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NÍVEIS SÉRICOS ELEVADOS DE ÁCIDO ÚRICO ESTÃO ASSOCIADOS À OXIDAÇÃO DE NUCLEOSÍDEOS EM PACIENTES COM DIABETES *MELLITUS* TIPO 2

Santa Maria, RS
2019

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Farmacêuticas, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciências Farmacêuticas**.

Orientadora: Profª. Dra. Maria Beatriz Moretto
Coorientador: Prof. Dr. Rafael Noal Moresco

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A educação é a arma mais poderosa para mudar o mundo.

Nelson Mandela

RESUMO

NÍVEIS SÉRICOS ELEVADOS DE ÁCIDO ÚRICO ESTÃO ASSOCIADOS À OXIDAÇÃO DE NUCLEOSÍDEOS EM PACIENTES COM DIABETES *MELLITUS* TIPO 2

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O ácido úrico, mesmo em concentrações fisiológicas, é capaz de causar danos a biomoléculas através de diversos mecanismos. Em especial, a hiperuricemia é capaz de favorecer a formação de espécies radicalares intracelulares, dessa forma, contribuindo para o surgimento de produtos da oxidação de nucleosídeos, como o 8-hidroxi-2'-deoxiguanosina (8-OHdG). Sabe-se que portadores de diabetes *mellitus* tipo 2 (DM2) possuem níveis mais elevados de oxidação de nucleosídeos e ácido desoxirribonucleico (DNA) em comparação a indivíduos saudáveis, porém, pouco foi explorado sobre o papel dos níveis séricos de ácido úrico na promoção desse dano em humanos. Dessa forma, o objetivo do presente estudo foi avaliar a relação entre os níveis séricos de ácido úrico e as concentrações urinárias de 8-OHdG, além da influência do controle glicêmico e de outros fatores sobre essa relação, em pacientes portadores de DM2 e indivíduos saudáveis. Foram recrutados, no Hospital Universitário de Santa Maria, 61 pacientes de ambos os gêneros, sendo 46 portadores de DM2, os quais foram atendidos no Ambulatório de Endocrinologia. Os níveis de ácido úrico, 8-OHdG e outros parâmetros foram mensurados, e então, os indivíduos foram estratificados conforme a mediana de ácido úrico sérico da população de estudo (< 5,3 mg/dL e ≥ 5,3 mg/dL). Os pacientes diabéticos que possuíam maiores níveis de ácido úrico demonstraram maiores níveis urinários de 8-OHdG comparados àqueles com menores níveis (20,0 [16,0–35,0] *versus* 16,6 [13,0–23,0] ng/mL, P=0.014, respectivamente). Essa diferença não foi observada nos indivíduos controles (P=0.483). Além disso, uma correlação positiva significativa foi encontrada entre esses dois parâmetros (r = 0.40, P<0.01). Ainda, foi observado que essa associação aconteceu de forma independente de fatores como hipertensão, creatinina sérica, índice de massa corporal e gênero. O controle glicêmico, parâmetros inflamatórios e oxidativos foram semelhantes entre os grupos, demonstrando que esses fatores não contribuíram para a elevação no 8-OHdG. Conforme os resultados obtidos, a associação entre os níveis séricos de ácido úrico e 8-OHdG urinário em pacientes portadores de DM2 indicam que elevações no ácido úrico podem estimular uma maior oxidação de nucleosídeos, independentemente de outros fatores.

Palavras-chave: Ácido úrico. Diabetes *mellitus* tipo 2. Oxidação de nucleosídeos. 8-hidroxi-2'-deoxiguanosina urinário.

ABSTRACT

HIGH SERUM URIC ACID LEVELS ARE ASSOCIATED WITH NUCLEOSIDE OXIDATION IN PATIENTS WITH TYPE 2 DIABETES

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Uric acid, even in physiologic levels, is capable of causing damage to biomolecules through some mechanisms. Especially, hyperuricemia is capable of favor the formation of intracellular radical species, thus, contributing to the occurrence of products of nucleoside oxidation, including the 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of this damage. It is known that patients with type 2 diabetes (T2D) have higher levels of nucleoside and deoxyribonucleic acid (DNA) oxidation compared to healthy individuals, however, little was explored in humans about the role of serum uric acid levels in promoting this damage in this population. Thus, the objective of this study was to evaluate the relationship between the levels of serum uric acid and the urinary concentrations of 8-OHdG, besides the influence of the glycemic control and other factors on this relationship, in patients with T2D and healthy individuals. In the University Hospital of Santa Maria, 61 patients were recruited, of those, 46 had T2D and were assisted at the Ambulatory of Endocrinology. The levels of uric acid, 8-OHdG and other parameters were measured. The patients were stratified according to the median of serum uric acid in the study population (< 5,3 mg/dL e ≥ 5,3 mg/dL). The patients with T2D who had higher levels of uric acid showed higher urinary levels of 8-OHdG compared to those with lower levels (20.0 [16.0–35.0] *versus* 16.6 [13.0–23.0] ng/mL, P=0.014, respectively). This association was not observed in the healthy individuals (P=0.483). Moreover, a significant positive correlation was observed between the two parameters (r = 0.40, P<0.01). Furthermore, the association was independent of factors such as hypertension, serum creatinine, body mass index, and gender. The glycemic control, inflammatory and oxidative parameters were similar in both groups, demonstrating that these factors did not contribute to the rise in 8-OHdG levels. According to the results, the association between the levels of serum uric acid and the urinary 8-OHdG in patients with T2D indicate that an elevation of uric acid can stimulate the nucleoside oxidation, independently of other factors.

Keywords: Uric acid. Type 2 diabetes. Nucleoside oxidation. Urinary 8-hydroxy2'-deoxyguanosine.

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ARTIGO CIENTÍFICO

LISTA DE ABREVIATURAS E SIGLAS

8-OHdG	8-hidroxi-2'-deoxiguanosina
8-oxodG	8-oxo-2'-deoxiguanosina
AOPP	Produtos proteicos de oxidação avançada
COX-2	Ciclooxigenase-2
DM	Diabetes *mellitus*
DM2	Diabetes *mellitus* tipo 2
DNA	Ácido desoxirribonucleico
ELISA	*Enzyme-Linked Immunosorbant Assay*
ERO	Espécies reativas de oxigênio
$HO^{\bullet}$	Radical hidroxila
IL-6	Interleucina-6
NADPH	Nicotinamida adenina dinucleotídeo fosfato
NF-κB	Fator nuclear *kappa*-B
TNF-α	Fator de necrose tumoral-*alfa*

SUMÁRIO

1 APRESENTAÇÃO

As seções **MATERIAIS E MÉTODOS**, **RESULTADOS** e **DISCUSSÃO** encontram-se no **ARTIGO CIENTÍFICO**, publicado no periódico *Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis*, e representam a íntegra deste estudo. O item **CONCLUSÕES**, encontrado no final desta dissertação, apresenta interpretações e comentários gerais sobre o manuscrito contido neste trabalho. As **REFERÊNCIAS BIBLIOGRÁFICAS** referem-se somente às citações contidas no item **INTRODUÇÃO** desta dissertação, de modo que as referências utilizadas para a elaboração do artigo estão mencionadas no mesmo.

2 INTRODUÇÃO

O ácido úrico, metabólito do catabolismo das purinas, exerce uma importante função antioxidante no plasma, porém, em níveis elevados, tem sido associado com atividades pró-oxidantes, o que nos últimos anos ligou esse metabólito ao desenvolvimento e complicações de diversas doenças, incluindo a hipertensão e o diabetes *mellitus* (DM) (LV et al., 2013; WU et al., 2017).

O DM, especialmente o tipo 2 (DM2), que representa a maioria dos casos, tornou-se um problema de saúde pública preocupante em todo o mundo, não somente pela própria etiologia e instalação da doença, mas também por suas complicações, que podem envolver danos cardiovasculares, renais e na retina, acarretados principalmente pela contínua hiperglicemia (FURUSYO; HAYASHI, 2013). No ano de 2017, haviam aproximadamente 425 milhões de pessoas portadoras de DM no mundo, e estima-se um crescimento para 693 milhões de indivíduos até o ano de 2045 (IDF, 2017).

Os mecanismos através dos quais o ácido úrico pode promover dano oxidativo a biomoléculas incluem a ativação de enzimas pró-oxidantes e a produção direta de espécies reativas de oxigênio (ERO), geralmente relacionados a altos níveis desse metabólito (CHOI; MOUNT; REGINATO, 2005). Nesse contexto, é de grande importância que seja investigada a atuação do ácido úrico como agente promotor de dano oxidativo no DM, uma vez que os pacientes diabéticos conhecidamente estão expostos a agentes oxidantes, e possuem maiores níveis de oxidação de DNA e nucleosídeos (TATSCH et al, 2015).

O dano aos nucleosídeos pode ser avaliado pela quantificação de biomarcadores tanto no soro quanto tecidos e urina, sendo esta última uma alternativa não-invasiva e de fácil obtenção, na qual pode ser avaliada a presença de 8-hidroxi-2’-deoxiguanosina (8-OHdG), um produto da oxidação da deoxiguanosina (WEIMANN et al., 2012). Essa molécula está associada a diferentes condições patológicas, incluindo o DM2 (TATSCH et al., 2015).

Estabelecer a relação entre os níveis de ácido úrico e a formação de 8-OHdG em pacientes diabéticos é uma importante ferramenta para a compreensão dos mecanismos de dano oxidativo no contexto do DM, bem como entender o papel do ácido úrico nessa doença. Assim, o presente trabalho busca verificar a existência de

uma relação entre níveis séricos elevados de ácido úrico e maior excreção urinária de 8-OHdG em pacientes portadores de DM2 e em indivíduos saudáveis.

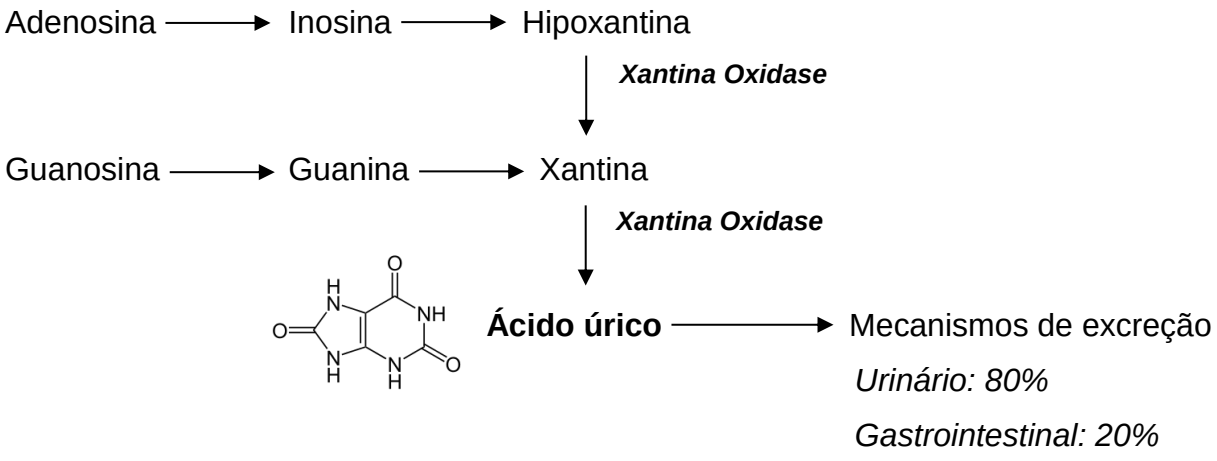
3 REVISÃO BIBLIOGRÁFICA

3.1 ÁCIDO ÚRICO

O ácido úrico (2,6,8-trioxipurina, $C_5H_4N_4O_3$) é um ácido fraco, encontrado no sangue, em pH fisiológico, principalmente sob a forma de urato, cujos níveis normais para homens encontram-se entre 3,5 e 7 mg/dL, e para mulheres, entre 2,6 e 6 mg/dL (BENN et al., 2018). A solubilidade do ácido úrico em água atinge níveis por volta de 6,8 mg/dL, e quando esse limiar é excedido, caracteriza-se a hiperuricemia, entretanto, proteínas como a albumina se ligam a essa molécula e aumentam sua solubilidade, permitindo com que valores superiores a 7 mg/dL sejam encontrados (JALAL et al., 2011).

O ácido úrico é o produto final do catabolismo das purinas endógenas e exógenas, podendo ser oriundo do fígado, em sua maior parte, e em uma menor parcela de tecidos periféricos como rins e intestino (JALAL et al., 2011). As purinas provenientes das células, além daquelas absorvidas da dieta via gastrintestinal, são transformadas em outros produtos, os quais sofrem ação da enzima xantina oxidase, a qual converte hipoxantina em xantina e essa em ácido úrico (BENN et al., 2018), como ilustrado na Figura 1. Na sua maior parte, a síntese de ácido úrico acontece no fígado, e sua excreção se dá através dos rins e intestinos (LI et al., 2011; OLIVEIRA; BURINI, 2012).

Figura 1. Mecanismos da homeostase do ácido úrico.



