Surg. 70 (2) (1955) 291–297.
[80] C. Spencer, S. Fatemi, Thyroglobulin antibody (TgAb) methods -strengths, pitfalls and clinical utility for monitoring TgAb-positive patients with differentiated thyroid cancer, Best Pract. Res. Clin. Endocrinol. Metab. 27 (2013) 701–712.
[81] C.A. Spencer, C.C. Wang, Thyroglobulin measurement. Techniques, clinical benefits, and pitfalls, Endocrinol. Metab. Clin. North Am. 24 (1995) 841–863.
[82] T. Karatzas, I. Vasileiadis, E. Zapanti, G. Charitoudis, E. Karakostas, G. Boutzios, Thyroglobulin antibodies as a potential predictive marker of papillary thyroid carcinoma in patients with indeterminate cytology, Am. J. Surg. 212 (5) (2016) 946–952.
[83] G.A. Lupoli, O.E. Okosieme, C. Evans, P.M. Clark, A.J. Pickett, L.D.K. Premawardhana, J.H. Lazarus, Prognostic significance of thyroglobulin antibody epitopes in differentiated thyroid cancer, J. Clin. Endocrinol. Metab. 100 (1) (2015) 100–108.
[84] S. Lee, J.W. Choe, H.K. Kim, J. Sung, High-sensitivity C-reactive protein and cancer, J. Epidemiol. 21 (2011) 161–168.
[85] B.H. Joshi, P. Leland, S. Lababidi, F. Varrichio, R.K. Puri, Interleukin-4 receptor alpha overexpression in human bladder cancer correlates with the pathological grade and stage of the disease, Cancer Med. 3 (6) (2014) 1615–1628.
[86] K.T. Venmar, K.J. Carter, D.G. Hwang, E.A. Dozier, B. Fingleton, IL4 receptor ILR4alpha regulates metastatic colonization by mammary tumors through multiple signaling pathways, Cancer Res. 74 (16) (2014) 4329–4340.
[87] B.H. Joshi, A. Suzuki, T. Fujisawa, P. Leland, F. Varrichio, S. Lababidi, R.K. Puri, Identification, characterization, and targeting of IL-4 receptor by IL-4-Pseudomonas exotoxin in mouse models of anaplastic thyroid cancer, Discov. Med. 20 (111) (2015) 273–284.
[88] L.L. Cunha, E.C. Morari, S. Nonogaki, M.A. Marcello, F.A. Soares, J. Vassallo, L.S. Ward, Interleukin 10 expression is related to aggressiveness and poor prognosis of patients with thyroid cancer, Cancer Immunol. Immunother. 66 (2) (2017) 141–148.
[89] Y. Li, G. Zhou, T. Ozaki, E. Nishihara, F. Matsuzuka, Y. Bai, Z. Liu, E. Taniguchi, A. Miyauchi, K. Kakudo, Distinct histopathological features of Hashimoto's thyroiditis with respect to IgG4-related disease, Mod. Pathol. 25 (8) (2012) 1086–1097.
[90] F. Tasli, G. Ozkok, A. Argon, D. Ersoz, A. Yagci, A. Uslu, N. Erkan, T. Salman, E. Vardar, The role of IgG4 plasma cells in the association of Hashimoto's thyroiditis with papillary carcinoma, APMIS 122 (12) (2014) 1259–1265.