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**ESTUDO DO PERFIL OXIDATIVO E AVALIAÇÃO DE
PARÂMETROS ENZIMÁTICOS EM PACIENTES COM
CÂNCER DE PRÓSTATA.**

TESE DE DOUTORADO

Vanessa Battisti

**Santa Maria, RS, Brasil
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PARÂMETROS ENZIMÁTICOS EM PACIENTES COM
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por

Vanessa Battisti

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elaborada por
Vanessa Battisti

Como requisito parcial para obtenção do grau de
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Santa Maria, 19 de dezembro de 2011

“A mente que se abre a uma nova idéia jamais voltará
ao seu tamanho original.”

Albert Einstein

DEDICATÓRIA

***Ao meu marido: Cleverson
Aos meus pais: Ireno e Terezinha***

Pelo amor incondicional

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Agradeço a Deus pela vida e por tudo que eu tenho.

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RESUMO
Tese de Doutorado
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Toxicológica
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ESTUDO DO PERFIL OXIDATIVO E AVALIAÇÃO DE PARÂMETROS
ENZIMÁTICOS EM PACIENTES COM CÂNCER DE PRÓSTATA.

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Local e Data da defesa: Santa Maria, 19 de dezembro de 2011

O câncer de próstata é reconhecido como um dos mais importantes problemas médicos enfrentados pela população masculina. Alterações inerentes ou adquiridas no metabolismo celular que ocorrem ao longo dos anos podem ter envolvimento no processo de desenvolvimento deste tipo de câncer. Dessa forma, o estresse oxidativo pode desempenhar um papel importante nos processos celulares associados com a iniciação e o desenvolvimento do câncer de próstata. Por outro lado, o câncer pode estar associado com alterações na agregação plaquetária e no metabolismo dos nucleotídeos. Além disso, as enzimas que degradam ésteres de colina desempenham funções não colinérgicas e parecem estar envolvidas com a proliferação e diferenciação celular. Levando em consideração tais afirmativas, este trabalho teve como objetivos avaliar o perfil oxidativo, através da determinação do conteúdo de substâncias reativas ao ácido tiobarbitúrico (TBARS) e de proteína carbonil, e as defesas antioxidantes, através da determinação das atividades das enzimas catalase (CAT) e superóxido dismutase (SOD) e dos níveis de tióis não-protéicos, vitamina C e vitamina E. Além disso, realizar a determinação das atividades das enzimas ectonucleotidases representadas pelas enzimas NTPDase, E-NPP, 5'nucleotidase e adenosina deaminase (ADA). Por fim, verificar as atividades das enzimas acetilcolinesterase (AChE), butirilcolinesterase (BuChE) e os parâmetros bioquímicos em pacientes e controles. Esse trabalho teve ainda como objetivo, avaliar a influência da escala de Gleason, da presença ou ausência de metástase e do tipo de tratamento a que foram submetidos os pacientes, nas dosagens realizadas. Os resultados demonstram que o conteúdo de TBARS e os níveis de carbonilação de proteínas aumentaram nos pacientes quando comparados com o grupo controle e mudanças no sistema de defesa antioxidante foram observadas. A atividade da CAT estava diminuída e a atividade da SOD aumentada nos pacientes quando comparados aos controles. Verificamos ainda um aumento nos níveis de tióis não-protéicos no plasma e eritrócitos e uma diminuição nos níveis séricos de vitamina C e de vitamina E em pacientes com câncer próstata. Os resultados mostraram que a hidrólise dos nucleotídeos ATP, ADP e AMP estava aumentada nos pacientes quando comparado ao grupo controle, o que pode ser comprovado pelo aumento nas atividades das enzimas NTPDase, E-NPP e 5'Nucleotidase. Por outro lado, nossos resultados revelaram uma diminuição na atividade da ADA no soro desses pacientes. Os resultados mostraram também que a agregação plaquetária foi diminuída apenas nos pacientes com câncer de próstata antes do início do tratamento. As atividades da AChE e BuChE foram diminuídas

nos pacientes em relação ao grupo controle. Com os dados podemos concluir que há um aumento na formação de espécies reativas e um desequilíbrio no sistema de defesa antioxidante enzimático e não-enzimático nos pacientes. Além disso, o câncer de próstata avançado pode estar associado com um estado de estresse oxidativo elevado. Os resultados refletem também o aumento na hidrólise de nucleotídeos e o conseqüente aumento nas concentrações de adenosina nos pacientes, que pode estar participando do processo de tumorigênese. A diminuição nas atividades das colinesterases (AChE e BuChE) e o conseqüente aumento nos níveis de acetilcolina sugerem a participação da mesma no processo de desenvolvimento do câncer. Finalmente, as alterações nas atividades das enzimas NTPDase, 5' Nucleotidase, E-NPP, ADA, BuChE e AChE quando os pacientes foram divididos em grupos considerando a escala de Gleason, a presença ou ausência de metástase óssea e o fato de estar ou não em tratamento, indicam que esses parâmetros devem ser considerados.

Palavras-chaves: Câncer de próstata; ectonucleotidasas; colinesterases; estresse oxidativo; defesas antioxidantes

ABSTRACT

Doctoral Thesis

Graduate Program in Biological Sciences: Toxicological Biochemistry
Federal University of Santa Maria, RS, Brazil

STUDY OF OXIDATIVE PROFILE AND ENZYMATIC PARAMETERS IN PROSTATE CANCER PATIENTS.

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Place and date of defense: Santa Maria, 19 december 2011

Prostate cancer is recognized as one of the most important medical problems in the male population. Inherent or acquired alterations in cellular metabolism that occur over the years may be involved in the process of developing this cancer. Thus, oxidative stress may play an important role in cellular processes associated with the initiation and development of prostate cancer. On the other hand, the cancer may be associated with changes in platelet aggregation and metabolism of nucleotides. In addition, enzymes that degrade esters of choline are well known as participating in non-cholinergic functions and intervene in important cell processes such as proliferation, differentiation and apoptosis. Considering such statements, this study aimed to evaluate the oxidative profile, through the determination of parameters such as the TBARS and protein carbonyl content and antioxidant defenses, by determine the catalase (CAT) and superoxide dismutase (SOD) activity and non-protein thiols, vitamin C and vitamin E levels. Also, determine ectonucleotidases enzyme activities represented by the enzymes NTPDase, E-NPP, 5'nucleotidase and adenosine deaminase (ADA) and verified the acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities. In addition, we evaluated the influence of Gleason score, the presence or absence of metastasis and type of treatment used by patients in the parameters analyzed. The results show that the content of TBARS and protein carbonylation levels increased in patients when compared with the control group. In addition, changes in the antioxidant defenses were observed. The CAT activity was decreased and SOD activity increased in patients compared to controls. We found also an increase in the levels of non-protein thiols in plasma and erythrocytes and a decrease in serum levels of vitamin C and vitamin E in patients. These results suggest an increased formation of reactive species and an imbalance in the enzymatic and non-enzymatic antioxidant system defense in patients with prostate cancer. Moreover, it was observed that advanced prostate cancer is associated with a state of high oxidative stress. The results showed that nucleotides hydrolysis (ATP, ADP and AMP) were increased in patients compared to controls, which maybe observed by the increase in the enzymes activity (NTPDase, E-NPP and 5'Nucleotidase). On the other hand, our results showed a decrease in ADA activity in serum of these patients. These results reflect the increase in the nucleotide hydrolysis and the consequent increase in the adenosine concentrations that may be involved in the tumorigenesis process. Our results also showed that inhibition of platelet aggregation was decreased only in patients with prostate cancer before starting treatment. The AChE and BuChE activities were decreased in patients in

relation to the control group. The decrease in the cholinesterases activities and the consequently increase in acetylcholine levels suggest the participation of this molecule in the process of cancer development. Finally, changes in the NTPDase, 5' nucleotidase, E-NPP, ADA, AChE and BuChE activities when patients were divided in groups considering the Gleason score, presence or absence of bone metastasis and the treatment, indicate that these parameters must be considered.

Key-words: Prostate cancer; ectonucleotidases; cholinesterase; oxidative stress; antioxidant defenses.

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LISTA DE ABREVIações

ACh- Acetilcolina
AChE – Acetilcolinesterase
ADA – Adenosina Deaminase
ADP- Adenosina Difosfato
ALT – Alanina Aminotransferase
AMP- Adenosina Monofosfato
AST – Aspartato Aminotransferase
ATP – Adenosina Trifosfato
BuChE – Butirilcolinesterase
CaP – Câncer de Próstata
CAT- Catalase
DHT - Dihidrotestosterona
DNPH - Dinitrofenilidrazina
EDTA – Ácido etilenodiaminotetracético
E-NPP- Ectonucleotídeo pirofosfatase/fosfodiesterase
E – NTPDase – Ecto Nucleosídeo Trifosfato Difosfohidrolase
EROs – Espécies Reativas de Oxigênio
FAL – Fosfatase Alcalina
GGT – Gamaglutamiltransferase
GpX – Glutathione Peroxidase
GSH – Glutathione Reduzida
INCA – Instituto Nacional do Câncer
LDH – Lactato Desidrogenase
LH – Hormônio Luteinizante
LHRH - Hormônio Liberador do Hormônio Luteinizante
MDA – Malondialdeído
PAS – Sítio Aniônico Periférico
PRP – Plasma Rico em Plaquetas
PSA – Antígeno Prostático Específico
SH – Grupo sulfidril
SNC – Sistema Nervoso Central

SOD – Superóxido Dismutase

TBA – Ácido Tiobarbitúrico

TBARS – Substâncias Reativas ao Ácido Tiobarbitúrico

TCA – Ácido Tricloroacético

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1. INTRODUÇÃO

Nas últimas décadas, o câncer de próstata tornou-se um problema relevante de saúde pública no Brasil e no mundo (CALVETE et al., 2003). O número de casos novos de câncer de próstata estimado para o Brasil no ano de 2012 é de 60.180. Estes valores correspondem a um risco estimado de 62 casos novos a cada 100 mil homens. Sem considerar os tumores de pele não melanoma, o câncer de próstata é o mais freqüente em todas as regiões do Brasil, com risco estimado de 68/100.000 na região Sul (INCA, 2012). No estado do Rio Grande do Sul, a estimativa para o ano de 2012 é de 79 novos casos a cada 100 mil homens, o que pode ser considerada uma taxa particularmente alta em comparação a outros estados.

A biópsia prostática permite ao patologista distinguir tumor benigno de maligno e identificar o grau de diferenciação celular. Atualmente o sistema de Gleason, que enfatiza a arquitetura glandular, é o mais utilizado para prever a evolução e predizer o estágio patológico do câncer de próstata (GLEASON, 1992). Lesões com escore de Gleason 2-4 (bem diferenciados) têm um comportamento mais ameno e as de escore 7-10 (indiferenciados) são agressivos, respondendo mal às diferentes opções de tratamento. O local mais comum de disseminação do câncer de próstata é o osso e a presença ou não de metástase óssea é um dado fundamental que direciona o tratamento. O tratamento deve ser individualizado para cada paciente levando-se em conta a idade, o estadiamento do tumor, o grau histológico, o tamanho da próstata, as comorbidades, a expectativa de vida, os anseios do paciente e os recursos técnicos disponíveis. Diferentes terapias hormonais que atuam através de diferentes mecanismos estão disponíveis, dentre elas o acetato de goserelina e acetato de ciproterona (ANDERSON, 2003). Além disso, o monitoramento de dano renal e hepático e do equilíbrio eletrolítico, entre outras determinações bioquímicas são de extrema importância em pacientes com câncer.

Assim como em outros cânceres, a idade é um fator de risco importante, uma vez que desenvolvimento do câncer de próstata aumenta drasticamente após os 40 anos (MIYAKE et al., 2004). O estudo do mecanismo envolvido no aumento da incidência do câncer de próstata com a idade é de grande interesse e importante para o entendimento desta doença. Uma explicação possível pode estar associada

ao desequilíbrio entre os agentes oxidantes e os mecanismos de defesa antioxidantes em vários tecidos, incluindo a próstata, que ocorre concomitantemente ao envelhecimento e leva a um estado oxidativo e a danos ao organismo (WARNER, 1994). Além disso, estudos mostraram que o tecido da próstata é particularmente vulnerável a estes danos oxidativos (BAKER et al., 1997; BOSTWICK et al., 2000).

Todos os componentes celulares são suscetíveis à ação das espécies reativas, porém a membrana é um dos mais atingidos em decorrência da peroxidação lipídica, que acarreta alterações na estrutura e na permeabilidade das mesmas (MELLO FILHO et al., 1984). A carbonilação de proteínas é outro exemplo de lesão biológica que pode ser promovida pelos radicais livres (TIAN et al., 1998).

Normalmente, o estresse oxidativo tem seus danos minimizados pelo sistema de defesa antioxidante enzimático, representado, principalmente, pelas enzimas superóxido dismutase (SOD) e catalase (CAT) (BONNEFOY et al., 2002). A detoxificação das espécies reativas envolve um mecanismo de elevada sincronia e as enzimas antioxidantes atuam de maneira cooperativa. Cabe a SOD a dismutação do radical superóxido a peróxido de hidrogênio, que é menos reativo e pode ser degradado por outras enzimas, como a CAT.

Além das defesas antioxidantes enzimáticas, possuem grande relevância os antioxidantes não enzimáticos. Dentre estes antioxidantes podem-se citar as vitaminas C, E e os compostos orgânicos contendo grupos sulfidril (SH), denominados tióis. A glutatona reduzida (GSH) é um tiol de baixo peso molecular que se encontra no interior celular, sendo um tripeptídeo importante na proteção contra o dano oxidativo.

A vitamina C apresenta, *in vitro*, uma multiplicidade de propriedades antioxidantes, protegendo várias biomoléculas contra o dano causado pelas espécies reativas (HALLIWELL e GUTTERIDGE, 2000). Além de sua ação direta contra radicais livres, o ácido ascórbico ou vitamina C afeta indiretamente o balanço entre oxidantes e antioxidantes, já que promove a regeneração do α -tocoferol, um importante antioxidante lipossolúvel. Foi observado que a ingestão de dietas contendo ácido ascórbico levou à diminuição da incidência de câncer (YOUNG et al., 1997). Os tocoferóis ou vitamina E são varredores de radicais peroxil, sendo, portanto, os inibidores mais importantes da peroxidação lipídica em animais. Devido as suas propriedades antioxidantes, acredita-se que esta vitamina possa prevenir

doenças associadas ao estresse oxidativo, dentre elas o câncer (BRIGELIUS-FLOHE et al., 2002).

Sabe-se que as células do câncer podem apresentar alterações nas vias de sinalização celular (SARKAR et al., 2010) e os nucleotídeos extracelulares produzem resposta em diversos processos biológicos, tais como apoptose, controle da proliferação e da diferenciação celular (BURNSTOCK, 2002). Vários trabalhos realizados em nosso laboratório de pesquisa evidenciam a estreita relação existente entre os nucleotídeos da adenina e o câncer (ARAÚJO et al., 2005; MALDONADO et al., 2008). O controle dos níveis de nucleotídeos na circulação é importante na manutenção do processo de sinalização celular mediado pelos mesmos e também na regulação da agregação plaquetária. Este controle é feito por uma família de enzimas que hidrolisa os nucleotídeos e, conseqüentemente, gera os seus respectivos metabólitos (BOROWIEC et al., 2006). Estas ecto-enzimas incluem as difosfohidrolases (Ecto-NTPDase), a família das ectonucleotídeos pirofosfato/fosfodiesterases (E-NPP), bem como a 5'-nucleotidase e a adenosina deaminase (ADA).

A NTPDase (EC 3.6.1.5, CD39) é uma enzima glicosilada ligada a membrana que hidrolisa adenosina trifosfato (ATP) e adenosina difosfato (ADP) em adenosina monofosfato (AMP) que é subsequentemente convertido à adenosina pela enzima 5'nucleotidase (EC 3.1.3.5, CD73). Ambas as enzimas desempenham um importante papel na regulação do fluxo sanguíneo pela regulação do catabolismo do ADP (KAWASHINA et al., 2000; MARCUS et al., 2003). A associação clínica entre o tromboembolismo venoso e o câncer é bem conhecida (DONATI et al., 2001), além disso, pacientes com câncer podem exibir aumento da ativação plaquetária (ARAÚJO et al., 2005).

A enzima ectonucleosídeo pirofosfatase/fosfodiesterase E-NPP (EC 3.1.4.1) é responsável pela hidrólise das ligações fosfodiésteres nos nucleotídeos, resultando na produção de nucleotídeos monofosfatos (GODING et al., 2003). Já a enzima adenosina deaminase (ADA) (EC 3.5.4.4) catalisa a desaminação da adenosina e desoxiadenosina em inosina e desoxinosina. Esta enzima tem sido encontrada alterada em certos tipos de tumores (SPYCHALA, 2000), além disso, é utilizada como um marcador de malignidade. Levando em consideração o fato de que a adenosina e os nucleotídeos da adenina possuem papel importante na fisiologia do câncer e que ambas as enzimas, ADA e E-NPPs estão alteradas em muitos

processos, é importante verificar o papel destas enzimas em pacientes com câncer de próstata.

Outro importante grupo de enzimas que tem sido foco de estudo em pacientes com câncer são as colinesterases. Apesar de ser bem conhecida como a enzima que hidrolisa a acetilcolina, a acetilcolinesterase (AChE) é agora também conhecida por ter funções não enzimáticas adicionais (SILMAN e SUSSMAN 2005). Várias observações têm também indicado à possibilidade de uma nova função da acetilcolinesterase em tecidos não-neuronais, incluindo tumores (SMALL et al., 1996; BATTISTI et al., 2009). Estudos relatam que parece haver uma associação entre mudanças na expressão e atividade das colinesterases e o desenvolvimento de vários tipos de câncer (RUIZ-ESPEJO et al., 2003). No entanto, ainda não existem muitos estudos relacionando modificações no comportamento das colinesterases à manifestação do câncer de próstata.

Estudos realizados anteriormente pelo nosso grupo de pesquisa demonstram a existência de alterações no perfil oxidativo (MALDONADO et al., 2006; BATTISTI et al., 2008) e no comportamento de várias enzimas (ARAÚJO et al., 2005; MALDONADO et al., 2008) em pacientes com diferentes tipos de câncer. Entretanto, o estudo enzimático e oxidativo em pacientes com câncer de próstata considerando a escala de Gleason e a presença ou ausência de metástase, bem como o tipo de tratamento a que os pacientes são submetidos, ainda não foi realizado. Considerando a possível relação dos parâmetros em estudo com o câncer de próstata e a alta frequência desta doença, torna-se importante avaliar o perfil de estresse oxidativo, através do estudo do sistema oxidante e antioxidante, além de determinar a atividade das enzimas NTPDase, 5' nucleotidase, E-NPP, ADA, AChE e BuChE em pacientes com diagnóstico comprovado de câncer de próstata. Finalmente, verificar a influência da escala de Gleason, da presença ou ausência de metástases e do tratamento nas determinações realizadas torna o trabalho mais relevante, já que estes parâmetros devem ser levados em consideração no câncer de próstata.

2. OBJETIVOS

Objetivo Geral:

Estudar o perfil oxidativo e avaliar parâmetros enzimáticos em plaquetas, soro ou sangue total de pacientes com câncer de próstata e controles, considerando a escala de Gleason, a presença ou ausência de metástases e o tratamento padrão utilizado.

Objetivos Específicos:

- Analisar os danos oxidativos causados pelos radicais livres através da dosagem de espécies reativas ao ácido tiobarbitúrico (TBARS) em plasma e da determinação da carbonilação protéica em soro de pacientes com câncer de próstata e de controles.
- Avaliar o estado das defesas antioxidantes enzimáticas através da determinação das enzimas catalase (CAT) e Superóxido dismutase (SOD) em sangue total.
- Avaliar o estado das defesas antioxidantes não enzimáticas através da determinação dos níveis de tióis não-protéicos em plasma e eritrócitos, Vitamina C e vitamina E em soro destes pacientes e controles.
- Verificar a atividade das enzimas NTPDase e 5'nucleotidase em plaquetas de pacientes com câncer de próstata e controles.
- Determinar a atividade das enzimas E-NPP e ADA em soro e plaquetas destes pacientes e controles.
- Avaliar a agregação plaquetária no plasma rico em plaquetas de pacientes e controles.
- Investigar possíveis alterações nas atividades das enzimas AChE e BuChE em sangue total e soro, respectivamente, de pacientes e controles.
- Determinar parâmetros bioquímicos, dentre eles, desidrogenase láctica, fosfatase alcalina, uréia, creatinina, ácido úrico, gama glutamil transferase, sódio, potássio, cálcio, magnésio, alanina aminotransferase, aspartato aminotransferase e albumina.
- Relacionar os resultados das atividades enzimáticas e do perfil de estresse oxidativo com alguns parâmetros importantes como: escore de Gleason, tratamento padrão e a presença ou ausência de metástase.

3. REVISÃO DE LITERATURA

3.1. Câncer de próstata

A próstata está localizada abaixo da bexiga e na frente do reto e sua função é produzir uma secreção líquida que faz parte do sêmen. No homem adulto, a próstata tem o tamanho aproximado de uma ameixa, pesando cerca de 20 gramas (Figura 1). O câncer de próstata é notadamente reconhecido como um problema de saúde pública, dado a sua magnitude no quadro de morbimortalidade masculina. A extensão do câncer de próstata é refletida pelas estatísticas publicadas pelo Instituto Nacional de Câncer (INCA, 2012). No estado do Rio grande do Sul, segundo o INCA, a taxa de incidência estimada para o ano 2012 é de 79 casos para cada 100.000 homens (INCA, 2012). Ainda segundo dados do INCA, no Brasil, após o câncer de pele, o de próstata é o que apresenta maior incidência, sendo a quarta causa de morte por neoplasias em homens. Além disso, o número de casos novos diagnosticados de câncer de próstata no mundo é de aproximadamente 543 mil casos por ano, representando 15,3% de todos os casos incidentes de câncer em países desenvolvidos e 4,3 % dos casos em países em desenvolvimento.

O câncer de próstata tem crescimento lento, e é raro antes dos 50 anos de idade, sendo que 85% dos casos são diagnosticados após os 65 anos (BRAWLEY et al., 2009; GREENE et al., 2009). A mortalidade por câncer de próstata é relativamente baixa, o que em parte reflete seu bom prognóstico em comparação a outros tipos de câncer. Nos países desenvolvidos, a sobrevida média estimada em cinco anos é de 64%, enquanto que para países em desenvolvimento, a sobrevida média é de 41% (INCA, 2010). O aumento observado nas taxas de incidência no Brasil pode ser parcialmente justificado pela evolução dos métodos diagnósticos, pela melhoria na qualidade dos sistemas de informação do país e pelo aumento na expectativa de vida (GONÇALVES et al., 2008). Os principais instrumentos diagnósticos utilizados para diagnosticar o câncer de próstata incluem o exame digital transretal da próstata, antígeno prostático específico (PSA) e a biópsia por ultrassonografia transretal (USTR).

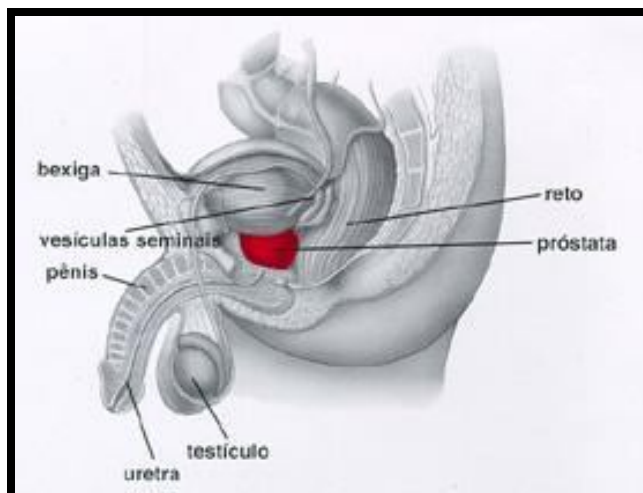


Figura 1: Próstata. Adaptado de www.inca.gov.br

A utilização de exames de rastreamento do câncer de próstata, como o PSA (antígeno específico prostático) é cercada por controvérsias na comunidade científica internacional, existindo diferentes recomendações das sociedades médicas e agências governamentais. Estudos realizados sobre o rastreamento do câncer de próstata mostraram-se discordantes quanto à eficácia do procedimento, dividindo a opinião dos pesquisadores e apontando a necessidade de maior investigação sobre o assunto (DJULBEGOVIC et al., 2010; WOLF et al., 2010).

3.2. Sintomatologia

Estudos sobre os sintomas evidenciam que, em geral, os pacientes com câncer de próstata têm descoberto a doença, por acaso, durante exames de rotina (GONÇALVES et al., 2008). No entanto, os sintomas de disfunção miccional são historicamente mencionados como os sintomas mais comumente relacionados a esta doença, porém não estão especificamente relacionados ao crescimento benigno ou maligno da glândula (RODHEN e AVERBECK, 2010). Esses sintomas incluem: polaciúria (aumento no número de micções), disúria (dificuldade para urinar), redução da força e calibre do jato urinário, noctúria (necessidade de urinar a noite), hesitação e sensação de repleção miccional.

Embora a hemospermia (presença de sangue no esperma) não seja uma manifestação característica do câncer de próstata, a sua ocorrência em indivíduos

acima de 50 anos deve levar o clínico a pensar nesta possibilidade diagnóstica (KIM e GRAYHACK, 2000).

Nos casos de doença metastática, a dor óssea e as fraturas patológicas são manifestações prevalentes; entretanto, um terço dos pacientes com metástases ósseas podem estar assintomáticos no momento do diagnóstico (RODHEN e AVERBECK, 2010).

O comprometimento linfonodal é assintomático nos estágios precoces da doença avançada regional, mas pode apresentar quadros clínicos exuberantes, com edema da genitália externa e dos membros inferiores, nos casos mais avançados. Além disso, sintomas neurológicos são observados em 20% dos pacientes com câncer de próstata. Dores em diferentes sítios, fraqueza, distúrbios esfinterianos urinários e retais são clinicamente os achados mais característicos (Rosenthal et al., 1992). A compressão medular aguda decorrente do comprometimento metastático das vértebras deve ser tratada emergencialmente.

3.3. Fatores de risco

Os fatores de risco para câncer de próstata são, na maioria, desconhecidos e inevitáveis (GOMES et al., 2008). Os dois fatores que apresentam certo consenso entre as fontes no que se refere ao aumento do risco de desenvolvimento do câncer de próstata são a idade e a história familiar. A grande maioria dos casos ocorre em homens com idade superior a 50 anos e naqueles com história de pai ou irmão com câncer de próstata. De acordo com SROUGI (2011), os riscos aumentam de 2,2 vezes quando um parente de 1º grau (pai ou irmão) é acometido pelo problema, de 4,9 vezes quando dois parentes de 1º grau são portadores do tumor e de 10,9 vezes quando três parentes de 1º grau têm a doença.

A incidência do câncer de próstata difere substancialmente entre os grupos étnicos. Alguns estudos apresentam faixas de riscos alta, intermediária e baixa para o desenvolvimento do câncer de próstata, situando-se os negros norte-americanos na primeira, os brancos na segunda e os japoneses na terceira faixa (GOMES et al., 2008). Alguns autores citam a dieta altamente calórica e os hormônios masculinos como fatores de risco para o câncer de próstata (EPSTEIN et al., 2005). Além disso, trabalhos sugerem que o tabagismo, o etilismo e o estilo de vida podem possuir relação com o desenvolvimento do câncer de próstata.

3.3.1. Idade

O envelhecimento é considerado o fator de risco mais significativo para o desenvolvimento do câncer de próstata (GONÇALVES et al., 2008). A incidência e a mortalidade por câncer de próstata estão fortemente associadas com a idade (MACEFIELD et al., 2009). A probabilidade de ocorrência de câncer de próstata em pacientes com idade inferior a 50 anos é muito baixa, sendo que, cerca de 60% dos casos de câncer de próstata diagnosticados são em homens com mais de 70 anos de idade.

Com o aumento da expectativa de vida dos homens, as possibilidades de se ter câncer de próstata podem ser aumentadas. Estima-se que, aos 80 anos, cerca de 50% dos homens sejam atingidos pelo câncer de próstata. Assim, é importante considerar que o risco de se desenvolver o câncer de próstata aumenta à medida que o homem envelhece (LOEB e SCHAEFFLE, 2009).

3.3.2. Tabagismo

A fumaça do cigarro contém centenas de substâncias cancerígenas, dentre elas o arsênico, polônio, formaldeído, acroleína, níquel, chumbo e cádmio, que têm efeitos comprovados em todas as fases de desenvolvimento de câncer (HECHT, 1997). As etapas de iniciação, promoção e progressão tumoral têm sido associado com compostos encontrados na fumaça do tabaco em uma variedade de modelos experimentais (RODGMAN et al., 2000). A associação entre o tabagismo e as neoplasias de pulmão, bexiga, cabeça e pescoço já está descrita em literatura, além disso, não se exclui uma possível associação entre o tabagismo e o câncer de próstata (FLESHNER, 2003).

Há uma série de mecanismos hipotéticos pelos quais a fumaça de cigarro pode aumentar o risco de desenvolvimento ou a progressão do câncer de próstata. Dentre estes mecanismos pode-se citar os efeitos do tabagismo na fisiologia hormonal, já que os hormônios sexuais desempenham um papel importante na etiologia e progressão do câncer de próstata. ENGLISH e colaboradores (2001) relatam que o tabagismo influencia os níveis de hormônios sexuais. Níveis dos hormônios estrogênio, testosterona e dehidroepiandrosterona têm se mostrado

significativamente diferentes entre homens fumantes, ex-fumantes e não-fumantes (HSIEH et al., 1998).

Outro mecanismo que pode ser citado na relação entre o cigarro e o câncer é a mutação genética, que é uma característica de todos os cânceres. A fumaça do cigarro contém muitos agentes mutagênicos conhecidos, como o benzopireno por exemplo, portanto, se considerarmos a fumaça do cigarro como causa de mutação genética associada à iniciação ou promoção da doença, pode existir uma associação entre o uso de tabaco e a incidência de câncer de próstata.

Por outro lado, o estresse oxidativo é considerado um mecanismo importante para a progressão do câncer de próstata (FLESHNER e KLOTZ, 1999). O cigarro é uma conhecida fonte de espécies reativas que podem causar dano celular se não forem neutralizados pelos antioxidantes. AGARWAL e RAO (2000) demonstraram que os níveis de antioxidantes importantes, como o licopeno por exemplo, podem ser reduzidos pelo uso do cigarro. Portanto, é plausível sugerir que o tabagismo, através de interação com antioxidantes, desempenha um papel importante na incidência e na progressão da doença.

3.3.3. Etilismo

O etanol é um conhecido agente cancerígeno e seu consumo é causalmente associado a diversas neoplasias, incluindo câncer de cabeça e pescoço, esôfago e hepático (BRESLOW e WEED, 1998). Porém, os estudos são contraditórios quando relacionam o risco de desenvolvimento de câncer de próstata e o consumo de bebidas alcoólicas (GONG et al., 2009). Estudos que possam melhor esclarecer a associação do consumo de álcool com o risco de desenvolvimento do câncer de próstata são importantes, já que não estão bem estabelecidos quais são os fatores de risco modificáveis associados a este tipo de câncer. O álcool altera o metabolismo de esteróides sexuais e, assim, teoricamente poderia desempenhar papel importante no desenvolvimento do câncer de próstata (TAVANI et al., 1994). Além disso, alguns autores sugerem que o consumo excessivo de álcool pode aumentar o risco relativo de desenvolvimento do câncer de próstata (GONG et al., 2009; YARNEI et al., 2011).

3.3.4. Profissão

Estudos sugerem a exposição ocupacional, como por exemplo, possíveis associações com a agricultura e com a exposição a substâncias perigosas, tais como pesticidas, cádmio e hidrocarbonetos aromáticos policíclicos como fatores de risco para o desenvolvimento do câncer de próstata (PARENT e SIEMIATYCKI, 2001; RAMIS et al., 2011).

Outros estudos trazem evidências de que os grupos ocupacionais potencialmente expostos a pesticidas, que incluem os agricultores e também os fabricantes de pesticidas podem apresentar riscos elevados de desenvolvimento de câncer de próstata (PARENT et al., 2009). Segundo PARENT et al. (2009) os agricultores expostos a altos níveis de pesticidas têm um risco duas vezes maior de desenvolvimento do câncer de próstata comparados aos agricultores não expostos.

3.4. Metástase óssea

O câncer de próstata é um tumor de crescimento lento, com progressão não linear. O local mais comum de disseminação hematogênica do câncer de próstata é o osso (Figura 2) (RIGAUD et al., 2002). A presença ou não de metástase óssea à época do diagnóstico é um dado fundamental que direciona o tratamento (LEE et al., 2000). A maioria das morbidades e mortalidade no câncer de próstata avançado deve-se direta ou indiretamente ao comprometimento ósseo metastático, incluindo dor óssea, fraturas e a imobilidade (CLARK e TORTI, 2003). A presença de metástase óssea é sabidamente um fator de mau prognóstico. Dos pacientes com câncer de próstata que desenvolvem metástase, 50% morrem dentro de 30 meses (RIGAUD et al., 2002). Na autópsia dos pacientes que morreram de câncer avançado de próstata, as metástases ósseas estavam presentes em 80% a 90% dos casos (CLARK e TORTI, 2003).