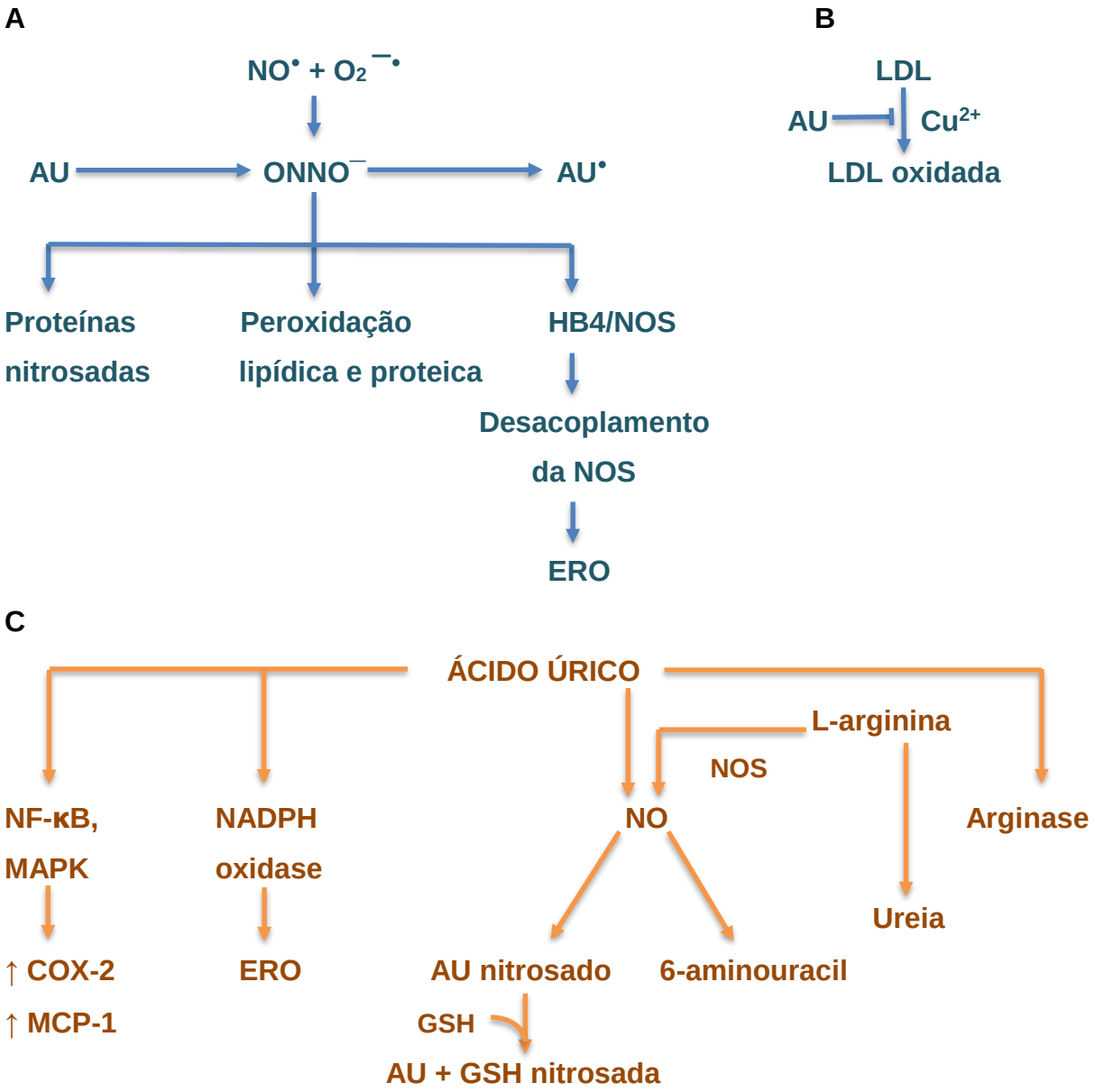
Fonte: Adaptado de Benn et al., 2018.

Apesar de uma alta taxa de filtração renal, mais de 90% do urato é reabsorvido nos túbulos proximais, contribuindo, assim, para as altas concentrações de ácido úrico encontradas no sangue (BENN et al., 2018). Outro fator que é determinante para a manutenção dos níveis de ácido úrico sanguíneo é a ausência da enzima uricase em humanos, fazendo com que a formação de ácido úrico seja a última etapa do catabolismo das purinas (CHOI; MOUNT; REGINATO, 2005). Esse acúmulo foi inicialmente visto como uma vantagem evolutiva por conta de sua capacidade antioxidante, porém, por sua associação com diversas doenças, essa visão vem sendo modificada nos últimos anos (CHOI; MOUNT; REGINATO, 2005).

Essa molécula possui ação antioxidante em níveis normais devido às suas duplas ligações, contribuindo para aproximadamente 50% dessa atividade no plasma, neutralizando radicais como oxigênio singleto e outros radicais de oxigênio (JOHNSON et al., 2005; SO; THORENS, 2010). Entretanto, em altos níveis e especialmente em condições hidrofóbicas, existem evidências de que o ácido úrico atua também como pró-oxidante (BAGNATI et al., 1999; DASEGOWDA et al., 2016). Alguns dos mecanismos antioxidantes e pró-oxidantes do ácido úrico estão ilustrados na Figura 2. O ácido úrico, quando atua como antioxidante, é capaz de interceptar a ação dos peroxinitritos (A) e da peroxidação lipídica mediada pelo cobre (B). Entretanto, sua capacidade pró-oxidante está envolvida na ativação de enzimas e vias oxidativas e inflamatórias (C), contribuindo para uma série de eventos capazes de danificar biomoléculas (SO; THORENS, 2010).

Alguns fatores que podem elevar a concentração sanguínea de ácido úrico incluem doenças renais (EJAZ et al., 2007), alguns medicamentos como ciclosporina e pirazinamida (OLIVEIRA; BURINI, 2012) e condições metabólicas que aumentam os níveis de ácido lático e corpos cetônicos (MOUNT; KWON; ZANDI-NEJAD, 2006). Outros fatores associados à hiperuricemia estão relacionados à dieta. O consumo de bebidas alcoólicas, principalmente de cerveja, foi associado à elevação dos níveis de ácido úrico e do risco de desenvolvimento de gota (CHOI; MOUNT; REGINATO, 2005). Além disso, o consumo de carnes em geral também pode aumentar a taxa de síntese do ácido úrico (SO; THORENS, 2010). O consumo de refrigerantes, bebidas ricas em frutose, também influencia positivamente na incidência de hiperuricemia (NAKAGAWA et al., 2006).

Figura 2. Mecanismos antioxidantes (A e B) e pró-oxidantes (C) do ácido úrico.

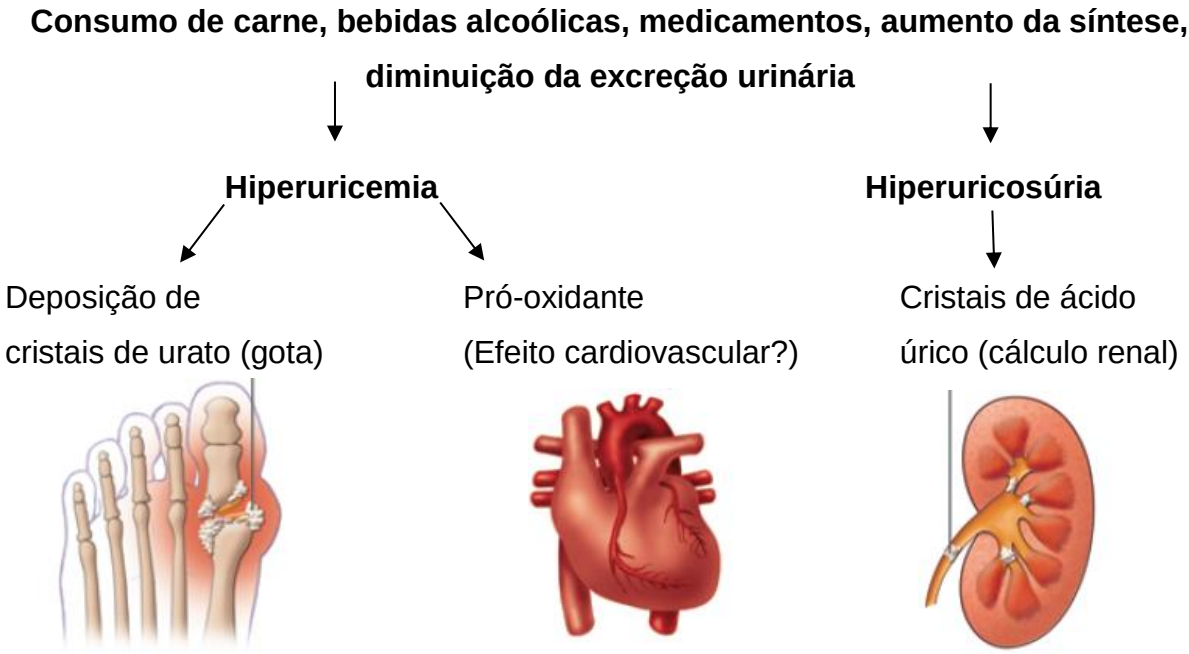


Fonte: Adaptado de So; Thorens, 2010. AU, ácido úrico; COX-2, ciclooxigenase-2; ERO, espécies reativas de oxigênio; GSH, glutationa; HB4, tetrahidrobiopterina; LDL, lipoproteína de baixa densidade; MCP-1, proteína de quimioatração de monócitos; MAPK, proteína quinase ativada por mitógeno; NADPH, nicotinamida adenina dinucleotídeo fosfato; NF-κB, fator nuclear *kappa*-B; NOS, óxido nítrico sintase; NO, óxido nítrico; $O_2^{-}\cdot$, ânion radical superóxido; $ONNO^{-}$, peroxinitrito;

3.2 HIPERURICEMIA E SUA PATOGENICIDADE

O ácido úrico, quando em elevadas concentrações no soro, possui uma forte associação com a etiologia de algumas doenças. A mais frequente é a gota, diretamente relacionada à deposição de cristais de urato nas articulações em situações de hiperuricemia, e na qual acontece um estado inflamatório (CHOI; MOUNT; REGINATO, 2005). A hipertensão arterial é outra patologia relacionada ao ácido úrico (WU et al., 2017), devido à sua capacidade de ativação direta do sistema renina-angiotensina (ZHANG et al., 2015) e promoção de disfunção endotelial (LI et al., 2016), resultando em uma associação positiva entre os níveis de ácido úrico e incidência de hipertensão. Algumas consequências da patogenicidade da hiperuricemia estão ilustradas na Figura 3.

Figura 3. Patogenicidade do ácido úrico.



Fonte: Adaptado de So; Thorens, 2010.

A inflamação é uma condição associada ao ácido úrico por múltiplos fatores. Condições hiperuricêmicas são responsáveis pela ativação de citocinas e vias pró-inflamatórias, como demonstrado por um estudo envolvendo jovens com hiperuricemia assintomática, no qual houve uma relação positiva entre a elevação

nos níveis de ácido úrico e o aumento de interleucina-6 (IL-6) e do fator de necrose tumoral-alfa (TNF-α) (ZHOU et al., 2018). Além disso, elevados níveis de ácido úrico podem mediar a ativação de vias inflamatórias mediadas pelo fator nuclear *kappa*-B (NF-κB) (SPIGA et al., 2017) e ciclooxigenase-2 (COX-2) (OĞUZ et al., 2017). A ativação de fatores pró-inflamatórios está relacionada ao desenvolvimento de doenças como a gota (KRISHNAN, 2010) e o DM (WANG et al., 2013).

3.2.1 Associação do ácido úrico com o DM

A relação entre DM e ácido úrico vem ganhando destaque nos últimos anos. O DM caracteriza-se por um conjunto de sinais e sintomas, resultantes da disfunção metabólica causada pela hiperglicemia, que se origina da subutilização da glicose em virtude da produção insuficiente ou nula de insulina, defeitos na sua ação ou ambos (ADA, 2016).

Os tipos mais frequentes de DM são o tipo 1 e o tipo 2. O primeiro é caracterizado por acometer, na sua maior parte, crianças e jovens, e acontece pela destruição das células beta-pancreáticas. Já o segundo, que representa a maioria dos casos de DM e acomete geralmente adultos, é um processo no qual existe uma diminuição na resposta dos receptores de glicose à insulina, levando à exaustão das células beta, culminando, nos dois tipos, na má utilização da glicose (ADA, 2016).

Sabe-se que fatores de risco para o desenvolvimento de DM2 incluem o estresse oxidativo causado por moléculas pró-oxidantes, incluindo a própria glicose, que são capazes de causar danos celulares no estado hiperglicêmico e, dessa forma, levando à resistência à insulina (MOON et al., 2017). Os produtos proteicos de oxidação avançada (AOPP), os quais são marcadores de oxidação, estão aumentados no DM (KALOUSOVÁ; SKRHA; ZIMA, 2002) e relacionados à disfunção endotelial nessa população (LIANG et al., 2014). A inflamação, mediada pela IL-6, também exerce um papel importante na etiologia da doença, contribuindo para o desenvolvimento e complicações do DM (REHMAN et al., 2017; TATSCH et al., 2012). Além disso, alguns grupos estão mais suscetíveis ao desenvolvimento de DM2, como obesos, idosos e algumas etnias (ADA, 2016).

Um estudo prospectivo conduzido com 2690 participantes acompanhados por aproximadamente 9 anos revelou que indivíduos que desenvolveram DM2 nesse período possuíam níveis mais elevados de ácido úrico (CHIEN et al., 2008). Essa

descoberta pode ligar o ácido úrico a diferentes fatores diabetogênicos, evidenciado por outros estudos, como a resistência à insulina (ZHI et al., 2016), desenvolvimento de obesidade (TSUSHIMA et al., 2013) e disfunção endotelial (LI et al., 2016). Além disso, o ácido úrico também foi relacionado às complicações do DM, como retinopatia e nefropatia (LIANG et al., 2016). Um estudo realizado por Čaušević e colaboradores (2010) revelou que os níveis de ácido úrico eram mais elevados no soro e na urina de indivíduos diabéticos quando comparados aos controles, resultado também alcançado por outros autores (KODAMA et al., 2009). Um estudo que comparou diversas outras pesquisas revelou que indivíduos diabéticos que foram acometidos por infarto cerebral possuíam níveis de ácido úrico 29% maior do que os que não apresentaram essa complicação do DM (DU; MA; ZHANG, 2017). Por último, elevados níveis de ácido úrico foram associados à mortalidade cardiovascular de portadores de DM2 independentemente de outros fatores (ZOPPINI et al., 2009), demonstrando que, apesar de ainda não estarem totalmente elucidados, existem mecanismos de patogenicidade do ácido úrico, especialmente em níveis elevados, contribuindo para o desenvolvimento, complicações e mortalidade no DM2.