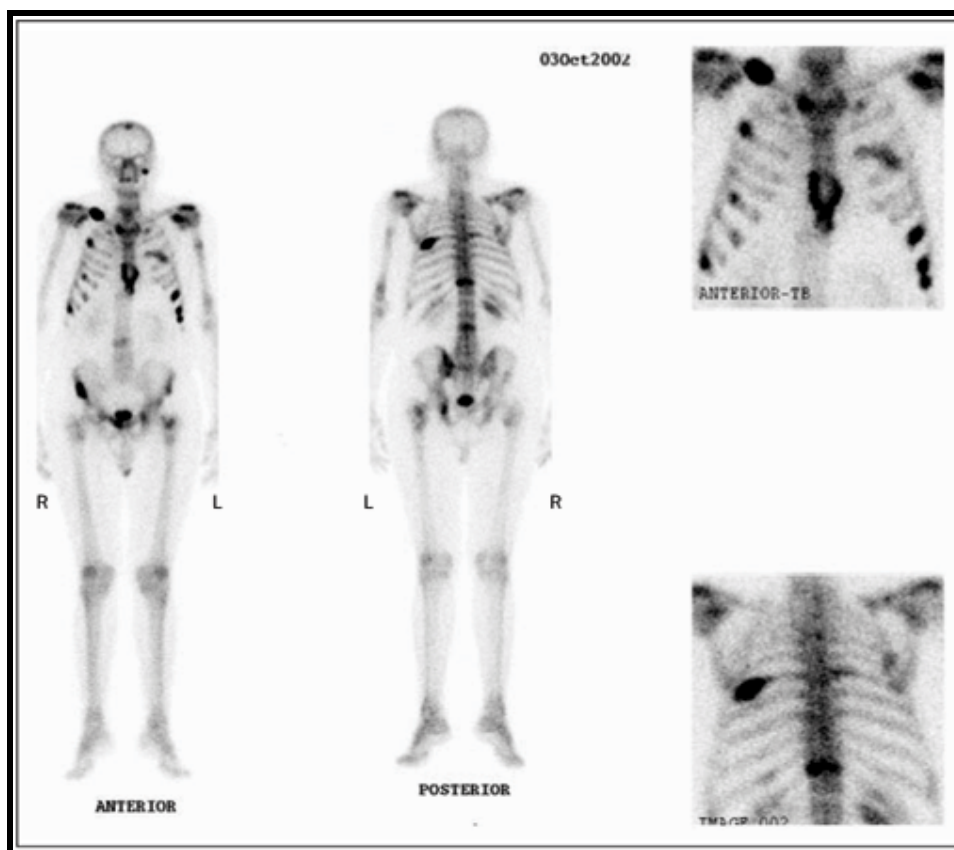


Figura 2: Múltiplas lesões focais de metástase óssea. Os pontos escuros representam metástases ósseas. Método: Cintilografia óssea. Adaptado de ABREU et al., 2005.

3.5. Escore de Gleason

O câncer de próstata é classificado usando o sistema de classificação de Gleason (Figura 3), que é baseado na aparência microscópica da arquitetura glandular da próstata (KIRBY e MADHAVAN, 2010). Segundo CALVETE e colaboradores (2003), o sistema de Gleason tem sido utilizado para ajudar a prever a evolução e prever o estágio patológico, sendo um critério adicional de prognóstico para se estabelecer com precisão as características biológicas das neoplasias prostáticas. O escore de Gleason é composto pelos dois padrões mais comuns de células cancerosas, expressas como números de um até cinco. O padrão celular mais comum é listado primeiro, em seguida lista-se o segundo tipo celular mais comum, a soma obtida é o valor da escala de Gleason final. Este sistema é

muito utilizado e seu resultado é dado por uma escala que varia de 2 a 10. Os tumores de graus 2 e 4 compreendem as lesões diferenciadas; já os de graus 5 e 6 incluem as lesões moderadamente diferenciadas; e, por último, os de graus de 7 a 10 relacionam-se às lesões indiferenciadas. Sabe-se que lesões com escore de Gleason 2-4 têm um comportamento mais indolente e as de escore 7-10 são agressivos, respondendo mal às diferentes opções de tratamento.

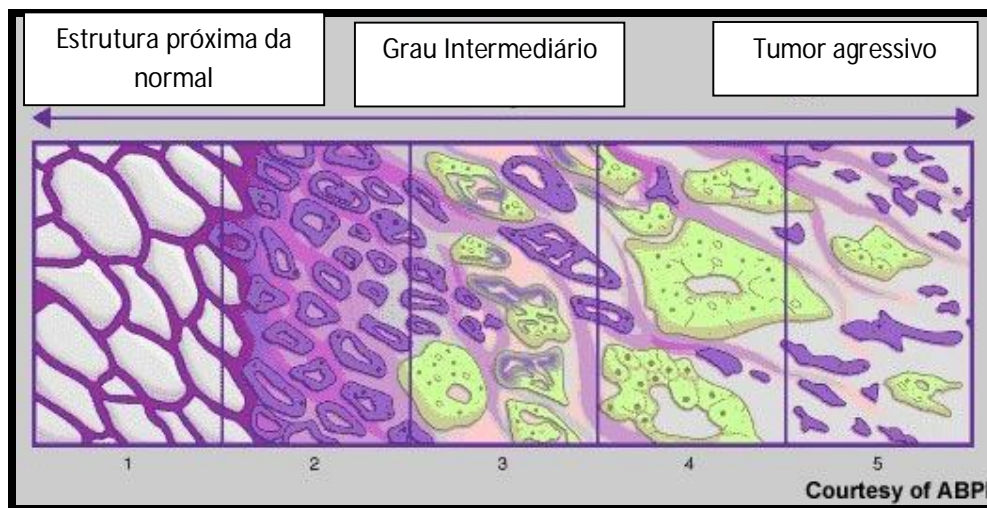


Figura 3: Escala de Gleason. Adaptado de <http://www.taringa.net/posts/info/5609275/Escala-de-Gleason---Cancer-de-prostata.html>

3.6. Tratamento

De acordo com CALVETE et al. (2003), as decisões acerca do tratamento do câncer de próstata compreendem muitos fatores. Quatro considerações são especialmente importantes para selecionar a opção de tratamento: o estágio do câncer (disseminação da doença), o grau ou Escore de Gleason, a idade e as escolhas do paciente (FAGUNDES et al., 2002).

3.6.1. Doença localizada:

As três opções geralmente aceitas para tratamento do câncer de próstata localizado são a prostatectomia radical (procedimento cirúrgico em que toda a próstata e os tecidos adjacentes são removidos), a radioterapia (tratamento local com um feixe de radiação direcionado) e a expectativa cuidadosa (não é realizado nenhum tratamento ativo). As diferentes modalidades utilizadas para o tratamento do câncer de próstata localizado segundo dados do CAPSURE (*Cancer of the Prostate Strategic Urologic Research Endeavor*) apresentam os seguintes percentuais: prostatectomia radical (51,6%), sementes de radiação implantadas na próstata, ou braquiterapia (21,7%), radioterapia externa (6,8%), observação (7,9%) e outros (12%). A conduta deve ser individualizada, em virtude dos efeitos deletérios associados à cirurgia e à radioterapia (externa e braquiterapia), custos e preferência do paciente (RODHEN e AVERBECK, 2010).

Apesar dos recentes estudos e melhorias nestes tratamentos, uma grande proporção de pacientes com doença localizada apresenta falha na terapia local e desenvolve doença metastática (ANDERSON e STERNBERG, 2008).

3.6.2. Doença metastática:

No caso de câncer metastático, a terapia endócrina ou hormonoterapia é o tratamento mais indicado (KALIKS e GIGLIO, 2008). Nesta terapia são empregados vários medicamentos à base de hormônios (estrógenos, análogos do hormônio liberador do hormônio luteinizante e antiandrógenos), que impedem a produção de testosterona ou bloqueiam as suas ações na próstata (TONON e SCHOFFEN, 2009). A orquiectomia ou retirada cirúrgica dos testículos, também é utilizada como hormonoterapia, já que elimina os órgãos que produzem o hormônio masculino, este apontado como principal responsável pelo crescimento do tumor (SHAHI e MANGA, 2006).

Muitas questões permanecem duvidosas em relação a estratégia de tratamento ideal para a doença localizada e para o câncer metastático. Com relação ao tratamento quimioterápico, diversos esquemas têm sido utilizados em tumores avançados ou metastáticos da próstata, porém, os resultados não são animadores.

A utilização de apenas uma droga ou a associação de várias drogas vem sendo proposta, entretanto, até o momento não se pode concluir quanto sua eficácia (WROCLAWSKI et al., 2003, NELIUS et al., 2011).

O tratamento hormonal para o câncer de próstata metastático, ou privação androgênica do câncer avançado de próstata é um tratamento paliativo, que melhora a expectativa e a qualidade de vida, mas não cura. Além disso, há um percentual de pacientes que, eventualmente, tornam-se refratários à terapia hormonal (HEIDENREICH et al., 2011).

3.6.2.1. Acetato de Goserelina

A testosterona tem muitas funções complexas, uma delas é influenciar, através de produto derivado conhecido como dihidrotestosterona (DHT), o crescimento do tecido prostático, incluindo o câncer (FAGUNDES et al., 2002). A produção de testosterona no testículo é regulada pela hipófise, através da liberação do hormônio luteinizante (LH). A utilização de estrógenos inibe em nível hipotalâmico e hipofisário a liberação de LH e, conseqüentemente, reduz os valores séricos da testosterona a níveis de castração (TONON e SCHOFFEN, 2009). Além da inibição da produção de testosterona, os estrógenos em doses elevadas têm uma ação citotóxica direta sobre as células prostáticas (SROUGI e SIMON, 1996).

Os medicamentos denominados análogos do hormônio liberador do hormônio luteinizante (LHRH), representados pelo acetato de goserelina, impedem a formação de testosterona no testículo, travando ou retardando o crescimento do câncer de próstata (SBU, 2011). A terapia com análogo do LHRH consiste na administração de uma droga denominada agonista do receptor do LHRH; este conduzindo a uma queda no nível de testosterona. São administrados como injeção subcutânea mensal (depósito ou depot), para ser liberado no período de 28 dias.

A administração de um análogo do LHRH produz um resultado tão bom quanto a remoção dos testículos, porém não envolve cirurgia. Esta classe de drogas pode oferecer uma alternativa para o paciente que não pode, ou opta por não realizar uma orquiectomia ou receber a terapia estrogênica (SCHELLHAMMER et al., 1997).

3.6.2.2. Acetato de Ciproterona

Outro tipo de hormonoterapia utilizada no combate ao câncer de próstata é a administração de drogas denominadas antiandrogênicas (representados pelo acetato de ciproterona), que bloqueiam os receptores da testosterona ao nível da próstata, impedindo, assim, a ação do hormônio masculino. Portanto, não atuam através da suspensão da produção de testosterona, mas impedindo a interação da dihidrotestosterona (derivado da testosterona) com as células cancerosas. Segundo MOTTET et al. (2011), a terapia com antiandrogênicos resultou em uma taxa de sobrevivência pior quando comparada a terapia com goserelina. Os resultados obtidos com os antiandrogênicos tem se mostrado conflitantes, portanto, esses agentes não têm sido muito utilizados como monoterapia no tratamento do câncer de próstata (MOTTET et al., 2011). Os antiandrogênicos quando usados em monoterapia podem preservar a libido e a potência sexual, sendo estas, as razões mais importantes para o uso isolado destes agentes.

3.7. Dosagens bioquímicas

Várias dosagens bioquímicas (lactato desidrogenase, dosagens para avaliação de função hepática e renal, equilíbrio eletrolítico e albumina) têm sido propostas como importantes para o prognóstico do câncer de próstata e devem ser monitoradas e avaliadas em pacientes com esta patologia.

A insuficiência renal aguda pode ser uma das muitas complicações associadas à malignidade (GIVENS et al., 2010) e o acompanhamento dos níveis de uréia, ácido úrico e creatinina são importantes em pacientes com câncer. Da mesma forma, a função hepática pode ser alterada no câncer e a dosagem de testes de função hepática, como, aspartato aminotransferase (AST), alanina aminotransferase (ALT), fosfatase alcalina (FAL) e gama-glutamilttransferase (GGT) se faz necessária. Além disso, evidências mostram que a GGT pode ser considerada um indicador do risco de câncer (TARGHER et al., 2010) e a FAL sérica total reflete a atividade osteoblástica e a tendência é estar mais elevada em pacientes com volume maior ou doença óssea metastática agressiva (SONPAVDE et al., 2010).

Níveis elevados de lactato desidrogenase (LDH) foram relatados como um fator prognóstico negativo para a sobrevida no câncer de próstata (SMALETZ et al., 2002). Por outro lado, o desequilíbrio eletrolítico pode ser induzido em pacientes com câncer por vários fatores. Assim, é importante verificar os níveis de íons no soro destes pacientes. Finalmente, as associações entre as concentrações de albumina e mortalidade têm sido amplamente relatadas em pacientes com várias doenças, incluindo o câncer (LIS et al., 2003).

3.8. Estresse oxidativo

O estresse oxidativo é definido como um desequilíbrio entre a produção de radicais livres, também conhecidos como oxidantes ou espécies reativas de oxigênio (EROs) e a sua eliminação por meio de mecanismos de proteção, definidos como antioxidantes (REUTER et al., 2010). Este desequilíbrio leva a danos às biomoléculas importantes para a célula, com potencial impacto sobre o organismo (Figura 4) (DURACKOVA, 2010). As EROs são produtos do metabolismo celular normal e desempenham papel importante na estimulação das vias de sinalização nas células, em resposta a mudanças intra e extracelulares e a condições ambientais. A maioria das EROs são geradas nas células nas reações endógenas da cadeia respiratória mitocondrial (POYTON et al., 2009). EROs como, por exemplo, o ânion superóxido (O_2^-), peróxido de hidrogênio (H_2O_2), radical hidroxila (OH^*) e peróxidos orgânicos são produtos da redução das moléculas de oxigênio na cadeia respiratória mitocondrial. As proteínas e os lipídios são alvos significativos para o dano oxidativo e modificações nestas moléculas podem aumentar o risco de mutagênese (SCHRAUFSTATTER et al., 1988).

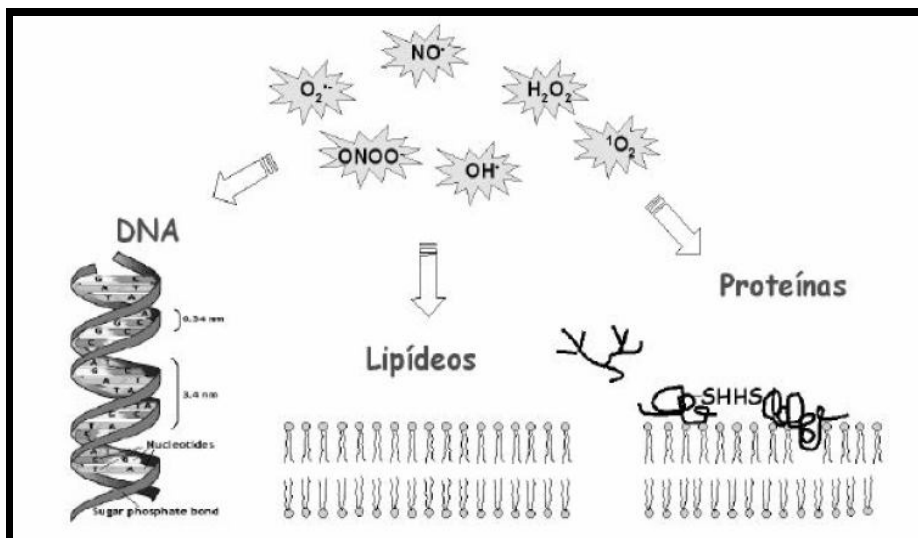


Figura 4 - Dano oxidativo a macromoléculas biológicas. Adaptado de TORRES, 2003.

3.8.1. Indicadores de dano oxidativo

3.8.1.1. Lipoperoxidação

Os ácidos graxos poliinsaturados, contendo duas ou mais duplas ligações, são facilmente oxidados pelas EROs formando radicais peróxil lipídicos e hidroperóxidos lipídicos, um processo chamado de peroxidação lipídica. Uma vez que o processo de peroxidação lipídica é iniciado, ele prossegue como uma reação em cadeia mediada pelos radicais livres envolvendo as etapas de iniciação, propagação e terminação (GAGO-DOMINGUEZ et al., 2005).

Um dos mais conhecidos produtos da lipoperoxidação é o malondialdeído (MDA), o qual é o produto final da degradação não enzimática de ácidos graxos poliinsaturados (VALKO et al., 2006; VALKO et al., 2007). Altos níveis de MDA elevam a formação de lipoperóxidos e indicam um aumento da lipoperoxidação (KASHYAP et al., 2005).

3.8.1.2. Oxidação Protéica

As proteínas são moléculas biológicas alvos para a modificação oxidativa e danos causados pelas EROs. A exposição das proteínas ao ataque dos radicais

livres resulta em múltiplas modificações nestas moléculas. As modificações nas proteínas incluem a oxidação dos grupos das cadeias laterais de aminoácidos, fragmentação, modificações na hidrofobicidade e na conformação e formação de novos grupos reativos (como os grupos carbonil). Estes processos podem resultar na perda da estrutura ou atividade enzimática das proteínas (HAWKINS e DAVIES, 2001). Portanto, os danos oxidativo às proteínas provocados pelas EROs podem ter consequências biológicas significativas (KLAUNIG et al., 2011). A carbonilação protéica parece ser um fenômeno comum durante a oxidação, e sua quantificação pode ser usada para medir a extensão do dano oxidativo (DONNE et al., 2003), já que é um marcador estável de oxidação protéica.

3.8.2. Defesas Antioxidantes

O sistema de defesa antioxidante tem a função de inibir e/ou reduzir os danos causados pela ação deletéria dos radicais livres ou das espécies reativas não-radicaais (BARBOSA et al., 2010). Tais ações podem ser alcançadas por meio de diferentes mecanismos de ação: impedindo a formação dos radicais livres ou espécies não-radicaais (sistemas de prevenção), impedindo a ação dessas espécies (sistemas varredores) ou, ainda, favorecendo o reparo e a reconstituição das estruturas biológicas lesadas (sistemas de reparo) (CLARKSON e THOMPSON, 2000). Usualmente, esse sistema é dividido em enzimático e não-enzimático.

3.8.2.1. Antioxidantes Enzimáticos

O sistema de defesa enzimático inclui as enzimas Superóxido Dismutase (SOD), Catalase (CAT) e Glutathione Peroxidase (GPx). As enzimas que atuam na detoxificação das espécies reativas agem em um mecanismo de elevada sincronia e atuam de forma altamente cooperativa. A SOD catalisa a dismutação do ânion radical superóxido ($O_2^{\cdot-}$) a peróxido de hidrogênio (H_2O_2) e O_2 , a CAT atua na decomposição de H_2O_2 a O_2 e H_2O e a GPx atua sobre peróxidos em geral, com utilização de glutathione como co-fator (Figura 5) (LEOPOLD e LOSCALZO, 2009).

Um dos mais eficazes antioxidantes enzimáticos intracelular é a enzima superóxido dismutase (EC 1.15.1.1). Embora esta enzima tenha sido isolada em 1939, foi somente em 1969 que MCCORD E FRIDOVICH comprovaram a sua atividade antioxidante. A SOD existe em várias isoformas, em humanos, existem três formas: citosólica (Cu, Zn-SOD), mitocondrial (Mn-SOD) e extracelular (SOD-CE).

Outro antioxidante enzimático importante é a CAT que está localizada em uma organela celular chamada de peroxissomo. A enzima promove de forma muito eficiente a conversão de peróxido de hidrogênio à água e oxigênio molecular. A capacidade da CAT de degradar o peróxido de hidrogênio se mostrou diminuída em uma variedade de tumores (VALKO et al., 2006).

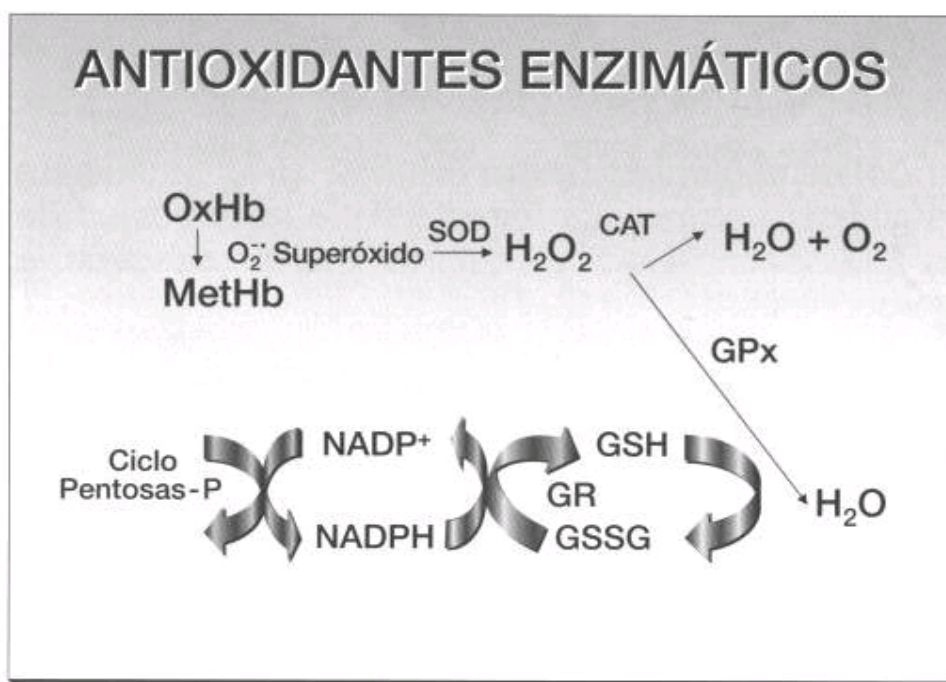


Figura 5 - Antioxidantes enzimáticos. Adaptado de <http://www.institutobiologico.com/seminarios/stop%20al%20envejecimiento.htm>.

3.8.2.2. Antioxidantes não – enzimáticos

Além das defesas antioxidantes enzimáticas, possuem grande relevância os antioxidantes não enzimáticos. O organismo possui a capacidade de produzir compostos que apresentam grande capacidade de defesa antioxidante, direta ou

indireta, atuando a fim de manter o estado de equilíbrio celular (HALLIWELL e GUTTERIDGE, 2000). Além disso, alguns compostos ingeridos na alimentação também atuam como antioxidantes. Entre os antioxidantes não enzimáticos podem-se citar as vitaminas, compostos fenólicos e os compostos de baixo peso molecular, como os tióis não protéicos.

A glutatona reduzida (GSH) está presente na maioria das células, é o tiol mais abundante no meio intracelular e sua capacidade redutora é determinada pelo grupamento –SH presente na cisteína. A GSH, que constitui um importante sistema de proteção endógena, é um tripeptídeo formado a partir dos resíduos dos aminoácidos glicina, glutamato e cisteína (RUBER e ALMEIDA, 2008).

A vitamina C ou ácido ascórbico é uma vitamina hidrossolúvel e antioxidante que reage diretamente com o oxigênio simples, radical hidroxila e radical superóxido. Além disso, coopera com a vitamina E na medida em que a regenera. A vitamina C tem papel importante na manutenção do tecido normal epitelial e na regeneração da epiderme, além de ter a função de evitar a formação de carcinógenos a partir de compostos precursores, podendo inibir a carcinogênese pelos seguintes mecanismos: alteração da estrutura do carcinógeno, inibição competitiva e prevenção de acesso do carcinógeno ao tecido-alvo por estabilidade crescente da membrana (ANTHONY e SCHORAH, 1992). Tem sido constatado em pesquisas que vários grupos de pacientes com câncer têm deficiência de vitamina C (SAMPAIO e ALMEIDA, 2009).

A família de vitaminas lipossolúveis E é composta pelos tocoferóis (alfa, beta, gama, os deltatoferóis) e os tocotrienóis, todos com provável atividade antioxidante. Essa função exercida por meio da inibição da peroxidação lipídica protege a integridade das membranas biológicas. A vitamina E é outro antioxidante de grande importância, e sua forma mais ativa biologicamente é o alfa-tocoferol. Sua função como antioxidante é proteger os tecidos adiposos do ataque de radicais livres, como por exemplo, a formação de radicais peróxidos a partir de ácidos graxos poliinsaturados nas membranas fosfolipídicas. Cabe ressaltar que a vitamina E tem sido citada como agente de prevenção para o câncer de próstata (BARDIA et al., 2009).

3.8.3. Estresse oxidativo *versus* câncer de próstata

O câncer de próstata possui uma etiologia complexa, sendo que fatores genéticos e ambientais contribuem para o aumento do risco de desenvolvimento da doença (HSING e CHOKKALINGAM, 2006; MINELLI et al., 2009). Entre os fatores ambientais, o estresse oxidativo tem recebido atenção como um fator que contribui para a patogênese do câncer de próstata, principalmente nos fenótipos de doença mais agressiva (KHANDRIKA et al., 2009)

As células prostáticas devem manter um equilíbrio entre a geração de EROs e as defesas antioxidantes. Um aumento na produção de EROs ou uma diminuição na capacidade antioxidante pode perturbar este equilíbrio e atuar na promoção da carcinogênese prostática (VALKO et al., 2006; KHANDRIKA et al., 2009).

Estudos revelam a associação inversa entre a ingestão de alimentos ricos em antioxidantes, como vitamina C, E, licopeno, entre outros e o risco de desenvolver o câncer de próstata (KIRSH et al., 2006). Portanto, fatores ambientais como, por exemplo, a dieta, inflamação, envelhecimento e desequilíbrio redox são possíveis mecanismos que contribuem para o aumento da geração de EROs. Este aumento de EROs pode estimular a proliferação celular, causar mutações somáticas no DNA e promover a instabilidade genética, senescência e, além disso, em células de câncer pode causar aumento da angiogênese e motilidade (KHANDRIKA et al., 2009).

3.9. Plaquetas

3.9.1. Estrutura

As plaquetas são fragmentos citoplasmáticos envolvidos por membranas e destituídos de núcleo (Figura 6), estão presentes no sangue e são produzidos a partir de megacariócitos na medula óssea (CASTRO et al., 2006). Medem de 1 a 3 μm de diâmetro e contém finos grânulos dispersos no citoplasma, ou concentrados no centro (BAIN, 2007). Morfologicamente, considerando o tamanho, densidade e coloração, apresentam marcada heterogeneidade (EYRE e GAMLIN, 2010).

As plaquetas possuem uma estrutura discóide complexa, apesar de parecerem ter aparência simples no esfregaço de sangue periférico (ANDREWS e BERNDT, 2004). Possuem estrutura interna dividida em quatro zonas: A zona periférica que inclui as membranas externa e interna e é onde se encontram os fosfolípidios de membrana; a zona sol-gel que é composta por citoesqueleto e pelo sistema contrátil; a zona de organelas que consiste de grânulos alfa, grânulos densos e componentes celulares e o sistema membranar que inclui o sistema tubular denso onde se concentra o cálcio (HARTWIG, 2002).

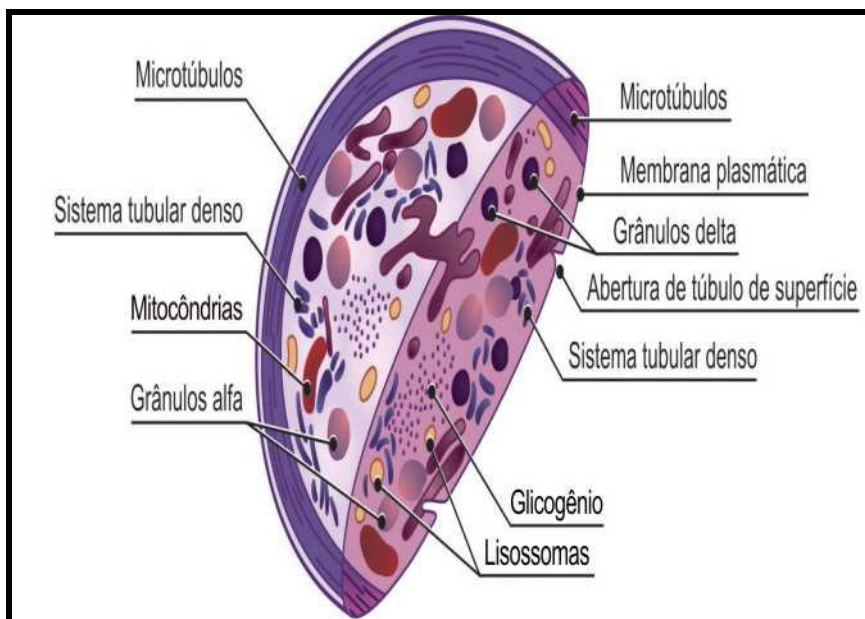


Figura 6 - Representação de uma célula plaquetária. Adaptado de LORENZI et al., 2003

3.9.2. Hemostasia e Agregação Plaquetária

A hemostasia é um processo fisiológico que tem como objetivo manter o sangue em estado fluido dentro dos vasos sanguíneos, sem que haja hemorragia ou trombose. A principal função das plaquetas é manter a hemostasia, na qual elas desempenham atividade mecânica e bioquímica.

Para que ocorra a coagulação, é fundamental que haja ativação plaquetária, que acontece como resposta ao dano vascular e/ou estímulo químico (pela trombina,

ADP, tromboxano A2 ou epinefrina). A adesão plaquetária resulta da ligação do complexo de superfície glicoproteína (Gp) Ib/Gp IX (GpIb/IX) (presente nas plaquetas) ao fator von Willebrand (FVW), presente no subendotélio vascular. O passo seguinte, a agregação plaquetária, é resultante da liberação de grânulos pelas plaquetas, tais como ADP e tromboxano A2, que ativam e recrutam outras plaquetas para o sítio de lesão. As plaquetas ativadas expressam, em sua superfície, o complexo plaquetário IIb/IIIa (GPIIb/IIIa), que se liga ao fibrinogênio circulante, mediando a formação do tampão plaquetário (MANN, 1999). A adesão e a ativação plaquetária são seguidas pela agregação plaquetária, levando à formação de um trombo rico em fibrinogênio no local da lesão. A agregação plaquetária é um complexo e dinâmico processo que envolve muitos ligantes (tais como fibrinogênio, fibronectina e FVW), receptores (como GPIIb α e aIIb β 3) e plaquetas em diferentes estados de ativação (BROOS et al., 2011).

3.9.3. Agregação plaquetária e o câncer

Desordens tromboembólicas são complicações bem conhecidas nos pacientes com câncer (CAMPELLO et al., 2011). Armand Trousseau (1801-1867) foi o primeiro a mostrar a relação entre câncer e trombose, através da observação do aparecimento de trombose venosa em pacientes com suspeita de neoplasia. No entanto, devido à deficiência de métodos diagnósticos eficazes na época, essa observação não pôde ser comprovada.

Segundo SABRKHANY (2011) além de estarem envolvidas no processo de hemostasia, as plaquetas também desempenham importante papel em doenças malignas. Não é incomum os pacientes com câncer desenvolverem alterações de coagulação, nas quais o funcionamento das plaquetas pode estar alterado, sugerindo o envolvimento das plaquetas no desenvolvimento e no crescimento do tumor. A angiogênese, que é o processo de crescimento de novos vasos sanguíneos a partir de vasos pré-existentes, é imprescindível para o crescimento do tumor. As plaquetas contêm várias moléculas pró e antiangiogênicas, além disso, as células do tumor são fenotipicamente e funcionalmente diferentes das células endoteliais do tecido saudável, estimulando a adesão plaquetária local e a subsequente ativação. TESSELAAR e colaboradores (2007) mostram que pacientes com câncer possuem

risco aumentado de eventos tromboembólicos, tais como: tromboembolismo venoso, incluindo trombose venosa profunda e embolia pulmonar.

3.10. Nucleosídeos e nucleotídeos de adenina

Os nucleosídeos são moléculas resultantes da união de uma base púrica ou pirimídica a um açúcar pentose. Exemplos de nucleosídeos são a citidina, uridina, adenosina, timidina, guanosina e inosina (ATKINSON et al., 2006). Os nucleosídeos são fosforilados por quinases específicas na célula, produzindo os nucleotídeos. Os nucleotídeos extracelulares de adenina, adenosina trifosfato (ATP), adenosina difosfato (ADP), adenosina monofosfato (AMP) e o nucleosídeo adenosina, modulam uma variedade de efeitos teciduais, que incluem o fluxo sanguíneo, a inflamação, a secreção, o tônus vascular e a função plaquetária (BURNSTOCK, 2002). Além disso, desempenham papéis importantes como moléculas carregadoras de energia celular e como segundo mensageiros intracelulares.

Os nucleosídeos e nucleotídeos exercem um papel de moléculas sinalizadoras extracelulares em vários tecidos, através dos receptores purinérgicos (BURNSTOCK e KNIGHT, 2004). A hipótese de um "sistema purinérgico de sinalização", usando nucleotídeos e nucleosídeos extracelulares como mediadores, foi pela primeira vez desenvolvido em estudos da neurotransmissão por BURNSTOCK e KNIGHT (2004). Os nucleotídeos são liberados no meio extracelular através de mecanismos como, por exemplo, a lise celular que ocorre frente a injúria celular. Também podem ser liberados por vesículas tais como os grânulos densos das plaquetas, que liberam o ATP e o ADP. A molécula de adenosina também pode ser liberada no meio extracelular como resultado da degradação do ATP e do ADP por enzimas específicas (YEGUTKIN, 2008).

A adenosina é produzida principalmente pela desfosforilação do AMP catalisada pela 5'-nucleotidase. Vários estudos sugerem a participação da adenosina no desenvolvimento do câncer (SPYCHALA, 2000). Devido ao seu rápido crescimento, os tumores sólidos rotineiramente, estão em um ambiente de hipóxia e necrose, o que leva a degradação de nucleotídeos da adenina e a liberação de adenosina (SPICHALA, 2000). A adenosina liberada pode exercer uma ampla gama de efeitos, como por exemplo, proteção contra a isquemia, a estimulação do

crescimento e da angiogênese e a supressão de respostas imunes, proporcionando o ambiente favorável para o acelerado crescimento do tumor.