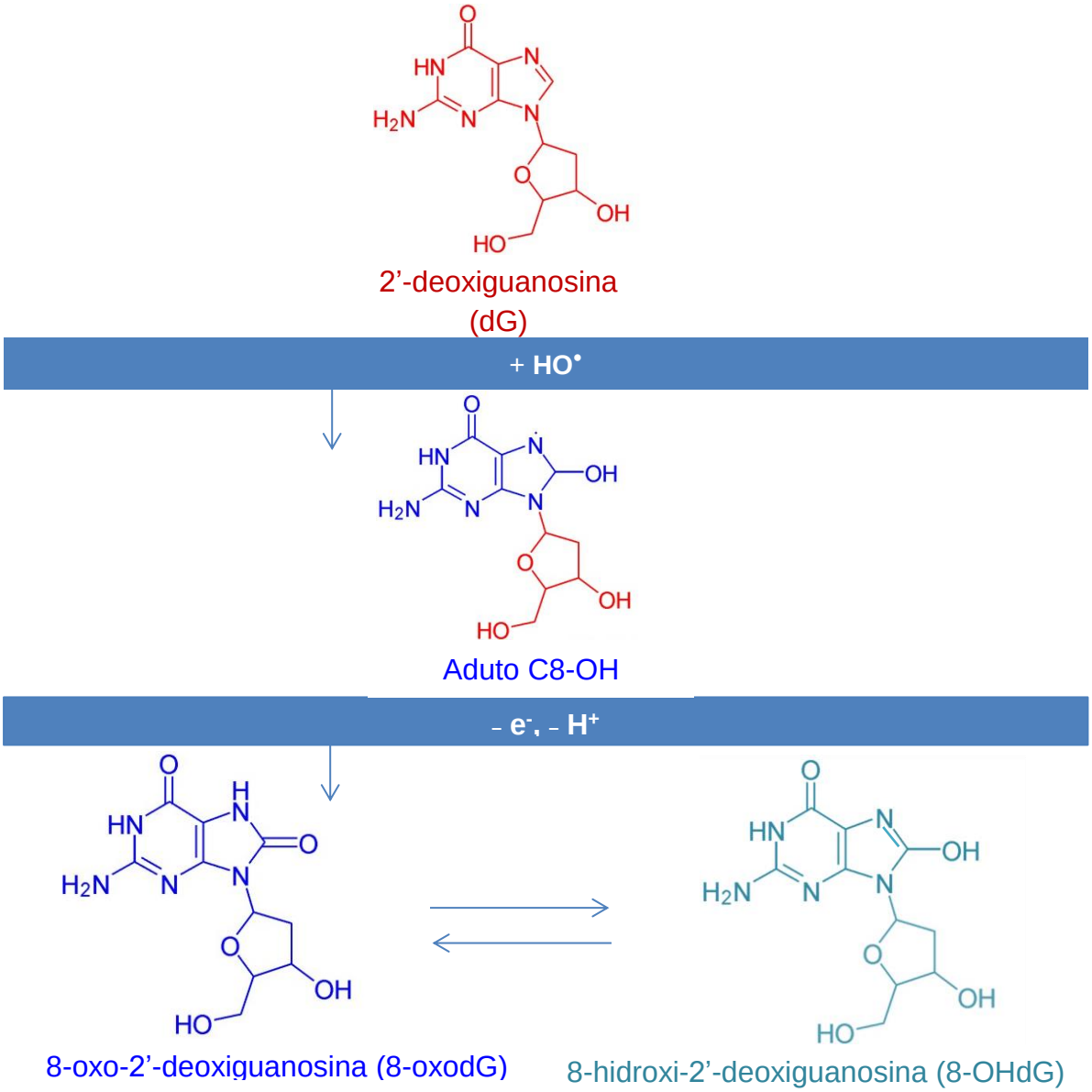
3.3 DANO OXIDATIVO DE NUCLEOSÍDEOS

O ácido desoxirribonucleico (DNA) é um polímero formado por cadeias de nucleotídeos, os quais, por sua vez, são constituídos de bases nitrogenadas, um açúcar pentose e um grupo fosfato; já nos nucleosídeos, o grupo fosfato está ausente (ZAHA; FERREIRA; PASSAGLIA, 2014). As bases nitrogenadas são classificadas em purinas, que possuem dois anéis heterocíclicos, e são adenina e guanina. Já as pirimidinas possuem somente um anel e são representadas pela citosina e timina (ZAHA; FERREIRA; PASSAGLIA, 2014). Diariamente, mais de 50.000 locais de dano podem ser observados em cada célula humana (LIU et al., 2016) provenientes de diferentes fontes exógenas e endógenas, radiação ultravioleta e radicais livres (COOKE; LUNEC; EVANS, 2002). Esses últimos podem se originar a partir das ERO, as quais possuem um papel fisiológico no organismo humano, porém, devido à sua natureza reativa, podem se tornar prejudiciais quando em desequilíbrio em relação às defesas antioxidantes, atacando diferentes

moléculas como proteínas, lipídeos e nucleosídeos (VALAVANIDIS; VLACHOGIANNI; FIOTAKIS, 2009).

O mais importante radical causador de danos a biomoléculas é o hidroxila ($HO^{\bullet}$), formado a partir de reações como a de Fenton, induzindo a entrada de um grupamento hidroxila na posição C8 da molécula de guanosina, formando um aduto C8-OH e rearranjo da molécula, formando inicialmente o 8-oxo-2'-deoxiguanosina (8-oxodG), o qual se interconverte no seu tautômero, 8-OHdG, caracterizando-o como um marcador de dano oxidativo da guanosina (LIU et al., 2016; VALAVANIDIS; VLACHOGIANNI; FIOTAKIS, 2009), como ilustrado na Figura 4.

Figura 4. Mecanismo de formação do 8-OHdG.



Fonte: Adaptado de Guo et al., 2017.

Esse tipo de dano requer constante reparo do local atingido e eliminação dos produtos formados a fim de evitar consequências como mutações (LINDAHL; WOOD, 1999). Mecanismos de reparo por excisão de bases e excisão de nucleotídeos são capazes de identificar os componentes danificados do DNA e substituí-los por outros íntegros, sendo responsáveis pelo aparecimento dessas moléculas na urina (COOKE; LUNEC; EVANS, 2002). Acreditava-se que a presença de 8-OHdG na urina se devia ao mecanismo de excisão de nucleotídeos de reparo do DNA (WEIMANN et al., 2012), porém, um estudo realizado por Evans et al. (2016) investigou um modelo animal com deficiência nesse mecanismo, revelando que não houve alteração na eliminação de 8-OHdG urinário nesses animais comparados aos *wildtype*, sugerindo que a fonte mais plausível desse marcador na urina pode ser o *pool* de 2'-desoxirribonucleotídeos. Devido à sua origem, a presença do 8-OHdG na urina é considerada como um marcador do estresse oxidativo global, em condições nas quais todas as células do organismo são atingidas, como no caso do DM e hiperuricemia (WEIMANN et al., 2012).

O ensaio de *Enzyme-Linked Immunosorbant Assay* (ELISA) é um dos mais empregados na avaliação do 8-OHdG em diferentes amostras, devido à sua fácil utilização e alta sensibilidade (WEIMANN et al., 2012). Esse método possui uma menor especificidade, porém, uma boa correlação com o método cromatográfico (ROSSNER et al., 2013), demonstrando que as técnicas imunológicas para mensuração dos produtos de danos aos nucleosídeos são confiáveis e comparáveis a outros métodos.

3.3.1 Associação do ácido úrico com o dano oxidativo aos nucleosídeos

O ácido úrico, devido ao seu comportamento pró-oxidante em algumas situações, foi relacionado ao dano oxidativo a biomoléculas. Em 1999, Bagnati e colaboradores evidenciaram que o ácido úrico foi capaz de potencializar a oxidação lipídica promovida pelo cobre, *in vitro*, especulando o seu potencial pró-oxidante. Outro estudo mostrou uma maior formação de 8-nitroguanina no soro de pacientes com artrite gotosa inflamatória, conforme aumento nos níveis de ácido úrico (CHANG et al., 2005). Ainda, um estudo em animais demonstrou que uma dieta rica em proteínas promoveu elevação nos níveis de ácido úrico concomitante com o aumento de 8-OHdG nas glândulas salivares (KOŁODZIEJ et al., 2017).

Alguns trabalhos propuseram os possíveis mecanismos pelos quais o ácido úrico pode estar envolvido com o aumento do dano oxidativo. Adipócitos expostos a diferentes concentrações de ácido úrico sofreram um aumento da produção de ERO, devido à ativação da enzima nicotinamida adenina dinucleotídeo fosfato (NADPH) oxidase, proporcionais às concentrações de ácido úrico (SAUTIN et al., 2007). Resultado semelhante foi encontrado em outras pesquisas utilizando células endoteliais aórticas (SÁNCHEZ-LOZADA et al., 2012), células tubulares (VERZOLA et al., 2014) e em um modelo animal de hiperuricemia (SÁNCHEZ-LOZADA et al., 2008). Outro mecanismo proposto foi a ativação do sistema renina-angiotensina por altas concentrações de ácido úrico e consequente mediação da produção de ERO, revelado por trabalho realizado em células endoteliais vasculares (YU et al., 2010). Corry e colaboradores (2008) também associaram o ácido úrico com a ativação desse sistema, porém em concentrações fisiológicas. Diante das evidências apresentadas, pode-se entender o papel que o ácido úrico, mesmo em concentrações fisiológicas, possui na ativação de enzimas e sistemas pró-oxidantes, além da promoção da produção de ERO, concentração-dependente.

Um estudo utilizando hepatócitos revelou que a incubação dessas células com crescentes concentrações de ácido úrico por um período de até 96 horas foi capaz de induzir um aumento na produção de 8-OHdG proporcional ao aumento da concentração de ácido úrico e maiores períodos de tempo (YANG et al., 2016). Houve um incremento nos níveis de 8-OHdG mesmo quando as células foram expostas ao ácido úrico na concentração de 5 mg/dL, um valor considerado normal em humanos, sendo uma forte evidência da relação entre altas concentrações de ácido úrico e dano a nucleosídeos.

Diante do apresentado, é possível estabelecer uma relação entre um incremento na concentração de ácido úrico e o aumento na produção de ERO, através de diferentes mecanismos, e consequente indução de dano aos nucleosídeos, porém, poucos estudos exploraram a existência dessa relação em humanos, tornando importante investigar se suas características pró-oxidantes podem contribuir para o dano aos nucleosídeos em portadores de DM2.

4 OBJETIVOS

4.1 OBJETIVO GERAL

Investigar a associação entre os níveis séricos de ácido úrico e o dano de nucleosídeos em pacientes portadores de DM2 e em indivíduos saudáveis.

4.2 OBJETIVOS ESPECÍFICOS

- Avaliar os níveis urinários de 8-OHdG nos pacientes com DM2 e nos indivíduos saudáveis, estratificados conforme os níveis séricos de ácido úrico (< 5,3 mg/dL e ≥ 5,3 mg/dL);
- Verificar a existência de correlação entre os níveis de ácido úrico sérico e 8-OHdG urinário na população do estudo;
- Examinar a influência do controle glicêmico, inflamação, oxidação proteica, hipertensão, índice de massa corporal, creatinina sérica e gênero sobre essa correlação.

5 ARTIGO CIENTÍFICO

High serum uric acid is associated with oxidative nucleoside damage in patients with type 2 diabetes

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High serum uric acid is associated with oxidative nucleoside damage in patients with type 2 diabetes

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Abstract

Uric acid presents different roles in an organism, since it can act as an antioxidant or a pro-oxidant molecule. High serum uric acid levels may cause damage to several structures, including nucleic acids and its components. Therefore, in this study the association between increased serum uric acid concentrations and oxidative nucleoside damage was investigated by assessment of urinary 8-hydroxydeoxyguanosine (8-OHdG) in patients with type 2 diabetes (T2D) and in healthy individuals. Urinary 8-OHdG and biochemical parameters were assessed in 61 patients who were initially grouped into 2 groups based on the median serum uric acid levels (< 5.3 mg/dL and ≥ 5.3 mg/dL). Urinary 8-OHdG was higher in patients with T2D and serum uric acid levels ≥ 5.3 mg/dL, when compared with the patients with serum uric acid levels < 5.3 mg/dL; however, co-occurrence of high serum uric acid with high urinary 8-OHdG was not observed in healthy individuals. A significant positive correlation between 8-OHdG and uric acid (r = 0.40, P < 0.01) was observed in patients with T2D. High serum uric acid levels were associated with high urinary 8-OHdG levels in patients with T2D, and this association was independent of gender, hypertension, body mass index, and serum creatinine.

Keywords: Uric acid; nucleoside oxidation; Urinary 8-hydroxydeoxyguanosine; Type 2 diabetes.

1. Introduction

Hyperuricemia is a condition that has been associated with certain pathological conditions, such as gout [1], diabetes mellitus (DM) [2], stroke [3], and hypertension [4]. Uric acid is a molecule that exhibits different activities in an organism [5], since it can act as an antioxidant [6] or a pro-oxidant molecule [7,8]. Uric acid at high levels in the serum can cause damage to several structures and cells [9-12], mainly by activating oxidative pathways mediated by the renin-angiotensin system (RAS) [11,13] and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [9,12,14,15]. Interestingly, the paradoxical pattern of uric acid manifests itself with respect to DNA damage, in which it can protect [16] or induce injury [17,18] to the DNA. Under certain conditions where an increase in pro-oxidant mechanisms is demonstrated (as observed in some pathologies), 8-hydroxydeoxyguanosine (8-OHdG) is formed, which indicates oxidative damage to guanine nucleoside [19].

Although evidence shows that uric acid may act as an antioxidant or as a pro-oxidant under certain circumstances, it is still not fully understood whether elevated serum uric acid concentrations are capable of promoting increased nucleoside oxidation in patients with type 2 diabetes (T2D). Therefore, the aim of the present study was to investigate the association between increased serum uric acid concentrations and oxidative nucleoside damage assessed via estimation of urinary 8-OHdG in patients with T2D and in healthy individuals.

2. Materials and Methods

2.1 Study population

Overall, 61 individuals were examined in this study, which included 46 patients with T2D enrolled at the University Hospital of Santa Maria (Rio Grande do Sul,

Brazil) and 15 healthy individuals. The individuals were grouped into 2 groups based on the median serum uric acid levels of this population i.e., < 5.3 mg/dL and ≥ 5.3 mg/dL. Clinical characteristics and medical histories of the patients were collected via a clinical and epidemiological assessment questionnaire or from the hospital's medical register. Height and weight were used to calculate the body mass index (BMI) by dividing the weight in kilograms with the square of the height in meters. Exclusion criteria included pregnancy, infectious diseases, liver diseases, fever, acute or chronic inflammatory diseases, and medical history of malignancy. The study protocol was approved by the Institutional Ethics Committee (12303113.0.0000.5346), and written informed consent was obtained from all patients.

2.2 Sample collection and laboratory assays

Blood samples were collected from all patients, after an overnight fast period of at least 8 h, via the venous puncture technique into Vacutainer® tubes (BD Diagnostics, Plymouth, UK) containing EDTA, sodium fluoride plus EDTA, or no anticoagulants. The samples were centrifuged at 2500 ×*g* for 15 min. Fasting glucose was measured using plasma, while serum was used to assess uric acid, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and high-sensitivity C-reactive protein (hs-CRP). These measurements were performed using standard methods via the Dimension RxL Max® automated analyzer (Siemens Healthcare Diagnostics Inc., Malvern, Pennsylvania, USA). Pro-inflammatory interleukin-6 (IL-6) in the serum was measured using commercial ELISA kits (R&D Systems Inc, Minneapolis, Minnesota, USA). The EDTA containing whole blood samples were used to measure glycated hemoglobin (HbA_{1c}) via the D-10® analyzer (Bio-Rad,

California, USA), and the EDTA containing plasma was used to measure advanced oxidation protein products (AOPPs) via the Cobas Mira® automated analyzer (Roche Diagnostics, Mannheim, Germany). First-morning urine samples were obtained from the patients and centrifuged at 1000 ×*g* for 5 min, and the supernatants were used to measure urinary albumin and 8-OHdG levels. Urinary 8-OHdG was measured using ELISA kits (Trevigen, Gaithersburg, USA), as per the manufacturer's instructions. The estimated glomerular filtration rate (eGFR) was calculated using the creatinine equation obtained from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) [20].