3.11. Receptores purinérgicos

Os nucleotídeos, uma vez presentes no meio extracelular, desempenham suas ações pela ligação a tipos específicos de receptores localizados na superfície de vários tipos de células (YEGUTKIN, 2008). Os nucleotídeos e nucleosídeos da adenina, que são moléculas sinalizadoras, medeiam seus efeitos por interações com diferentes receptores de superfície celular denominados purinoreceptores (Figura 7). Existem duas principais famílias de purinoreceptores, os receptores de adenosina ou do tipo P1, e os receptores do tipo P2, que reconhecem principalmente o ATP e o UTP e seus análogos difosfonados. Os receptores P1 respondem principalmente à adenosina e são divididos em quatro subtipos A1, A2a, A2b, A3, todos acoplados à proteína G, e classificados desta forma com base em evidências moleculares, bioquímicas e farmacológicas.

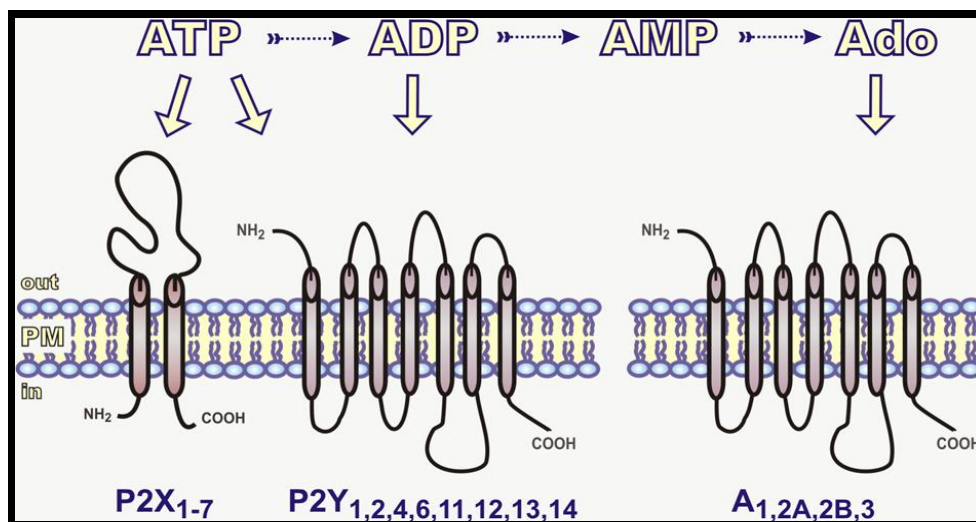


Figura 7: Vias de sinalização purinérgica – receptores. Adaptado de YEGUTKIN, 2008

3.12. Enzimas que degradam nucleotídeos e nucleosídeos

Os eventos induzidos pelos nucleotídeos extracelulares são regulados pela ação das ectonucleotidases, que são importantes moduladores da transmissão da sinalização purinérgica. As ectonucleotidases são enzimas que atuam em conjunto, formando uma cadeia enzimática e hidrolisando os nucleotídeos extracelulares aos seus respectivos nucleosídeos (SCHETINGER et al., 2007). Na última década, várias ectonucleotidases foram descobertas e caracterizadas (ROBSON et al., 2006). Dentre estas enzimas podem-se destacar as ENTPDases (Ecto-Nucleosídeo Trifosfato Difosfohidrolase), a família das E-NPPs (Ecto-Nucleotídeo Pirofosfatase/Fosfodiesterases), 5'-nucleotidase e a adenosina deaminase (ADA) (Figura 8) (ROBSON et al., 2006; YEGUTKIN, 2008). A cadeia enzimática se inicia com a ação da E-NTPDase e da E-NPP, as quais catalisam a hidrólise do ATP e do ADP formando AMP (ZIMMERMANN, 2000). A seguir a enzima 5'-nucleotidase hidrolisa a molécula de AMP formando adenosina, a qual posteriormente é degradada pela ação da ADA gerando inosina (YEGUTKIN, 2008).

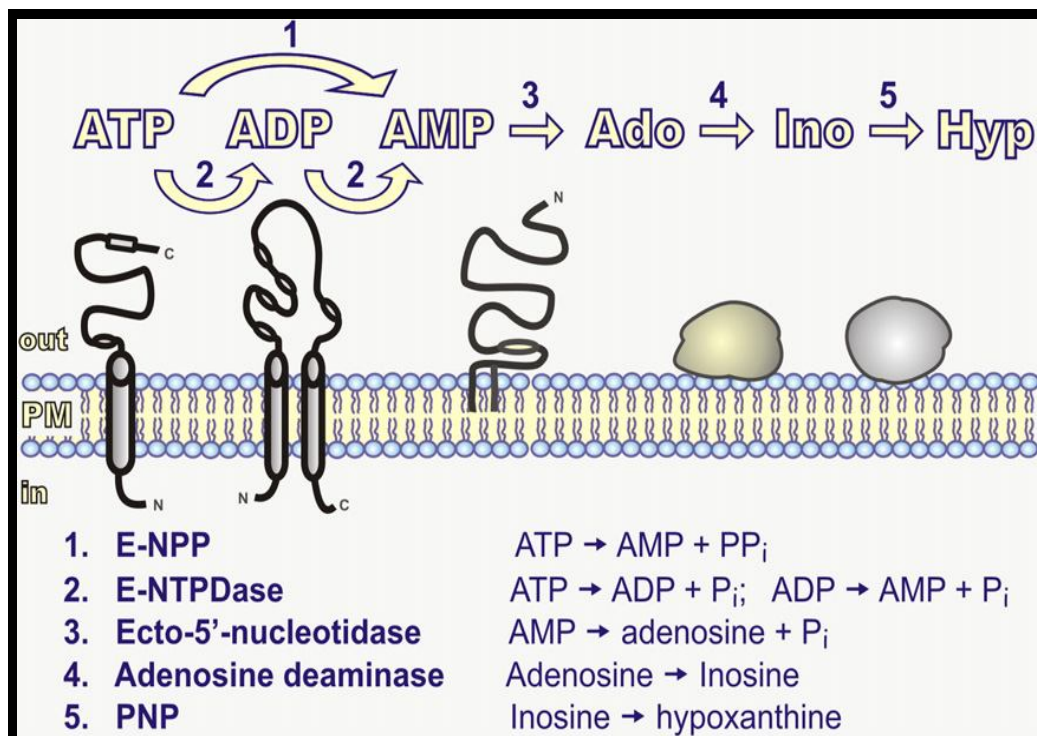


Figura 8 - Enzimas envolvidas na degradação extracelular de nucleotídeos e nucleosídeos da adenina. Adaptado de Yegutkin, 2008.

3.12.1. NTPDase

As NTPDases são um grupo de enzimas glicosiladas extracelulares que catalisam a hidrólise do fosfato terminal dos nucleosídeos trifosfato (por exemplo, ATP e UTP) e difosfatos (ADP e UDP). As NTPDases precisam de um meio com pH alcalino e que contenha concentrações milimolares de Ca^{2+} e Mg^{2+} , na ausência destes cátions as NTPDases não apresentam atividade.

Ao menos oito diferentes membros da família de E-NTPDases, com propriedades enzimáticas distintas, foram descobertos e estudados nos últimos anos (Figura 9) (ROBSON et al., 2006; SCHETINGER et al., 2007). As NTPDases 1, 2, 3 e 8 estão ligadas a membrana plasmática e possuem um domínio extracelular (Figura 10) (ELIANHU et al., 2010). A NTPDase 1 (CD39/ATPDase/ectoapyrase /ecto-ADPase) hidrolisa ATP e ADP igualmente bem e na proporção de 1/1, enquanto a NTPDase 2 (ecto-ATPase/CD39L1) hidrolisa preferencialmente o ATP. Ambas NTPDase 3 (CD39L3/HB6) e NTPDase 8 são intermediárias e funcionais entre a NTPDase 1 e a NTPDase 2 mostrando preferência pelo ATP sobre o ADP (KUKULSKI et al., 2005). As NTPDases 4-7 estão associadas principalmente com organelas intracelulares. O AMP, produto da atividade da NTPDase, é hidrolisado pela ecto-5'-nucleotidase (CD73), liberando a adenosina.

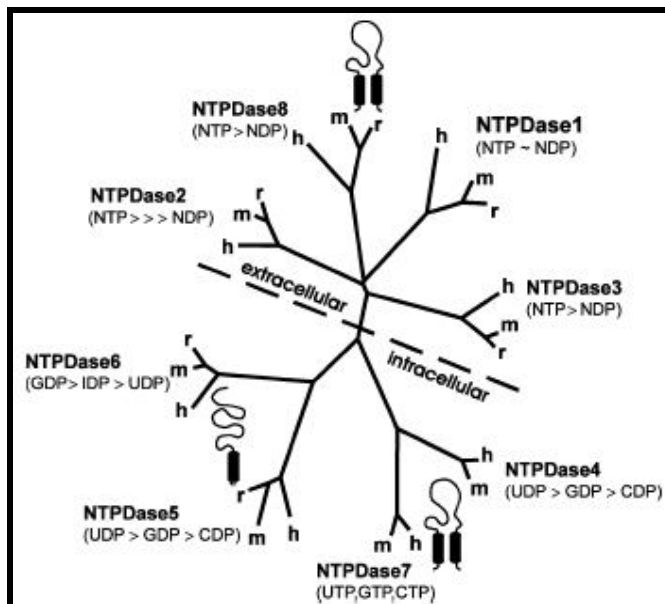


Figura 9 - Membros da Família das NTPDases. Adaptado de ROBSON et al., 2006

As NTPDases estão envolvidas em uma série de processos fisiológicos e patológicos em uma multiplicidade de tecidos. De um modo geral, as ectonucleotidases limitam o efeito de ATP nos receptores P2. Além disso, possuem grande importância na síntese de adenosina. A adenosina produzida pela 5'-nucleotidase pode atuar em quatro tipos diferentes de receptores P1 (A1, A2A, A2B e A3).

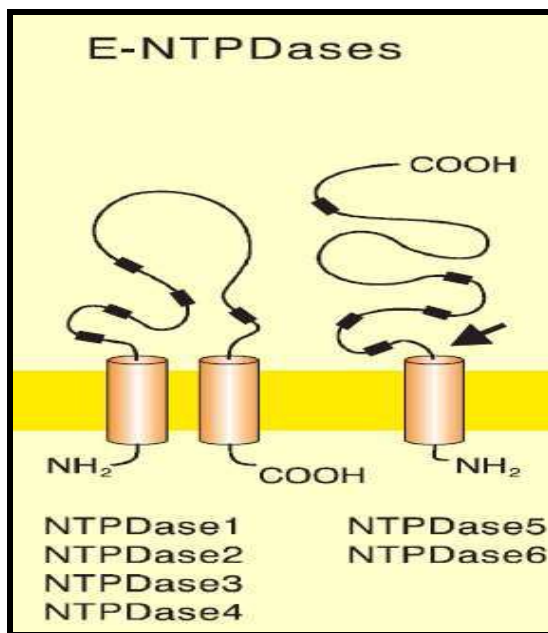


Figura 10 - Topografia de membrana de ectonucleotidases. Adaptado de ZIMMERMANN, 2001.

3.12.2. Ecto-Nucleotídeo Pirofosfatase/Fosfodiesterase (E-NPP)

A família de E-NPP é composta por sete enzimas estruturalmente relacionadas que estão localizadas na superfície das células e são expressas como proteínas transmembranas ou enzimas secretoras (BUFFON et al., 2010). Estas enzimas estão envolvidas numa grande variedade de atividades biológicas, que incluem: a formação dos ossos, a motilidade celular, as metástases tumorais e a resistência à insulina em diabetes do tipo II (GODING et al., 2003; STEFAN et al., 2006). Estas proteínas hidrolisam ligações pirofosfato ou fosfodiéster das moléculas, tais como nucleotídeos e dinucleotídeos (NPP1-3), fosfolipídios e nucleotídeos (NPP2), e os ésteres de colina (NPP6 e NPP7). Relata-se também a existência das

NPPs 4 e 5, porém pouco se sabe sobre sua atividade catalítica (GODING et al., 2003). As E-NPP hidrolisam uma ampla gama de substratos, tais como UDP-galactose, + NAD, AMPc, ATP e ADP (STEFAN et al., 2005, 2006).

Devido ao fato de seu sítio catalítico ser voltado para o meio extracelular essa família de enzimas é denominada ecto-NPPs (E-NPPs) e funcionam, *in vitro*, em pH alcalino entre 8.5-8.9 (GODING, 2000). A porção extracelular contém ainda um resíduo de treonina que é transitoriamente adenilado e/ou fosforilado durante o mecanismo catalítico (MALDONADO, 2008).

A catálise realizada pelas E-NPP afeta processos importantes, tais como a proliferação celular, motilidade celular e angiogênese (STEFAN et al. 2005). Evidências apontam o envolvimento da NPP2 na progressão do câncer. A NPP2 é secretada por células tumorais de vários tipos, incluindo pele, pulmões e as células de câncer de mama. Além disso, em reação acoplada com a 5'-nucleotidase, a NPP2 tem o potencial de hidrolisar ATP a adenosina, que é um importante fator de crescimento tumoral (STEFAN et al. 2006).

3.12.3. Ecto 5'- nucleotidase

A enzima ecto-5'-nucleotidase (E.C. 3.1.3.5, Ecto-5'-NT) desfosforila nucleótidos monofosfatados não cíclicos (ZIMERMANN, 2000), através da hidrólise da ligação fosfodiéster de 5'-ribonucleótidos, levando à formação do correspondente ribonucleosídeo e fosfato. A principal função em animais é a hidrólise de AMP até adenosina (ZIMMERMANN, 2000). Esta atividade enzimática é dependente de cátions divalentes, como cálcio e magnésio. A ecto-5'-nucleotidase é uma enzima ancorada à membrana plasmática por glicosil-fosfatidil inositol (GPI), sendo que formas solúveis da enzima podem ser originadas mediante a ação de uma fosfolipase específica (ZIMMERMANN et al., 1993).

Vários tipos desta enzima têm sido descritos, ao todo são sete subtipos isolados e caracterizados em humanos, com localização subcelular variada, sendo 5 delas localizadas no citosol, uma na matriz mitocondrial e uma anexada a membrana plasmática externa. Enquanto essas enzimas compartilham a habilidade em hidrolisar 5'- nucleosídeos monofosfatos e se sobrepõem na especificidade ao

substrato, elas variam na afinidade por 5'- monofosfatos (HUNSUCKER et al., 2005). A Ecto 5'-NT produz a adenosina extracelularmente e é uma enzima presente e expressa em muitos tecidos e em diferentes níveis, variando muito a sua atividade e concentração em fibroblastos e em alguns carcinomas.

3.12.4. Adenosina Deaminase (ADA)

A ADA é uma importante enzima que catalisa a desaminação irreversível da adenosina e 2'-desoxiadenosina em inosina e 2'-desoxinosina respectivamente (Figura 11). É uma enzima amplamente expressa no intestino, timo, baço e outros tecidos linfóides e não linfóides (SPYCHALA, 2000) possuindo funções importantes na imunidade celular. Em humanos a ADA existe na forma de duas isoenzimas classificadas como: ADA1 e ADA2 (SHAROYAN et al., 2006). A ADA1 é uma proteína monômera encontrada em todo o organismo, que está localizada no citosol e possui massa molecular de aproximadamente 40 kDa. A ADA1 pode ser expressa como uma ectoenzima na superfície de linfócitos (FRANCO et al., 1998) e células dendríticas (DESROSIERS et al., 2007).

Por outro lado, a ADA2 apresenta diferenças tanto estruturais quanto cinéticas. Possui massa molecular de aproximadamente 100 kDa e representa a menor parte da atividade da ADA em tecidos, sendo abundante no plasma. Além disso, a ADA2 tem menor afinidade para a adenosina e menor atividade catalítica com a deoxiadenosina que a ADA1 (IWAKI-EGAWA et al., 2006).

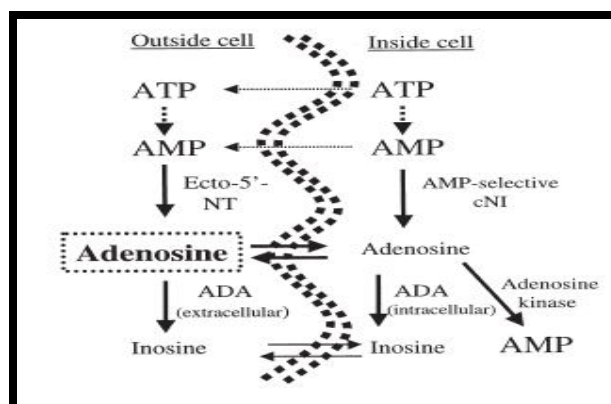


Figura 11 - Catabolismo dos nucleotídeos da adenina e geração de adenosina. Adaptado de SPYCHALA, 2000.

A diminuição na atividade da ADA foi relacionada com vários distúrbios imunológicos e imunoproliferativos (HERSHFIELD e MITCHELL, 1995). A ADA tem sido considerada um marcador de malignidade e a diminuição da sua atividade tem sido observada em vários carcinomas, incluindo o câncer de cólon (BALIS, 1985).

3.13. Enzimas que degradam ésteres de colina

3.13.1. Acetilcolina (ACh)

A acetilcolina (ACh) (Figura 12) é um mediador químico de sinapses no sistema nervoso central (SNC), no sistema nervoso periférico (SNP) e também na junção neuromuscular. A ACh, seus receptores e as enzimas responsáveis por sua síntese e degradação constituem o sistema de neurotransmissão colinérgica. A ACh foi descoberta por Henry Dale em 1914 e a sua função como neurotransmissora foi confirmada em 1921 por Otto Loewi. O ciclo vital de síntese, armazenamento e liberação da ACh é similar em todas as sinapses colinérgicas e a ACh desempenha um papel fundamental na sinalização neuronal tanto no SNC quanto no SNP (WESSLER et al., 1998).

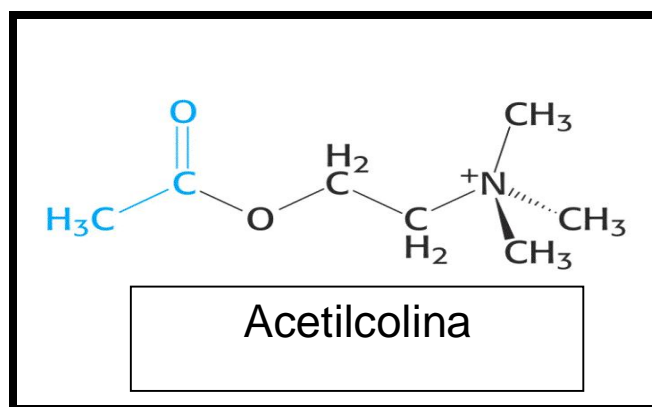


Figura 12 - Estrutura química do neurotransmissor acetilcolina. Adaptado de http://www.unizar.es/departamentos/bioquimica_biologia/docencia/Biofvirtual/Tema-ImN/ImpNerv.htm

A colina acetiltransferase catalisa o estágio final da síntese de ACh a partir da colina, um importante produto do metabolismo dos lipídios da dieta, e acetil-CoA, um produto do metabolismo celular (SOREQ e SEIDMAN, 2001). Após sua síntese a

ACh é captada por vesículas de armazenamento, principalmente nas terminações nervosas onde fica armazenada até a sua liberação (RAND, 2007).

Depois de ser liberada, a ACh se difunde na fenda sináptica e ativa receptores específicos, posicionados nas células pós sinápticas. Com base nos subtipos de receptores colinérgicos capazes de se ligar à nicotina e à muscarina e que respondem à ativação colinérgica com alta afinidade, as ações da ACh foram classificadas em muscarínicas e nicotínicas (DALE, 1914). A ação da acetilcolina cessa quando é hidrolisada em acetato e colina pela enzima acetilcolinesterase (AChE), presente na fenda sináptica (Figura 13). A colina liberada é, em parte, recaptada para o terminal pré-sináptico, através de um mecanismo de recaptação de alta afinidade (SOREQ e SEIDMAN, 2001) onde poderá ser reutilizada para a síntese de novas moléculas de ACh.

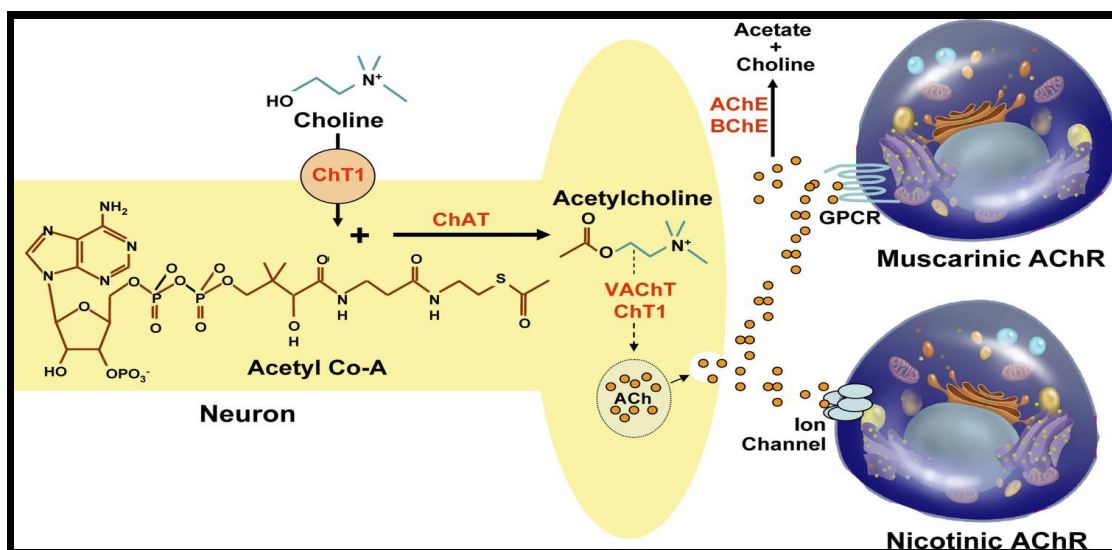


Figura 13 - Produção, liberação, interação com os receptores e degradação da Ach. Adaptado de SHAH et al., 2009

3.13.2. Colinesterases

São descritos dois tipos de colinesterases que incluem: a) enzimas com alta afinidade para a ACh ligadas à membrana neuronal e presentes em todas as sinapses colinérgicas, a acetilcolinesterase (AChE); b) enzima com alta afinidade para a butirilcolina, também chamada de butirilcolinesterase (BuChE) sintetizada principalmente no fígado e sendo também encontrada no plasma, músculo liso,

pâncreas, adipócitos, pele, substância branca do encéfalo e coração (WESCOE et al., 1947).

A AChE e a BuChE são tipicamente diferenciadas pelas suas taxas relativas de hidrólise de ACh e de butirilcolina e pelo efeito distinto de inibidores seletivos (GOODMAN e GILMAN, 2006). Genes diferentes e únicos codificam a AChE e a BuChE em mamíferos, porém, a sequência de aminoácidos da BuChE e da AChE tem 53% de identidade, sendo bem conservada no sítio ativo.

3.13.2.1. Acetilcolinesterase (AChE)

A função da acetilcolinesterase (AChE) consiste na interrupção da ação da ACh nas junções de várias terminações nervosas colinérgicas com seus órgãos efetores ou locais pós-sinápticos (GOODMANN e GILMANN, 2006). Com relação a sua estrutura, a AChE ocorre como duas classes gerais de formas moleculares: oligômeros homoméricos simples de subunidades catalíticas (monômeros, dímeros, tetrâmeros) e associações heteroméricas de subunidades catalíticas, com subunidades estruturais (Figura 14) (MASSOULIÉ et al., 1993). As formas homoméricas são encontradas solúveis na célula ou associadas à membrana externa da célula através de um glicofosfolípídio fixado. As formas homoméricas incluem a forma globular monomérica (G1), dimérica (G2) e tetrâmera (G4) que diferem uma da outra quanto às suas características e quanto ao grau de hidrofobicidade, consequentes à adição pós-transducional de um glicofosfolípídeo no aminoácido carboxi-terminal (TAYLOR e BROWN, 1999).

As formas heteroméricas, por sua vez, consistem em uma montagem heteromérica das subunidades estrutural e catalítica, onde a ligação através de pontes dissulfeto de uma molécula tríplice helicoidal de colágeno a um, dois ou três tetrâmeros catalíticos resulta nas formas estruturais assimétricas A4, A8 e A12 (MASSOULIÉ et al., 1993).

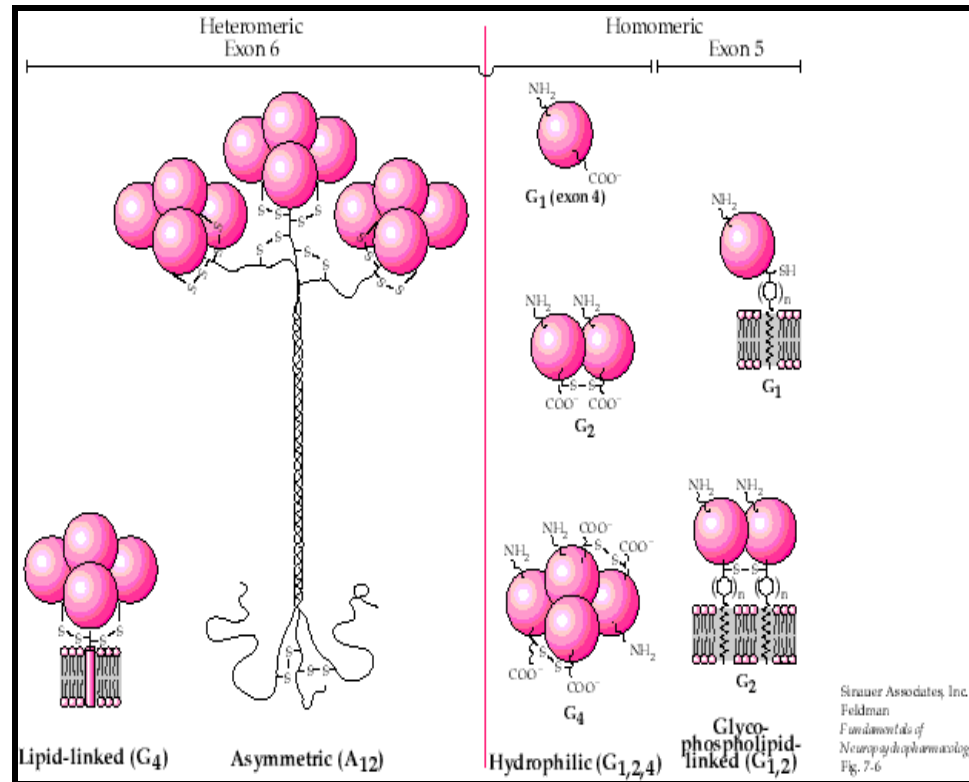


Figura 14 - Isoformas da enzima AChE. Adaptado de http://www.chemistry.emory.edu/justice/test/ach_inactivation.htm

As estruturas tridimensionais da AChE mostram que o centro ativo da enzima é quase centrossimétrico a cada subunidade, residindo na base de uma garganta estreita de cerca de 20 Å de profundidade (SUSSMAN et al., 1991). Na base da garganta situam-se os resíduos da tríade catalítica: serina 200, histidina 440 e glutamato 327 (Figura 15). A AChE é classificada como uma serina hidrolase e o mecanismo catalítico assemelha-se ao de outras hidrolases.

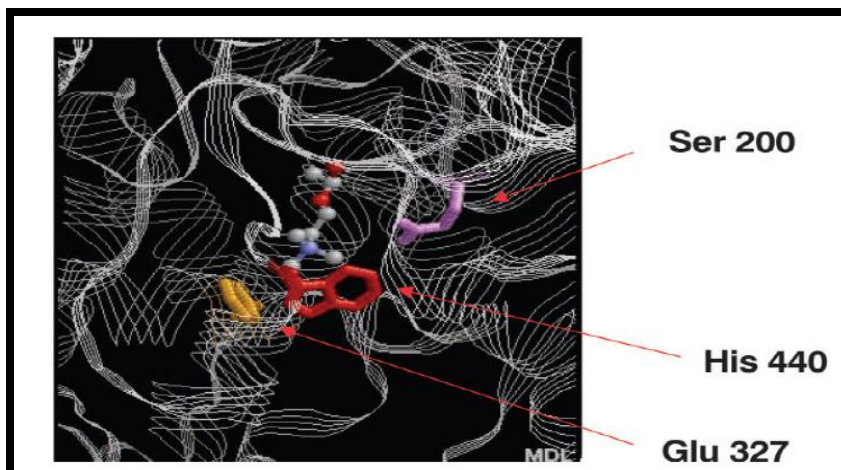


Figura 15 - Visão do sítio ativo da AChE e dos resíduos de aminoácidos que constituem a tríade catalítica. Adaptado de VIEGAS JÚNIOR et al., 2004.

Durante o ataque enzimático da ACh, forma-se um éster com geometria trigonal, um intermediário tetraédrico entre a enzima e o substrato, que sofre colapso em um conjugado acetilenzima, com liberação concomitante da colina. A acetilenzima é muito lábil à hidrólise, resultando na formação de acetato e enzima ativa (ROSENBERRY, 1975).

A AChE é uma das enzimas mais eficientes conhecidas, já que uma molécula de AChE tem a capacidade de hidrolisar 6×10^5 moléculas de ACh por minuto. Já se propôs que a AChE possui múltiplas funções biológicas não-clássicas, que em muitos casos, ainda são controversas (SOREQ E SEIDMAN, 2001). Essas funções não-clássicas incluem a adesão celular, a sinaptogênese, a montagem de fibras amilóides, a ativação de receptores, hematopoiese e a trombopoiese (GOOMAN e GILMAN, 2006).

3.13.2.2. Butirilcolinesterase (BuChE)

No plasma, a BuChE ocorre predominantemente como um tetrâmero, formado por dímero de dímeros (Figura 16). Na formação de cada dímero, os monômeros estão ligados por pontes de dissulfeto e, ao formar o tetrâmero, os dímeros ligam-se entre si por ligações não covalentes. Outras formas moleculares, como monômeros e dímeros, também estão presentes no plasma em menores proporções. Os

monômeros são constituídos de 574 aminoácidos e nove cadeias de carboidratos, com peso molecular aproximado de 85 kDa (LOCKRIDGE et al., 1987 a, b).

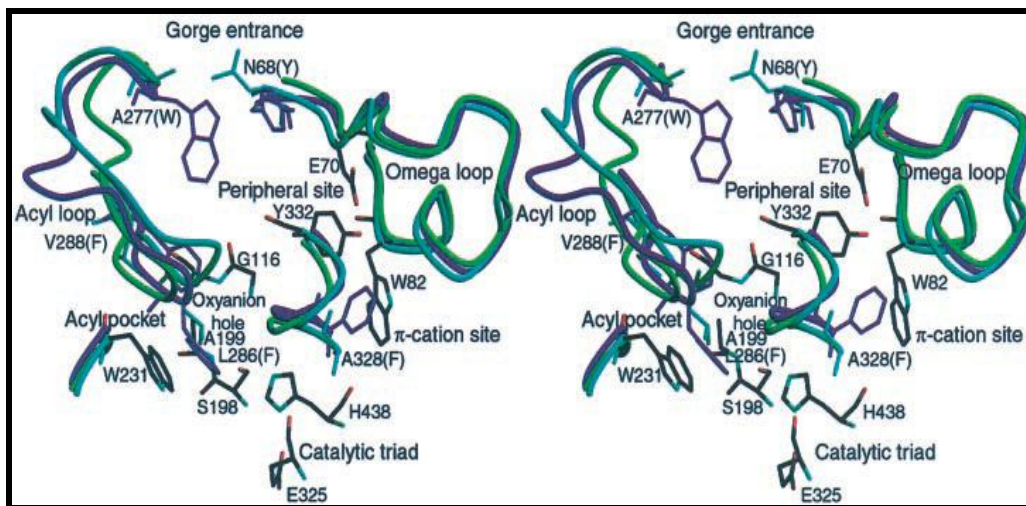


Figura 16 – Detalhes da estrutura da BuChE. Adaptado de NICOLET et al., 2003.

A BuChE possui seu sítio ativo em forma de garganta composto por aproximadamente 55 aminoácidos. O sítio aniônico periférico (PAS), localizado na boca da garganta, é formado pelos aminoácidos D70 (ácido aspártico) e Y332 (tirosina) os quais estão envolvidos na ligação inicial de substratos carregados positivamente. O sítio esterásico da BuChE é formado pela tríade catalítica dos aminoácidos S198 (serina), H438 (histidina) e E325 (ácido glutâmico) responsáveis pela hidrólise. Sugere-se a participação da BuChE na regulação da proliferação celular e também na expressão da AChE, além da participação desta última no processo de diferenciação celular (LAYER e WILLBOLD, 1995)

A BuChE é abundante no fígado e plasma. Ela hidrolisa eficientemente a butirilcolina e tem um papel importante no metabolismo de alguns agentes anestésicos clinicamente importantes (por exemplo, succinilcolina, e procaína) (BERNARDI et al., 2010).

3.13.2.3. Colinesterases e o câncer

A AChE e a BuChE, em menor grau, limitam a ação da ACh nas sinapses colinérgicas e junções neuromusculares. Além disso, as colinesterases parecem possuir funções não-colinérgicas importantes (NIETO-CERON et al., 2010). Elas intervêm em processos celulares como a proliferação, diferenciação, apoptose e reconhecimento celular (SOREQ e SEIDMAN, 2001), o que sugere uma possível influência das colinesterases na tumorigênese. Estudos mostram que há alterações nos genes da AChE em patologias como na leucemia (LAPIDOT-LIFSON et al., 1989) e no carcinoma de ovário (ZAKUT et al., 1990). Segundo Ruiz-Espejo e colaboradores (2002) as atividades da AChE e da BuChE se comportam de forma oposta em pacientes com câncer de mama. Além disso, mudanças na estrutura e/ou expressão bem como a presença de enzimas com propriedades anormais foram relatadas em vários tipos de tumores (VIDAL, 2005).

APRESENTAÇÃO

Os resultados desta tese estão apresentados sob a forma de artigos e manuscritos, os quais se encontram no item “**ARTIGOS E MANUSCRITOS**”. As seções Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas encontram-se nos próprios artigos e manuscritos e representam a íntegra deste estudo. Os itens **DISCUSSÃO** e **CONCLUSÕES**, encontrados no final desta tese, apresentam interpretações e comentários gerais a respeito dos resultados demonstrados nos artigos e manuscritos contidos neste trabalho. As **REFERÊNCIAS BIBLIOGRÁFICAS** referem-se somente as citações que aparecem nos itens **INTRODUÇÃO**, **REVISÃO BIBLIOGRÁFICA** e **DISCUSSÃO** desta tese.

4. ARTIGOS E MANUSCRITOS

4.1 Artigo 1

**Estresse Oxidativo e status antioxidante em pacientes com câncer de próstata:
Relação com a escala de Gleason, tratamento e metástase óssea**

Oxidative stress and antioxidant status in prostate cancer patients: Relation to Gleason score, treatment and bone metastasis

Vanessa Battisti, Liési D.K. Maders, Margarete D. Bagatini, Luiz Gustavo B. Reetz, Juarez Chiesa, Iara E. Battisti, Jamile F. Gonçalves, Marta M.F. Duarte, Maria R.C. Schetinger, Vera M. Morsch

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Original article

Oxidative stress and antioxidant status in prostate cancer patients: Relation to Gleason score, treatment and bone metastasis

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ABSTRACT

Over the last decade, epidemiological, experimental and clinical studies have implicated oxidative stress in the development and progression of prostate cancer. In the present study, we evaluated the oxidative status and antioxidant defense in patients with prostate cancer (PCa) taking into consideration: treatment, Gleason score and bone metastasis. For this, we measured concentrations of plasmatic thiobarbituric acid reactive substances (TBARS), serum protein carbonylation, whole blood catalase (CAT) and superoxide dismutase (SOD) activities, as well as the plasma and erythrocyte thiol levels and serum vitamin C and E concentration. This study was performed on 55 patients with PCa and 55 healthy men. TBARS levels and serum protein carbonylation were higher in PCa patients than in controls and altered levels of antioxidants were found in these patients. CAT activity was decreased and SOD activity was higher in PCa patients when compared with controls. Non-protein thiol levels were increased, however, serum vitamin C and vitamin E content were reduced in PCa patients when compared with controls. In addition, different parameters analyzed in PCa patients based on metastasis, treatment and Gleason score showed changes in oxidative stress biomarkers and antioxidant defenses. These findings may indicate an imbalance in the oxidant/antioxidant status, supporting the idea that oxidative stress plays a role in PCa, moreover, the oxidative profile appear to be modified by bone metastasis, treatment and Gleason score.

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1. Introduction

Prostate cancer (PCa) is an important worldwide health problem. It remains unknown why older men have a greater risk of PCa and poorer outcomes compared with younger men. PCa is a major age-related malignancy with most incidences occurring between 54 and 75 years [1]. The histologic differentiation grade is the strongest prognosticator of PCa. Different histologic grading systems for PCa have been developed historically, and the Gleason grading system [2,3] is now the most extensively used.

Since bone metastases will occur in 65–75% of patients with prostate cancer, early diagnosis and treatment would help to prevent skeletal-related events, in turn prolonging survival [4]. Although great advances have been made in the treatment of PCa in recent years, questions still remain concerning the optimal treatment strategy for both localized disease and metastatic disease.

This lack of a standard treatment approach may be partly attributed to variations in the risk of disease progression among patients [5]. Several different hormonal therapies, which act by different mechanisms, are available. Luteinizing hormone-releasing hormone (LHRH) agonists such as goserelin downregulate the pituitary LHRH receptors thereby suppressing the luteinizing hormone (LH) and testosterone secretion, while antiandrogens (such as cyproterone acetate) act at the androgen receptor to block the effects of androgens, irrespective of their source [6].

The cause of this disease is not well understood; however, certain factors are commonly linked to its development [7]. Many of the factors that are associated with PCa such as aging, imbalance of androgens, the antioxidant system, dietary fat, and pre-malignant conditions such as high grade prostate intraepithelial neoplasia, etc. may be linked to oxidative stress [8]. In addition, PCa is commonly associated with a shift in the antioxidant–prooxidant balance resulting in increased oxidative stress [7].

Oxidative stress is an inevitable consequence of aerobic life, and there is growing understanding that the endogenous generation of toxic products of oxidative stress plays a major role in aging and

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age-related diseases such as PCa [9]. Reactive oxygen species (ROS), such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are capable of causing lipid peroxidation, altering the activity of sulfhydryl (SH)-dependent enzymes, and damaging DNA [10]. Under normal conditions, a dynamic equilibrium exists between the production of ROS and the antioxidant capacity of the cell [11]. Oxidative stress occurs when ROS levels exceed the antioxidant capacity of a cell [12]. To control the effect of ROS, aerobic cells have antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), as well as non-enzymatic antioxidants such as non-protein thiols, vitamin C and vitamin E to defend against oxidative stress. SOD catalyses the dismutation of the superoxide anion into hydrogen peroxide (H_2O_2). H_2O_2 can be transformed into H_2O and O_2 by CAT. Additionally, non-protein thiols have a variety of functions in bioreduction and detoxification processes [13]. The main antioxidant function of vitamin E is to protect against lipid peroxidation [14] while ascorbic acid, or vitamin C, is an essential water-soluble vitamin [15].

Recently, the presence of oxidative stress was observed in studies carried out in our laboratory with acute lymphoblastic leukemia [13] and uterine cervix neoplasia patients [16], but information about the activities of antioxidant enzymes have been conflicting in patients with cancer. Moreover, the influence of the standard treatment, Gleason grading system and bone metastasis in the levels of oxidative damage and antioxidant defenses have not been investigated in patients with PCa in previous studies. Therefore, the novelty of the present study is that oxidative profiles were investigated considering these parameters. Toward this end, we determined the intensity of biological damage caused by free radicals in lipids and proteins through the measurement of lipid peroxidation and protein carbonylation levels. Additionally, we verified the antioxidant profile in PCa patients and controls by assessing the main enzymatic (CAT and SOD) and non-enzymatic antioxidants (thiols, vitamin C and vitamin E).

2. Patients and methods

2.1. Patients

The sample consisted of 55 PCa patients at the Oncology-Hematology Laboratory of the Hospital of the Federal University of Santa Maria. The controls consisted of 55 healthy volunteers with ages and social conditions similar to those of the patients. They presented no acute or chronic diseases such as diabetes, parasitosis or immune dysfunction. Also, the controls used in this study were not under any pharmacological therapy.

Patients included in this study were diagnosed with PCa based on histological evaluation (positive biopsy results). For purposes of analysis, patients with prostate cancer were further divided into different groups for each of four distinct parameters: presence or absence of metastasis, standard treatment (without previous treatment, goserelin acetate or cyproterone acetate) and Gleason score. Consent was given by family members of all the patients included in this work. The Human Ethics Committee from the Federal University of Santa Maria approved the protocol under number 23081.09047/2008-47. The patient's general characteristics are shown on Table 1.

2.2. Sample collection

The blood was collected in vacutainer tubes without an anticoagulant system, centrifuged at 5000 rpm for 10 min, the precipitate was discarded and the serum was used to determine protein carbonyl, vitamin C and vitamin E contents. CAT and SOD

Table 1
General characteristics of the patients.

Characteristics	No. of patients (percent)
Age	
<70 years	12 (22)
70–80 years	31 (56)
>80 years	12 (22)
Gleason	
≤6	20 (36)
=7	21 (38)
≥8	14 (26)
Metastasis	
Yes	23 (42)
No	32 (58)
Treatment	
No	10 (18)
Goserelin acetate	35 (64)
Cyproterone acetate	10 (18)
Family history	
Yes	36 (65)
No	19 (35)
Smoker	
Yes	35 (64)
No	20 (36)
Alcohol	
Yes	23 (42)
No	32 (58)

activities were determined using whole blood collected in citrated vacutainer tubes and diluted to 1:10 in saline solution. For plasmatic thiobarbituric acid reactive substances (TBARS) and non-protein thiols, the blood was collected using EDTA as anticoagulant. The sample was then centrifuged (5000 rpm for 10 min) and the plasma was used to determine MDA and thiols and the erythrocytes were used only to verify thiol levels.

2.3. Determination of lipid peroxidation and carbonylation of serum protein

Lipid peroxidation was estimated by measuring TBARS in plasma samples according to a modified method of Jentszsch [17]. Briefly, 0.2 mL of serum was added to the reaction mixture containing 1 mL of 1% ortho-phosphoric acid, 0.25 mL alkaline solution of thiobarbituric acid-TBA (final volume 2.0 mL) followed by 45 min heating at 95 °C. The results were expressed as nanomole MDA per milliliter of plasma.

The carbonylation of serum proteins was determined by a modified Levine's method [18]. Firstly, from 1 mL of serum, the proteins were precipitated using 0.5 mL of 10% trichloroacetic acid (TCA) and centrifuged at 5000 rpm for 5 min discarding the supernatant. One half milliliter of 10 mmol/L 2,4-dinitrophenylhydrazine (DNPH) in 2 mol/L HCl was added to this protein precipitate and incubated at room temperature for 30 min. During incubation, the samples were mixed vigorously every 15 min. After incubation, 0.5 mL of 10% TCA was added to the protein precipitate and centrifuged at 5000 rpm for 5 min. After discarding the supernatant, the precipitate was washed twice with 1 mL of ethanol/ethylacetate (1:1), centrifuging out the supernatant in order to remove the free DNPH. The precipitate was dissolved in 1.5 mL of protein dissolving solution (2 g SDS and 50 mg EDTA in 100 mL 80 mmol/L phosphate buffer, pH 8.0) and incubated at 37 °C water bath for 10 min. The color intensity of the supernatant was measured using a spectrophotometer at 370 nm against 2 mol/L HCl. Carbonyl content was calculated by using the molar extinction

coefficient (21×10^{-3} mol cm) and results were expressed as nanomoles per milligram protein.

2.4. Catalase (CAT) and superoxide dismutase (SOD) activities

The determination of CAT activity was carried out in accordance with a modified method of Nelson and Kiesow [19]. This assay involves the change in absorbance at 240 nm due to CAT-dependent decomposition of hydrogen peroxide. An aliquot (0.02 mL) of blood was homogenized in potassium phosphate buffer, pH 7.0. The spectrophotometric determination was initiated by the addition of 0.07 mL in an aqueous solution of hydrogen peroxide 0.3 mol/L. The change in absorbance at 240 nm was measured for 2 min. CAT activity was calculated using the molar extinction coefficient ($0.0436 \text{ cm}^2/\mu\text{mole}$) and the results were expressed as picomoles per milligram protein.

SOD activity measurement was based on the inhibition of the radical superoxide reaction with adrenalin as described by Mc Cord and Fridovich [20]. In this method, SOD present in the sample competes with the detection system for the superoxide radical. A unit of SOD is defined as the amount of enzyme that inhibits by 50% the oxidation speed of adrenalin. The oxidation of adrenalin leads to the formation of the colored product, adrenochrome, which is detected by spectrophotometer. SOD activity is determined by measuring the speed of adrenochrome formation, observed at 480 nm, in a reaction medium containing glycine-NaOH (50 mM, pH 10) and adrenalin (1 mM).

2.5. Determination of non-protein thiols

Non-protein thiols were assayed in plasma and erythrocytes by the method of Ellman [21]. Aliquots (0.1 mL) of plasma were added to a phosphate buffer 0.3 mol/L (0.85 mL), pH 7.4 and the reaction was read at 412 nm after the addition of 10 mM 5-5'-dithio-bis(2-nitrobenzoic acid) (DTNB) (0.05 mL). Results were expressed as $\mu\text{mol/mL}$ of plasma.

Aliquots of erythrocytes (0.3 mL) were hemolyzed with 10% Triton X-100 (0.1 mL) and, after 10 min, precipitated with 0.2 mL of 20% TCA. After centrifugation at 5,000 rpm for 10 min, the supernatant aliquots reacted with 50 μL of DTNB (10 mM) and the reaction product was read at 412 nm. Results were expressed as $\mu\text{mol/mL}$ of erythrocyte.

2.6. Serum vitamin C and vitamin E quantification

Vitamin C analysis was performed by the method described by Jacques-Silva et al. [22]. The samples were deproteinized with trichloroacetic acid (TCA) 15%, using 0.5 mL of sample and 0.5 mL of TCA, vortex-mixed for 15 s and centrifuged at 1800 g for 15 min. Then, 0.4 mL of supernatant was removed and incubated at 37 °C in a medium containing 4.5 mg/mL dinitrophenylhydrazine, 0.6 mg/mL thiourea, 0.075 mg/mL CuSO_4 , and 0.675 mol/L H_2SO_4 . After 3 h, 1 mL of 65% H_2SO_4 was added. Then, the samples were read in spectrophotometer at 520 nm. Calibration curves with L(+)-ascorbic acid were used to determine the concentration, following the same procedure as used for the samples.

Serum vitamin E was estimated by a modified method of Hansen and Warwick [23]. In a cover tube, 140 μL of Milli-Q water (Millipore, Bedford, MA, USA) was added to 20 μL of butylated hydroxytoluene 10 mM (BHT), 140 μL of sample and 2.1 mL of ethanol solution (66%). After this, it was vortex-mixed for 10 s and 3.5 mL of n-hexane was added and mixed for 1 min. It was then centrifuged at 1800 g for 10 min and 3 mL of superior phase transferred to fluorimeter cuvettes and the vitamin E was measured in the fluorimeter: excitation: 295 nm; emission: 340 nm. All samples were analyzed in duplicate. Calibration

curves with d-tocopherol (Sigma-Aldrich Inc, USA) were used to determine the concentration, following the same procedure as used for the samples.

2.7. Protein determination

Protein was measured by the method of Bradford [24] using bovine serum albumin as standard.

2.8. Statistical analysis

Data are presented as mean \pm standard error. The distribution of all dependent variables was examined by the Shapiro-Wilk test and was found not to differ significantly from normal. Data were analyzed statistically by one-way ANOVA followed by the Duncan's multiple test. Differences were considered significant when the probability was $P < 0.05$. The SPSS version 13.0 was used for all analyses (SPSS Inc., USA).

3. Results

PCa patients showed higher TBARS levels in plasma (Fig. 1A) when compared with controls. In addition, protein oxidation, determined by protein carbonyl content in serum, was increased in patients in relation to the control group (Fig. 1B). CAT and SOD activities are shown in Fig. 1C and D. CAT in PCa patients was significantly decreased when compared to control group. On the other hand, SOD activity was higher in PCa patients versus controls.

Fig. 2A and B showed non-protein thiol levels in plasma and erythrocytes of PCa and the control group. Non-protein thiol levels were increased in the plasma and erythrocytes of PCa patients when compared with the control group. Serum vitamin C (Fig. 2C) and serum vitamin E content (Fig. 2D) were reduced in PCa patients when compared with controls.

3.1. Oxidative status considering metastasis

Figs. 3 and 4 show a comparison of patients with localized prostate cancer and bone metastatic disease. TBARS and protein carbonyl levels were increased for both (Fig. 3A and B). SOD activity was increased only in patients with localized prostate cancer, but CAT activity was reduced in both groups in relation to the control (Fig. 3C and D). Non-protein thiol levels were increased in the plasma and erythrocytes of patients with bone metastasis (Fig. 4A and B). Vitamin C and E content were altered in both groups in relation to the control group (Fig. 4C and D).

3.2. Oxidative status considering treatment

Patients were then separated considering the treatment used and Fig. 5A shows that TBARS was increased in patients who had not received any therapy and in patients treated with goserelin acetate. CAT activity (Fig. 5C) was reduced in patients not treated and treated with cyproterone acetate when compared to controls. Plasma thiol levels were increased only in patients not treated and erythrocyte thiols were increased for all groups (Fig. 6A and B). Vitamin C was reduced in all groups and vitamin E content was reduced only in patients not treated and in treatment with cyproterone acetate compared to the control group (Fig. 6C and D).

3.3. Oxidative status considering Gleason scale

Figs. 7 and 8 show patients divided in groups based on Gleason score. Level of TBARS (Fig. 7A) was increased in all

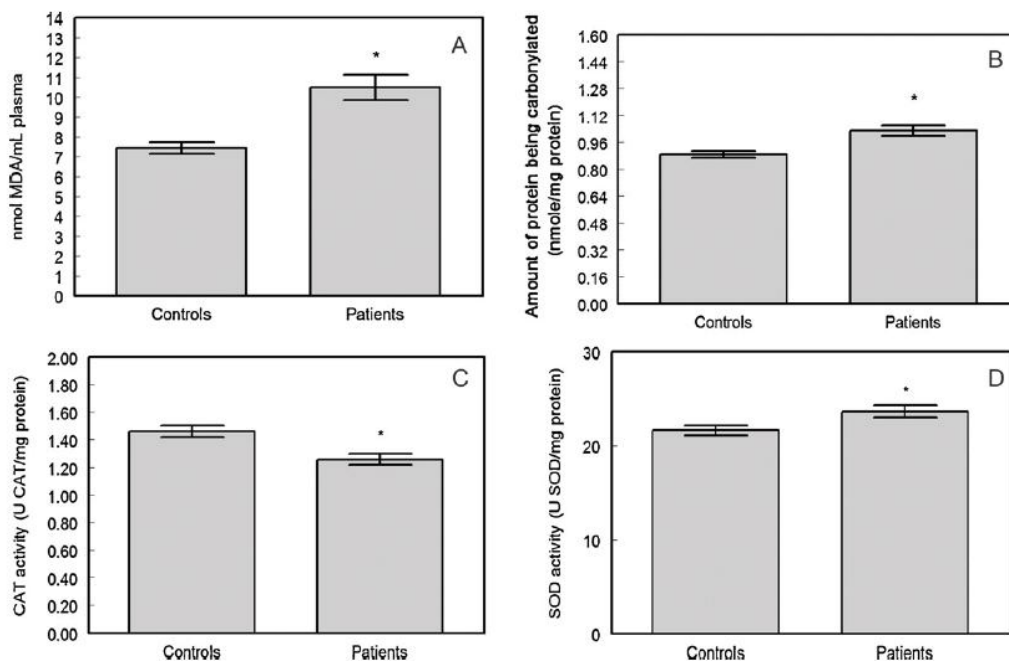


Fig. 1. A. TBARS levels in plasma of PCa patients. B. Protein carbonylation content in serum of PCa patients. C. CAT activity in total blood of PCa patients. D. SOD activity in total blood of PCa patients. Each column represents mean \pm SE. Differences are considered significant when $P < 0.05$ (*).

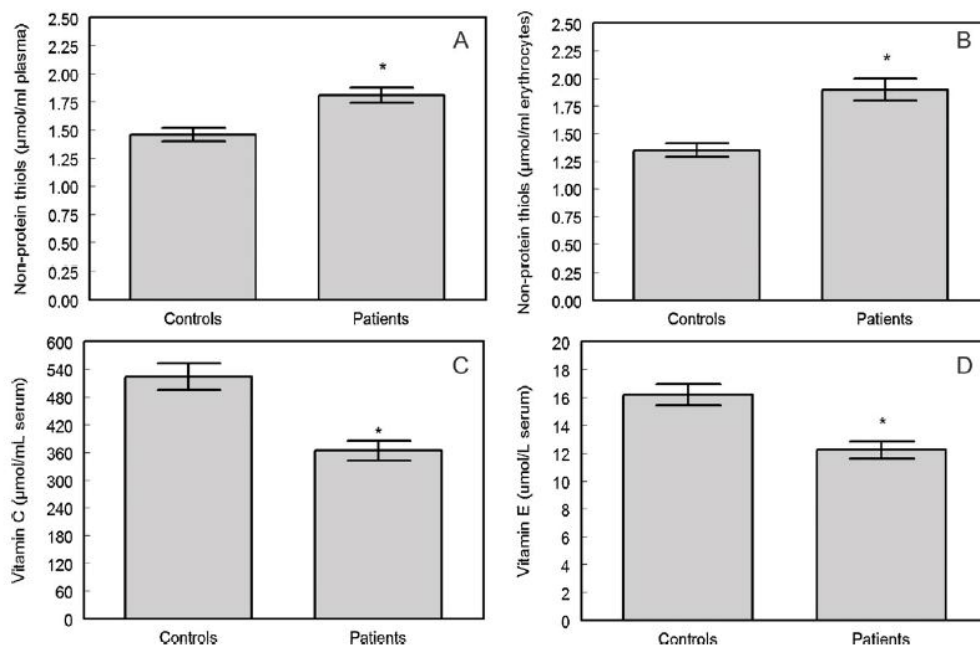


Fig. 2. A. Non-protein thiol levels in plasma of PCa patients. B. Non-protein thiol levels in erythrocytes of PCa patients. C. Vitamin C content in serum of PCa patients. D. Vitamin E content in serum of PCa patients. Each column represents mean \pm SE. Differences are considered significant when $P < 0.05$ (*).

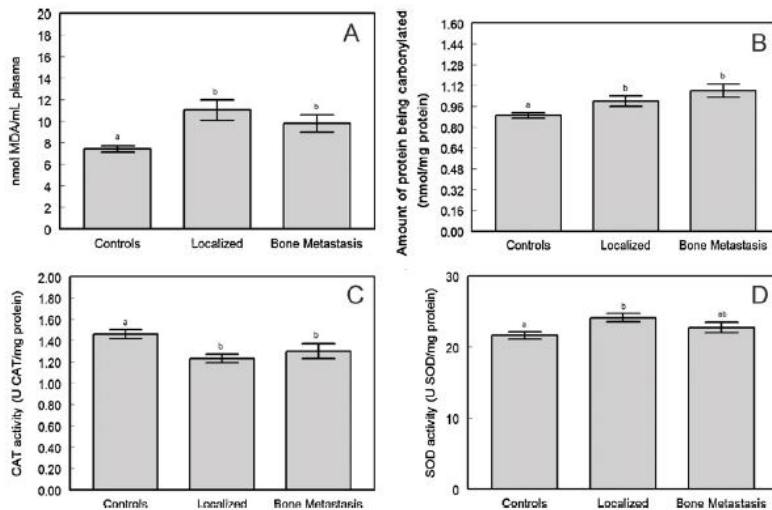


Fig. 3. Patients were divided in groups considering the presence of bone metastasis. A. TBARS levels in plasma. B. Protein carbonylation content in serum. C. CAT activity in total blood. D. SOD activity in total blood. Each column represents mean ± SE. Differences are considered significant when $P < 0.05$ (*).

patients group. Moreover, the major protein damage (protein carbonyl) occurred in the group with Gleason scores greater than 8 (Fig. 7B). Moreover, CAT activity was reduced (Fig. 7C) and plasma thiol levels were increased in this group when

compared to the control group (Fig. 8A). Erythrocyte thiol level was increased in all groups (Fig. 8B). On the other hand, vitamin C and vitamin E content were reduced in all groups in relation to the control group (Fig. 8C and D).

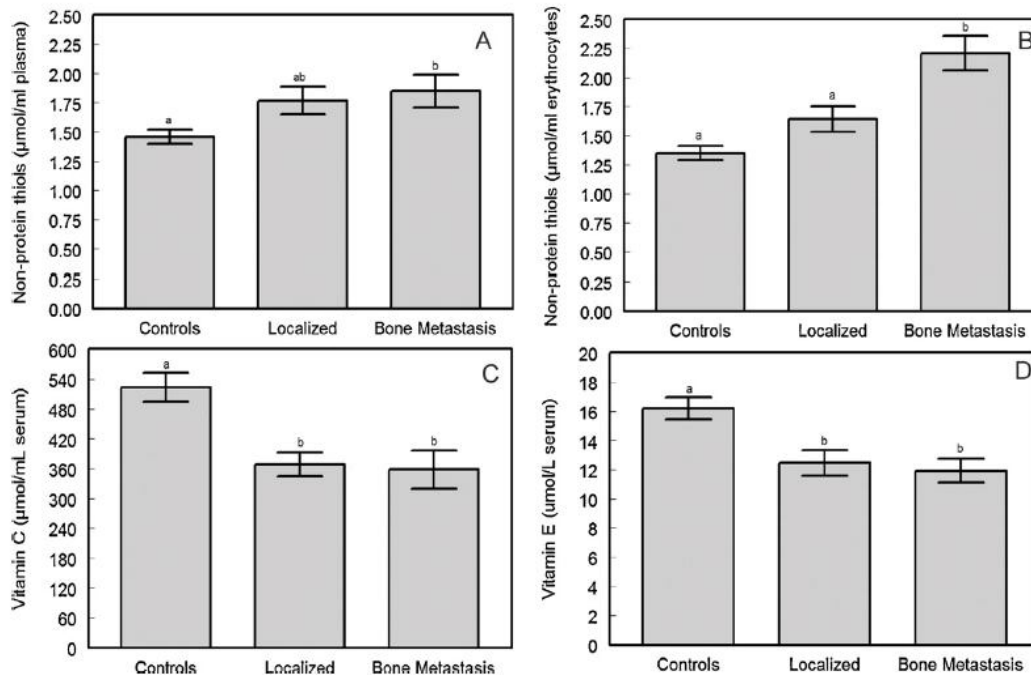


Fig. 4. Patients were divided in groups considering the presence of bone metastasis. A. Non-protein thiol levels in plasma. B. Non-protein thiol levels in erythrocytes. C. Vitamin C content in serum. D. Vitamin E content in serum. Each column represents mean ± SE. Differences are considered significant when $P < 0.05$ (*).

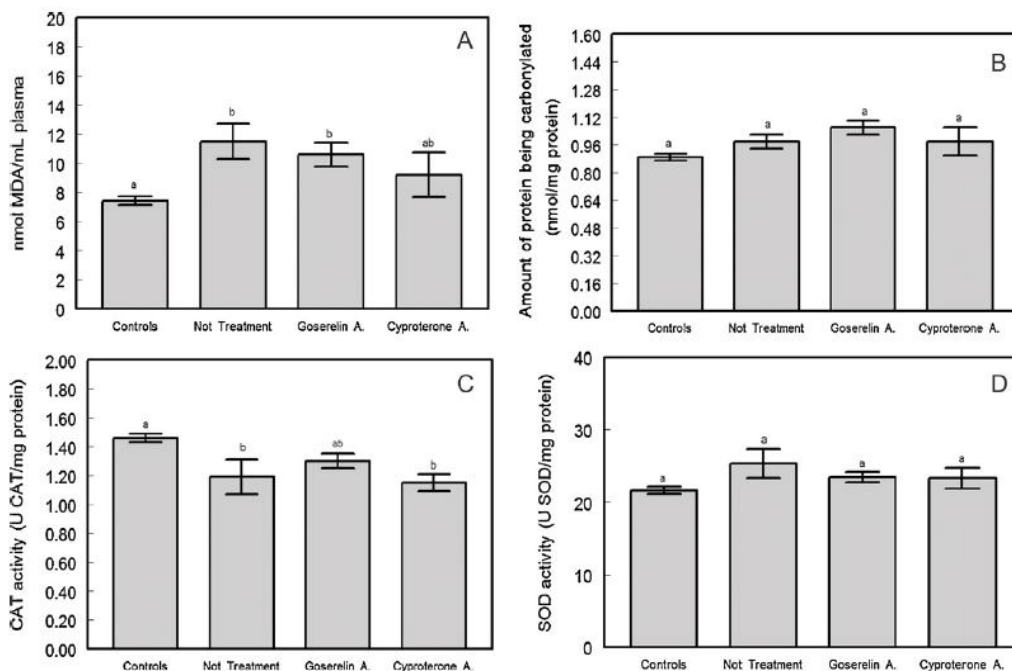


Fig. 5. Patients were divided in groups considering treatment type. A. TBARS levels in plasma. B. Protein carbonylation content in serum. C. CAT activity in total blood. D. SOD activity in total blood. Each column represents mean \pm SE. Differences are considered significant when $P < 0.05$ (*).

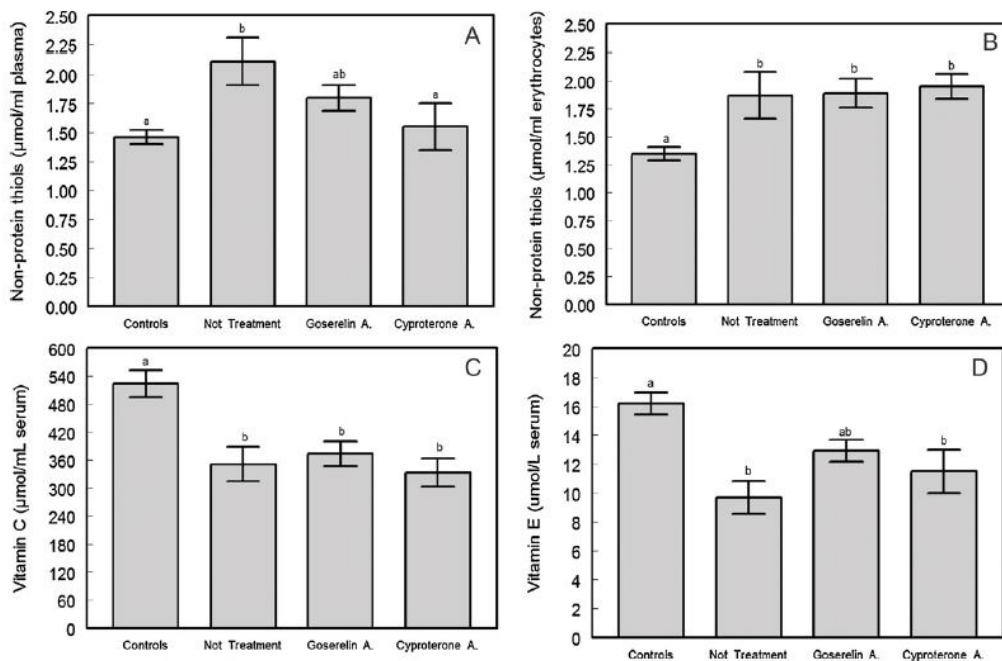


Fig. 6. Patients were divided in groups considering treatment type. A. Non-protein thiol levels in plasma. B. Non-protein thiol levels in erythrocytes. C. Vitamin C content in serum. D. Vitamin E content in serum. Each column represents mean \pm SE. Differences are considered significant when $P < 0.05$ (*).

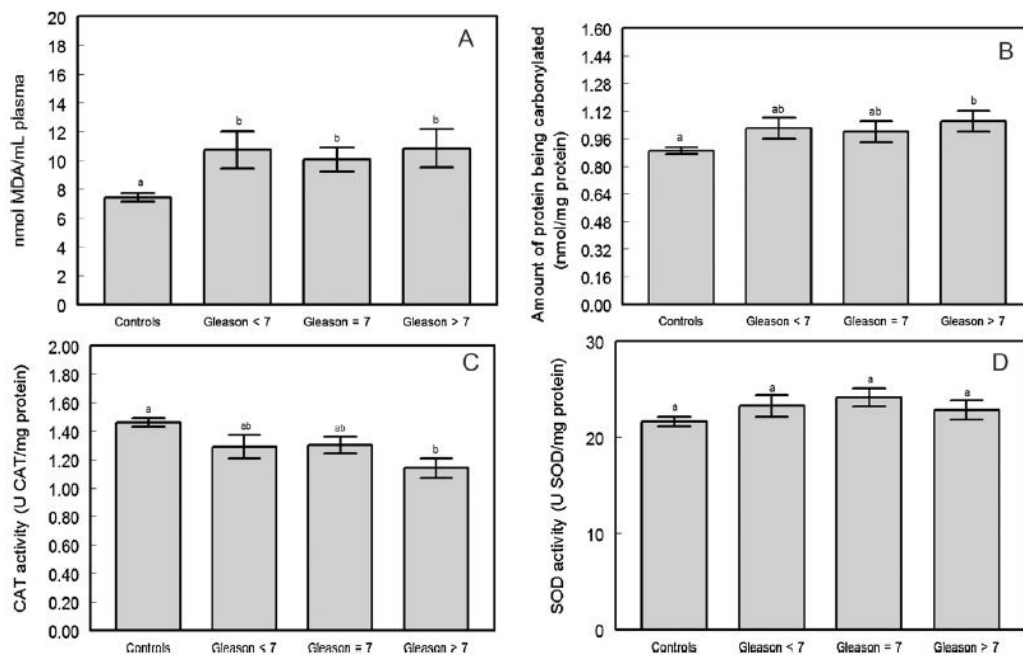


Fig. 7. Patients were divided in groups considering Gleason grading system. A. TBARS levels in plasma. B. Protein carbonylation content in serum. C. CAT activity in total blood. D. SOD activity in total blood. Each column represents mean \pm SE. Differences are considered significant when $P < 0.05$ (*).