2.3 Statistical analysis

The variables were tested for normality using the D'Agostino-Pearson omnibus test. The parametric variables are presented as mean ± standard deviation (SD), and the non-parametric variables are presented in terms of median and interquartile range (IQR). Statistical differences between the groups were analyzed using Student's *t*-test or the Mann-Whitney test. The categorical data are expressed as percentages, and the groups were compared using Fisher's exact test. Spearman's correlation was performed to evaluate the relationship between the serum uric acid and urinary 8-OHdG values. Additionally, a multiple regression analysis was performed to investigate the influence of some variables on urinary 8-OHdG levels. Results were considered to be statistically significant when two-tailed P values were < 0.05. All results were analyzed using GraphPad Prism® version 6.01 (GraphPad Software, La Jolla, California, USA) and Statistica® version 9.1 (StatSoft Inc., Tulsa, Oklahoma, USA).

3. Results

The baseline characteristics of the participants included in the study are shown in Table 1. No differences in age, proportion of smokers, proportion of patients with T2D, diabetes duration, fasting glucose, HbA_{1c}, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, hs-CRP, IL-6, AOPPs, and eGFR were detected between the groups. However, significant differences were observed with respect to gender, hypertension, BMI, serum creatinine, serum uric acid, and 8-OHdG levels. The prevalence of hypertension was higher in patients with uric acid ≥ 5.3 mg/dL, when compared with patients with serum uric acid < 5.3 mg/dL (76.7% *versus* 48.3%, P = 0.033). The group with higher uric acid had slightly higher BMI values when compared with the group with lower uric acid (30.9 [28.0–35.7] *versus* 28.7 [23.0–36.6] kg/m², P = 0.032). Serum creatinine levels were also higher in the group with uric acid ≥ 5.3 mg/dL, when compared with the group with serum uric acid < 5.3 mg/dL (88.4 [75.1–114.9] *versus* 76.0 [66.3–88.4] μmol/L, P = 0.008).

Furthermore, urinary 8-OHdG levels were significantly higher in patients with high serum uric acid when compared with those with low serum uric acid (20.0 [16.0–35.0] *versus* 16.6 [13.0–23.0] ng/mL, P = 0.014). Urinary 8-OHdG levels were also analyzed separately in patients with T2D and in healthy individuals. Interestingly, the co-occurrence of high serum uric acid with high urinary 8-OHdG was only demonstrated in patients with T2D, as shown in Figure 1. A significant positive correlation between serum uric acid and urinary 8-OHdG (r = 0.40, P < 0.01; Figure 2) was also observed in patients with T2D. However, this correlation was not statistically significant in the healthy individuals (r = 0.24, P = 0.37). Furthermore, multiple linear regression analysis showed that the association between urinary 8-

OHdG and serum uric acid concentrations was independent of other variables such as gender, hypertension, BMI, and serum creatinine, as shown in Table 2.

4. Discussion

The association between serum uric acid and urinary 8-OHdG in patients with T2D and healthy individuals was investigated in the present study. Interestingly, we observed the co-occurrence of high serum uric acid with high urinary 8-OHdG in patients with T2D only, and not in healthy individuals. Increase in urinary 8-OHdG was independent of gender, hypertension, BMI, and serum creatinine. We had previously reported a rise in urinary 8-OHdG in patients with T2D when compared with their respective controls [21]. However, in the present study, we investigated the influence of serum uric acid on nucleoside oxidation in patients with T2D and observed that it occurred most evidently in patients with T2D with higher serum uric acid concentrations. Moreover, this study showed no association between high serum uric acid and high urinary 8-OHdG in healthy individuals. Thus, we speculate that the isolated increase in serum uric acid (at the concentrations estimated in the present study) does not appear to have the potential to cause oxidative damage to nucleosides. However, serum uric acid at high concentrations, when combined with other metabolic and pro-oxidant changes observed in diabetes, appears to potentiate the formation of 8-OHdG in T2D patients.

Several pathways have been associated with oxidative stress and diabetes, including the activation of protein kinase C and formation of advanced glycation end products [22], all of which can lead to DNA damage. Analysis of measures related to the pro-oxidant environment was limited in this study as only serum AOPPs, which showed no significant differences between the groups, when measured in the study

population. AOPPs, markers of protein oxidation and mediators of inflammation, are formed as a result of reactions between plasma proteins (mostly albumin) and chlorinated oxidants produced by myeloperoxidase [23]. Other pathways such as Fenton's reaction may also contribute to the formation of AOPPs [24]. Furthermore, pro-inflammatory cytokines such as IL-6 can also activate pro-oxidant mediators such as NADPH oxidase and increase reactive oxygen species (ROS) production [25]. However, no significant differences with respect to the pro-inflammatory biomarkers, IL-6 and hs-CRP, were observed between the groups investigated in the present study. Thus, these findings indicate that the association between high serum uric acid and high urinary 8-OHdG in patients with T2D does not seem to directly involve the activation of the pro-inflammatory mechanisms investigated here.

Several reports suggest increased serum [26,27] and urinary [28] levels of 8-OHdG in patients with pre-DM [26,29] and DM [28,30], as well as in patients with diabetes with complications [21,31]. Most of these studies propose that increased 8-OHdG levels in DM occur due to the oxidative environment promoted by uncontrolled glycaemia. However, in our observation, no difference in HbA_{1c} and fasting glucose levels was observed between the groups, which led us to believe that other factors contributed to the increase in nucleoside oxidation in T2D. In this context, uric acid deserves attention once it initiates pro-oxidant pathways. High uric acid levels have been reported to induce intracellular oxidative stress in different cell types [9,11-14,32] and rats [15] by activating RAS and NADPH oxidase. Interestingly, recent evidence indicates that, during uric acid metabolism, the enzyme xanthine oxidoreductase produces free radicals, which can cause the increase of ROS and 8-OHdG production [33].

In the presence of oxidants, uric acid can turn into an oxidant as well [34,35]. DNA damage under oxidative conditions has also been linked to uric acid levels [17,18]. There is evidence of oxidative damage to guanine in patients with gouty arthritis as uric acid levels increase [36]. Strong evidence of the relationship between hyperuricemia and nucleoside oxidative damage was also provided by an *in vitro* study [10]. Hepatocytes incubated with increasing concentrations of uric acid showed intracellular oxidative stress and increasing 8-OHdG levels as the uric acid concentrations increased with longer incubation periods. Interestingly, significant amounts of 8-OHdG were observed even at 5 mg/dL of uric acid, which is considered to be a normal concentration in the plasma [10]. For these reasons, we speculate that high serum uric acid, combined with other pro-oxidant changes related to diabetes, could activate and/or potentiate some pro-oxidant pathways that contribute to the enhanced production of 8-OHdG as observed in patients with T2D having serum uric acid ≥ 5.3 mg/dL.

Unfortunately, this study has some limitations. Firstly, the number of subjects enrolled was relatively small. Although we reported an association between high serum uric acid and high urinary 8-OHdG in patients with T2D, it was not very strong, which may have been influenced by this relatively small number of investigated individuals. Secondly, oxidative nucleoside damage was assessed in relation to 8-OHdG levels in urine, which was measured using commercial ELISA kits. It is relevant to note that this assay presents low specificity since it does not discriminate between oxidized guanine nucleosides originating from the DNA, RNA, or oxidized base. Despite these limitations, this study reported an association between increased serum uric acid concentrations and oxidative nucleoside damage assessed in terms of urinary 8-OHdG in T2D patients. It is already known that such damage can occur

in patients with T2D due to inadequate glycemic control. However, this study demonstrated the co-occurrence of high serum uric acid with high urinary 8-OHdG levels in patients with T2D, independent of other factors such as glucose and glycated hemoglobin levels. However, further research involving a larger population and other measures of pro-oxidant environments, such as NADPH oxidase and xanthine oxidoreductase activities, is required to investigate the mechanisms involved in this association.

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Conflict of Interest

The authors declare no conflicts of interest.

References

1. T. Bardin, P. Richette, Definition of hyperuricemia and gouty conditions, Curr. Opin. Rheumatol. 26 (2014) 186-191.

2. K.L. Chien, M.F. Chen, H.C. Hsu, W.T. Chang, T.C. Su, Y.T. Lee, et al., Plasma uric acid and the risk of type 2 diabetes in a Chinese community, Clin. Chem. 54 (2008) 310-316.

3. M. Li, W. Hou, X. Zhang, L. Hu, Z. Tang, Hyperuricemia and risk of stroke: a systematic review and meta-analysis of prospective studies, Atheroscler. 232 (2014) 265-270.

4. W. Zhang, K. Sun, Y. Yang, H. Zhang, F.B. Hu, R. Hui, Plasma uric acid and hypertension in a Chinese community: prospective study and meta-analysis, Clin. Chem, 55 (2009) 2026-2034.

5. D.K. Kang, S.K. Ha, Uric acid puzzle: dual role as anti-oxidant and pro-oxidant, Electrolyte Blood Press. 12 (2014) 1-6.

6. R. Yoshida, I. Shioji, A. Kishida, Y. Ogawa, Moderate alcohol consumption reduces urinary 8-hydroxydeoxyguanosine by inducing of uric acid, Ind. Health 39 (2001), 322-329.

7. M.S. Convento, E. Pessoa, M.A. Dalboni, F.T. Borges, N. Schor, Pro-inflammatory and oxidative effects of noncrystalline uric acid in human mesangial cells: contribution to hyperuricemic glomerular damage, Urol. Res. 39 (2011) 21-27.

8. J.X. Zhang, Y.P. Zhang, Q.N. Wu, B. Chen, Uric acid induces oxidative stress via an activation of the renin-angiotensin system in 3T3-L1 adipocytes, Endocr. 48 (2015) 135-142.

9. D. Verzola, E. Ratto, B. Villaggio, E.L. Parodi, R. Pontremoli, G. Garibotto, et al., Uric acid promotes apoptosis in human proximal tubule cells by oxidative stress and the activation of NADPH oxidase NOX 4, PLoS One 9 (2014) e115210.

10. Y. Yang, Y. Zhou, S. Cheng, J.L. Sun, H. Yao, L. Ma, Effect of uric acid on mitochondrial function and oxidative stress in hepatocytes, Genet. Mol. Res. 15 (2016) gmr8644.

11. M.A. Yu, L.G. Sánchez-Lozada, R.J. Johnson, D.H. Kang, Oxidative stress with an activation of the renin-angiotensin system in human vascular endothelial cells as a novel mechanism of uric acid-induced endothelial dysfunction, J. Hypertens. 28 (2010) 1234-1242.

12. L.G. Sánchez-Lozada, M.A. Lanaspa, M. Cristóbal-García, F. García-Arroyo, V. Soto, D. Cruz-Robles, et al., Uric acid-induced endotelial dysfunction is associated with mitochondrial alterations and decreased intracellular ATP concentrations, Nephron Exp. Nephrol. 121 (2012) e71-e78.

13. D.B. Corry, P. Eslami, K. Yamamoto, M.D. Nyby, H. Makino, M. L. Tuck, Uric acid stimulates vacular smooth muscle cell proliferation and oxidative stress via the vascular renin-angiotensin system, J. Hypertens. 26 (2008) 269-275.

14. Y.Y. Sautin, T. Nakagawa, S. Zharikov, R.J. Johnson, Adverse effects of the classic antioxidant uric acid in adipocytes: NADPH oxidase-mediated oxidative/nitrosative stress, Am. J. Physiol. Cell Physiol. 293 (2007) C584-C596.

15. L.G. Sánchez-Lozada, V. Soto, E. Tapia, C. Avila-Casado, Y.Y. Sautin, T. Nakagawa, et al., Role of oxidative stress in the renal abnormalities induced by experimental hyperuricemia, Am. J. Physiol. Renal Physiol. 295 (2008) F1134-F1141.

16. S. Burkhardt, R.J. Reiter, D.X. Tan, R. Hardeland, J. Cabrera, M. Karbownik, DNA oxidatively damaged by chromium(III) and H_2O_2 is protected by the antioxidants melatonin, N^1-acetyl-N^2-formyl-5-methoxykynuramine, resveratrol and uric acid, Int. J. of Biochem. & Cell Biol. 33 (2001) 775-783.

17. F.A. Shamsi, S.M. Hadi, Photoinduction of strand scission in DNA by uric acid and Cu(II), Free Radic. Biol. Med. 19 (1995) 189-196.

18. F. Shamsi, S. Husain, S.M. Hadi, DNA breakage by uric acid and Cu(II): binding of uric acid to DNA and biological activity of the reaction, J. Biochem. Toxicol. 11 (1996) 67-71.

19. A. Valavanidis, T. Vlachogianni, C. Fiotakis, 8-hydroxy-2'-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis, J. Environ. Sci. Health, Part C: Environ. Carcinog. Ecotoxicol. Rev. 27 (2009) 120-139.

20. A.S. Levey, L.A. Stevens, C.H. Schmid, Y. Zhang, A.F. Castro 3rd, H.I. Feldman, et al., A new equation to estimate glomerular filtration rate, Ann. Intern. Med. 150 (2009) 604-612.

21. E. Tatsch, J.A.M. de Carvalho, B.S. Hausen, Y.S. Bollick, V.D. Torbitz, T. Duarte, et al., Oxidative DNA damage is associated with inflammatory response, insulin resistance and microvascular complications in type 2 diabetes, Mutat. Res. 782 (2015) 17-22.

22. F. Giacco, M. Brownlee, Oxidative stress and diabetic complications, Circ. Res. 107 (2010) 1058-1070.

23. V. Witko-Sarsat, M. Friedlander, C. Capeillère-Blandin, T. Nguyen-Khoa, A.T. Nguyen, J. Zingraff, P., et al., Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int. 49 (1996) 1304-1313.

24. G.V. Bochi, V.D. Torbitz, L.P. Cargnin, J.A. de Carvalho, P. Gomes, R.N. Moresco, An alternative pathway through the Fenton reaction for the formation of advanced oxidation protein products, a new class of inflammatory mediators, Inflamm. 37 (2014) 512-521.

25. J.L. Mehta, N. Rasouli, A.K. Sinha, B. Molavi, Oxidative stress in diabetes: A mechanistic overview of its effects on atherogenesis and myocardial dysfunction, Int. J. Biochem. Cell Biol. 38 (2006) 794-803.

26. H. Al-Aubaidy, H.F. Jelinek, Oxidative DNA damage and obesity in type 2 diabetes mellitus, Eur. J. Endocrinol. 164 (2011) 899-904.