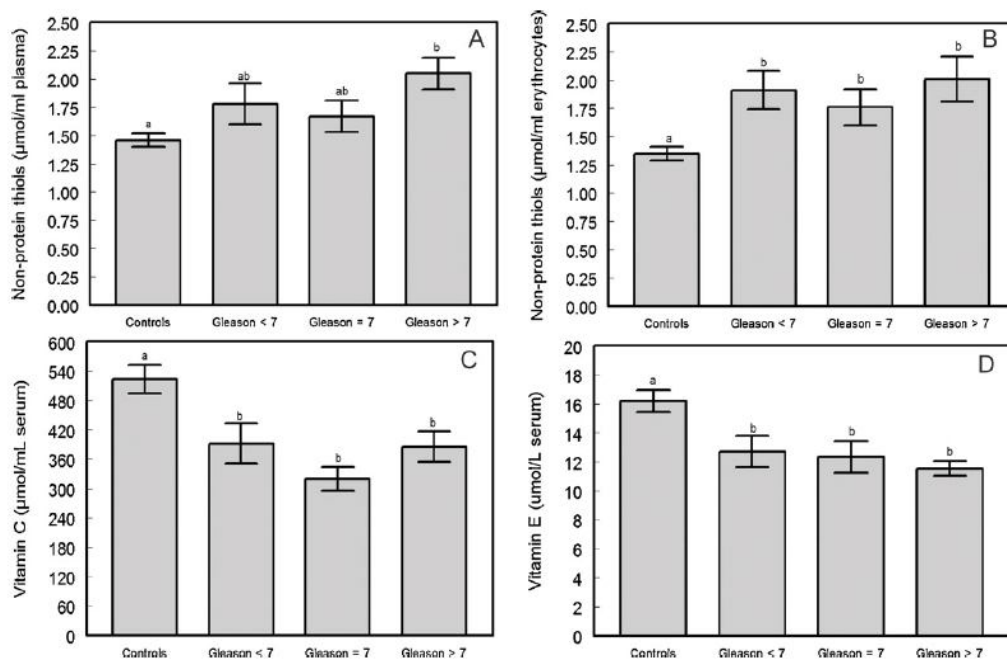


Fig. 8. Patients were divided in groups considering Gleason grading system. A. Non-protein thiol levels in plasma. B. Non-protein thiol levels in erythrocytes. C. Vitamin C content in serum. D. Vitamin E content in serum. Each column represents mean \pm SE. Differences are considered significant when $P < 0.05$ (*).

4. Discussion and conclusion

Progressive inherent or acquired changes in cellular metabolism occurring over the years may play a very important role in the development of PCa [8]. Thus, excessive production of ROS or inadequacy in a normal cell's antioxidant defense system (or both) can cause the cell to experience oxidative stress and increased ROS may play a role in cellular processes associated with the initiation and development of many cancers including PCa [25].

In the present study, TBARS contents and protein carbonylation levels were shown to be increased in patients when compared with the control group. These results are in accordance with earlier studies of Goswami et al. [26] and Yilmaz et al. [27] who confirmed significantly increased lipid peroxides and protein carbonyls in carcinoma prostate cases when compared to benign prostate growth and controls. Our results reveal an alteration in lipid peroxidation and protein carbonylation, besides, changes in the antioxidant defense system in PCa patients were observed. With respect to SOD and CAT activities, our findings revealed that CAT activity in PCa patients was significantly decreased when compared to the control group. These results were in agreement with others authors that have shown lower CAT immunoreactivity [28,29] and erythrocyte CAT activity [30] in PCa when compared with benign prostate tissue. These results are interesting since Bostwick et al. [29] reported low levels of CAT in PCa, thereby implicating oxidative DNA damage in prostate carcinogenesis.

On the other hand, we observed that SOD activity was higher in PCa patients versus controls. Then, there was a different modulation of SOD and CAT in PCa patients when compared to the control group. SOD activity increased in PCa patients, probably as a response to oxidative stress. Evidence supports an integrated model of inherited and acquired deficiencies in cellular defense mechanisms against oxidative stress in the genesis of PCa [31]. The most striking finding was the remarkable increase of SOD activity observed in PCa without a proportional increase of CAT activity. Studies suggest that the overexpression of SOD without a compensatory increase in CAT has deleterious effects upon the cell [32]. This imbalance could be explained through the association of inflammation with the development of human cancers [33] and consequently increased SOD mRNA attributed to pro-inflammatory molecules [34]. On the other hand, previous investigators have documented that O_2^- produced during cancer can inactivate CAT and peroxidase [35]. This is one of the probable reasons for the decreased activity of CAT.

Among non-protein thiols, GSH plays an important role in the antioxidant defense system by scavenging reactive species and regenerating other antioxidants. Our study demonstrated a rise in non-protein thiol levels in plasma and erythrocytes of PCa patients when compared with the healthy group. These findings are in agreement with another study that showed increased antioxidant defenses in the beginning of tumor development and also showed rapid GSH synthesis in tumor cells [36]. The possible explanation for this is that the rise in non-protein thiols levels could be a compensatory mechanism to prevent tissue damage caused by oxidative stress.

In the present study, a significant decrease in serum vitamin C and vitamin E levels in PCa patients was observed. Previous observations correlated the risk of PCa with decreased serum vitamin E [37]. Decrease in vitamin E in these patients could be due to the possibility that vitamin E reacts very rapidly with molecular oxygen and free radicals generated during prostate carcinogenesis. This argument was supported by the fact that lipid peroxidation products were significantly higher in the PCa patients compared to the control group. It is therefore suggested that vitamin E acts as a free radical scavenger protecting polyunsaturated fatty acids from peroxidation reactions in the PCa patients. In relation to serum

vitamin C, it has been shown to be inversely correlated with biomarkers of oxidative stress, suggesting that vitamin C may decrease oxidative stress. Moreover, epidemiological data showing ethnic and geographic variations in PCa incidence and mortality have suggested that the consumption of dietary antioxidants, i.e. vitamins C and E, may be protective [38]. Thus, the possible application of agents capable of modulating the oxidant-antioxidant balance in PCa treatment is of interest [39].

Additional studies that investigate other risk factors associated with PCa and oxidative stress may clarify some important issues. Our study analyzed some important factors (such as metastasis, standard treatment and Gleason score) in relation to oxidative stress in PCa patients. An important aspect to be analyzed is that the skeleton is the most common site of tumor metastasis ROS may have contrasting effects on metastasis [40,41]. In some cancers, the formation of ROS contributes to the metastatic phenotype while in other cancers, reduced ROS are linked with metastasis [42,43]. In general, however, increased ROS levels are considered to be closely linked to the accelerated formation of metastasis, and the removal of ROS is a rational strategy to inhibit metastasis [44]. In our study, TBARS and protein carbonyl levels were increased and CAT activity, vitamin C and Vitamin E levels were reduced in both groups regardless of the presence or absence of bone metastasis. However, SOD activity was increased only in patients with localized disease and non-protein thiol levels in plasma and erythrocytes were elevated only in patients with bone metastasis. However, unlike with we expected, the results found no indicate significant changes in oxidative status of patients with localized disease or bone metastasis.

PCa is a sex hormone-responsive tumor. Several different hormonal therapies, which act by different mechanisms, are available. In our study, we analyzed patients who had not received any therapy, patients in treatment with goserelin and in treatment with cyproterone acetate. In patients not treated and in patients in treatment with goserelin acetate can be observed the major changes in oxidative biomarkers like TBARS. On the other hand, the antioxidant defenses (CAT and vitamin E) showed alterations in patients not treated and those treated with cyproterone acetate. We can suggest that the imbalance in the oxidative status seems not to be a result of the treatment with chemotherapeutic agents but may be involved with the pathogenesis of prostate cancer, since this imbalance can be observed mainly in patients not treated. Levels of oxidative damage and antioxidant defenses have not been investigated in PCa patients under different treatments in comparison with patients who had not received any therapy. More studies are necessary to verify the relation between the type of treatment used and the oxidative status in PCa patients.

The remarkable heterogeneity of PCa can be represented by the identification of five different growth patterns from 1 (most differentiated) to 5 (least differentiated). By adding the most dominant growth pattern (the primary Gleason pattern) to the next most dominant growth pattern (the secondary Gleason pattern) a tiered total score of ascending aggressiveness from 2 to 10 is obtained [45]. Interestingly, we have observed an increased oxidative stress (as evidenced by elevated protein carbonylation) and alterations in CAT activity and in plasmatic non-protein thiol levels in the cancer group with a Gleason score greater than 8. Furthermore, these findings confirm previous observations [46,47] and indicate that advanced PCa is associated with a state of high oxidative stress.

So, the present work provides evidence for the increased levels of oxidative damage and changes in the antioxidant system in PCa patients, suggesting a possible link between oxidative stress and this type of cancer. In addition, we hope that our results contribute to the study of the oxidative profile in PCa patients, since they demonstrate that the presence of metastasis, standard treatment

used and Gleason score modify the oxidative profile of PCA patients. Furthermore, our study showed that aggressiveness PCA is associated with a high oxidative stress status. However, more studies are necessary to confirm the exact connection between the type of treatment and bone metastasis in the oxidative stress observed in PCA patients.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgement

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4.2 Manuscrito 1

Enzimas que degradam nucleotídeos e agregação plaquetária em pacientes com câncer de próstata: Influência da escala de Gleason, terapia hormonal e metástase óssea

Nucleotide degrading enzymes and platelet aggregation in prostate cancer patients: influence of Gleason score, hormonal therapy and bone metastasis

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Abstract

Background: To analyze the NTPDase and 5'-nucleotidase activities in platelets from prostate cancer (PCa) patients and to verify the platelet aggregation considering clinicopathological parameters.

Methods: Patients were divided according to Gleason score, bone metastasis and hormonal therapy. This study was performed with 66 patients with PCa and 40 controls.

Results: Platelet enzyme activities increased in PCa patients in relation to the control group. Gleason score, metastasis and hormonal therapy influenced NTPDase and 5'-nucleotidase activities. In addition, the hormonal therapy modified the platelet aggregation in the PCa patients.

Conclusions: Our findings indicate an involvement of extracellular nucleotides in the pathophysiology of PCa, since NTPDase and 5'-nucleotidase act together to control nucleotide levels. We demonstrate for the first time that enzyme activities are changed in platelets from PCa patients with distinct Gleason score, with bone metastasis, as well as in patients under hormonal therapy.

Keywords: Prostate cancer; NTPDase, 5'-nucleotidase, platelet aggregation, Gleason score; bone metastasis; hormonal therapy

1. Introduction

Members of the E-NTPDase/CD39 family, previously classified as E-type ATPases, constitute a class of ecto-enzymes characterized by their capacity to hydrolyze nucleoside tri and diphosphates [1, 2]. NTPDase (EC 3.6.1.5, CD39, ecto-apyrase, ATP diphosphohydrolase) is a glycosylated membrane-bound enzyme that hydrolyzes ATP and ADP to AMP, which is subsequently converted to adenosine by 5'-nucleotidase (EC 3.1.3.5, CD73). NTPDase and CD73 play an important role in the regulation of blood flow and thrombogenesis by regulating ADP catabolism [3]. Both NTPDase and 5'-nucleotidase are located in the platelet membrane [3, 4].

Thromboregulation is a process or a group of processes by which circulating blood cells and vessel wall cells interact to regulate or inhibit thrombus formation [5, 6]. Platelets are one of the most important blood components that participate in and regulate thrombus formation by releasing active substances such as ADP [5]. Platelets are important for the haemostatic process as these cells adhere to sites of vascular injury, releasing ADP, Ca^{2+} , thromboxane A_2 , and other active substances [7]. ADP and Ca^{2+} promote the recruitment of platelets and adhesion to the vessel wall [8]. Moreover, the presence of ADP stimulates platelet aggregation and contributes to thrombus formation [9].

The association between clinical venous thromboembolism (VTE) and cancer has been recognized in the medical literature [10, 11]. The interactions between components of the hemostatic system and cancer cells are multifaceted. Tumorigenesis may be promoted by several changes in cellular components, including proteins exposed on the cell surface, such as ecto-enzymes [12]. It is known that adenosine accumulates in solid tumors at high concentrations and it has been shown to stimulate tumor growth and angiogenesis as well as to inhibit cytokine

synthesis, adhesion of immune cells to the endothelial wall, and the function of T-cells, macrophages, and natural killer cells [13].

Prostate cancer (PCa) is a worldwide health problem. It remains unknown why older men have a greater risk of PCa and poorer outcomes compared with younger men. PCa is a major age-related malignancy with most incidences occurring between 54 and 75 years [14]. The histologic differentiation grade is the strongest prognosticator of PCa. Different histologic grading systems for PCa have been developed historically, and the Gleason grading system [15, 16] is now the most extensively system used.

Although great advances have been made in the treatment of PCa in recent years, questions still remain concerning the optimal treatment strategy for both localized disease and metastatic disease (mainly bone metastasis). The lack of a standard treatment approach may be partly attributed to variations in the risk of disease progression among patients [17]. Several different hormonal therapies, which act by different mechanisms, are available. Luteinizing hormone-releasing hormone (LHRH) agonists, such as goserelin, downregulate the pituitary LHRH receptors thereby suppressing the luteinizing hormone (LH) and testosterone secretion [18].

In studies from our research group, it has been observed that ectonucleotidase's activities are modified in some diseases such as diabetes, hypertension [19], breast cancer [20] and uterine cervical neoplasia [21]. One aspect that must be emphasized here is the fact that adenine nucleotides and nucleosides play a complex role in cancer growth [2]. Of particular importance, adenosine and ATP stimulates the proliferation of certain tumor cells, whereas AMP inhibits the growth of tumor cells [13, 22]. Thus, in an attempt to investigate the potential role of enzymes that

participate in the hydrolysis of ATP, ADP, and AMP in the etiology of PCa, as well as the possible interference of the Gleason score, bone metastasis and hormonal therapy with adenine nucleotide hydrolysis, we assayed the NTPDase and CD73 activities in platelets of PCa patients considering these different parameters. Furthermore, we also verified the platelet aggregation in this pathological condition.

2. Materials and Methods

2.1. Chemicals

Nucleotides, HEPES, and Trizma base were purchased from Sigma (St. Louis, MO, USA). All the other reagents used in the experiments were of analytical grade and of the highest purity.

2.2. Patients

The sample consisted of 66 PCa patients aged 72.96 ± 5.64 years under treatment at the Oncology-Hematology Laboratory of the Hospital of the Federal University of Santa Maria. Patients included in this study were diagnosed with PCa based on histological evaluation (positive biopsy results). The diagnosis and estimation of cancer volume and Gleason Score relies in the biopsy procedures by sampling the prostate at a number of locations via biopsy needles. The biopsy-sampled prostate tissue then undergoes microscopic examinations, revealing cancer presence/ absence and cancer aggressiveness [23].

For purposes of analysis, patients with prostate cancer were further divided into different groups for each of 3 distinct parameters: metastasis, hormonal therapy with goserelin acetate and Gleason score. Patients were divided based on the presence

of metastasis, since the majority of patients with advanced prostate cancer develops bone metastasis that causes considerable morbidity, including pain and pathologic fractures. Bone scan is the used imaging method to detect PCa bone metastases. The PCa patients were grouped according to hormonal therapy since for many patients LHRH agonists such as goserelin acetate, provides a more acceptable method of treatment. In addition, the patients were divided into groups based on the Gleason score, since the Gleason system is the prevalent prostate cancer grading system worldwide. Consent was given by family members of all the patients included in this work.

The control group consisted of 40 individuals aged 71.40 ± 3.64 years, who presented normal blood pressure, were free from diabetes mellitus, alcoholism, cigarette smoking, chronic diseases and who had not been submitted to any pharmacological therapy during the month before the study. All subjects gave written informed consent to participate in the study. The protocol was approved by the Human Ethics Committee of the Health Science Center from the Federal University of Santa Maria, protocol number 23081.09047/2008-47. Ten milliliters of blood was obtained from each patient and used for platelet-rich plasma preparation and biochemical determinations. The same procedure was carried out for the control group. PCa patients were interviewed to obtain information such as the alcohol consumption, cigarette smoke and family history. Patient general characteristics are shown in Table 1.

2.3. Platelet aggregation

The platelet aggregation profile was evaluated by the method of Born [24] by measuring turbidity with a Chrono-log optical aggregometer (AGGRO/IINK® Model

810-CA software for Windows ver. 5.1) using ADP at a concentration of 2.5, 5 and 7.5 $\mu\text{mol/l}$ as agonist. The results were expressed as percentage of aggregation.

2.4. Platelet rich plasma preparation (PRP)

The PRP was prepared according to Pilla et al. 1996 [25], with minor modifications. Briefly, the blood was collected into 0.129 M citrated vacutainer tubes and centrifuged at 1000 rpm for 10 minutes. After this, the PRP was centrifuged at 3500 rpm for 30 minutes and washed twice with 3.5 mM HEPES buffer, pH 7.0, which contained 142 mM NaCl, 2.5 mM KCl and 5.5 mM glucose.

The platelet pellets were resuspended in HEPES buffer and the protein was adjusted to 0.4–0.6 mg/mL for the determination of ectonucleotidase activities. The integrity of the platelet preparation was confirmed by determining the lactate dehydrogenase (LDH) activity using the labtest kit (Labtest, Lagoa Santa, MG, Brasil).

2.5. Ectonucleotidase activities

Ectonucleotidase activities were determined using a PRP preparation according to Pilla et al., 1996 [25]. Briefly, to determine CD39 activity, 20 μL of PRP preparation were added to the system mixture, which contained 5 mM CaCl_2 , 100 mM NaCl, 5 mM KCl, 6 mM glucose and 50 mM tris-HCl buffer, pH 7.4. The reaction was started by the addition of 20 μL of ATP or ADP (1mM) as substrate. For AMP hydrolysis, CD73 activity was determined as described above, except that 5 mM CaCl_2 was replaced by 10 mM MgCl_2 and the nucleotide added was 2 mM AMP [25].

Both reactions were stopped by the addition of 200 μL of 10% trichloroacetic acid (TCA) to provide a final concentration of 5%. After this, the inorganic phosphate

released by ATP, ADP and AMP hydrolysis was determined in triplicate by the method of Chan et al. [26] using KH_2PO_4 as standard. The same process was carried out in control tubes to exclude non-enzymatic hydrolysis, by adding 20 μL of protein to the reaction medium after TCA the results were expressed as nmol inorganic phosphate released/minute/milligram of protein (nmol Pi/min/mg protein).

2.6. Protein determination

Protein was determined by the Coomassie blue method according to Bradford [27] using bovine serum albumin as standard.

2.7. Statistical analysis

Data are presented as mean \pm standard error of mean (SEM). The distribution of all dependent variables was examined by the Shapiro-Wilk test and was found not to differ significantly from normal. Data were analyzed statistically by one-way ANOVA followed by the Duncan's multiple test. Differences were considered significant when the probability was $P < 0.05$. The SPSS version 13.0 was used for all analyses (SPSS Inc., USA).

3. Results

3.1. Characteristics of patients

The clinical characteristics of the patients group are shown in Table 1. Most of the patients were classified in the Gleason score < 7 , were between 70 and 80 years old, had localized disease, and were under hormonal therapy with goserelin acetate. Risk factor analysis showed prevalence of smoking and family history of cancer in the patient groups studied.

3.2. Platelet aggregation

Platelet aggregation using ADP at 2.5 and 7.5 μM was decreased in PCa patients compared to control. Post-hoc comparisons made by Duncan's test revealed that the platelet aggregation using ADP at all concentrations was significantly decreased in patients in hormonal therapy with goserelin acetate. However, when patients were grouped according to the Gleason score, and presence or absence of the metastasis, there was no significant difference between the patient groups and controls. Results are shown in Table 2.

3.3. ATP, ADP, and AMP hydrolysis

The results described here are from one-way ANOVA followed by Duncan's post-hoc comparisons. Almost 4% of the platelets were disrupted indicating that the preparation was predominantly intact, as observed by LDH determination (data not shown).

The results obtained in the present study show that both CD39 and CD73 activities were altered in platelets from PCa patients. As can be observed in Figure 1, ATP, ADP, and AMP hydrolysis were significantly increased in platelets from patients when compared with the control group ($P < 0.05$).

3.4. Influence of Gleason score on platelet ATP, ADP, and AMP hydrolysis

Considering Gleason score (Fig. 2), it can be observed that ADP hydrolysis was modified by the Gleason score of PCa, and post-hoc comparisons by Duncan's test revealed that ADP hydrolysis was increased in PCa patients with Gleason score = 7.

ATP and AMP hydrolysis were not significantly altered by Gleason score in the PCa patients.

3.5. Influence of metastasis on platelet ATP, ADP, and AMP hydrolysis

Figure 3 shows a comparison of patients with localized PCa and bone metastatic disease with the control group. Post-hoc comparisons made by Duncan's test revealed that ATP and ADP hydrolysis were increased in the patients with localized disease in relation to the control group, while it was not significantly different for AMP hydrolysis.

3.6. Influence of hormonal therapy on platelet ATP, ADP, and AMP hydrolysis

Post-hoc comparisons made by Duncan's test revealed that ATP, ADP, and AMP hydrolysis were not significantly altered in patients under treatment. On the other hand, ATP, ADP, and AMP hydrolysis were significantly higher in patients who had not received any therapy when compared to control subjects (Fig. 4).

4. Discussion

Prostate Cancer (PCa) is now recognized as one of the most important medical problems facing the male population [28]. It has been proposed that there may be a hereditary component to PCa risk, since family history of PCa is a well established risk factor for PCa risk in men [29]. Our study demonstrated that most PCa patients included in this study have a family history of any cancer or PCa. Furthermore, in our study, we observed that most PCa patients were former or current smokers. The important relationship between tobacco smoking and cancer is well established [30].

Cancer has been reported to be associated with problems in platelet aggregation and, most frequently, with blood coagulation. The association between cancer and thromboembolism was first described by Trousseau in 1865 [31], who noted an association between venous thrombosis and malignant disease [32]. Moreover, cancer has also been associated with platelet activation and with disorders in blood coagulation [33].

Our results show that platelet aggregation was decreased in PCa patients compared to the control. However, when patients were grouped according to receiving hormonal therapy or not, it could be observed that only the patients that received therapy showed a decrease in platelet aggregation in relation to the control group. The reduced platelet aggregation found in the group that received hormonal therapy compared to the control may indicate that this treatment was effective in controlling tumor cell proliferation, since tumor cells may cause an activation of coagulation. PCa is frequently hormone dependent and therefore anti-androgen therapy has been an important aspect of treatment for PCa patients [34]. Munson et al. [35] suggested that platelets may secrete growth factors that could stimulate cancer proliferation; however, patients involved in this study appeared not to present any significant platelet activation, as confirmed by the decreased platelet aggregation profile.

Tumorigenesis may be promoted by several changes in cellular components, including proteins exposed on the cell surface, such as ecto-enzymes [12]. These enzyme activities are extremely important for maintaining physiological levels of extracellular adenine nucleotides (ATP, ADP and AMP) in almost all tissues and cells.

Studies have indicated that adenine nucleotides and adenosine have an important role in tumor growth [2, 13, 36, 37]. Due to rapid growth, solid tumors routinely experience severe hypoxia and necrosis, which causes adenine nucleotide degradation and adenosine release [13]. Of particular importance, there are lines of evidence showing that adenosine can function as a stimulant of tumor growth, whereas AMP has the opposite effect [2]. However, little is known about the effects of these nucleotides on the prostate or prostatic cell lines.

The present results clearly demonstrate that the hydrolysis of nucleotides is changed in PCa patients. We verified that both CD39 and CD73 activities were altered in platelets from PCa patients. ATP, ADP, and AMP hydrolysis were significantly increased in platelets from patients when compared with the control group ($p < 0.05$). Buffon and cols [1] demonstrated that the presence of CD39 and CD73 enzymes in Walker 256 tumor cells may be important for the regulation of the extracellular adenine nucleotide/adenine nucleoside ratio therefore leading to tumor growth. Kittel and cols [38] showed that the presence of enzymes, such as ectonucleotidases, in the plasma membrane of different tumors, has been correlated with proliferative and metastatic activities.

Our results show an increase in the enzyme activities and consequently in the nucleotide hydrolysis in PCa patients. Inhibitory effects of extracellular ATP on tumor growth have been described in cells and tissues, including prostate carcinoma cells [39]. On the other hand, Chen et al. [40] showed that extracellular ATP had a pro-invasion effect in PCa cells. However, the results of ATP hydrolysis agree with previously published data showing that expression of ecto-ATPases is increased during cancer progression [41, 42]. We can suggest that CD39 can protect the

organism against an increase in the platelet aggregation, while CD73 is more involved in the control of adenosine formation.

One interesting observation to be made in the present study is that ATP, ADP, and AMP hydrolysis were modulated differently in relation to the Gleason score, presence or absence of metastasis and use of hormonal therapy. This may indicate that the CD39 and CD73 activities are being affected differently by these conditions.

Considering Gleason score, ADP hydrolysis was increased in PCa patients with Gleason score 7. These findings suggest that there is a mechanism to prevent platelet aggregation, since ADP acts upon platelets regulating their aggregation and modifying their shapes. On the other hand, ATP has been postulated to be a competitive inhibitor of ADP-induced platelet aggregation [43]. The released ADP can interact with receptors on platelets and induce platelet aggregation. However, further studies are needed to evaluate the role of nucleotides in the aggressiveness of PCa.

When the patients were grouped according to the presence of localized disease or bone metastasis, our results showed that ATP and ADP hydrolysis were increased in the patients with localized disease in relation to the other group, while it was not significantly different for AMP hydrolysis. There are no studies in literature relating CD39 and CD73 activities with the development of metastasis in PCa patients. Tumor invasion and metastasis are multistep processes in which loss of cell-cell adhesion and increased proteolysis and cell motility have been shown to be critical steps [44]. Factors that accelerate steps above may promote tumor invasion and then affect metastasis [20]. Stagg et al (2010) [45], suggested a role for CD73-derived adenosine in tumor metastasis in patients with breast cancer. Already, Zhang et al. [22] indicated that ATP may increase the risk of metastasis in PCa cells. In fact,

our work shows that CD39 activity is lower, thus suggesting higher ATP concentrations in patients with metastasis.

In order to evaluate the possible influence of hormonal therapy use in enzyme activities, we grouped PCa patients in two groups: patients who had not received any therapy preceding the blood sampling and PCa patients undergoing hormonal therapy. Our results revealed that ATP, ADP, and AMP hydrolysis were significantly higher in patients who had not received any hormonal therapy when compared to control subjects. Consequently, we may suggest that the increase observed in CD39 and CD73 activities is not due to the hormonal therapy used by the PCa patients. Also, these findings support the argument that it is the pathological condition that generates the increase in these enzyme activities. Furthermore, our results suggest that adenosine production is increased in patients who had not received any hormonal therapy. Thus, the increased production of adenosine could be acting as tumor promoting in these PCa patients.

These results are supported by findings from the literature which affirm that CD73 activity is elevated in the presence of certain tumors. Sychala and Kitajewski [46] suggested that the increase in the generation of adenosine by tumors could be correlated with the development of drug resistance and the more aggressive course of the disease. In this context, Baqui et al (2010) [47] investigated compounds that have been found to be relatively potent CD73 inhibitors that may exhibit anticancer effects *in vivo* by reducing the production of adenosine and inhibiting angiogenesis. This is significant since these compounds may be important to obtain potentially novel anticancer drugs.

As we previously described, adenosine, produced by CD73, has many functions that favor tumor development. Furthermore, it may stimulate angiogenesis in hypoxic

solid tumors. This effect results from the fact that it stimulates the proliferation of human endothelial cells and the expression of the vascular endothelial growth factor [13, 48]. In addition, hypoxia, resulting from the tumor presence, may increase the expression of the enzyme which generates adenosine [49].

The results of this study are interesting and have not been reported in the literature before therefore they need to be investigated with more details. It is plausible to suggest that changes in the enzyme activities that metabolize adenine nucleotides may be involved in platelet activation and in tumor development, since NPTDase seems to be involved in controlling platelet aggregation and 5'-nucleotidase appears to be related with the control of adenosine concentrations. The increase in the both nucleotide hydrolysis (ATP and ADP) may be preventing excessive platelet aggregation induced by ADP, while the increased production of adenosine could be acting as tumor promoting in PCa patients. In addition, our results suggest that the PCa stage (Gleason score), bone metastasis, and hormonal therapy have a rather complex influence on nucleotide hydrolysis by platelets. Furthermore, our study demonstrated for the first time that these clinicopathological parameters analyzed may influence the enzyme activities.

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Fig. 1: ATP, ADP, and AMP hydrolysis in the platelets of PCa patients (n = 66) and controls (n = 40). NTPDase is specified by ATP and ADP hydrolysis and 5'nucleotidase by AMP hydrolysis. Each column represents mean \pm S.E.M. Differences are considered significant when $P < 0.05$ (*).

Fig. 2: ATP, ADP, and AMP hydrolysis in the platelets of PCa patients divided by Gleason scale. NTPDase is specified by ATP and ADP hydrolysis and 5'nucleotidase by AMP hydrolysis. Patients, considering Gleason scale, were divided into: Gleason <7 (n = 26), Gleason =7 (n= 25) and Gleason >7 (n=15). Controls consisted of 40 healthy subjects. Each column represents mean \pm S.E.M. Duncan's multiple range tests: groups that show different letters are statistically different ($P < 0.05$).

Fig. 3: ATP, ADP, and AMP hydrolysis in the platelets of PCa patients divided by presence or no of metastasis. NTPDase is specified by ATP and ADP hydrolysis and 5'nucleotidase by AMP hydrolysis. Patients, considering metastasis, were divided into: localized disease (localized, n = 42) and bone metastasis (bone metastasis, n=24). Controls consisted of 40 healthy subjects. Each column represents mean \pm S.E.M. Duncan's multiple range tests: groups that show different letters are statistically different ($P < 0.05$).

Fig. 4: ATP, ADP, and AMP hydrolysis in the platelets of PCa patients divided by treatment. NTPDase is specified by ATP and ADP hydrolysis and 5'nucleotidase by AMP hydrolysis. Patients, considering treatment, were divided into: patients who had not received any therapy preceding the blood sampling (n=14) and patients undergoing hormonal therapy (n=52). Controls consisted of 40 healthy subjects.

Each column represents mean \pm S.E.M. Duncan's multiple range tests: groups that show different letters are statistically different ($P < 0.05$).