27. H.Z. Pan, D. Chang, L.G. Feng, F.J. Xu, H.Y. Kuang, M.J. Lu, Oxidative damage to DNA and its relationship with diabetic complications, Biomed. Environ. Sci. 20 (2007) 160-163.

28. J. Leinonen, T. Lehtimäki, S. Toyokuni, K. Okada, T. Tanaka, H. Hiai, et al., New biomarker evidence of oxidative DNA damage in patients with non-insulin dependent diabetes mellitus, FEBS Lett. 417 (1997) 150-152.

29. H. Al-Aubaidy, H.F. Jelinek, 8-hydroxy-2-deoxy-guanosine identifies oxidative DNA damage in a rural prediabetes cohort, Redox Rep. 15 (2010) 155-160.

30. K. Krapfenbauer, R. Birnbacher, H Vierhapper, K. Herkner, D. Kampel, G. Lubec, Glycoxidation, and protein and DNA oxidation in patients with diabetes mellitus, Clin. Sci. 95 (1998) 331-337.

31. X. Ye, R. Jiang, Q. Zhang, R. Wang, C. Yang, J. Ma, et al., Increased 8-hydroxy-2’-deoxyguanosine in leukocyte DNA from patients with type 2 diabetes and microangiopathy, J. Int. Med. Res. 44 (2016) 472-482.

32. C. Luo, X. Lian, L. Hong, J. Zou, Z. Li, Y. Zhu, et al., High uric acid activates the ROS-AMPK pathway, impairs CD68 expression and inhibits OxLDL-induced foam-

cell formation in a human monocytic cell line, THP-1, Cell. Physiol. Biochem. 40 (2016) 538-548.

33. M. Yisireyili, M. Hayashi, H. Wu, Y. Uchida, K. Yamamoto, R. Kikuchi, et al., Xanthine oxidase inhibition by febuxostat attenuates stress-induced hyperuricemia, glucose dysmetabolism, and prothrombotic state in mice. Sci. Rep. 7 (2017) 1266.

34. M. Bagnati, C. Perugini, C. Cau, R. Bordone, E. Albano, G. Bellomo, When and why a water-soluble antioxidant becomes pro-oxidant during copper-induced low-density lipoprotein oxidation: a study using uric acid, Biochem. J. 340 (1999) 143-152.

35. R.A. Patterson, E.T.M. Horsley, D.S. Leake, Prooxidant and antioxidant properties of human serum ultrafiltrares toward LDL: important role of uric acid, J. Lipid Res. 44 (2003) 512-521.

36. H.R. Chang, C.C. Lai, J.D. Lian, C.C. Lin, C.J. Wang, Formation of 8-nitroguanine in blood of patients with inflammatory gouty arthritis, Clin. Clim. Acta 362 (2005) 170-175.

Table 1. Baseline characteristics and biochemical parameters of the study participants stratified using serum uric acid values.

Parameters	Serum uric acid < 5.3 mg/dL	Serum uric acid ≥ 5.3 mg/dL	P-value
Age (y)	62.0 ± 9.4	58.3 ± 14.4	0.241
Male (%)	20.0	54.8	0.008
Smokers (%)	8.7	24.1	0.268
Hypertension (%)	48.3	76.7	0.033
Type 2 diabetes mellitus (%)	73.3	77.4	0.772
Diabetes duration (years)	13.1 ± 6.5	13.7 ± 9.4	0.844
BMI (kg/m²)	28.7 (23.0–36.6)	30.9 (28.0–35.7)	0.032
Fasting glucose (mmol/L)	6.4 (5.3–8.2)	6.8 (5.6–8.4)	0.874
HbA_{1c} (mmol/mol)	42.6 (36.1–72.9)	44.8 (39.1–63.4)	0.894
HbA_{1c} (%)	6.4 (5.8–9.2)	6.6 (6.1–8.3)	0.892
Total cholesterol (mmol/L)	4.6 ± 0.7	4.4 ± 0.9	0.250
HDL cholesterol (mmol/L)	1.3 (1.1-1.6)	1.2 (1.0-1.5)	0.328
LDL cholesterol (mmol/L)	2.5 ± 0.6	2.2 ± 0.7	0.112
Triglycerides (mmol/L)	1.3 (1.0–1.9)	1.4 (1.1–1.7)	0.355
hs-CRP (mg/L)	0.4 (0.2–0.7)	0.8 (0.2–1.4)	0.225
IL-6 (pg/mL)	152.0 (83.0–197.3)	175.0 (137.5-257.0)	0.156
AOPPs (µmol/L)	65.0 (55.7–80.8)	63.6 (48.2–90.8)	0.969
eGFR (mL/min/1.73m^2)	84.7 ± 17.8	79.7 ± 26.8	0.413
Serum creatinine (µmol/L)	76.0 (66.3–88.4)	88.4 (75.1–114.9)	0.008
Serum uric acid (mg/dL)	3.9 ± 0.8	6.6 ± 1.1	<0.001
Urinary 8-OHdG (ng/mL)	16.6 (13.0–23.0)	20.0 (16.0–35.0)	0.014

Data are expressed as mean ± SD or median and IQR. AOPPs, advanced oxidation protein products; BMI, body mass index; HbA_{1c}, glycated hemoglobin; hs-CRP, high-sensitive C-reactive protein; eGFR, estimated glomerular filtration rate; IL-6, interleukin-6; 8-OHdG, 8-hydroxydeoxyguanosine.

Table 2. Multiple linear regression analysis of urinary 8-OHdG as a dependent variable adjusted for gender, hypertension, body mass index (BMI), and serum creatinine.

	B	**SE_B**	**t**	**P-value**
Male	-3.281	3.944	-0.832	0.409
Hypertension	4.640	3.369	1.377	0.175
Body mass index	0.232	0.184	1.264	0.212
Serum creatinine	0.331	7.207	0.046	0.963

Regression coefficients (B), standard error of B (SE_B), and t statistic with corresponding *P*-value.

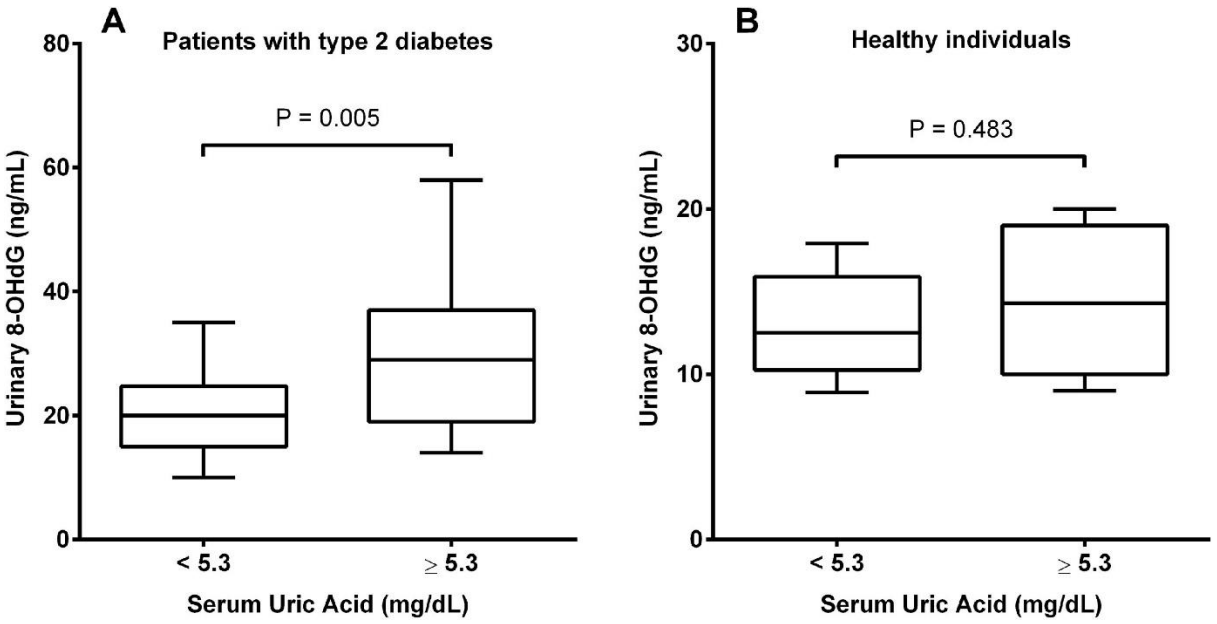


Figure 1. Box-and-whisker plots showing the urinary values of 8-OHdG in (A) patients with type 2 diabetes and (B) healthy individuals. Subjects were stratified based on serum uric acid levels < 5.3 mg/dL and ≥ 5.3 mg/dL. The box contained 50% of all values (from the 25th to 75th percentile) and was divided by the horizontal bar representing the median value (50th percentile).

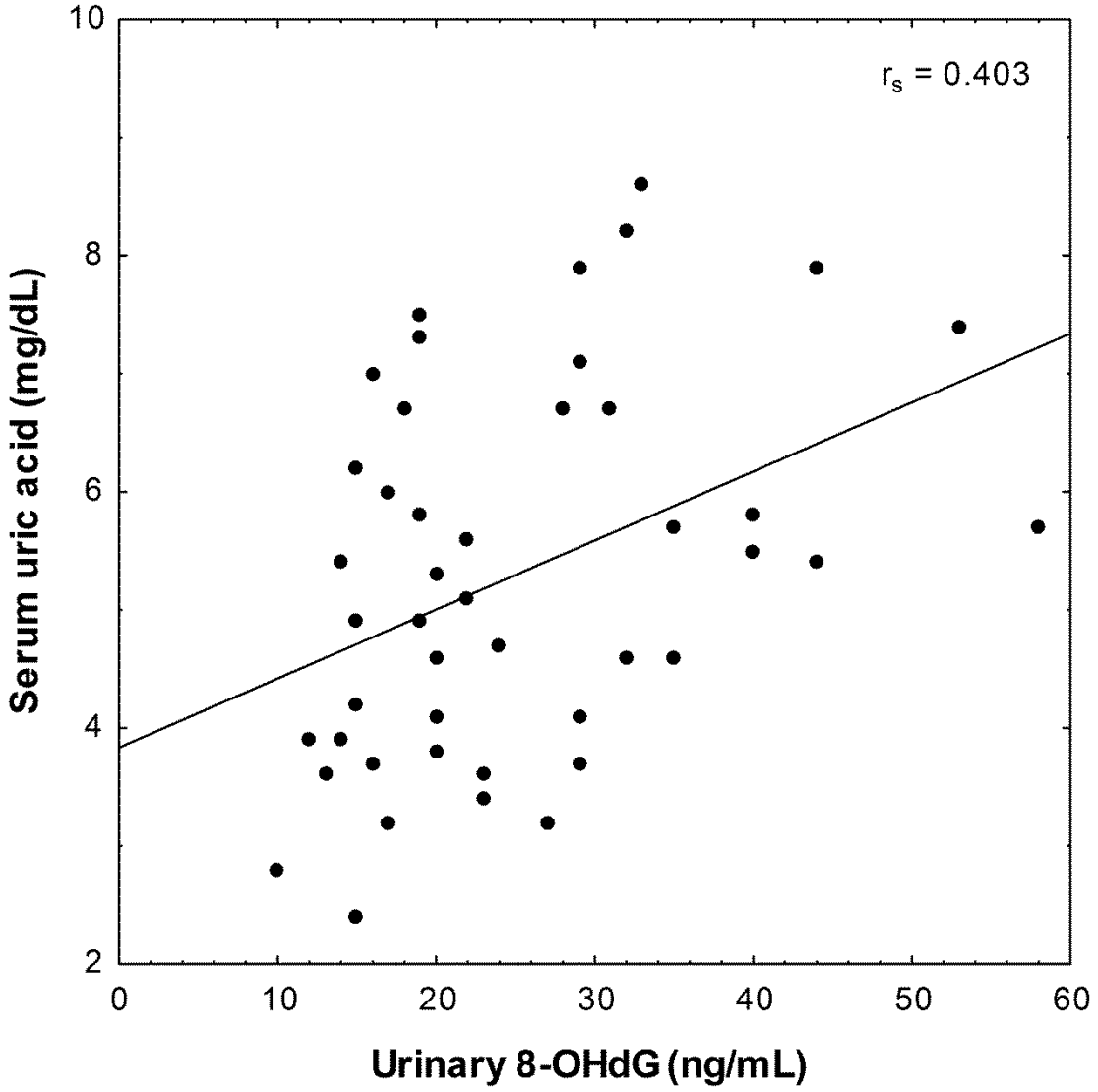
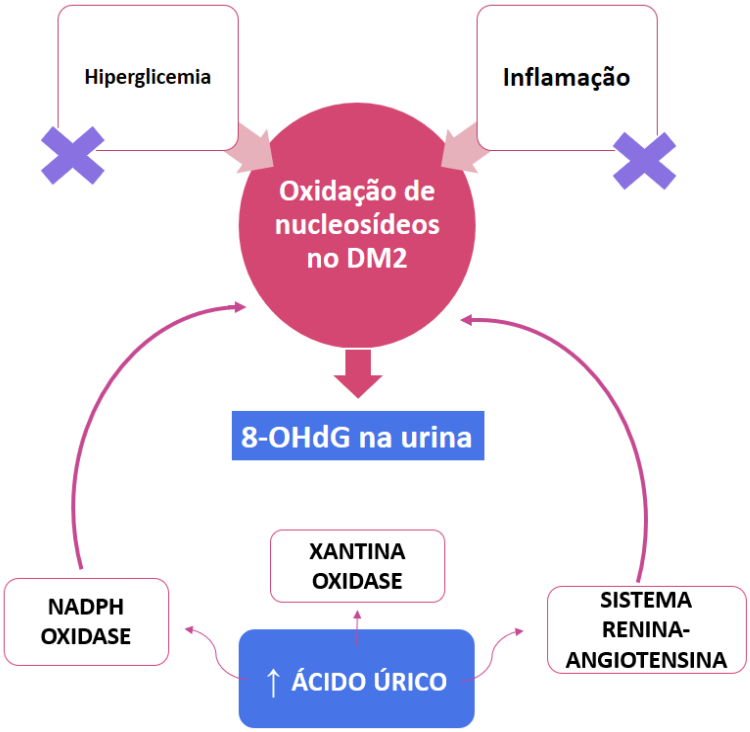


Figure 2. Positive correlation between urinary 8-OHdG and serum uric acid values (*r* = 0.40, P < 0.01) was observed in patients with type 2 diabetes.