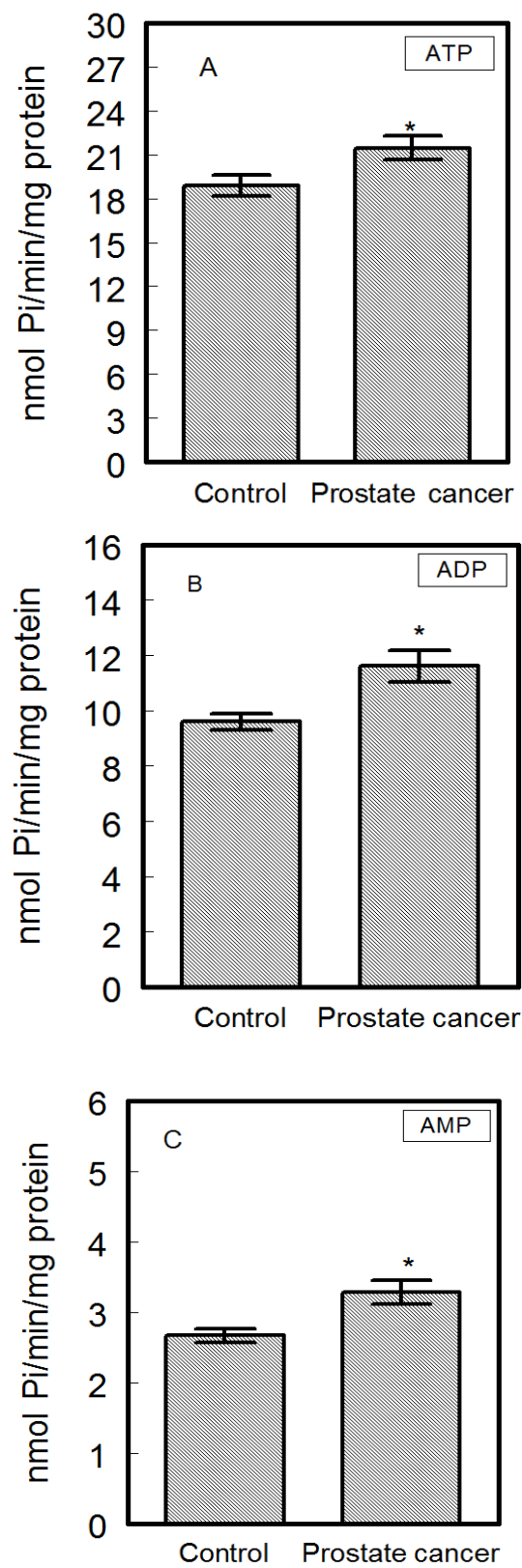


Figure 1

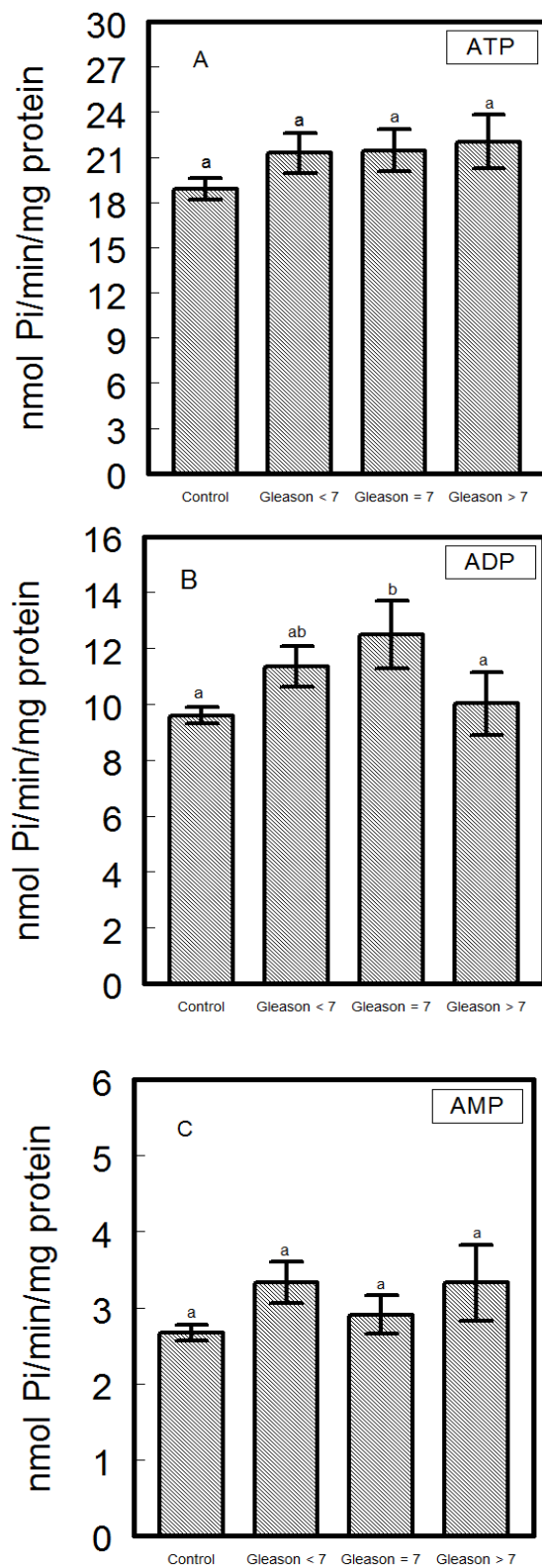


Figure 2

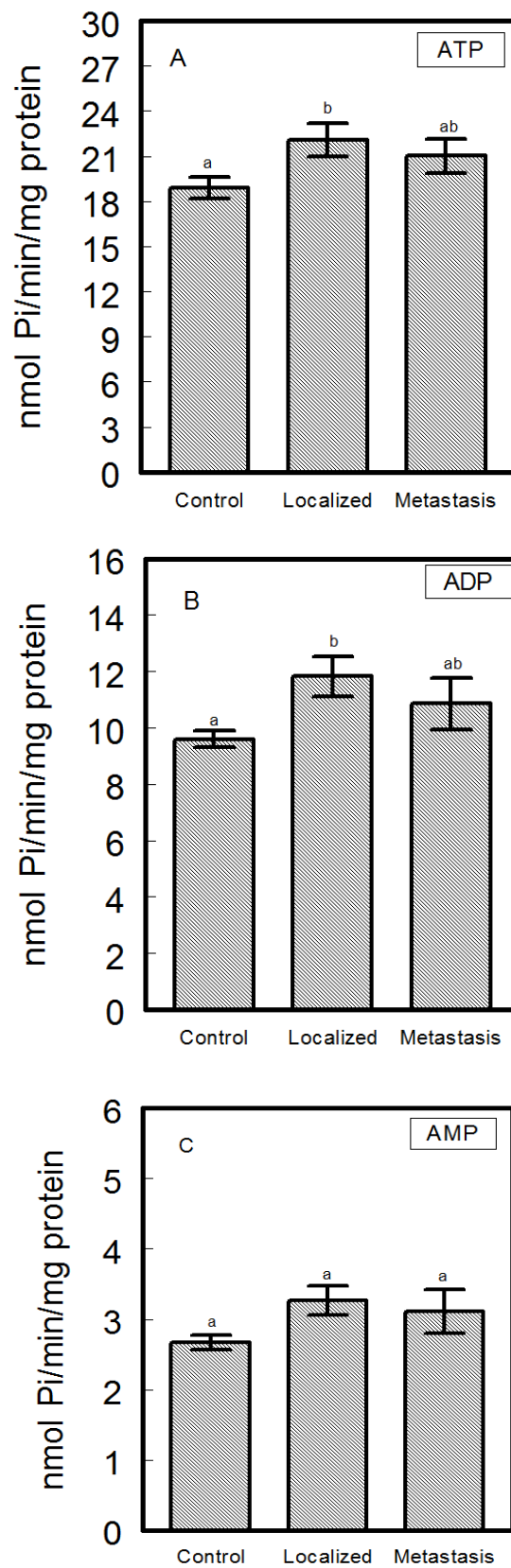


Figure 3

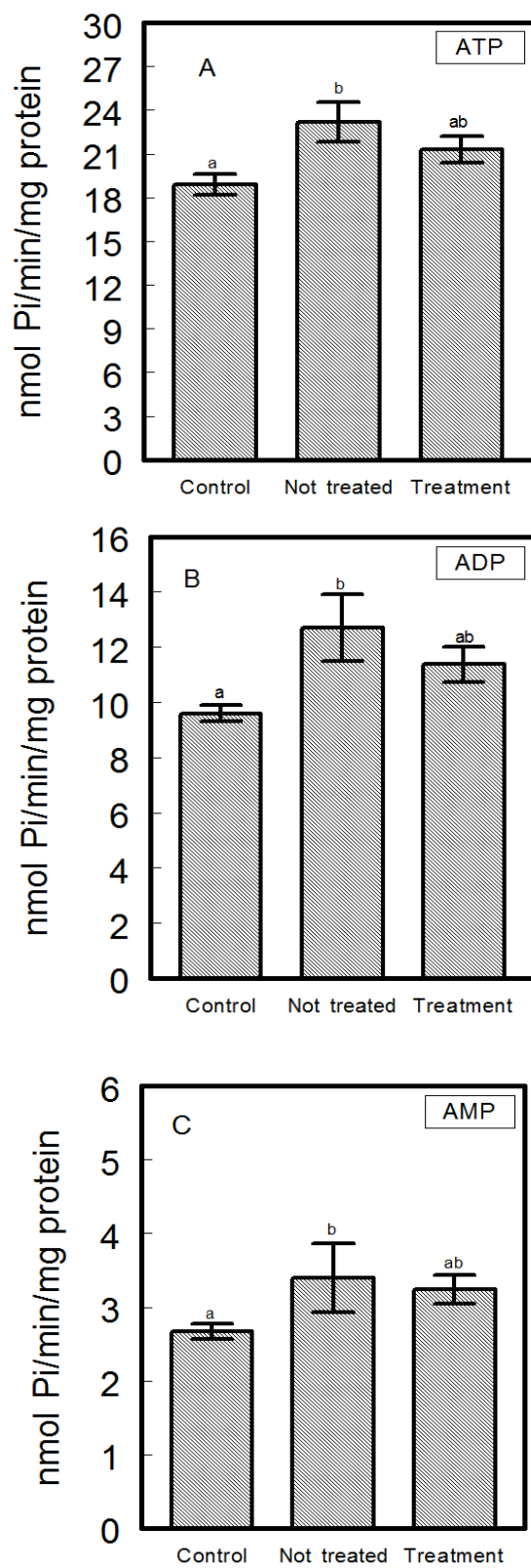


Figure 4

Table 1: Clinical characteristic of prostate cancer patients (PCa)

Variable	Patients
Gleason	< 7 (40%; n= 26)
	= 7 (37%; n=25)
	>7 (23%; n=15)
Age (y)	< 70 years (24%; n=16)
	70-80 years (53%; n=35)
	>80 years (23%; n=15)
Metastasis	Localized disease = 64% (n=42)
	Bone metastasis = 36% (n= 24)
Treatment	Not treated = 21% (n= 14)
	Goserelin acetate = 79% (n= 52)
Family history	Yes = 66% (n= 44)
	No = 34% (n= 22)
Smoker	Yes = 64% (n= 42)
	No = 36% (n= 24)
Alcohol	Yes = 42% (n= 28)
	No = 58% (n= 38)

Table 2: Platelet aggregation profile.

Groups	Agonist		
	ADP (2.5 μ M)	ADP (5 μ M)	ADP (7.5 μ M)
Control	75.42 \pm 1.83	75.57 \pm 2.52	74.14 \pm 2.50
Prostate Cancer	67.71 \pm 1.98 ^a	69.34 \pm 1.94	67.50 \pm 1.50 ^a
Gleason			
< 7	69.54 \pm 3.71	71.00 \pm 2.64	67.81 \pm 2.15
= 7	66.53 \pm 2.98	70.15 \pm 3.30	68.69 \pm 2.71
>7	67.12 \pm 4.08	65.75 \pm 4.49	65.12 \pm 3.09
Metastasis			
Yes	67.66 \pm 3.42	67.58 \pm 3.68	66.33 \pm 2.68
No	67.75 \pm 2.48	70.40 \pm 2.22	68.20 \pm 1.83
Treatment			
Yes	64.22 \pm 2.00 ^a	67.13 \pm 2.51 ^a	65.81 \pm 1.96 ^a
No	75.40 \pm 3.63	74.20 \pm 2.32	71.20 \pm 1.78

Platelet aggregation was evaluated by using ADP at concentrations of 2.5, 5 and 7.5 μ M as agonist. The results are expressed as percentage of aggregation. Data were analyzed statistically by one-way ANOVA, followed by Duncan's multiple range test.

^a Significantly reduced related to control ($P < 0.05$, $n = 20$).

4.3 Manuscrito 2

Atividades das enzimas E- NPP e ADA em pacientes com câncer de próstata:
Influência da escala de Gleason, tratamento e metástase óssea

Ectonucleotide Pyrophosphatase/ Phosphodiesterase (E-NPP) and Adenosine
Deaminase (ADA) activities in prostate cancer patients: Influence of Gleason score,
treatment and bone metastasis

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Ectonucleotide Pyrophosphatase/ Phosphodiesterase (E-NPP) and Adenosine Deaminase (ADA) activities in prostate cancer patients: Influence of Gleason score, treatment and bone metastasis

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Abstract

Objectives: The relation between adenine nucleotides and cancer has already been described in literature. Considering that the enzymes ectonucleotide pyrophosphatase/ phosphodiesterase (E-NPP) and adenosine deaminase (ADA) act together to control nucleotide levels, we aimed to investigate the role of these enzymes in prostate cancer (PCa) patients.

Design and methods: E-NPP and ADA activities were determined in serum and platelets of PCa patients and controls. We also verified the influence of the Gleason score, bone metastasis and treatment in the enzyme activities.

Results: Platelets and serum E-NPP activity increased, whereas ADA activity in serum decreased in PCa patients. In addition, Gleason score, metastasis and treatment influenced E-NPP and ADA activities.

Conclusions: We may propose that E-NPP and ADA are involved in the development of PCa. Moreover, E-NPP and ADA activities are modified in PCa patients with distinct Gleason score, with bone metastasis, as well as in patients under treatment.

Keywords: Prostate cancer, ectonucleotide pyrophosphatase/ phosphodiesterase, adenosine deaminase

1. Introduction

Prostate cancer (PCa) is the most common non-cutaneous cancer in men and the second most common form of cancer death [1]. The factors that determine the risk of developing clinical prostate cancer and particularly the risk of developing aggressive disease are not well characterized [2]. The mechanism of prostate carcinogenesis involves a combination of both acquired factors and inherited genetic predisposition, a concept supported by extensive evidences [3, 4]. The greatest known risk factor is age, with a very low risk of developing the disease in men under 50 and rising risk with increasing age thereafter; nearly 65% of cases occur in men aged 65 and older [5]. The morphological appearance of cancer cells reflecting distinct degrees of differentiation was elegantly analyzed and classified by Gleason [6, 7] into the currently used standard grading system, which is based on the degree of glandular differentiation and the interaction of the malignant glands with stroma [8].

Although great advances have been made in the treatment of PCa in recent years, questions still remain concerning the optimal treatment strategy for both localized disease and metastatic disease (mainly bone metastases). This lack of a standard treatment may be partly attributed to variations in the risk of disease progression among patients [9]. A vast majority (90%) of PCa deaths occurs in patients with bone metastases [10]. Men with advanced PCa are at high risk of developing bone metastases resulting in clinically significant skeletal morbidity and severe bone pain.

Extracellular adenine nucleotides and nucleosides act as signaling molecules involved in a wide spectrum of biological effects [11]. A variety of pathophysiological responses to nucleotides have been described, including roles in cell proliferation and growth [12] as well as induction of apoptosis [13]. The control of circulating

nucleotide levels is important in the maintenance of the physiological nucleotide-mediated signaling process. This control is exerted by a family of enzymes that hydrolyzes such nucleotides and consequently generates their respective metabolites [14]. These ecto-enzymes include the ecto-nucleoside triphosphate diphosphohydrolase (Ecto-NTPDase) and ectonucleotide pyrophosphatase/phosphodiesterase (E-NPP) families as well as 5'-nucleotidase and adenosine deaminase (ADA) [15].

E-NPP (EC 3.1.4.1) is responsible for hydrolyzing 5'- phosphodiester bonds in nucleotides and their derivatives, where both purines and pyrimidines serve as substrates, resulting in the production of nucleotide monophosphate [16]. E-NPP hydrolyzes a broad range of substrates and influences many physiological processes. Dysfunctions of E-NPP have been shown to be involved in the pathophysiology of several diseases, including cancer. Aberrant expressions of E-NPP have been demonstrated in several conditions including cell motility and migration, angiogenesis and tumor cell invasion [17].

Adenosine, which is a metabolite of adenine nucleotides, plays important roles that depend on the type of receptor in each tissue and on the origin of the damage. Adenosine presents tumor promoting functions [18] by stimulating angiogenesis and cytoprotection as well as by reducing hypoxia through vasodilatation.

Adenosine deaminase (ADA, E.C 3.5.4.4) catalyses the deamination of adenosine and deoxyadenosine into inosine and deoxyinosine [19]. Due to the irreversibility of the reaction catalyzed by ADA, the reaction appears to be one of the rate-limiting steps in adenosine degradation [20]. In several studies, ADA activities have presented controversial results regarding cancerous tissues and cells [21-23].

Considering the fact that adenosine and adenine nucleotides play an important role in the physiology of cancer and both ADA and E-NPP are altered in many physiological processes, the aim of this study was to investigate the ADA and E-NPP activities in serum and platelets of patients with prostate cancer. We also aimed to evaluate the possible relationship or influence of Gleason score, metastases and treatment in these enzyme activities.

2. Materials and methods

2.1. Selection of the patients

The sample consisted of 68 PCa patients under treatment at the Oncology-Hematology Laboratory of the Hospital of the Federal University of Santa Maria. The controls consisted of 40 healthy volunteers with ages and social conditions similar to those of the patients. The average age of controls was 71.46 ± 7.61 (mean \pm SD) years and the average age of PCa patients was 72.57 ± 8.76 (mean \pm SD). The controls presented no acute or chronic diseases such as diabetes, parasitosis or immune dysfunction. Also, the controls studied here were not under any pharmacological therapy.

Patients included in this study were diagnosed with PCa based on histological evaluation (positive biopsy results). For purposes of analysis, patients with PCa were divided into different groups considering 3 distinct parameters: presence or absence of metastasis, standard treatment or without any type of treatment and Gleason score. Consent was given by family members of all patients included in this work. The Human Ethics Committee from the Federal University of Santa Maria approved the protocol under number 23081.09047/2008-47.

2.2. Platelet isolation

The platelet rich plasma was prepared according to Pilla et al., [24] with the following minor modifications. In short, the blood was collected into 0.129 M citrated vacutainer tubes and centrifuged at 500 rpm for 10 min. After this, the platelet rich plasma was centrifuged at 3700 rpm for 30 min and washed twice with 3.5 mM HEPES buffer, pH 7.0, which contained 142 mM NaCl, 2.5 mM KCl and 5.5 mM glucose. The platelet pellets were resuspended in HEPES buffer and used to determine E-NPP and ADA activities.

Soon after the separation of the platelets, the cell viability was determined by measuring the activity of the enzyme lactate dehydrogenase (LDH) present in the sample, using the labtest kit (Labtest, Lagoa Santa MG, Brasil). The procedure was repeated before and after the incubation period and samples with more than 5% of disrupted cells were excluded.

2.3. Ecto-NPP activity determination—measurement of p-Nph-5'-TMP hydrolysis in platelets and serum

The ectonucleotide pyrophosphatase/phosphodiesterase (ENPP) activity, from platelets, was assessed using p-nitrophenyl 5'-thymidine monophosphate (p-Nph-5'-TMP) as substrate as described by Fürstenau et al. [25]. The reaction medium containing 50 mM Tris-HCl buffer, 120 mM NaCl, 5.0 mM KCl, 60 mM glucose, 5.0 mM CaCl₂, pH 8.9, was preincubated with approximately 20 µg per tube of platelet protein for 10 min at 37 °C in a final volume of 200 µL.

The enzyme reaction was started by the addition of p-Nph-5'-TMP to a final concentration of 0.5 mM. After 80 min of incubation, 200 µL NaOH 0.2 N was added to the medium to stop the reaction. The amount of p-nitrophenol released from the

substrate was measured at 400 nm using a molar extinction coefficient of $18.8 \times 10^{-3} \text{ M/cm}$. The enzymatic reaction, for serum samples, was determined as described by Sakura et al. 1998 [26]. The reaction medium containing p-Nph-5'-TMP as substrate (at a final concentration of 0.5 mM) in 100 mM Tris-HCl PH 8.9 was incubated with 1 mg of serum protein at 37 °C for 5 min in a final volume of 200 μL and the reaction was stopped by 200 μL of NaOH 0.2 N. Controls to correct nonenzymatic substrate hydrolysis were performed by adding platelet preparations and serum after the reaction had been stopped with NaOH as described for platelets. All samples were performed in triplicate.

Enzyme activities were generally expressed as nanomol of p-nitrophenol released per minute per milligram of protein (nmol p-nitrophenol released/min/mg protein).

2.4. Adenosine deaminase determination

Adenosine deaminase (ADA), both in serum and platelets, was determined according to Guisti and Galanti [27]. Briefly, 50 μL of serum or platelets reacted with 21 mmol/L of adenosine pH 6.5 and was incubated at 37 °C for 60 min. This method is based on the direct production of ammonia when ADA acts in excess of adenosine. The protein content used for the platelet experiment was adjusted to 0.7–0.9 mg/mL. This concentration was chosen after a pilot experiment in which we used several protein concentrations. This range gave the best results, compared with ADA activity found in the erythrocytes.

Results were expressed in units per liter (U/L). One unit (1 U) of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia per minute from adenosine at standard assay conditions.

2.5. Protein determination

Protein content was determined according to Bradford (1976) [28], using bovine serum albumin as standard.

2.6. Statistical analysis

Statistical analysis was performed with the commercial SPSS package for Windows©. All data are expressed as the mean \pm standard error. The distribution of all dependent variables was examined by the Shapiro-Wilk test and was found not to differ significantly from normal. Data were analyzed statistically by one-way ANOVA followed by the Duncan's multiple test. Differences were considered significant when the probability was $P < 0.05$.

3. Results

The results described here were compared using the one-way ANOVA test followed by Duncan's post-hoc comparisons. LDH revealed that nearly 5% of the platelets were disrupted indicating that the sample was predominantly intact (data not shown). E-NPP activity from serum (Fig. 1A) and platelets (Fig. 1B) was significantly increased in PCa patients when compared with the control group. On the other hand, ADA activity in serum (Fig. 1C) was significantly decreased in PCa patients compared with the control group. For the ADA activity in platelets, no significant changes were observed (Fig. 1D).

Considering Gleason score, E-NPP activity in serum (Fig. 2A) was significantly increased only in the group with Gleason score lower than 7 in relation to the other groups. However, E-NPP activity in the platelets (Fig. 2B) was not altered by different

Gleason scores. ADA activity from platelets (Fig. 2C) and serum (Fig 2D) was significantly decreased in patients with Gleason score higher than 7 in relation to the control group (serum activity) and the other groups (platelet activity).

Figure 3 shows a comparison of patients with localized PCa and bone metastatic disease with the control group. Post-hoc comparisons made by Duncan's test revealed that the E-NPP activity in serum (Fig. 3A) was increased in the patients with localized disease in relation to the control group. ADA activity in serum (Fig. 3C) of patients with bone metastases was significantly decreased when compared with the control group. Nevertheless, for the ADA and E-NPP activities in platelets (Fig. 3B and D), there was no significant difference between the groups.

E-NPP activity in the serum (Fig. 4A) was increased in the group of patients who had not received any therapy in relation to the control group. Post-hoc comparisons made by Duncan's test revealed that the serum ADA activity (Fig. 4C) was decreased in the group of patients who had not received any therapy preceding the blood sampling when compared to the control group. However, no significant changes for ADA and E-NPP activities in platelets (Fig 4B and D) were observed.

4. Discussion

In recent years, significant efforts have been made to understand the biological and molecular mechanisms involved in the PCa development and progression. Cancer cells are known to have alterations in multiple cellular signaling pathways [29]. Extracellular nucleotides, particularly ATP and adenosine, produce responses in diverse biological processes, such as apoptosis and control of cell proliferation and differentiation [30]. Nucleotides have been shown to affect the proliferation of different cell types, although the results are not always clear and the

mechanisms may be complex and indirect, depending on the cell type and the presence of ecto-nucleotidases [18]. These enzymes include members of the E-NPP and ADA families which hydrolyze ATP into ADP, AMP and adenosine [31].

E-NPP has been detected in almost all tissues [32], although individual isoforms are usually restricted to specific substructures and/or cell types. In this study, E-NPP evaluated in PCa patients was visibly altered. E-NPP is frequently altered by pathological situations and its activity may become a useful tool to evaluate the disease progression [15]. Such activity has been found to be increased in normal pregnancy and in some types of cancer and cholestatic liver disease [33, 34]. Our results demonstrate that E-NPP activity in serum and platelets was significantly increased in PCa patients in relation to the control, possibly as a consequence of the enhanced nucleotide degradation caused by the tumor presence, generating more AMP. On the other hand, we may suggest a compensatory mechanism in attempting to inhibit tumor growth, since studies have showed that AMP has an inhibitory effect on the growth of breast cancer cells [35].

Aiming to elucidate the involvement of nucleotide degradation in PCa, we analyzed the activity of E-NPP in patients divided in groups considering some important factors. Taking into account parameters such Gleason score, metastasis and treatment in PCa patients, we could observe interesting and unexpected results. Regarding the Gleason score, the E-NPP activity in serum was increased only in patients with Gleason score lower than 7. In the groups of patients with localized disease and in the patients who had not received any treatment, an increase in E-NPP activity in serum was also observed. In these groups, we observed enhanced nucleotide degradation consequently a lower concentration of ATP is available. We may suggest that the increase observed in E-NPP activity is not due to the hormonal

therapy used by the PCa patients. Also, these findings support the argument that it is the pathological condition that generates the increase in this enzyme activity.

In addition, we can suggest that extracellular ATP participates in the regulation of cell migration and may have effects in PCa. Zhang et. al (2010) [36] showed that ATP promoted cell migration of PCa cells. They suggest that extracellular ATP promoted invasion of PCa cells by increasing the motility of cells and the expression of Matrix metalloproteinases, which is from the family of enzymes closely related to tumor invasion. However, in our study, E-NPP activity in platelets was not altered by Gleason scores, metastasis and treatment. Therefore, further studies are suggested to elucidate the relationship between E-NPP and the parameters studied.

Alterations in total ADA activity have been largely described in patients with malignant diseases, although the results are still contradictory [15]. Few published clinical studies about the value of ADA activity in PCa patients are available in the literature.

Our results reveal that ADA activity was decreased in the serum of PCa patients. However, ADA activity in platelets was not significantly changed, when PCa patients were compared with controls. As it was demonstrated, the changes in the ADA activity appear to be more sensitive in serum than in platelets. On the other hand, decreases in the ADA activity in serum and platelets revealed a significant involvement with prognostic factors, such as Gleason score, metastasis and treatment.

ADA contributes to the regulation of intra- and extracellular adenosine concentrations by the conversion into inosine, and the irreversibility of the reaction makes it the limiting step in adenosine degradation [37]. Adenosine accumulates at high concentrations in solid tumors, exerting a range of malignancy-promoting effects

such as protection against ischemia, stimulation of growth and angiogenesis, as well as inhibition of cytokine synthesis. Some authors therefore conclude that adenosine is a major factor promoting tumor growth [18].

Our results are in agreement with other authors that showed low ADA activity values in tumor tissues or lymphocytes from cancer patients [22, 23, 38]. On the other hand, in some studies ADA activity was found to be increased in cancerous tissue, suggesting that increased ADA activity may be a compensatory mechanism against toxic accumulation of its substrates [39]. In order to explain the discrepancy, further investigation is required.

Most of the studies found in the literature have evaluated ADA activity in serum and cells such as lymphocytes, monocytes and erythrocytes, but none until now had determined ADA activity in platelets. Our present study determined E-NPP and ADA activities in platelets and serum of patients, as well as verified whether clinicopathological parameters may influence these enzymatic activities in PCa. Interestingly, we have observed a decrease in the ADA activities in platelets and serum in the cancer group with a Gleason score greater than 7. Furthermore, these findings indicate that advanced PCa is associated with a low ADA activity and consequently high adenosine concentration.

When the patients were divided considering the presence or absence of the metastasis, a reduction in the ADA activity only in patients with metastasis was observed. These results are interesting and support the idea that adenosine may be involved in the process of metastasis. Moreover, our results showed that patients who did not receive any previous treatment presented the ADA activity in serum diminished. This fact probably contributes to the increased availability of adenosine in this group. This suggestion is based on the properties of adenosine as a tumor

facilitating agent, since this nucleoside causes induction of vasodilatation, neovasculogenesis and the reduction of hypoxia and inflammation [14, 18].

5. Conclusions:

The experimental data showed in this work strongly support the hypothesis that extracellular nucleotide hydrolysis has an important role in PCa. In addition, we may propose that ATP and adenosine may have tumor-promoting activities. An interesting and important aspect is that clinicopathological parameters analyzed in PCa patients based on Gleason score, metastasis and treatment may influence E-NPP and ADA activities.

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Fig. 1: E-NPP activity in serum (A) and platelets (B) and ADA activity in serum (C) and platelets (D) of PCa patients and controls. Each column represents mean \pm S.E. Differences are considered significant when $p < 0.05$ (*).

Fig. 2: E-NPP activity in serum (A) and platelets (B) and ADA activity in serum (C) and platelets (D) of PCa patients divided by Gleason score. Patients, considering Gleason score, were divided into: Gleason lower than 7 (<7 , $n = 25$), Gleason equal to 7 ($=7$, $n = 25$) and Gleason higher than 7 (>7 , $n=18$). Controls consisted of 40 healthy subjects. Each column represents mean \pm S.E. Duncan's multiple range tests: groups that show different letters are statistically different ($p < 0.05$).

Fig. 3: E-NPP activity in serum (A) and platelets (B) and ADA activity in serum (C) and platelets (D) of PCa patients divided by presence or no of metastasis. Patients, considering metastasis, were divided into: localized disease ($n = 44$) and bone metastasis ($n=24$). Controls consisted of 40 healthy subjects. Each column represents mean \pm S.D. Duncan's multiple range tests: groups that show different letters are statistically different ($p < 0.05$).

Fig. 4: E-NPP activity in serum (A) and platelets (B) and ADA activity in serum (A) and platelets (B) of PCa patients divided by treatment. Patients, considering treatment, were divided into: patients who had not received any therapy preceding the blood sampling (no treatment, $n=13$) and patients undergoing treatment (treatment, $n=55$). Controls consisted of 40 healthy subjects. Each column represents mean \pm S.D. Duncan's multiple range tests: groups that show different letters are statistically different ($p < 0.05$).