6 CONSIDERAÇÕES FINAIS

- Os pacientes portadores de DM2 que apresentaram níveis de ácido úrico sérico ≥ 5,3 mg/dL também demonstraram uma elevação nos níveis urinários de 8-OHdG, relação que não foi percebida nos indivíduos saudáveis;
- Os níveis urinários de 8-OHdG foram positivamente correlacionados com o ácido úrico sérico nos pacientes avaliados;
- Essa associação foi independente dos parâmetros hipertensão, índice de massa corporal, creatinina sérica e gênero, além do controle glicêmico, IL-6, proteína C-reativa ultrassensível e AOPP, que foram semelhantes nos grupos de estudo;
- Conforme os resultados encontrados nesse estudo, propõe-se uma conexão entre o ácido úrico e os mecanismos de dano aos nucleosídeos no DM2, apresentada na Figura 5:

Figura 5. Associação entre o ácido úrico e o dano aos nucleosídeos no DM2.



A hiperglicemia e a inflamação podem ser responsáveis pela oxidação de nucleosídeos em pacientes portadores de DM2, porém, foram semelhantes nos grupos de estudo, dessa forma, o ácido úrico pode estar ativando vias pró-oxidantes e levando ao aparecimento do 8-OHdG na urina.
Fonte: a autora.

7 CONCLUSÃO

Em conclusão, os pacientes portadores de DM2 que possuíam níveis elevados de ácido úrico sérico demonstraram maior oxidação de nucleosídeos independentemente de outros fatores. Dessa forma, apesar de algumas limitações, esse estudo contribui para uma melhor compreensão do envolvimento do ácido úrico em processos oxidativos, especialmente no dano aos nucleosídeos.

REFERÊNCIAS BIBLIOGRÁFICAS

ADA - American Diabetes Association. Standards of medical care in diabetes. **Diabetes Care**, v. 39, supplement 1, 2016.

BAGNATI, M. et al. When and why a water-soluble antioxidant becomes pro-oxidant during copper-induced low-density lipoprotein oxidation: a study using uric acid. **Biochemical Journal**, v. 340, p. 143-152, 1999.

BENN, C. L. et al. Physiology of hyperuricemia and urate-lowering treatments. **Frontiers in Medicine**, v. 5, 160, 2018.

ČAUŠEVIĆ, A. et al. Relevance of uric acid in the progression of type 2 diabetes mellitus. **Bosnian Journal of Basic Medical Sciences**, v. 10, n. 1, p. 54-59, 2010.

CHAN, H. R. et al. Formation of 8-nitroguanine in blood of patients with inflammatory gouty arthritis. **Clinica Chimica Acta**, v. 362, p. 170-175, 2005.

CHIEN, K. L. et al. Plasma uric acid and the risk of type 2 diabetes in a Chinese community. **Clinical Chemistry**, v. 54, n. 2, p. 310-316, 2008.

CHOI, H. K.; MOUNT, D. B.; REGINATO, A. M. Pathogenesis of gout. **Annals of Internal Medicine**, v. 143, n. 7, p. 499-516, 2005.

COOKE, M. S.; LUNEC, J.; EVANS, M. D. Progress in the analysis of urinary oxidative DNA damage. **Free Radical Biology and Medicine**, v. 33, n. 12, p. 1601-1614, 2002.

CORRY, D. B. et al. Uric acid stimulates vascular smooth muscle cell proliferation and oxidative stress via the vascular renin-angiotensin system. **Journal of Hypertension**, v. 26, n. 2, p. 269-275, 2008.

DASEGOWDA, S. M. et al. Serum uric acid and its relation to adenosine deaminase, lipid profile and oxidative stress in Diabetes Mellitus type 2. **Journal of Investigational Biochemistry**, v. 5, n. 2, p. 32-36, 2016.

DU, L.; MA, J.; ZHANG, X. Higher serum uric acid may contribute to cerebral infarction in patients with type 2 Diabetes Mellitus: A meta-analysis. **Journal of Molecular Neuroscience**, v. 61, n. 1, p. 25-31, 2017.

EJAZ, A. A. et al. Could uric acid have a role in acute renal failure? **Clinical Journal of the American Society of Nephrology**, v. 2, p. 17-21, 2007.

EVANS, M. D. et al. Nucleotide excision repair of oxidized genomic DNA is not a source of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine. **Free Radical Biology and Medicine**, v. 99, p. 385-391, 2016.

FURUSYO, H.; HAYASHI, N. Glycated albumin and diabetes mellitus. **Biochimica et Biophysica Acta**, v. 1830, n. 12, p. 5509-5514, 2013.

GUO, C. et al. Potential application of the oxidative nucleic acid damage biomarkers in detection of diseases. **Oncotarget**, v. 8, n. 43, p. 75767-75777, 2017.

IDF – International Diabetes Federation. **IDF Diabetes Atlas**, 8th. edition, 2017.

JALAL, D. I. et al. Uric acid as a mediator of diabetic nephropathy. **Seminars in Nephrology**, v. 31, n. 5, p. 459-465, 2011.

JOHNSON, R. J. et al. Essential hypertension, progressive renal disease, and uric acid: a pathogenetic link? **Journal of the American Society of Nephrology**, v. 16, n. 7, p. 1909-1919, 2005.

KALOUSOVÁ, M.; SKRHA, J.; ZIMA, T. Advanced glycation end-products and advanced oxidation protein products in patients with type 2 diabetes mellitus. **Physiological Research**, v. 51, n. 6, p. 597-604, 2002.

KODAMA, S. et al. The association between uric acid and the development of type 2 diabetes mellitus – A meta analysis. **Diabetes Care**, v. 32, n. 9, p. 1737-1742, 2009.

KOŁODZIEJ, U. et al. Chronic high-protein diet induces oxidative stress and alters the salivar gland function in rats. **Archives of Oral Biology**, v. 84, p. 6-12, 2017.

KRISHNAN, E. Inflammation, oxidative stress and lipids: the risk triad for atherosclerosis in gout. **Rheumatology (Oxford)**, v. 49, n. 7, p. 1229-1238, 2010.

LI, P. et al. Uric acid enhances PKC-dependent eNOS phosphorylation and mediates cellular ER stress: A mechanism for uric acid-induced endothelial dysfunction. **International Joural of Molecular Medicine**, v. 37, n. 4, p. 989-997, 2016.

LI , Q. et al. Serum uric acid level and its association with metabolic syndrome and carotid atherosclerosis in patients with type 2 diabetes. **Cardiovascular Diabetology**, v. 10, n. 72, p. 1-7, 2011.

LIANG, M. et al. Increased plasma advanced oxidation protein products is an early marker of endothelial dysfunction in type 2 diabetes patients without albuminuria 2. **Journal of Diabetes**, v. 6, n. 5, p. 417-426, 2014.

LIANG, C. C. et al. Association of serum uric acid concentration with diabetic retinopathy and albuminuria in Taiwanese patients with type 2 diabetes mellitus. **International Journal of Molecular Sciences**, v. 17, 1248, 2016.

LINDAHL, T.; WOOD, R. D. Quality control by DNA repair. **Science**, v. 286, p. 1897-1905, 1999.

LIU, B. et al. Mechanisms of mutagenesis: DNA replication in the presence of DNA damage. **Mutation Research – Reviews in Mutation Research**, v. 768, p. 53-67, 2016. (A)

LV, Q. et al. High serum uric acid and increased risk of type 2 diabetes: A systematic review and meta-analysis of prospective cohort studies. **PLoS One**, v. 8, n. 2, e56864, 2013.

MOON, J. S. et al. Metformin prevents glucotoxicity by alleviating oxidative and ER stress-induced CD36 expression in pancreatic beta cells. **Journal of Diabetes and its Complications**, v. 31, n. 1, p. 21-30, 2017.

MOUNT, D. B.; KWON, C. Y.; ZANDI-NEJAD, K. Renal urate transport. **Rheumatic Disease Clinics of North America**, v. 32, p. 313-331, 2006.

NAKAGAWA, T. et al. A causal role of uric acid in fructose-indiced metabolic syndrome. **American Journal of Physiology – Renal Physiology**, v. 290, n. 3, F625-631, 2006.

OĞUZ, N. et al. Effect of uric acid on inflammatory COX-2 and ROS pathways in vascular smooth muscle cells. **Journal of Receptor and Signal Transduction Research**, v. 37, n. 5, p. 500-505, 2017.

OLIVEIRA, E. P.; BURINI, R. C. High plasma uric acid concentration: causes and consequences. **Diabetology and Metabolic Syndrome**, v. 4, n. 12, p. 2-7, 2012.

REHMAN, K. et al. Role of interleukin-6 in development of insulin resistance and type 2 diabetes mellitus. **Critical Reviews in Eukaryotic Gene Expression**, v. 27, n. 3, p. 229-236, 2017.

ROSSNER, P. et al. Urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine values determined by a modified ELISA improves agreement with HPLC–MS/MS. **Biochemical and Biophysical Research Communications**, v. 440, n. 4, p. 725-730, 2013.

SÁNCHEZ-LOZADA, L. G. et al. Role of oxidative stress in the renal abnormalities induced by experimental hyperuricemia. **American Journal of Physiology – Renal Physiology**, v. 295, F1134-F1141, 2008.

SÁNCHEZ-LOZADA, L. G. et al. Uric acid-induced endothelial dysfunction is associated with mitochondrial alterations and decreased intracellular ATP concentrations. **Nephron Experimental Nephrology**, v. 121, e71-e78, 2012.

SAUTIN, Y. Y. et al. Adverse effects of the classic antioxidant uric acid in adipocytes: NADPH oxidase-mediated oxidative/nitrosative stress. **American Journal of Physiology: Cell Plysiology**, v. 293, n. 2, p. C584-C596, 2007.

SO, A.; THORENS, B. Uric acid transport and disease. **The Journal of Clinical Investigation**, v. 120, n. 6, p. 1791-1799, 2010.

SPIGA, R. et al. Uric acid is associated with inflammatory biomarkers and induces inflammation *via* activating the NF-κB signaling pathway in HepG2 cells. **Arteriosclerosis, Thrombosis, and Vascular Biology**, v. 37, n. 6, p. 1241-1249, 2017.

TATSCH, E. et al. Association between DNA strand breakage and oxidative, inflammatory and endothelial biomarkers in type 2 diabetes. **Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis**, v. 732, p. 16-20, 2012.

TATSCH, E. et al. Oxidative DNA damage is associated with inflammatory response, insulin resistance and microvascular complications in type 2 diabetes. **Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis**, v. 782, p. 17-22, 2015.

TSUSHIMA, Y. et al. Uric acid secretion from adipose tissue and its increase in obesity. **The Journal of Biological Chemistry**, v. 288, n. 38, p. 27138-27149, 2013.

VALAVANIDIS, A.; VLACHOGIANNI, T.; FIOTAKIS, C. 8-hydroxy-2'-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. **Journal of Environmental Science and Health Part C**, v. 27, p. 120-139, 2009.

VERZOLA, D. et al. Uric acid promotes apoptosis in human proximal tubule cells by oxidative stress and the activation of NADPH oxidase NOX 4. **PLoS One**, v. 9, n. 12, e115210, 2014.

WANG, X. et al. Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. **Diabetes Care**, v. 36, n. 1, p. 166-175, 2013.

WEIMANN, A. et al. Assays for urinary biomarkers of oxidatively damaged nucleic acids. **Free Radical Research**, v. 46, n. 4, p. 531-540, 2012.

WU, L. et al. Association between serum uric acid level and hypertension in a Chinese elderly rural population. **Clinical and Experimental Hypertension**, v. 39, n. 6, p. 505-512, 2017.

YANG, Y. et al. Effect of uric acid on mitochondrial function and oxidative stress in hepatocytes. **Genetics and Molecular Research**, v. 15, n. 2, gmr15028644, 2016.

YU, M. A. et al. Oxidative stress with an activation of the renin-angiotensin system in human vascular endothelial cells as a novel mechanism of uric acid-induced endothelial dysfunction. **Journal of Hypertension**, v. 28, p. 1234-1242, 2010.

ZAHA, A.; FERREIRA, H. B.; PASSAGLIA, L. M. P. **Biologia Molecular Básica**. 5 ed. Porto Alegre. Artmed, 2014.

ZHANG, J. X. et al. Uric acid induces oxidative stress via na activation of the renin-angiotensin system in 3T3-L1 adipocytes. **Endocrine**, v. 48, p. 135-142, 2015.

ZHI, L. et al. High uric acid induces insulin resistance in cardiomyocytes *in vitro* and *in vivo*. **PLoS One**, v. 11, n. 2, e0147737, 2016.

ZHOU, Y. et al. Relationship between oxidative stress and inflammation in hyperuricemia: Analysis based on asymptomatic young patients with primary hyperuricemia. **Medicine (Baltimore)**, v. 97, n. 49, e13108, 2018.

ZOPPINI, G. et al. Elevated serum uric acid concentrations independently predict cardiovascular mortality in type 2 diabetic patients. **Diabetes Care**, v. 32, p. 1716-1720, 2009.

ANEXO A – PARECER DE APROVAÇÃO DO COMITÊ DE ÉTICA INSTITUCIONAL

UNIVERSIDADE FEDERAL DE SANTA MARIA/ PRÓ-REITORIA DE PÓS-GRADUAÇÃO E

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO DO PERFIL DE BIOMARCADORES ASSOCIADOS A PROCESSOS METABÓLICOS, INFLAMATÓRIOS, OXIDATIVOS E GENOTÓXICOS EM PACIENTES COM DIABETES MELLITUS E SUA ASSOCIAÇÃO COM O DESENVOLVIMENTO DE COMPLICAÇÕES CRÔNICAS

Pesquisador: RAFAEL NOAL MORESCO

Área Temática:

Versão: 2

CAAE: 12303113.0.0000.5346

Instituição Proponente: Universidade Federal de Santa Maria/ Pró-Reitoria de Pós-Graduação e

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 236.696

Data da Relatoria: 11/03/2013

Apresentação do Projeto:

A prevalência do diabetes mellitus (DM) vem aumentando significativamente nas últimas décadas, sendo que as complicações crônicas representam
a maior causa de morbidade e mortalidade em pacientes com esta patologia a longo prazo. Como o exato mecanismo pelo qual o DM leva ao
desenvolvimento destas complicações é complexo, e não está ainda totalmente elucidado, o objetivo deste projeto será avaliar o perfil de
biomarcadores associados a processos metabólicos, inflamatórios, oxidativos e genotóxicos em pacientes com DM e em pré-diabéticos, bem como
sua associação com o desenvolvimento de complicações crônicas. Para isto, será realizado um estudo transversal prospectivo envolvendo 300
pacientes adultos, de ambos os sexos, com o diagnóstico de DM tipo 1 e 2. Também serão recrutados para o estudo cerca de 100 indivíduos prédiabéticos
e 100 indivíduos saudáveis. Serão avaliados os seguintes parâmetros: níveis de insulina, creatinina, uréia, colesterol total, HDL colesterol,
LDL colesterol, triglicérides, PCR-us, IL-6, IL-10, albumina, proteínas totais, IMA, NOx, ácido úrico, TNF-¿, AST, ALT, ferro total, ferritina,

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transferrina, sTfR, NTBI, TIBC, xantina, hipoxantina, xantina oxidase, xantina desidrogenase, NAG, AOPP, glutationa redutase, HbA1c, FRAP, TOS,
índices hematimétricos, dano no DNA, albumina urinária, creatinina, GGT, FAL, KIM-1, NGAL, NAG. Também será avaliado o potencial prognóstico
em relação ao desenvolvimento das complicações crônicas do DM e mortalidade durante um período de 24 meses dos biomarcadores envolvidos
neste estudo.

Objetivo da Pesquisa:

Avaliar o perfil de biomarcadores associados a processos metabólicos, inflamatórios, oxidativos e genotóxicos em pacientes com diabetes mellitus, bem como em pré-diabéticos, a fim de investigar o potencial diagnóstico e prognóstico destes biomarcadores para o desenvolvimento de complicações crônicas do diabetes.

Avaliação dos Riscos e Benefícios:

adequados para o tipo de pesquisa realizada

Comentários e Considerações sobre a Pesquisa:

Pesquisa com tema relevante, bem estruturada, com justificativa, objetivos e metodologia coerentes.

Considerações sobre os Termos de apresentação obrigatória:

adequados em sua nova versao

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

aprovar o proejeto

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

UNIVERSIDADE FEDERAL DE SANTA MARIA/ PRÓ-REITORIA DE PÓS-GRADUAÇÃO E

SANTA MARIA, 03 de Abril de 2013

Assinador por:
Félix Alexandre Antunes Soares
(Coordenador)

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ANEXO B – ARTIGO CIENTÍFICO PUBLICADO

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High serum uric acid is associated with oxidation of nucleosides in patients with type 2 diabetes

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Type 2 diabetes

ABSTRACT

Uric acid presents different roles in an organism, since it can act as an antioxidant or a pro-oxidant molecule. High serum uric acid levels may cause damage to several structures, including nucleic acids and its components. Therefore, in this study the association between increased serum uric acid concentrations and oxidation of nucleosides was investigated by assessment of urinary 8-hydroxydeoxyguanosine (8-OHdG) in patients with type 2 diabetes (T2D) and in healthy individuals. Urinary 8-OHdG and biochemical parameters were assessed in 61 patients who were initially divided into 2 groups based on the median serum uric acid levels (< 5.3 mg/dL and ≥5.3 mg/dL). Urinary 8-OHdG was higher in patients with T2D and serum uric acid levels ≥5.3 mg/dL, when compared with the patients with serum uric acid levels < 5.3 mg/dL; however, co-occurrence of high serum uric acid with high urinary 8-OHdG was not observed in healthy individuals. A significant positive correlation between 8-OHdG and uric acid ($r = 0.40$, $P < 0.01$) was observed in patients with T2D. High serum uric acid levels were associated with high urinary 8-OHdG levels in patients with T2D, and this association was independent of gender, hypertension, body mass index, and serum creatinine.

1. Introduction

Hyperuricemia is a condition that has been associated with certain pathological conditions, such as gout [1], diabetes mellitus (DM) [2], stroke [3], and hypertension [4]. Uric acid is a molecule that exhibits different activities in the organism [5], since it can act as an antioxidant [6] or a pro-oxidant molecule [7,8]. Uric acid at high levels in the serum can cause damage to several structures and cells [9–12], mainly by activating oxidative pathways mediated by the renin-angiotensin system (RAS) [11,13] and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [9,12,14,15]. Interestingly, the paradoxical pattern of uric acid manifests itself with respect to DNA damage, in which it can protect [16] or induce injury [17,18] to the DNA. Under certain conditions where an increase in pro-oxidant mechanisms is demonstrated (as observed in some pathologies), 8-hydroxydeoxyguanosine (8-OHdG) is formed, which indicates oxidative damage to guanine nucleoside [19].

Although evidence shows that uric acid may act as an antioxidant or as a pro-oxidant under certain circumstances, it is still not fully understood whether elevated serum uric acid concentrations are capable of promoting increased nucleoside oxidation in patients with type 2 diabetes (T2D). Therefore, the aim of the present study was to investigate the association between increased serum uric acid concentrations and oxidation of nucleosides assessed *via* estimation of urinary 8-OHdG in patients with T2D and in healthy individuals.

2. Materials and methods

2.1. Study population

Overall, 61 individuals were examined in this study, which included 46 patients with T2D enrolled at the University Hospital of Santa Maria

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(Rio Grande do Sul, Brazil) and 15 healthy individuals. The individuals were grouped into 2 groups based on the median serum uric acid levels of this population *i.e.*, < 5.3 mg/dL and ≥5.3 mg/dL. Clinical characteristics and medical histories of the patients were collected *via* a clinical and epidemiological assessment questionnaire or from the hospital's medical register. Height and weight were used to calculate the body mass index (BMI) by dividing the weight in kilograms with the square of the height in meters. Exclusion criteria included pregnancy, infectious diseases, liver diseases, fever, acute or chronic inflammatory diseases, and medical history of malignancy. The study protocol was approved by the Institutional Ethics Committee (12303113.0.0000.5346), and written informed consent was obtained from all patients.

2.2. Sample collection and laboratory assays

Blood samples were collected from all patients, after an overnight fast period of at least 8 h, *via* the venous puncture technique into Vacutainer® tubes (BD Diagnostics, Plymouth, UK) containing EDTA, sodium fluoride plus EDTA, or no anticoagulants. The samples were centrifuged at 2500 × *g* for 15 min. Fasting glucose was measured using plasma, while serum was used to assess uric acid, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and high-sensitivity C-reactive protein (hs-CRP). These measurements were performed using standard methods *via* the Dimension RxL Max® automated analyzer (Siemens Healthcare Diagnostics Inc., Malvern, Pennsylvania, USA). Pro-inflammatory interleukin-6 (IL-6) in the serum was measured using commercial ELISA kits (R&D Systems Inc, Minneapolis, Minnesota, USA). The EDTA containing whole blood samples were used to measure glycated hemoglobin (HbA_{1c}) *via* the D-10 analyzer (Bio-Rad, California, USA), and the EDTA containing plasma was used to measure advanced oxidation protein products (AOPPs) *via* Cobas Mira® automated analyzer (Roche Diagnostics, Mannheim, Germany). First-morning urine samples were obtained from the patients and centrifuged at 1000 × *g* for 5 min and the supernatants were used to measure urinary albumin and 8-OHdG levels. Urinary 8-OHdG was measured using ELISA kits (Trevigen, Gaithersburg, USA), as per the manufacturer's instructions. The estimated glomerular filtration rate (eGFR) was calculated using the creatinine equation obtained from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) [20].

2.3. Statistical analysis

The variables were tested for normality using the D'Agostino-Pearson omnibus test. The parametric variables are presented as mean ± standard deviation (SD), and the non-parametric variables are presented in terms of median and interquartile range (IQR). Statistical differences between the groups were analyzed using Student's *t*-test or the Mann–Whitney test. The categorical data are expressed as percentages, and the groups were compared using Fisher's exact test. Spearman's correlation was performed to evaluate the relationship between the serum uric acid and urinary 8-OHdG values. Additionally, a multiple regression analysis was performed to investigate the influence of some variables on urinary 8-OHdG levels. Results were considered to be statistically significant when two-tailed P values were < 0.05. All results were analyzed using GraphPad Prism® version 6.01 (GraphPad Software, La Jolla, California, USA) and Statistica® version 9.1 (StatSoft Inc., Tulsa, Oklahoma, USA).

3. Results

The baseline characteristics of the participants included in the study are shown in Table 1. No differences in age, proportion of smokers, proportion of patients with T2D, diabetes duration, fasting glucose, HbA_{1c}, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, hs-CRP, IL-6, AOPPs, and eGFR were detected between the groups.

Table 1
Baseline characteristics and biochemical parameters of the study participants stratified using serum uric acid values.

Parameters	Serum uric acid < 5.3 mg/dL	Serum uric acid ≥5.3 mg/dL	P-value
Age (y)	62.0 ± 9.4	58.3 ± 14.4	0.241
Male (%)	20.0	54.8	0.008
Smokers (%)	8.7	24.1	0.268
Hypertension (%)	48.3	76.7	0.033
Type 2 diabetes mellitus (%)	73.3	77.4	0.772
Diabetes duration (years)	13.1 ± 6.5	13.7 ± 9.4	0.844
BMI (kg/m^2)	28.7 (23.0–[illegible].6)	30.9 (28.0–35.7)	0.032
Fasting glucose (mmol/L)	6.4 (5.3–8.2)	[illegible] (5.6–8.4)	0.874
HbA_{1c} (mmol/mol)	42.6 [illegible].1–72.5)	4[illegible].8 (39.1–63.4)	0.894
HbA_{1c} (%)	6.4 [illegible].8–8.2)	[illegible].6 (6.1–8.3)	0.892
Total cholesterol (mmol/L)	4.6 ± 0.7	4.4 ± 0.9	0.250
HDL cholesterol (mmol/L)	1.3 (1.1–1.6)	1.2 (1.0–1.5)	0.328
LDL cholesterol (mmol/L)	[illegible] ± 0.6	2.2 ± 0.7	0.112
Triglycerides (mmol/L)	1.3 (1.0–1.9)	1.4 (1.1–1.7)	0.355
hs-CRP (mg/L)	0.4 (0.2–0.7)	0.8 (0.2–1.4)	0.225
IL-6 (pg/mL)	152.0 (83.0–197.3)	175.0 (137.5–257.0)	0.156
AOPPs (μmol/L)	65.0 (55.7–80.8)	63.6 (48.2–90.8)	0.969
eGFR (ml/min/1.73 m^2)	84.7 ± 17.8	79.7 ± 26.8	0.413
Serum creatinine (μmol/L)	76.0 (66.3–88.4)	88.4 (75.1–114.9)	0.008
Serum uric acid (mg/dL)	3.9 ± 0.8	6.6 ± 1.1	< 0.001
Urinary 8-OHdG (ng/mL)	16.6 (13.0–23.0)	20.0 (16.0–35.0)	0.014

Data are expressed as mean ± SD or median and IQR. AOPPs, advanced oxidation protein products; BMI, body mass index; HbA_{1c}, glycated hemoglobin; hs-CRP, high-sensitive C-reactive protein; eGFR, estimated glomerular filtration rate; IL-6, interleukin-6; 8-OHdG, 8-hydroxydeoxyguanosine.

However, significant differences were observed with respect to gender, hypertension, BMI, serum creatinine, serum uric acid, and 8-OHdG levels. The prevalence of hypertension was higher in patients with uric acid ≥5.3 mg/dL, when compared with patients with serum uric acid < 5.3 mg/dL (76.7% *versus* 48.3%, P = 0.033). The group with higher uric acid had slightly higher BMI values when compared with the group with lower uric acid (30.9 [28.0–35.7] *versus* 28.7 [23.0–36.6] kg/m^2, P = 0.032). Serum creatinine levels were also higher in the group with uric acid ≥5.3 mg/dL, when compared with the group with serum uric acid < 5.3 mg/dL (88.4 [75.1–114.9] *versus* 76.0 [66.3–88.4] μmol/L, P = 0.008).

Furthermore, urinary 8-OHdG levels were significantly higher in patients with high serum uric acid when compared with those with low serum uric acid (20.0 [16.0–35.0] *versus* 16.6 [13.0–23.0] ng/mL, P = 0.014). Urinary 8-OHdG levels were also analyzed separately in patients with T2D and in healthy individuals. Interestingly, the co-occurrence of high serum uric acid with high urinary 8-OHdG was only demonstrated in patients with T2D, as shown in Fig. 1. A significant positive correlation between serum uric acid and urinary 8-OHdG (r = 0.40, P < 0.01; Fig. 2) was also observed in patients with T2D. However, this correlation was not statistically significant in the healthy individuals (r = 0.24, P = 0.37). Furthermore, multiple linear regression analysis showed that the association between urinary 8-OHdG and serum uric acid concentrations was independent of other variables such as gender, hypertension, BMI, and serum creatinine, as shown in Table 2.

4. Discussion

The association between serum uric acid and urinary 8-OHdG in patients with T2D and healthy individuals was investigated in the present study. Interestingly, we observed the co-occurrence of high serum uric acid with high urinary 8-OHdG in patients with T2D only,

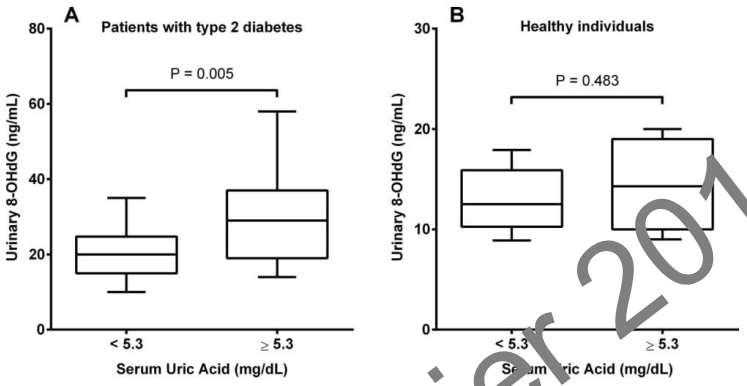

Fig. 1. Box-and-whisker plots showing the urinary values of 8-OHdG in (A) patients with type 2 diabetes and (B) healthy individuals. Subjects were stratified based on serum uric acid levels < 5.3 mg/dL and ≥ 5.3 mg/dL. The box contained 50% of all values (from the 25th to 75th percentile) and was divided by the horizontal bar representing the median value (50th percentile).

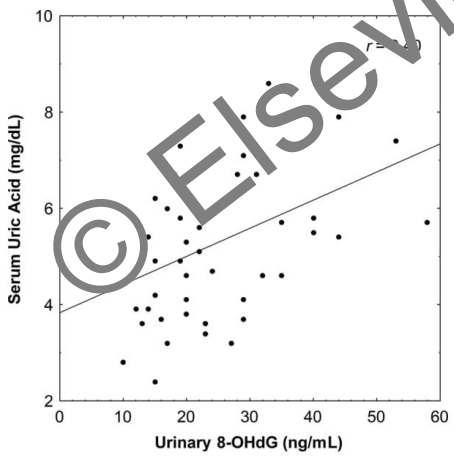

Fig. 2. Positive correlation between urinary 8-OHdG and serum uric acid values (r = 0.40, P < 0.01) was observed in patients with type 2 diabetes.