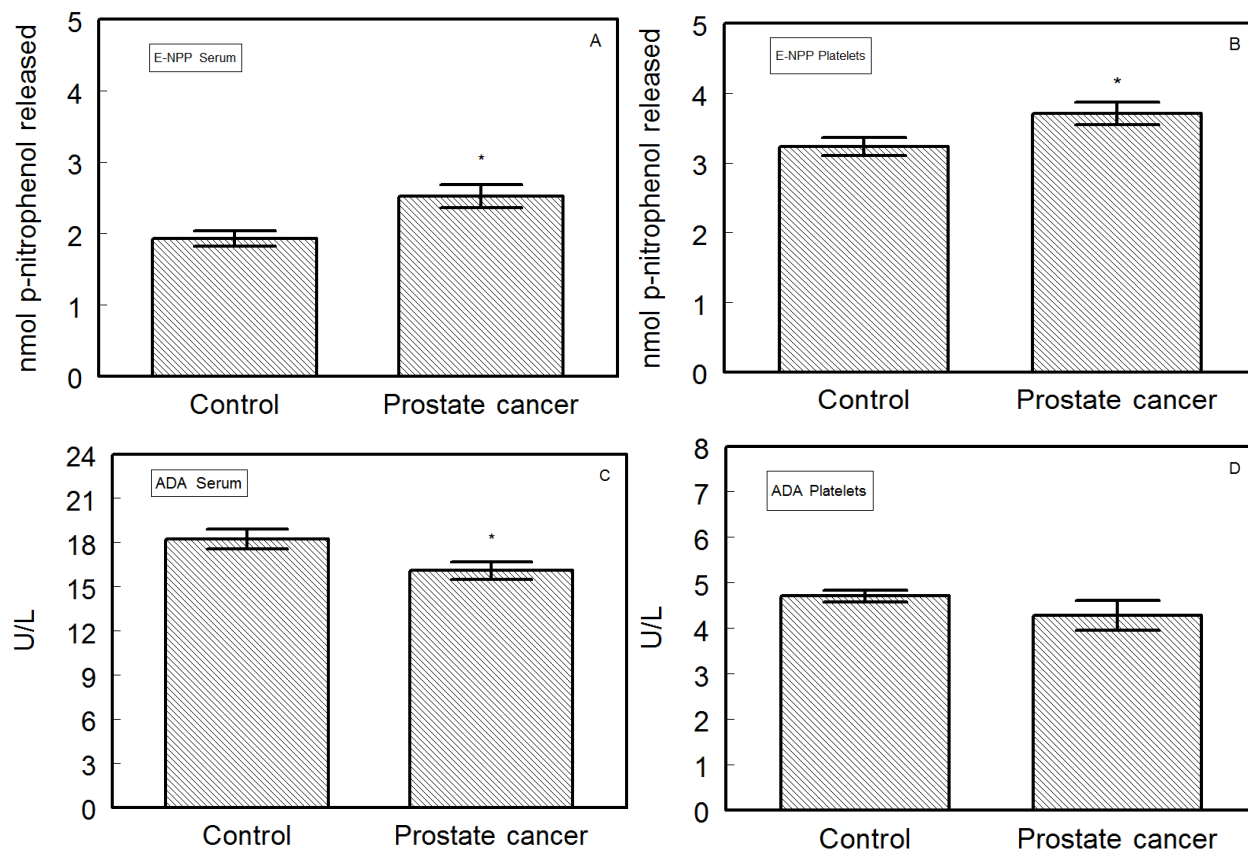


Figure 1

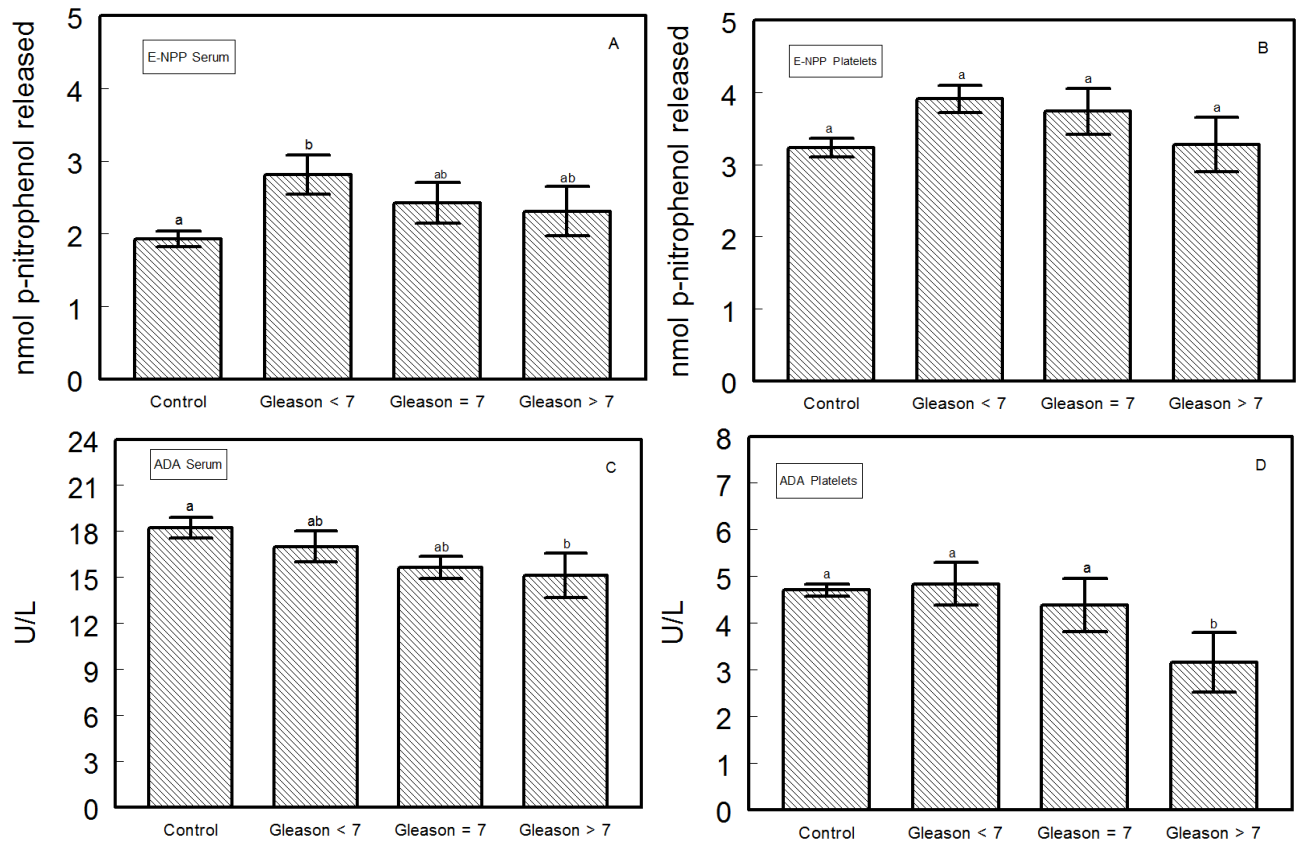


Figure 2

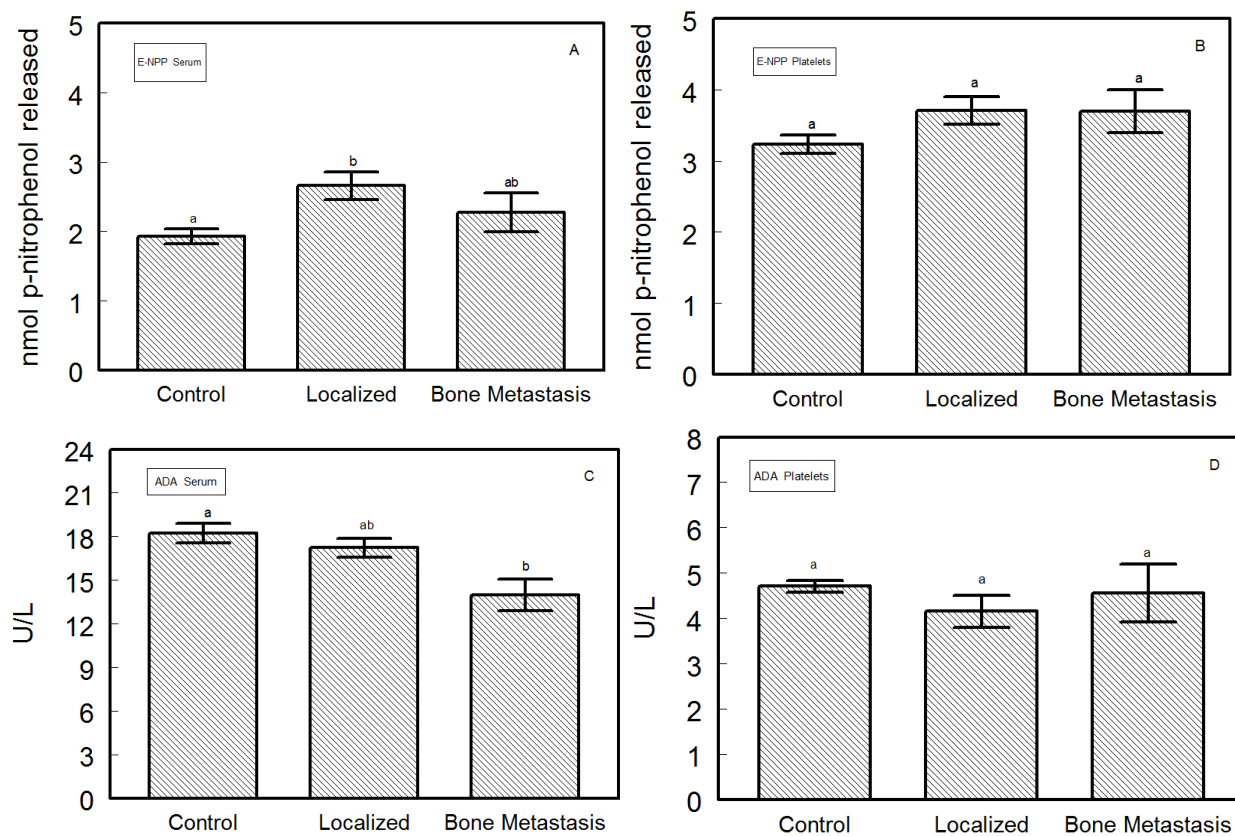


Figure 3

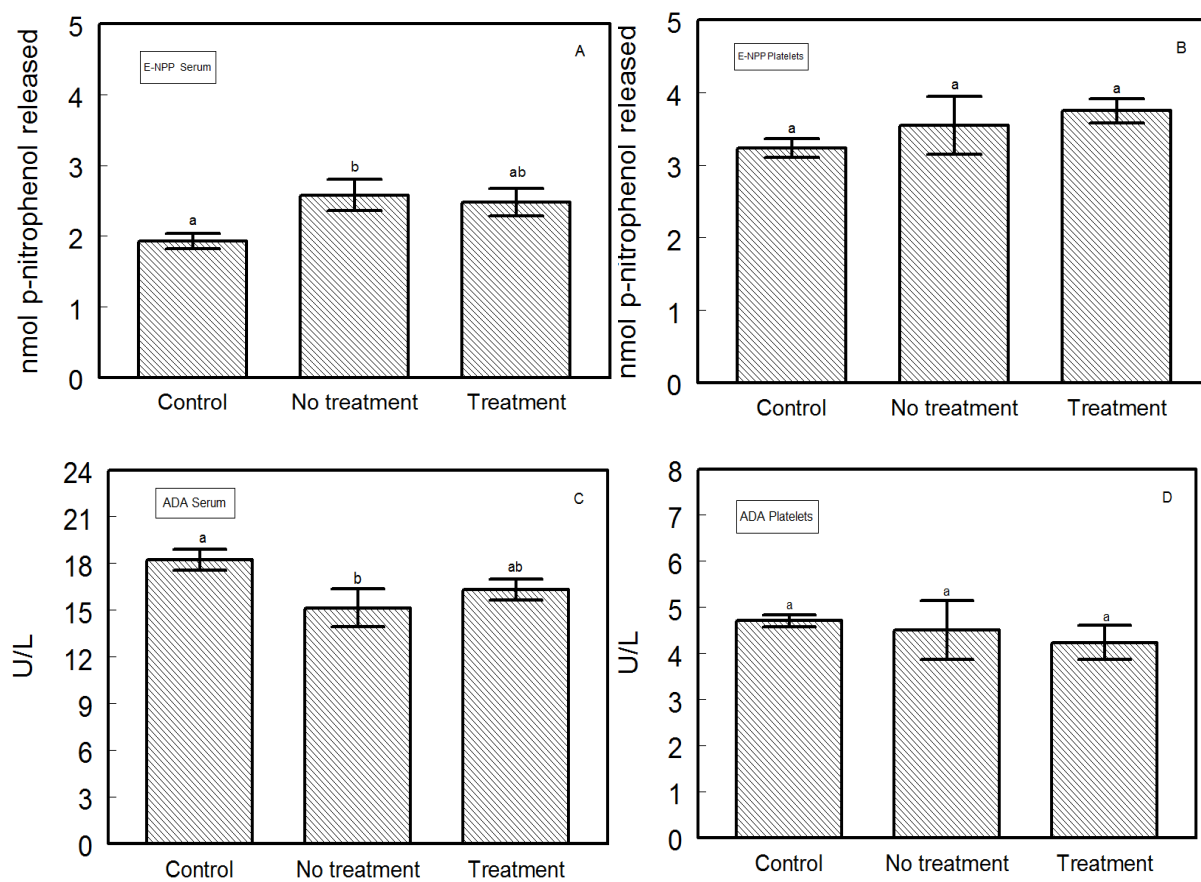


Figure 4

4.4. Artigo 2

Atividade das enzimas colinesterases em pacientes com câncer de próstata:
Influência da escala de Gleason, tratamento e metástase óssea

Cholinesterase activities in patients with prostate cancer: influence of Gleason score,
treatment and bone metastasis

Vanessa Battisti^a, Margarete D. Bagatini^b, Liési D.K. Maders^a, Juarez Chiesa^c, Karen F. Santos^a, Jamile F. Gonçalves^d, Fátima H. Abdalla^a, Iara E. Battisti^e, Maria R.C. Schetinger^a, Vera M. Morsch

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Original article

Cholinesterase activities and biochemical determinations in patients with prostate cancer: Influence of Gleason score, treatment and bone metastasis

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ABSTRACT

Prostate cancer (PCa) is the sixth most common type of cancer worldwide. Cholinesterase is well known as having non-cholinergic functions such as cellular proliferation and differentiation, suggesting a possible influence of cholinesterase in tumorigenesis. Thus, the aim of this study was to investigate the whole blood acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BChE) activities and some biochemical parameters in PCa patients. This study was performed in 66 PCa patients and 40 control subjects. AChE and BChE activities were determined in PCa patients and the influence of the Gleason score; bone metastasis and treatment in the enzyme activities were also verified. Furthermore, we also analyzed possible biochemical alterations in these patients. AChE and BChE activities decreased in PCa patients in relation to the control group and various biochemical changes were observed in these patients. Moreover, Gleason score, metastasis and treatment influenced cholinesterase activities and biochemical determinations. Our results suggest that cholinesterase activities and biochemical parameters are altered in PCa. These facts support the idea that the drop in the cholinesterase activity and the consequent increased amount of acetylcholine could lead to a cholinergic overstimulation and increase the cell proliferation in PCa.

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1. Introduction

Prostate cancer (PCa) is an important cause of mortality and morbidity worldwide and it is the most common noncutaneous malignancy in men [1]. The standard prognostic approach to PCa patients includes some clinical and pathological characteristics, such as Gleason score, presence of metastasis and response to hormonal therapy. PCa is a major age-related malignancy with most incidences occurring between 54 and 75 years [2]. The histologic differentiation grade is the strongest prognosticator of PCa. The morphological appearance of cancer cells reflecting distinct degrees of differentiation was elegantly analyzed and classified by Gleason [3,4] into the currently used standard grading system, which is based on the degree of glandular differentiation and the interaction of the malignant glands with stroma.

Little is known about the risk factors for PCa progression [5]. Family history of PCa is a well-established risk factor for PCa risk in men. Several prospective [6] and retrospective [7] studies have

reported two to four fold increased risk of PCa in men with a positive family history of PCa especially in a first-degree relative. Many other factors have been studied for their potential role as risk factors for PCa, but the evidence for or against each have been contradictory. These factors have included alcohol use [8], tobacco use [9] and occupation [10]. A large number of studies have assessed the risk of PCa in various occupational groups. There are some hints that occupational groups potentially exposed to pesticides, which include farmers might experience excess risks [10].

Although great advances have been made in the treatment of PCa in recent years, questions still remain concerning the optimal treatment strategy for both localized disease and metastatic disease (mainly bone metastasis). The lack of a standard treatment approach may be partly attributed to variations in the risk of disease progression among patients [11]. Several different hormonal therapies, which act by different mechanisms, are available. Luteinizing hormone-releasing hormone (LHRH) agonists such as goserelin down regulate the pituitary LHRH receptors suppressing the luteinizing hormone (LH) and testosterone secretion [12]. The introduction of LHRH agonists revolutionized the treatment of advanced PCa [13].

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Increasingly, evidence supports the involvement of cholinesterases in nonclassic functions [14,15], such as cellular proliferation and differentiation [16], suggesting a possible influence of cholinesterases in tumorigenesis. Indeed, either the cholinesterase genes are structurally altered or their products are aberrantly expressed in a variety of tumor types [17–19]. Butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) catalyze the hydrolysis of choline esters. BChE is abundant in liver and plasma [20]. It efficiently hydrolyses butyrylcholine and has a major role in the metabolism of some clinically important anesthetic agents. AChE is found in many tissues, including erythrocytes, nerve endings, lungs, spleen, and brain [21]. It has relative specificity to the neurotransmitter acetylcholine, playing a crucial role in the nervous system function. Although the function of “non-neuronal cholinergic system” is not yet clarified, it appears to intervene in important cell processes such as proliferation, differentiation, apoptosis and cell–cell recognition [22].

In patients with PCa, various biological (family history), biochemical (lactate dehydrogenase, hepatic function tests, renal function tests, electrolyte balance, albumine), and other prognostic factors have been proposed, including tobacco use, alcohol use, the extent of disease (metastatic or localized), Gleason score and response to hormonal therapy. However, the real prognostic value of such factors has not been fully defined [23].

Acute renal failure can be one of the many complications associated with malignancy [24] and the monitoring of urea, uric acid and creatinine levels are important in PCa patients. Similarly, the hepatic function can be altered in PCa and the dosage of hepatic function tests as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Gamma-glutamyltransferase (GGT) is necessary. Elevated lactate dehydrogenase (LDH) levels are consistently reported as a prognostic factor for poor survival in PCa [25], and the total serum ALP partly reflects osteoblastic activity, which is likely to be more pronounced in patients with larger volume or aggressive bony metastatic disease [26]. Furthermore, there is some evidence to use GGT as an indicator of cancer risk [27]. On the other hand, the electrolyte imbalance in cancer patients can be induced by various factors. Thus, it is important to verify the ion levels in the serum of PCa patients. Finally, associations between albumin concentrations and mortality have been widely reported in patients with various diseases, including cancer [28].

Recently, the role of cholinesterases was investigated in studies carried out in our laboratory with acute lymphoblastic leukemia [29] and in hypertensive and ischemic patients [30], but information about the activities of these enzymes have been conflicting in patients with PCa. Then, in attempt to investigate the potential role of AChE and BChE in the etiology of PCa, as well as the possible interference of different parameters in the enzyme activities, we examined the influence of Gleason score, bone metastasis and hormonal therapy on plasma BChE and total blood AChE activities, two enzymes involved in biological functions, such as proliferation, and cellular differentiation. Furthermore, we also verified various biochemical (LDH, hepatic function tests, renal function tests, electrolyte balance, albumine), and other prognostic factors (tobacco use, alcohol use, the extent of disease, Gleason score and response to hormonal therapy) in PCa patients.

2. Patients and methods

2.1. Patients

The sample consisted of 66 PCa patients aged 72.43 ± 5.89 years from the Oncology-Hematology Laboratory of the Hospital of the Federal University of Santa Maria. Patients included in this study were diagnosed with PCa based on histological evaluation (positive biopsy

results). For purposes of analysis, patients with PCa were further divided into different groups for each of three distinct parameters: metastasis, hormonal therapy with goserelin or cyproterone acetate and Gleason score. Consent was given by family members of all the patients included in this work.

The control group consisted of 40 individuals aged 71.40 ± 3.64 years, who presented normal blood pressure, were free from diabetes mellitus, alcoholism, cigarette smoking, chronic diseases, and had not been submitted to any pharmacological therapy. All subjects gave written informed consent to participate in the study. The protocol was approved by the Human Ethics Committee of the Health Science Center from the Federal University of Santa Maria, protocol number 23081.09047/2008-47. Ten milliliters of blood was obtained from each patient and used for platelet-rich plasma preparation and biochemical determinations. The same procedure was carried out for the control group. Patient general characteristics are shown in Table 1.

2.2. Chemicals

Acetylthiocholine iodide (ASCh), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB, Ellman's reagent) and Triton X-100 were obtained from Sigma (Deisenhofen, Germany), ethopropazine hydrochloride from Aldrich (Steinheim, Germany). All the other reagents used in the experiments were of analytical grade and of the highest purity.

2.3. Sample preparation

The blood was collected in vacutainer tubes using EDTA as anticoagulant. For AChE activity in whole blood, the samples were hemolyzed with phosphate buffer 0.1 M, pH 7.4 containing Triton X-100 (0.03%) and stored at -20°C for 1 week. For BChE activity the plasma was separated and stored at -20°C to posterior analyses. The blood was collected in vacutainer tubes without an anticoagulant system and centrifuged at 5000 rpm for 10 minutes. The precipitate was discarded and the serum was used to biochemical determinations.

2.4. Biochemical determinations

A blood sample was obtained and serum was used to the determination of LDH, hepatic function tests, renal function tests, electrolyte balance and albumin. AST, ALT, ALP and GGT were measured for the hepatic function testing. Urea, uric acid and creatinine were used to determine the renal function.

Table 1
Characteristics of patients.

Variable	Patients
Gleason	< 7 (40%) = 7 (37%) > 7 (23%)
Age (years)	< 70 (24%) 70–80 (53%) > 80 (23%)
Metastasis	Localized disease = 64% Bone metastasis = 36%
Treatment	Not treated = 17% Goserelin A. = 68% Cyproterone A. = 15%
Family history	Yes = 66% No = 34%
Smoker	Yes = 64% No = 36%
Alcohol	Yes = 42% No = 58%
Occupation	Farmer = 42% Others = 58%

All measurements were performed using standard methods on Cobas MIRA[®] (Roche Diagnostics, Basel, Switzerland) automated analyzer.

2.5. Whole blood acetylcholinesterase and plasma butyrylcholinesterase activities

Whole blood AChE activity was determined by the method of Elmam et al. [31] modified by Worek et al. [32]. To achieve temperature equilibration and complete reaction of sample matrix sulfhydryl groups with DTNB, the mixture was incubated for 10 minutes prior to addition of substrate. Enzyme activity was corrected for spontaneous hydrolysis of the substrate and DTNB degradation. The butyrylcholinesterase (BChE EC 3.1.1.8) was inhibited by ethopropazine. AChE activity was measured at 436 nm and 37 °C using polystyrene cuvetts. The activity of whole blood AChE was calculated from the quotient between AChE activity and protein content and the results are expressed as $\mu\text{mol/h/mg}$ of protein. The same method was used for the determination of BChE activity in the plasma, except that the acetylcholine substrate was replaced by butyrylthiocholine and the results were expressed in the $\mu\text{moles BcSch/h/mg}$ of protein.

2.6. Protein determination

Protein was determined by the Coomassie blue method according to Bradford [33] using bovine serum albumin as standard.

2.7. Statistical analysis

Data are presented as mean \pm standard error. The distribution of all dependent variables was examined by the Shapiro-Wilk test and

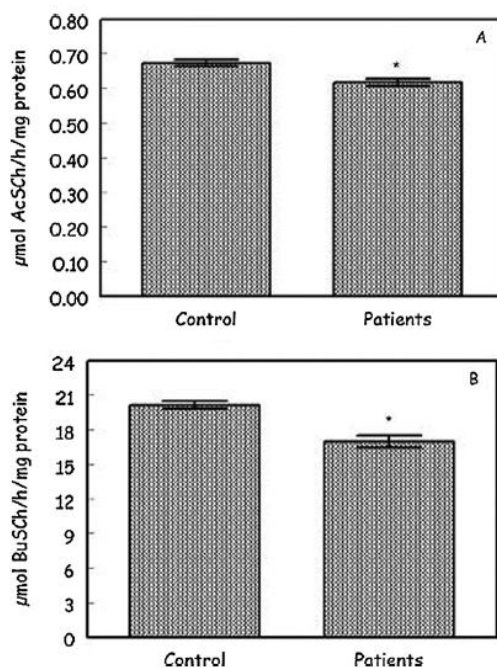


Fig. 1. Acetylcholinesterase (A) and butyrylcholinesterase (B) activities of prostate cancer patients ($n = 66$) and controls ($n = 40$). Each column represents mean \pm S.E. Differences are considered significant when $P < 0.05$ (*).

was found not to differ significantly from normal. Data were analyzed statistically by one-way Anova followed by the Duncan's multiple tests. Differences were considered significant when the probability was $P < 0.05$. The SPSS version 13.0 was used for all analyses (SPSS Inc., USA).

3. Results

3.1. Characteristics of patients

The clinical characteristics of the patient group are shown in Table 1. Most of the patients were classified in the Gleason score less than 7, were between 70 and 80-years-old, had localized disease and were under hormonal therapy with goserelin acetate. Risk factor analysis showed prevalence of smoking and family history of cancer in the patient groups studied. In addition, a great part of PCA patients involved in this study were farmers and reported alcohol use.

3.2. Acetylcholinesterase and butyrylcholinesterase activities

Post-hoc comparisons made by Duncan's test revealed that the AChE in total blood and BChE in the plasma activities were decreased in the PCA patients compared to the control group (Fig. 1A and B).

Fig. 2A and B shows patients divided in groups based on Gleason score. Both AChE and BChE activities were significantly decreased in patients with Gleason score equal and higher than 7 in relation to the other groups.

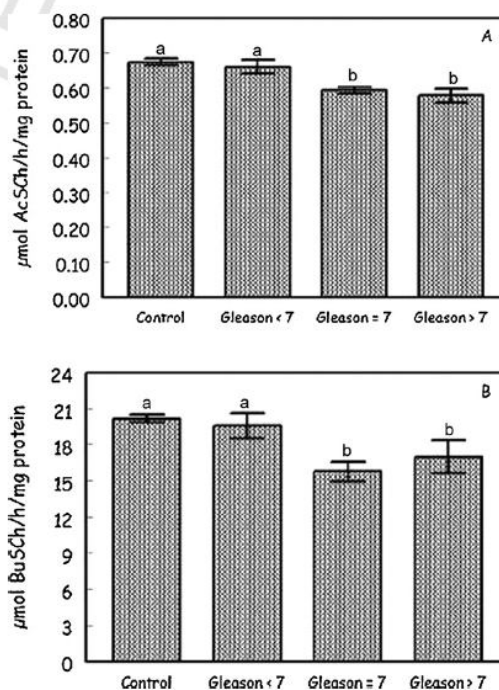


Fig. 2. Acetylcholinesterase (A) and butyrylcholinesterase (B) activities of prostate cancer patients divided by Gleason score. Patients, considering Gleason score, were divided into: Gleason lower than 7 (< 7, $n = 26$), Gleason equal to 7 (= 7, $n = 25$) and Gleason higher than 7 (> 7, $n = 15$). Controls consisted of 40 healthy subjects. Each column represents mean \pm S.E. Duncan's multiple range tests: groups that show different letters are statistically different ($P < 0.05$).

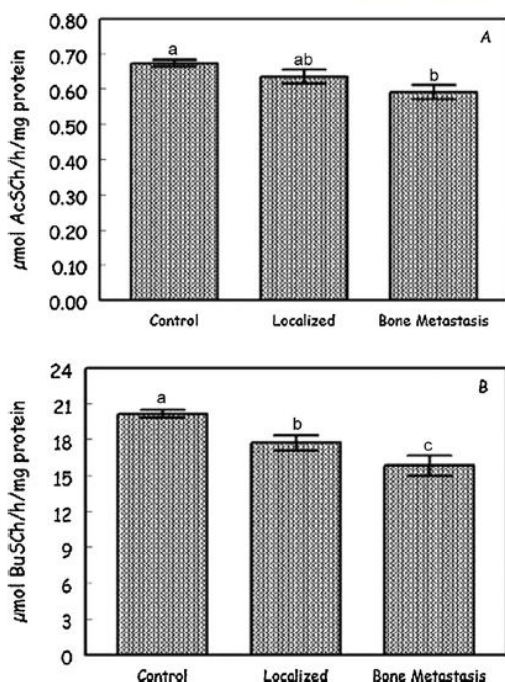


Fig. 3. Acetylcholinesterase (A) and butyrylcholinesterase (B) activities of prostate cancer patients divided by presence or no of metastasis. Patients, considering metastasis, were divided into: localized disease ($n=42$) and bone metastasis ($n=24$). Controls consisted of 40 healthy subjects. Each column represents mean \pm S.D. Duncan's multiple range tests; groups that show different letters are statistically different ($P < 0.05$).

Fig. 3 shows a comparison of patients with localized PCa and bone metastatic disease with the control group. Post-hoc comparisons made by Duncan's test revealed that the AChE activity (Fig. 3A) was decreased only in patients with bone metastasis in relation to the other groups. Regarding the BChE activity, Fig. 3B shows a reduced activity in all patients in relation to the controls, however, the highest reduction was observed in patients with bone metastasis.

AChE activity in patients divided by treatment is shown in Fig. 4A. No significant difference was observed between groups of patients and controls. On the other hand, post-hoc comparisons

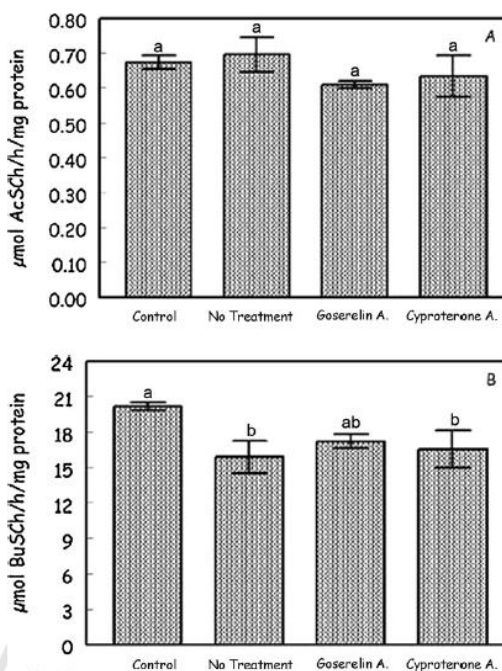


Fig. 4. Acetylcholinesterase (A) and butyrylcholinesterase (B) activities of prostate cancer patients divided by treatment. Patients, considering treatment, were divided into: patients who had not received any therapy preceding the blood sampling (no treatment, $n=11$), patients undergoing treatment with Goserelin Acetate ($n=45$) and patients undergoing treatment with Cyproterone Acetate ($n=10$). Controls consisted of 40 healthy subjects. Each column represents mean \pm S.D. Duncan's multiple range tests; groups that show different letters are statistically different ($P < 0.05$).

made by Duncan's test revealed that the BChE activity was decreased in patients who had not received any therapy or treated with cyproterone acetate when compared to the controls.

3.3. Biochemical determinations

Tables 2 and 3 show the biochemical determinations in patients divided in different groups considering the presence or absence of metastasis, standard treatment (without previous treatment, goserelin acetate or cyproterone acetate) and Gleason score.

Table 2

Biochemical determination (LDH, GGT, ALP, urea, creatinine and uric acid) in prostate cancer patients divided in groups based on type of treatment, Gleason score and bone metastasis.

	LDH (U/L)	GGT (U/L)	ALP (U/L)	Urea (mg/dL)	Creatinine (mg/dL)	Uric Acid (mg/dL)
Control	144.42 \pm 3.76	29.15 \pm 1.40	88.42 \pm 3.31	26.00 \pm 1.04	0.88 \pm 0.04	4.73 \pm 0.14
Type of Treatment						
No treatment	231.33 \pm 36.45 ^a	49 \pm 3.43 ^a	259.66 \pm 120.23 ^a	49.95 \pm 12.67 ^a	1.03 \pm 0.16	5.55 \pm 1.05
Goserelin A.	223.69 \pm 12.26 ^a	39 \pm 5.83	150.41 \pm 31.28	47.16 \pm 2.74 ^a	1.43 \pm 0.21	5.27 \pm 0.24
Cyproterone A.	261.11 \pm 27.47 ^a	22.2 \pm 1.24	88.77 \pm 13.45	42.73 \pm 5.04 ^a	1.02 \pm 0.19	4.6 \pm 1.54
Gleason Score						
<7	248 \pm 17.66 ^a	19.8 \pm 1.56	123.43 \pm 29.43	43.05 \pm 2.41 ^a	1.32 \pm 0.32	5.3 \pm 0.40
=7	245.43 \pm 22.45 ^a	36.63 \pm 7.3	157.13 \pm 53.07	47.51 \pm 5.73 ^a	1.18 \pm 0.23	4.87 \pm 0.32
>7	183.37 \pm 21.98	66.5 \pm 25.58 ^a	269.25 \pm 43.65 ^a	50.33 \pm 8.59 ^a	1.22 \pm 0.16	5.37 \pm 0.73
Bone Metastasis						
Yes	219.88 \pm 15.83 ^a	43.23 \pm 9.73	211.26 \pm 51.18 ^a	49.22 \pm 4.56 ^a	1.51 \pm 0.28 ^a	4.88 \pm 0.30
No	239.86 \pm 14.46 ^a	35.75 \pm 7.76	101.03 \pm 18.11	44.63 \pm 2.27 ^a	1.18 \pm 0.98	5.48 \pm 0.32

^a LDH=lactate dehydrogenase; GGT=gamma-glutamyltransferase; ALP=alkaline phosphatase.

Table 3

Biochemical determination (Na, K, Ca, Mg, AST, ALT and albumin) in prostate cancer patients divided in groups based on type of treatment, Gleason score and bone metastasis.

	Na (mEq/L)	K (mEq/L)	Ca (mg/dL)	Mg (mg/dL)	AST (U/L)	ALT (U/L)	Albumin (g/100 mL)
Control	140.95 ± 0.51	4.59 ± 0.05	9.44 ± 0.06	2.05 ± 0.02	23.55 ± 0.78	22.80 ± 1.09	4.12 ± 0.06
Type of Treatment							
No treatment	139.66 ± 1.08	4.46 ± 0.16	9.11 ± 0.23	1.90 ± 0.1	55.43 ± 2.4 ^a	37.5 ± 1.5 ^a	4.67 ± 0.04
Goserelin A.	138.74 ± 0.61	4.39 ± 0.06	9.16 ± 0.09	2.13 ± 0.03	24.48 ± 4.4	22.61 ± 1.88	4.11 ± 0.11
Cyproterone A.	139.12 ± 1.80	4.51 ± 0.23	9.21 ± 0.11	1.97 ± 0.12	23.4 ± 4.3	23.8 ± 3.77	4.16 ± 0.28
Gleason Score							
< 7	139.05 ± 1.19	4.49 ± 0.12	9.14 ± 0.52	2.08 ± 0.05	20.6 ± 2.55	20.4 ± 2.07	4.22 ± 0.13
= 7	138.60 ± 0.56	4.42 ± 0.11	9.10 ± 0.12	2.11 ± 0.07	21.14 ± 1.79	24.71 ± 2.31	4.04 ± 0.17
> 7	137.77 ± 3.23 ^a	4.40 ± 0.15	9.25 ± 0.26	2.11 ± 0.09	28.00 ± 2.78	50.32 ± 4.72	4.02 ± 0.41
Bone Metastasis							
Yes	138.53 ± 0.58 ^a	4.38 ± 0.08	9.14 ± 0.12 ^a	2.09 ± 0.04	30.22 ± 4.91	21.55 ± 2.36	3.95 ± 0.15
No	139.24 ± 0.88	4.45 ± 0.09	9.18 ± 0.10	2.11 ± 0.05	22.10 ± 1.81	25.35 ± 2.38	4.27 ± 0.10

AST = aspartate aminotransferase; ALT = alanine aminotransferase.

Post-hoc comparisons made by Duncan's test revealed that DHL activity and levels of urea in the serum were increased in all PCa patients. Our results show that ALP activity was elevated only in patients that had not received any therapy preceding the blood sampling, with Gleason score higher than 7 and in patients with bone metastasis compared with other groups. In relation to the GGT activity, our results show elevated activity in patients that had not received any therapy and in patients with Gleason score higher than 7. The activities of ALT and AST were increased in patients that had not received any therapy. Creatinine level was increased only in patients with bone metastasis. On the other hand, calcium level was decreased in these patients. Levels of sodium were decreased in patients with Gleason score higher than 7 and with bone metastasis. Nevertheless, for the uric acid, potassium, magnesium and albumin, there was no significant difference between the groups.

4. Discussion and conclusion

Prostate cancer is the sixth most common type of cancer worldwide and it is a considerable health risk for men throughout the world. It has been proposed that there may be a hereditary component to PCa risk, since family history of PCa is a well-established risk factor for PCa risk in men [34]. In accordance, our study demonstrated an increased PCa risk for men with a family history of any cancer or PCa. In our study, we observed that most PCa patients were current smokers. One important relationship between tobacco smoking and cancer is well established [35]. Cigarette smoke contains hundreds of carcinogenic substances that have demonstrable effects on all phases of cancer development. Tumor initiation, promotion and progression have been associated with compounds found in tobacco smoke in a variety of experimental tumor model systems [36]. Moreover, a significant number of PCa patients were farmers and reported alcohol consumption. According to Parent et al. [10], farmers exposed to high levels of pesticides had a two-fold excess risk of PCa compared to unexposed farmers. Moreover, alcohol consumption has been shown to alter sex steroid metabolism and thus could theoretically play a role in PCa development [37].

AChE is an essential enzyme in the nervous system that plays a key role in terminating neurotransmission at cholinergic synapses. AChE is a constituent of various cell types and tissues that include nervous tissues, human fibroblasts, rat kidney cells, human erythrocytes and human megakaryocytes [38,39]. In these tissues, the biological role of AChE is not limited to its classical role in hydrolyzing the neurotransmitter acetylcholine, but includes non-classical roles, such as cellular proliferation and differentiation, suggesting a possible influence of cholinesterases in tumorigenesis.

Our results show that the AChE and BChE activities were decreased in PCa patients in relation to the control group. These

results support the hypothesis that the alteration in cholinesterase activity in tumors may arise from an increased degradation or release of these enzymes. Another important aspect to be discussed is that a decrease in the cholinesterase would affect mainly the hydrolysis of acetylcholine. According to Song et al. [40], this chemical compound stimulates cancerous cells growing in lung tumors. The drop in cholinesterase activity and the consequent increased amount of acetylcholine could lead to a cholinergic overstimulation and increase the cell proliferation in cancer. In the context of cancer, the opposite variation of AChE and BChE activities in human breast carcinoma [41], the decrease of AChE activity in metastatic lymph nodes [42], the aggressiveness of human astrocytoma linked to changes in the composition of AChE variants [43], the correlation between the high levels of cytoplasmic AChE with decreased survival in ovarian cancer patients [44] and the function of cholinesterases in the male reproductive physiology [45] are worth mentioning.

When the patients were divided in groups based on clinicopathological characteristics interesting results were observed. AChE and BChE activities were modulated differently in relation to the Gleason score, presence or absence of metastasis and use of hormonal therapy. This may indicate that the cholinesterase activities are being affected differently by these conditions. Interestingly, we have observed a decrease in cholinesterase activities in the cancer group with a Gleason score greater than 7. Furthermore, these findings indicate that advanced PCa is associated with a decreased cholinesterase activity and consequently high acetylcholine concentration.