Table 2
Multiple linear regression analysis of urinary 8-OHdG as a dependent variable adjusted for gender, hypertension, body mass index (BMI), and serum creatinine.

	B	SE_B	t	P-value
Male	−3.281	3.944	−0.832	0.409
Hypertension	4.640	3.369	1.377	0.175
Body mass index	0.232	0.184	1.264	0.212
Serum creatinine	0.331	7.207	0.046	0.963

Regression coefficients (B), standard error of B (SE_B), and t statistic with corresponding *P*-value.

and not in healthy individuals. Increase in urinary 8-OHdG was independent of gender, hypertension, BMI, and serum creatinine. We had previously reported a rise in urinary 8-OHdG in patients with T2D when compared with their respective controls [21]. However, in the present study, we investigated the influence of serum uric acid on nucleoside oxidation in patients with T2D and observed that it occurred most evidently in patients with T2D with higher serum uric acid concentrations. Moreover, this study showed no association between high serum uric acid and high urinary 8-OHdG in healthy individuals. Thus, we speculate that the isolated increase in serum uric acid (at the concentrations estimated in the present study) does not appear to have the potential to cause oxidative damage to nucleosides. However, serum uric acid at high concentrations, when combined with other metabolic and pro-oxidant changes observed in diabetes, appears to potentiate the formation of 8-OHdG in T2D patients.

Several pathways have been associated with oxidative stress and diabetes, including the activation of protein kinase C and formation of advanced glycation end products [22], all of which can lead to DNA damage. Analysis of measures related to the pro-oxidant environment was limited in this study as only serum AOPPs, which showed no significant differences between the groups, when measured in the study population. AOPPs, markers of protein oxidation and mediators of inflammation, are formed as a result of reactions between plasma proteins (mostly albumin) and chlorinated oxidants produced by myeloperoxidase [23]. Other pathways such as Fenton's reaction may also contribute to the formation of AOPPs [24]. Furthermore, pro-inflammatory cytokines such as IL-6 can also activate pro-oxidant mediators such as NADPH oxidase and increase reactive oxygen species (ROS) production [25]. However, no significant differences with respect to the pro-inflammatory biomarkers, IL-6 and hs-CRP, were observed between the groups investigated in the present study. Thus, these findings indicate that the association between high serum uric acid and high urinary 8-OHdG in patients with T2D does not seem to directly involve the activation of the pro-inflammatory mechanisms investigated here.

Several reports suggest increased serum [26,27] and urinary [28] levels of 8-OHdG in patients with pre-DM [26,29] and DM [28,30], as well as in patients with diabetes with complications [21,31]. Most of these studies propose that increased 8-OHdG levels in DM occur due to the oxidative environment promoted by uncontrolled glycaemia. However, in our observation, no difference in HbA_{1c} and fasting glucose levels were observed between the groups, which led us to believe that other factors may contribute to the increase of nucleoside oxidation in T2D. In this context, uric acid deserves attention once it initiates pro-oxidant pathways. High uric acid levels have been reported to induce intracellular oxidative stress in different cell types [9,11–14,32] and rats [15] by activating RAS and NADPH oxidase. Interestingly, recent evidence indicates that, during uric acid metabolism, the enzyme xanthine oxidoreductase produces free radicals, which can cause the increase of ROS and 8-OHdG production [33].

In the presence of oxidants, uric acid can turn into an oxidant as well [34,35]. DNA damage under oxidative conditions has also been linked to uric acid levels [17,18]. There is evidence of oxidative damage to guanine in patients with gouty arthritis as uric acid levels increase [36]. Strong evidence of the relationship between hyperuricemia and nucleoside oxidative damage was also provided by an *in vitro* study

[10]. Hepatocytes incubated with increasing concentrations of uric acid showed intracellular oxidative stress and increasing 8-OHdG levels as the uric acid concentrations increased with longer incubation periods. Interestingly, significant amounts of 8-OHdG were observed even at 5 mg/dL of uric acid, which is considered to be a normal concentration in the plasma [10]. For these reasons, we speculate that high serum uric acid, combined with other pro-oxidant changes related to diabetes, can activate and/or potentiate some pro-oxidant pathways that contribute to the enhanced production of 8-OHdG as observed in patients with T2D having serum uric acid ≥ 5.3 mg/dL.

Unfortunately, this study has some limitations. Firstly, the number of subjects enrolled was relatively small. Although we reported an association between high serum uric acid and high urinary 8-OHdG in patients with T2D, it was not very strong, which may have been influenced by this relatively small number of investigated individuals. Secondly, oxidation of nucleosides was assessed in relation to 8-OHdG levels in urine, which was measured using commercial ELISA kits. It is relevant to note that this assay presents low specificity since it does not discriminate between oxidized guanine nucleosides originating from the DNA, RNA, or oxidized base. Despite these limitations, this study reported an association between increased serum uric acid concentrations and oxidation of nucleosides assessed in terms of urinary 8-OHdG in T2D patients. It is already known that such damage can occur in patients with T2D due to inadequate glycemic control. However, this study demonstrated the co-occurrence of high serum uric acid with high urinary 8-OHdG levels in patients with T2D, independent of other factors such as glucose and glycated hemoglobin levels. However, further research involving a larger population and other measures of pro-oxidant environments, such as NADPH oxidase and xanthine oxidoreductase activities, is required to investigate the mechanisms involved in this association.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

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References

[1] T. Bardin, P. Richette, Definition of hyperuricemia and gouty conditions, Curr. Opin. Rheumatol. 26 (2014) 186–191.
[2] K.L. Chien, M.F. Chen, H.C. Hsu, W.T. Chang, T.C. Su, Y.T. Lee, et al., Plasma uric acid and the risk of type 2 diabetes in a Chinese community, Clin. Chem. 54 (2008) 310–316.
[3] M. Li, W. Hou, X. Zhang, L. Hu, Z. Tang, Hyperuricemia and risk of stroke: a systematic review and meta-analysis of prospective studies, Atherosclerosis 232 (2014) 265–270.
[4] W. Zhang, K. Sun, Y. Yang, H. Zhang, F.B. Hu, R. Hui, Plasma uric acid and hypertension in a Chinese community: prospective study and meta-analysis, Clin. Chem. 55 (2009) 2026–2034.
[5] D.K. Kang, S.K. Ha, Uric acid puzzle: dual role as anti-oxidant and pro-oxidant, Electrolyte Blood Press. 12 (2014) 1–6.
[6] R. Yoshida, I. Shioji, A. Kishida, Y. Ogawa, Moderate alcohol consumption reduces urinary 8-hydroxydeoxyguanosine by inducing of uric acid, Ind. Health 39 (2001) 322–329.
[7] M.S. Convento, E. Pessoa, M.A. Dalboni, F.T. Borges, N. Schor, Pro-inflammatory and oxidative effects of noncrystalline uric acid in human mesangial cells: contribution to hyperuricemic glomerular damage, Urol. Res. 39 (2011) 21–27.
[8] J.X. Zhang, Y.P. Zhang, Q.N. Wu, B. Chen, Uric acid induces oxidative stress via an activation of the renin-angiotensin system in 3T3-L1 adipocytes, Endocrine 48 (2015) 135–142.
[9] D. Verzola, E. Ratto, B. Villaggio, E.L. Parodi, R. Pontremoli, G. Garibotto, et al., Uric acid promotes apoptosis in human proximal tubule cells by oxidative stress and the activation of NADPH oxidase NOX 4, PLoS One 9 (2014) e115210.
[10] Y. Yang, Y. Zhou, S. Cheng, J.L. Sun, H. Yao, L. Ma, Effect of uric acid on mitochondrial function and oxidative stress in hepatocytes, Genet. Mol. Res. 15 (2016) gmr8644.
[11] M.A. Yu, L.G. Sánchez-Lozada, R.J. Johnson, D.H. Kang, Oxidative stress with an activation of the renin-angiotensin system in human vascular endothelial cells as a novel mechanism of uric acid-induced endothelial dysfunction, J. Hypertens. 28 (2010) 1234–1242.
[12] L.G. Sánchez-Lozada, M.A. Lanaspa, M. Cristóbal-García, F. García-Arroyo, V. Soto, D. Cruz-Robles, et al., Uric acid-induced endotelial dysfunction is associated with mitochondrial alterations and decreased intracellular ATP concentrations, Nephron Exp. Nephrol. 121 (2012) e71–e78.
[13] D.B. Corry, P. Eslami, K. Yamamoto, M.D. Nyby, H. Makino, M.L. Tuck, Uric acid stimulates vacular smooth muscle cell proliferation and oxidative stress via the vascular renin-angiotensin system, J. Hypertens. 26 (2008) 269–275.
[14] Y.Y. Sautin, T. Nakagawa, S. Zharikov, R.J. Johnson, Adverse effects of the classic antioxidant uric acid in adipocytes: NADPH oxidase-mediated oxidative/nitrosative stress, Am. J. Physiol. Cell Physiol. 293 (2007) C584–C596.
[15] L.G. Sánchez-Lozada, V. Soto, E. Tapia, C. Avila-Casado, Y.Y. Sautin, T. Nakagawa, et al., Role of oxidative stress in the renal abnormalities induced by experimental hyperuricemia, Am. J. Physiol. Renal Physiol. 295 (2008) F1134–F1141.
[16] S. Burkhardt, R.J. Reiter, D.X. [illegible], R. Hardeland, F. Cabrera, M. Karbownik, DNA oxidatively damaged by chromium(III) and its protected by the antioxidants melatonin, N^1-acetyl-N^2-formyl-5-methoxykynuramine, resveratrol and uric acid, Int. J. Biochem. Cell Biol. [illegible] (2001) 775–783.
[17] F.A. Shamsi, M. [illegible]di, Photoinduction of strand scission in DNA by uric acid and Cu(II), Free Rad. Biol. Med. 19 (1995) 189–196.
[18] F.A. Shamsi, S. Hussain, [illegible]di, DNA breakage by uric acid and Cu(II): binding of uric acid to DNA and biological activity of the reaction, J. Biochem. Toxicol. 11 (1996) 67–71.
[19] A. Kalavanidis, T. Vlachogianni, C. Fiotakis, 8-hydroxy-2'-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis, J. Environ. Sci. Health Part C: Environ. Carcinog. Ecotoxicol. Rev. 27 (2009) 120–139.
[20] A.S. Levey, L.A. Stevens, C.H. Schmid, Y. Zhang, A.F. Castro III, H.I. Feldman, et al., A new equation to estimate glomerular filtration rate, Ann. Intern. Med. 150 (2009) 604–612.
[21] E. Tatsch, J.A.M. de Carvalho, B.S. Hausen, Y.S. Bollick, V.D. Torbitz, T. Duarte, et al., Oxidative DNA damage is associated with inflammatory response, insulin resistance and microvascular complications in type 2 diabetes, Mutat. Res. 782 (2015) 17–22.
[22] F. Giacco, M. Brownlee, Oxidative stress and diabetic complications, Circ. Res. 107 (2010) 1058–1070.
[23] V. Witko-Sarsat, M. Friedlander, C. Capeillère-Blandin, T. Nguyen-Khoa, A.T. Nguyen, J. Zingraff, et al., Advanced oxidation protein products as a novel marker of oxidative stress in uremia, Kidney Int. 49 (1996) 1304–1313.
[24] G.V. Bochi, V.D. Torbitz, L.P. Cargnin, J.A. de Carvalho, P. Gomes, R.N. Moresco, An alternative pathway through the Fenton reaction for the formation of advanced oxidation protein products, a new class of inflammatory mediators, Inflammation 37 (2014) 512–521.
[25] J.L. Mehta, N. Rasouli, A.K. Sinha, B. Molavi, Oxidative stress in diabetes: a mechanistic overview of its effects on atherogenesis and myocardial dysfunction, Int. J. Biochem. Cell Biol. 38 (2006) 794–803.
[26] H. Al-Aubaidy, H.F. Jelinek, Oxidative DNA damage and obesity in type 2 diabetes mellitus, Eur. J. Endocrinol. 164 (2011) 899–904.
[27] H.Z. Pan, D. Chang, L.G. Feng, F.J. Xu, H.Y. Kuang, M.J. Lu, Oxidative damage to DNA and its relationship with diabetic complications, Biomed. Environ. Sci. 20 (2007) 160–163.
[28] J. Leinonen, T. Lehtimäki, S. Toyokuni, K. Okada, T. Tanaka, H. Hiai, et al., New biomarker evidence of oxidative DNA damage in patients with non-insulin dependent diabetes mellitus, FEBS Lett. 417 (1997) 150–152.
[29] H. Al-Aubaidy, H.F. Jelinek, 8-hydroxy-2-deoxy-guanosine identifies oxidative DNA damage in a rural prediabetes cohort, Redox Rep. 15 (2010) 155–160.
[30] K. Krapfenbauer, R. Birnbacher, H. Vierhapper, K. Herkner, D. Kampel, G. Lubec, Glycoxidation, and protein and DNA oxidation in patients with diabetes mellitus, Clin. Sci. 95 (1998) 331–337.
[31] X. Ye, R. Jiang, Q. Zhang, R. Wang, C. Yang, J. Ma, et al., Increased 8-hydroxy-2'-deoxyguanosine in leukocyte DNA from patients with type 2 diabetes and microangiopathy, J. Int. Med. Res. 44 (2016) 472–482.
[32] C. Luo, X. Lian, L. Hong, J. Zou, Z. Li, Y. Zhu, et al., High uric acid activates the ROS-AMPK pathway, impairs CD68 expression and inhibits OxLDL-induced foam-cell formation in a human monocytic cell line, THP-1, Cell. Physiol. Biochem. 40 (2016) 538–548.
[33] M. Yisireyili, M. Hayashi, H. Wu, Y. Uchida, K. Yamamoto, R. Kikuchi, et al., Xanthine oxidase inhibition by febuxostat attenuates stress-induced hyperuricemia, glucose dysmetabolism, and prothrombotic state in mice, Sci. Rep. 7 (2017) 1266.
[34] M. Bagnati, C. Perugini, C. Cau, R. Bordone, E. Albano, G. Bellomo, When and why a water-soluble antioxidant becomes pro-oxidant during copper-induced low-density lipoprotein oxidation: a study using uric acid, Biochem. J. 340 (1999) 143–152.
[35] R.A. Patterson, E.T.M. Horsley, D.S. Leake, Prooxidant and antioxidant properties of human serum ultrafiltrares toward LDL: important role of uric acid, J. Lipid Res. 44 (2003) 512–521.
[36] H.R. Chang, C.C. Lai, J.D. Lian, C.C. Lin, C.J. Wang, Formation of 8-nitroguanine in blood of patients with inflammatory gouty arthritis, Clin. Clim. Acta 362 (2005) 170–175.