When the patients were divided considering the presence or absence of the metastasis, a reduction in the AChE activity only in patients with metastasis was observed. In relation to the BChE activity the major decrease was observed in patients with bone metastasis as well. These results are interesting and support the idea that acetylcholine may be involved in the process of metastasis. Both cholinesterases have been implicated in the development processes as a cellular proliferation stimulator [46,47]. AChE can be considered a marker of early differentiation [48], while BChE may be involved in cellular migration and fiber guidance [49].

On the other hand, there was no change in AChE activity when considered the treatment type. Patients who did not receive any previous treatment or in treatment with cyproterone presented BChE activity decreased. Furthermore, it is possible to suppose that the change of BChE activities in tumor is a consequence of the neoplastic transformations and that it contributes to the maintenance of the tumorigenic process. However, in order to explain these modifications in the BChE activity in different treatment, further investigation is required, since is the first time that cholinesterase activities were determined based on treatment type in PCa patients.

In relation to biochemical determinations in PCa patients, several changes were verified. LDH activity and levels of urea in the serum were increased in all PCa patients. The prognostic role of LDH has been widely investigated in special cancer groups [50]. Elevated LDH is consistently reported as a prognostic factor for poor survival in lung cancer, pancreatic cancer and PCa [25]. Regarding the renal damage represented by increased urea level, it is difficult to quantify the extent of renal complications associated with malignancy, as renal dysfunction can be present before the identification of malignancy, coincide with the diagnosis of malignancy, or be a secondary or tertiary effect of treatment [24].

Our results show that ALP activity were elevated only in patients that had not received any therapy preceding the blood sampling, with Gleason score higher than 7 and in patients with bone metastasis when compared with other groups. Total serum ALP is a commonly and serially measured marker that is inexpensive and readily available. ALP partly reflects osteoblastic activity, which is likely to be more pronounced in patients with larger volume or aggressive bony metastatic disease [26]. In relation to the GGT activity, our results show an elevated activity in patients that had not received any therapy and in patients with Gleason score higher than 7. Our results are in accordance with Hemelrijck et al. [51] that show a positive association between GGT levels and tumor incidence that may be explained by the link between GGT and the cell redox state. The activities of AST and ALT were increased in patients that had not received any therapy. The liver enzymes ALT and AST are biomarkers known to be specific for liver damage as they mainly appear in the liver and their levels increase when there is injury to the liver. Thus, an increase in these enzyme activities indicates a liver damage in patients that had not received any therapy.

In addition, creatinine level was increased only in patients with bone metastasis. Therefore, a marked increase in serum creatinine confirms an indication of functional damage to the kidney in this group of patients. On the other hand, calcium level was decreased in these patients. Tandon and Rizvi reported that hypocalcemia can be a manifestation of PCa metastatic to bone [52]. Levels of sodium were decreased in patients with Gleason score higher than 7 and with bone metastasis. Hyponatremia is often observed in patients with breast cancer, renal cancer, prostate cancer, and paraneoplastic syndrome. According to Shibata [53], one of the causes of hyponatremia is antidiuretic hormone-producing tumor.

The medical literature shows several examples of an inverse relationship between serum albumin levels and survival in patients with advanced cancer [28]. However, in our study no significant difference was observed in the albumin levels between the PCa and controls. Although the breakdown of cells, observed in some patients with cancer, may increase the blood levels of uric acid, magnesium and potassium, in our study there were no changes in these parameters in patients with PCa.

Finally, our study indicates that biochemical prognostic factors, like lactate dehydrogenase, hepatic function tests, renal function tests and electrolyte balance are altered in PCa patients. In addition, our results could show a possible relationship between the inhibition of cholinesterase activities and cellular proliferation in PCa, indicating that these enzymes may be functionally important in neoplastic cell transformation. All the clinical and pathological characteristics are fundamental to the PCa prognosis, and our findings show alterations in the cholinesterase activities and in the biochemical determinations considering such characteristics like the presence of metastasis, standard treatment used and Gleason score. Therefore, the role of the non-neuronal cholinergic system in different diseases (including PCa) needs to be clarified in more detail in order to optimize a future targeted-therapy.

Disclosure of interest

The authors have not supplied their declaration of conflict of interest.

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5.DISCUSSÃO

O Câncer de próstata (CaP) é reconhecido como um dos mais importantes problemas médicos enfrentados pela população masculina (HEIDENREICH et al., 2010). Tem sido proposto um componente hereditário ao risco de CaP, já que a história familiar é um fator de risco bem estabelecido (SRIDHAR et al., 2010). Nosso estudo demonstrou haver um risco aumentado para o CaP em homens com história familiar de câncer. Observamos também que a maioria dos pacientes envolvidos no estudo eram fumantes ou ex-fumantes. A importante relação entre tabagismo e câncer está bem estabelecida (DITRE et al., 2011). Além disso, um número significativo de pacientes com CaP eram agricultores e relataram consumo de álcool. De acordo com PARENT (2001), os agricultores expostos a altos níveis de pesticidas têm um risco duas vezes maior de desenvolver o CaP e o consumo de álcool pode alterar o metabolismo de esteróides sexuais e, portanto, poderia, teoricamente, ter um papel na causa do CaP (LIU et al., 2000).

Nos últimos anos, esforços significativos têm sido feitos para compreender os mecanismos biológicos e moleculares envolvidos no desenvolvimento e progressão do CaP. Alterações progressivas inerentes ou adquiridas no metabolismo celular que ocorrem ao longo dos anos podem ter um papel muito importante no desenvolvimento desta patologia (KHANDRIKA, 2009). Assim, a produção excessiva de EROs ou a diminuição no sistema de defesa antioxidante podem levar ao estresse oxidativo. Esse estado de estresse oxidativo pode desempenhar papel importante nos processos celulares associados com a iniciação e o desenvolvimento do CaP (FLESHNER e KLOTZ, 1999).

No presente estudo, o conteúdo de TBARS e os níveis de carbonilação de proteínas mostraram-se aumentados em pacientes quando comparado com o grupo controle. Além disso, mudanças no sistema de defesa antioxidante foram observadas. Com relação as atividades da SOD e da CAT, nossos resultados revelaram que a atividade CAT estava diminuída e a atividade da SOD aumentada nos pacientes, quando comparados aos controles. Esses resultados foram interessantes já que estudos sugerem que a superexpressão de SOD sem um aumento compensatório na CAT tem efeitos deletérios sobre as células (PINHO et al., 2006). Este desequilíbrio pode ser explicado através da associação da inflamação com o desenvolvimento do câncer (O_BYRNE et al., 2001) e,

consequentemente, o aumento da SOD atribuído as moléculas pró-inflamatórias (VISNER et al., 1990). Por outro lado, autores relataram que o O_2^- produzido durante o câncer pode inativar a CAT (KENO, 1997), o que é uma das prováveis razões para a diminuição da atividade desta enzima.

Os tióis não-protéicos desempenham um papel importante no sistema de defesa antioxidante, realizando a detoxificação das espécies reativas e a regeneração de outros antioxidantes. Verificamos um aumento nos níveis de tióis não-protéicos no plasma e eritrócitos dos pacientes, o que está de acordo com outros autores que sugerem um aumento nos níveis de tióis não-protéicos em pacientes com CaP e a síntese aumentada de tióis pelas células tumorais (CHIOU, 1999). Uma diminuição significativa nos níveis séricos de vitamina C e de vitamina E também foi verificado nos pacientes envolvidos neste estudo. Sugere-se que a vitamina E age protegendo os ácidos graxos poliinsaturados da peroxidação lipídica causada pelos radicais livres. Estudos anteriores relacionaram o aumento do risco de desenvolver CaP com a redução dos níveis séricos de vitamina E (HUANG et al., 2003). Com relação aos níveis séricos de vitamina C, tem-se observado uma correlação inversa dos níveis desta vitamina com a presença de biomarcadores de dano oxidativo, sugerindo que a vitamina C pode diminuir o estresse oxidativo (PATHAK et al., 2003).

A heterogeneidade notável de CaP pode ser representada pela identificação de cinco diferentes padrões de crescimento de 1 (mais diferenciado) a 5 (menos diferenciado). Ao adicionar o padrão de crescimento predominante (o padrão de Gleason primário) ao segundo padrão de crescimento mais dominante (o padrão de Gleason secundário) uma escala de agressividade de 2 a 10 é obtida (VIS et al., 2007). Curiosamente, temos observado um aumento do stress oxidativo (como evidenciado pela carbonilação de proteínas elevada), alterações na atividade da CAT plasmática e nos níveis de tióis não protéicos no grupo de pacientes classificados na escala de Gleason superior a 8. Esses resultados confirmam observações anteriores (CHAN et al., 1999; YOSSEPOWITCH et al., 2007) e indicam que o CaP avançado está associado a um estado de estresse oxidativo elevado.

Um aumento dos níveis de EROs pode estar relacionado com a formação acelerada de metástases, e a remoção dessas EROs é uma estratégia racional para inibir esse processo (NISHIKAWA, 2006). No entanto, em nosso estudo, os níveis de TBARS e de proteína carbonil foram aumentados, já a atividade da CAT e os níveis

de vitamina C e E foram reduzidos em ambos os grupos, independentemente da presença ou ausência de metástase óssea. Por outro lado, a atividade da SOD aumentou apenas nos pacientes com doença localizada e os níveis de tióis não protéicos no plasma e eritrócitos foram elevados apenas nos pacientes com metástase óssea. Portanto, ao contrário do esperado, os resultados encontrados não indicam mudanças significativas no estado oxidativo de pacientes com doença localizada ou metástase óssea.

O câncer de próstata é um tumor sensível a hormônios. Diversas terapias hormonais, que atuam por diferentes mecanismos, estão disponíveis. Neste trabalho, foram analisados os pacientes que não tinham recebido nenhum tipo de tratamento, os pacientes em tratamento com goserelina e em tratamento com acetato de ciproterona. Nos pacientes não tratados e nos pacientes em tratamento com acetato de goserelina puderam ser observadas as maiores alterações nos biomarcadores oxidativos como, por exemplo, o TBARS. Por outro lado, as defesas antioxidantes (CAT e Vitamina E) estavam alteradas nos pacientes não tratados e naqueles tratados com acetato de ciproterona. Sugerimos que o desequilíbrio no status oxidativo não parece ser resultado do tratamento, mas pode estar envolvido com a patogênese do câncer de próstata, uma vez que esse desequilíbrio pode ser observado principalmente no grupo de pacientes não tratados. Mais estudos são necessários para verificar a relação entre o tipo de tratamento utilizado e o estado oxidativo em pacientes com CaP.

Estudos mostram que o câncer pode estar associado com problemas na agregação plaquetária. A associação entre o câncer e tromboembolismo foi primeiramente descrita por Trousseau em 1865, que observou uma associação entre trombose venosa e doença maligna (LIP et al., 2002). Além disso, o câncer também tem sido associado com a ativação plaquetária e com distúrbios na coagulação do sangue (PRANDONI et al., 2005). Nossos resultados mostraram que a agregação plaquetária foi diminuída em pacientes com CaP em relação aos controles. No entanto, quando os pacientes foram agrupados em pacientes não tratados e em tratamento com terapia hormonal, pode-se observar que apenas os pacientes em tratamento mostraram uma diminuição na agregação plaquetária em relação ao grupo controle. A agregação de plaquetas reduzida no grupo que recebeu terapia hormonal pode indicar, em parte, que este tratamento foi eficaz, uma vez que as células do tumor podem causar a ativação da coagulação. MUNSON et al. (1995)

sugeriram que as plaquetas podem secretar fatores de crescimento que poderiam estimular a proliferação do câncer, no entanto, os pacientes envolvidos neste estudo não apresentaram qualquer ativação plaquetária significativa.

As células cancerosas são conhecidas por apresentarem alterações nas várias vias de sinalização celular (SARKAR et al., 2010). Os nucleotídeos extracelulares, produzem resposta em diversos processos biológicos, tais como apoptose, controle da proliferação e diferenciação celular (BURNSTOCK, 2002) e, portanto, podem ter um papel importante no crescimento do tumor (BURNSTOCK, 2002; SPYCHALA 2000). Há estudos mostrando que a adenosina pode funcionar como um estimulante do crescimento do tumor, enquanto o AMP tem o efeito contrário (SPYCHALA et al., 2004; MAZUREK et al., 1997). No entanto, pouco se sabe sobre os efeitos desses nucleotídeos nas células prostáticas.

Os eventos induzidos pelos nucleotídeos extracelulares são regulados pela ação das ectonucleotidases, que são importantes moduladores da neurotransmissão purinérgica. Dentre estas enzimas pode-se destacar as NTPDases (Ecto-Nucleosideo Trifosfato Difosfoidrolase), a família das E-NPPs (Ecto-Nucleotideo Pirofosfatase/Fosfodiesterases), a 5'-nucleotidase e a adenosina deaminase (ADA) (ROBSON et al., 2006; YEGUTKIN, 2008).

Nossos resultados demonstram claramente que a hidrólise dos nucleotídeos ATP, ADP e AMP está aumentada nos pacientes quando comparado ao grupo controle, o que pode ser comprovado pelo aumento nas atividades das enzimas NTPDase, E-NPP e 5' Nucleotidase. Nossos resultados estão de acordo com outros autores que mostraram que a expressão das ecto-ATPases está aumentada durante o câncer (SAPHNER et al., 1991; RATHBONE et al., 1992). Efeitos inibitórios do ATP extracelular no crescimento do tumor foram descritos em células e tecidos, incluindo células de carcinoma da próstata (JANSSENS e BOEYNAEMS, 2001). Por outro lado, CHEN et al. (2004) mostraram que o ATP extracelular teve um efeito pró-invasivo nas células de CaP. Pode-se sugerir que a enzima NTPDase pode proteger o organismo de um possível aumento da agregação plaquetária, já que degrada o ADP que é um indutor deste processo, enquanto que a 5'nucleotidase está mais envolvida no controle da formação de adenosina. Já a enzima E-NPP é freqüentemente alterada por situações patológicas e sua atividade pode tornar-se uma ferramenta útil para avaliar a progressão da doença. O aumento verificado na atividade das 2 enzimas que são responsáveis pela degradação do ATP (NTPDase

e E-NPP) confirma a teoria de que estas enzimas trabalham de forma conjunta e em sintonia na hidrólise dos nucleotídeos.

Alterações na atividade total da ADA também têm sido amplamente descritas em pacientes com doenças malignas, embora os resultados ainda sejam contraditórios. Poucos estudos clínicos publicados sobre o valor da atividade da ADA em pacientes com CaP estão disponíveis na literatura.

Nossos resultados revelaram que a atividade da ADA estava diminuída no soro de pacientes com CaP. No entanto, a atividade da ADA nas plaquetas não se alterou significativamente, portanto, as mudanças na atividade da ADA parecem ser mais sensíveis no soro do que em plaquetas. Nossos resultados estão de acordo com outros autores que verificaram uma diminuição na atividade da ADA em tecidos tumorais ou linfócitos de pacientes com câncer (NAMIoT et al., 1996; BIRI et al., 1999). A ADA contribui para a regulação das concentrações de adenosina pela conversão da mesma em inosina e pela irreversibilidade desta reação, o que faz com que seja o passo limitante da reação (VANNONI et al., 2004). A adenosina se acumula em altas concentrações nos tumores sólidos, promovendo efeitos como a proteção contra a isquemia, a estimulação do crescimento e da angiogênese, bem como a inibição da síntese de citocinas. Alguns autores, portanto, concluem que a adenosina é um fator importante na promoção do crescimento do tumor (SPYCHALA, 2000). A maioria dos estudos encontrados na literatura têm avaliado a atividade da ADA em soro e em células como linfócitos, monócitos e eritrócitos, mas poucos determinaram a atividade desta enzima na superfície das plaquetas, que são células circulantes, e, portanto, de grande importância.

Com o objetivo de elucidar o envolvimento de degradação de nucleotídeos em plaquetas e soro de pacientes com CaP, analisamos a atividade das enzimas envolvidas neste processo, considerando alguns parâmetros importantes. Levando em conta o escore de Gleason, a presença ou ausência de metástases e tratamento pudemos observar resultados interessantes. A hidrólise dos nucleotídeos ATP, ADP, AMP e adenosina foram modulados de forma diferente quando consideramos o escore Gleason, a presença ou ausência de metástase e o uso de terapia hormonal. Isso pode indicar que as atividades enzimáticas estão sendo afetados de forma distinta por essas condições.

Considerando-se o escore de Gleason, a hidrólise do ADP foi aumentada em pacientes com escore de Gleason igual a 7. Estes resultados podem indicar um

mecanismo para inibir a agregação plaquetária, já que o ADP pode interagir com receptores nas plaquetas e induzir a agregação plaquetária. No entanto, mais estudos são necessários para avaliar o papel da NTPDase na agressividade do CaP. Por outro lado, a atividade da E-NPP em soro foi aumentada apenas nos pacientes com escore de Gleason inferior a 7. Neste grupo uma menor concentração de ATP está disponível. ZHANG et. al (2010) mostraram que ATP promove a invasão das células de CaP através do aumento da motilidade. Além disso, há uma maior concentração de AMP em pacientes com doença menos agressiva, indicando que este nucleotídeo pode ter um efeito inibitório sobre o crescimento das células em pacientes com CaP. Com relação a atividade da ADA, observamos uma diminuição em plaquetas e soro de pacientes com um escore de Gleason maior que 7. Esses resultados indicam que CaP avançado está associada a uma atividade de ADA baixa e, conseqüentemente, a uma alta concentração de adenosina.

Quando os pacientes foram agrupados de acordo com a presença de doença localizada ou metástase óssea, nossos resultados mostraram que a hidrólise do ATP e do ADP foram aumentadas nos pacientes com doença localizada em relação ao grupo com metástase óssea. Além disso, um aumento na atividade de E-NPP em soro também foi observado. Não existem estudos na literatura relacionando as atividades destas enzimas com o desenvolvimento de metástases em pacientes com CaP. A invasão tumoral e a metástase são processos constituídos por várias etapas em que a perda de adesão célula-célula, a proteólise aumentada e a motilidade celular tem se mostrado passos críticos (STEEG, 2006). Fatores que aceleram esses passos podem promover a invasão do tumor e afetar o processo de metástase.

STAGG et al (2010), sugeriram um papel para a 5' nucleotidase nas metástases do tumor em pacientes com câncer de mama. Já, Zhang et al. indicam que o ATP pode aumentar o risco de metástase em células de CaP. Nosso trabalho mostra que a atividade da NTPDase e da E-NPP é menor em pacientes com metástases, sugerindo maiores concentrações de ATP nestes pacientes. No entanto, mais estudos são necessários, visto que as concentrações de nucleotídeos não só dependem das atividades das enzimas, mas também de nucleotídeos pré-existentes, que pode ser variável entre os pacientes. Finalmente, uma redução na atividade de ADA foi verificada apenas em pacientes com metástase. Estes

resultados são interessantes e apoiam a idéia de que a adenosina podem estar envolvida no processo de metástase.

Para avaliar a possível influência do uso da terapia hormonal nas atividades enzimáticas, agrupamos os pacientes com CaP em dois grupos: pacientes que não tinham recebido qualquer tratamento anterior à coleta de sangue e pacientes submetidos à terapia hormonal. Nossos resultados revelaram que a hidrólise dos nucleotídeos ATP, ADP, AMP promovidas pela enzima NTPDase, E-NPP e 5' Nucleotidase foram significativamente maiores em pacientes que não receberam qualquer terapia hormonal quando comparado aos controles. Nossos resultados sugerem ainda que a produção de adenosina é aumentada nos pacientes que não receberam qualquer terapia hormonal. Além disso, a atividade da ADA em soro se mostrou diminuída nos pacientes que não receberam qualquer tratamento prévio. Este fato provavelmente contribui para o aumento da disponibilidade de adenosina neste grupo, que atua como um agente de progressão tumoral, uma vez que induz a neovascularização, a redução da hipóxia e a inflamação (BOROWIEC et al., 2006).

As colinesterases (AChE e BChE) são enzimas essenciais no sistema nervoso, que desempenham um papel fundamental nas sinapses colinérgicas. Porém, o papel biológico destas enzimas não se limita à sua função clássica e inclui funções não-clássicas, como proliferação e diferenciação celular, o que sugere uma possível influência das colinesterases na tumorigênese. Nossos resultados mostram que as atividades da AChE e BChE foram diminuídas em pacientes com CaP em relação ao grupo controle. A diminuição nas atividades das colinesterases afeta principalmente a hidrólise da acetilcolina. De acordo com SONG et al. (2003), este composto estimula o crescimento das células do câncer em tumores de pulmão. A queda nas atividades das colinesterases e o conseqüente aumento da acetilcolina poderia levar a uma superestimulação colinérgica e aumentar a proliferação celular no câncer.

No contexto do câncer, a variação oposta de atividades da AChE e BChE em carcinoma de mama (RUIZ-ESPEJO et al., 2002), a queda na atividade da AChE nos linfonodos metastático (RUIZ-ESPEJO et al., 2003), a correlação entre os altos níveis AChE com a diminuição da sobrevida em pacientes com câncer de ovário (MOTAMED-KHORASANI et al., 2007) e a função das colinesterases na fisiologia reprodutiva masculina (NIETO-CERÓN et al., 2010) devem ser mencionados.

Quando os pacientes foram divididos considerando a presença ou ausência de metástase, uma redução na atividade da AChE apenas nos pacientes com metástase foi observada. Em relação à atividade de BChE a maior diminuição foi observada em pacientes com metástase óssea também. Estes resultados são interessantes e sugerem que a acetilcolina pode estar envolvida no processo de metástase. Por outro lado, não houve alteração na atividade da AChE quando considerado o tipo de tratamento. Pacientes que não receberam qualquer tratamento prévio ou em tratamento com ciproterona apresentaram uma diminuição na atividade da BChE. É possível supor que as mudanças na atividades da BChE é uma consequência das transformações neoplásicas e que contribui para a manutenção do processo de tumorigênese. No entanto, a fim de explicar essas modificações da atividade da BChE em pacientes em tratamento com terapias diferenciadas, mais estudos são necessários, uma vez que esta é a primeira vez que as atividades das colinesterases foram determinadas em pacientes com CaP com base no tipo de tratamento utilizado.

As atividades de AChE e BChE foram modulados de forma diferente em relação ao escore Gleason, observamos uma diminuição nas atividades das colinesterase no grupo de pacientes com um escore de Gleason maior que 7. Esses resultados indicam que o CaP avançado está associado a uma diminuição da atividade das colinesterases e, conseqüentemente, a uma concentração de acetilcolina alta.

Finalmente, verificamos o perfil bioquímico dos pacientes, através das determinações dos principais “marcadores” que devem ser monitorados e várias mudanças foram verificadas. A atividade da enzima lactato desidrogenase (LDH) e os níveis de uréia no soro foram aumentados em todos os pacientes. O papel prognóstico da LDH tem sido amplamente investigada em grupos especiais de câncer (SUH et al., 2007). A LDH elevada é relatada como um fator prognóstico para a sobrevida no câncer de pulmão, câncer de pâncreas e CaP (SMALETZ et al., 2002). Foi possível verificar também a presença de possível dano renal, que pode ser quantificado pelos níveis de uréia aumentada nos pacientes. Porém, é difícil quantificar o grau de complicações renais associados com malignidade, já que a disfunção renal pode estar presente antes da identificação de malignidade, coincidirem com o diagnóstico de malignidade, ou ser um efeito secundário ou terciário do tratamento (GIVENS et al., 2010).

Nossos resultados mostram ainda que a atividade da fosfatase alcalina (FAL) foi elevada apenas nos pacientes que não tinham recebido qualquer tratamento anterior à coleta de sangue, com escore de Gleason superior a 7 e em pacientes com metástase óssea, quando comparado com outros grupos. A FAL sérica total é um marcador medido com frequência e, em parte, reflete a atividade osteoblástica, o que tende a ser mais pronunciado em pacientes com volume maior ou doença óssea metastática agressiva (SONPAVDE et al., 2010). Com relação à atividade da gama glutamil transferase (GGT), nossos resultados mostram uma atividade elevada em pacientes que não receberam tratamento e em pacientes com escore de Gleason superior a 7. Nossos resultados estão de acordo com HEMELRIJCK et al., (2011) que mostram uma associação positiva entre os níveis de GGT e a incidência de tumores. Já as atividades da aspartato aminotransferase (AST) e alanina aminotransferase (ALT) foram aumentadas em pacientes que não receberam nenhuma terapia. As enzimas hepáticas ALT e AST são biomarcadores conhecido por serem específicas para lesão hepática e seus níveis aumentam quando há lesão no fígado. Assim, essa alteração pode indicar lesão hepática em pacientes que não receberam nenhuma terapia.

Além disso, dosamos os níveis de creatinina, que foram maiores apenas em pacientes com metástase óssea, o aumento na creatinina sérica sugere um prejuízo funcional para o rim. Por outro lado, o nível de cálcio foi diminuído nestes pacientes. TANDON e RIZVI (2005) relataram que a hipocalcemia pode indicar uma manifestação de metástase óssea. Os níveis de sódio estavam diminuídos nos pacientes com escore de Gleason superior a 7 e com metástase óssea. A hiponatremia é frequentemente observada em pacientes com câncer de mama, câncer renal, câncer de próstata, e síndrome paraneoplásica (SHIBATA, 2010).

Os resultados deste trabalho caracterizam a persistência do estresse oxidativo no câncer de próstata, o que pôde ser evidenciado pelo aumento nos níveis de danos oxidativos e pela diminuição das defesas antioxidantes. Por outro lado, este trabalho mostrou alterações nas atividades das enzimas ectonucleotidases e colinesterases nos pacientes com câncer, o que pode estar relacionado com a participação destas enzimas no desenvolvimento do câncer. Além disso, fatores prognósticos como a escala de Gleason, a presença ou ausência de metástase óssea e o tratamento mostraram ter influencia sobre os parâmetros enzimáticos e marcadores de estresse oxidativo avaliados. Cabe ressaltar ainda que o

envolvimento da adenosina e da acetilcolina com o câncer de próstata pode representar um importante ponto de investigação e um potencial alvo terapêutico em pacientes com câncer.

Nossos resultados representam uma importante contribuição no estudo do perfil oxidativo, na investigação do papel das enzimas ectonucleotidases e colinesterases, agregação plaquetária e determinações bioquímicas em pacientes com câncer de próstata, já que levou em consideração a escala de Gleason, a presença de metástases e o tratamento utilizado, fatores importantes envolvidos nesta doença.

6. CONCLUSÕES

- O aumento de dano oxidativo, representado pelo TBARS e conteúdo de proteína carbonil, nos pacientes com câncer de próstata sugere um aumento na formação de EROs nos mesmos.
- O aumento na atividade da SOD e a diminuição na atividade da CAT podem indicar dano celular e refletem o desequilíbrio no sistema de defesa antioxidante enzimático nos pacientes com câncer de próstata.
- Os níveis aumentados de tióis não-protéicos sugerem que este antioxidante pode atuar como um mecanismo compensatório frente ao aumento do estresse oxidativo. Por outro lado, os níveis diminuídos de vitamina C e vitamina E, indicam uma deficiência destes antioxidantes nos pacientes com câncer de próstata.
- Os maiores danos oxidativos observados em pacientes classificados na escala de Gleason maior de que 7, indicam que o câncer de próstata mais avançado está associado com um estado de estresse oxidativo elevado.
- O aumento observado nas atividades das enzimas NTPDase e 5' Nucleotidase (plaquetas) e da enzima E-NPP (soro e plaquetas) e a diminuição na atividade da enzima ADA em soro de pacientes com câncer de próstata refletem o aumento na hidrólise de nucleotídeos e o consequente aumento nas concentrações de adenosina que pode estar relacionada e/ou participando do processo de tumorigênese.
- As alterações nas atividades das enzimas NTPDase, 5' Nucleotidase e E-NPP quando os pacientes foram divididos em grupos considerando a escala de Gleason, a presença ou ausência de metástase óssea e o fato de estar ou não em tratamento, indicam que esses parâmetros devem ser considerados e podem ter uma influência complexa na hidrólise de nucleotídeos.
- A acentuada diminuição na atividade da ADA observada nos pacientes com escala de Gleason maior do que 7, nos pacientes com metástase óssea e que não receberam nenhum tipo de tratamento, indica que nestes grupos pode estar havendo uma maior influência da adenosina sobre as células do câncer, promovendo efeitos que favorecem o crescimento tumoral.

- A diminuição na agregação plaquetária observada no grupo de pacientes com câncer de próstata em tratamento sugere, em parte, a eficácia terapêutica
- A diminuição nas atividades das colinesterases (AChE e BuChE) e o consequente aumento nos níveis de ACh corroboram a sugestão da participação da Ach no processo de desenvolvimento do câncer.
- As atividades diminuídas da AChE e BuchE nos pacientes classificados na escala de Gleason igual ou maior do que 7 e nos pacientes com metástase óssea, indiretamente refletem níveis aumentados de Ach, que pode estar envolvida no processo de metástase e no avanço do câncer de próstata.
- As alterações nas dosagens bioquímicas (LDH, FAL, GGT, AST, ALT, creatinina, uréia, cálcio e sódio) observadas nos pacientes com câncer de próstata indicam que estes parâmetros devem ser avaliados e monitorados rotineiramente.

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8.ANEXOS

Anexo 1 - Carta de aprovação pelo Comitê de Ética

 <p>MINISTÉRIO DA SAÚDE Conselho Nacional de Saúde Comissão Nacional de Ética em Pesquisa (CONEP)</p>	<p>UNIVERSIDADE FEDERAL DE SANTA MARIA Pró-Reitoria de Pós-Graduação e Pesquisa Comitê de Ética em Pesquisa - CEP - UFSM REGISTRO CONEP: 243</p> 
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CARTA DE APROVAÇÃO

O Comitê de Ética em Pesquisa – UFSM, reconhecido pela Comissão Nacional de Ética em Pesquisa – (CONEP/MS) analisou o protocolo de pesquisa:

Título: Estudo do perfil oxidativo e avaliação de parâmetros enzimáticos em pacientes com câncer de próstata.
Número do processo: 23081.009047/2008 -47
CAAE (Certificado de Apresentação para Apreciação Ética): 0115.0.243.000-08
Pesquisador Responsável: Vera Maria Morsch

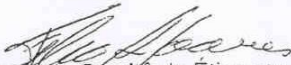
Este projeto foi APROVADO em seus aspectos éticos e metodológicos de acordo com as Diretrizes estabelecidas na Resolução 196/96 e complementares do Conselho Nacional de Saúde. Toda e qualquer alteração do Projeto, assim como os eventos adversos graves, deverão ser comunicados imediatamente a este Comitê. O pesquisador deve apresentar ao CEP:

Janeiro/2009	Relatório parcial
Janeiro/2010	Relatório parcial
Janeiro/2011	Relatório final

Os membros do CEP-UFSM não participaram do processo de avaliação dos projetos onde constam como pesquisadores.

DATA DA REUNIÃO DE APROVAÇÃO: 14/07/2008

Santa Maria, 14 de julho de 2008.


 Vice-Coordenador do Comitê de Ética em Pesquisa – UFSM
 Registro CONEP N. 243.

Comitê de Ética em Pesquisa - UFSM - Av. Roraima, 1000 – Prédio da Reitoria - 7º andar - Campus Universitário
 97105-900 – Santa Maria – RS - - Tel: 0 xx 55 3220 9362 – email: comiteeticapesquisa@mail.ufsm.br

Anexo 2 - Termo de consentimento Livre e Esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

O Programa de Pós Graduação em Bioquímica Toxicológica da UFSM está desenvolvendo o projeto de pesquisa “ESTUDO DO PERFIL OXIDATIVO E AVALIAÇÃO DE PARÂMETROS ENZIMÁTICOS EM PACIENTES COM CÂNCER DE PRÓSTATA”, através da doutoranda Vanessa Battisti, orientada pela prof^a Vera Maria Morsch. Este projeto tem como objetivo avaliar a atividade de componentes sanguíneos em pacientes com câncer de próstata e em indivíduos controles saudáveis, com a finalidade de colaborar para um melhor entendimento desta doença, além de proporcionar mais informações aos pacientes, já que estes terão acesso aos resultados da pesquisa.

Será realizada uma coleta de sangue (punção venosa) para obtenção de soro e plasma. O desconforto se resume à picada da agulha, sendo que após a coleta o local poderá ficar dolorido ou arroxado, mas não requer nenhum cuidado especial voltando ao normal em poucos dias. Todo material utilizado para a coleta será descartável e/ou desinfectado. Este estudo não envolve risco adicional de vida ou contaminação aos pacientes. As amostras serão tratadas de acordo com os protocolos experimentais estabelecidos.

Fica garantido que os dados coletados ficarão sob responsabilidade do pesquisador e que os mesmos serão utilizados apenas para fins científicos, sem que o paciente seja identificado, garantindo assim o anonimato.

A participação deste estudo é livre e voluntária, sendo que não haverá nenhuma forma de compensação financeira ou custos para o participante. A recusa na participação não leva nenhum prejuízo ou comprometimento dos cuidados médicos aos pacientes.

Pelo presente Termo de Consentimento Livre e Esclarecido, declaro que estou de acordo em participar deste projeto de pesquisa, livre de qualquer constrangimento, pois fui informado de forma clara e detalhada dos objetivos e dos procedimentos que serão realizados. Fui igualmente informado da garantia de receber respostas a qualquer dúvida que ainda puder ter sobre assuntos

relacionados com a pesquisa, e da liberdade de retirar meu consentimento a qualquer momento, sem que haja prejuízo de qualquer ordem.

Ciente e de acordo com o que foi anteriormente exposto, eu _____ estou de acordo em participar desta pesquisa, assinando este consentimento.

Santa Maria, ____ de _____ 200__.

Nome Paciente

Identidade

Assinatura do pesquisador

Em caso de dúvidas, entrar em contato com Prof^a Dr^a orientadora Vera Maria Morsch, 3220 8978, ou com Vanessa Battisti (pesquisadora), 055 3333 5462.