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**PROLACTINA COMO TOXINA URÊMICA: relação entre prolactina e citocinas
inflamatórias em pacientes em hemodiálise**

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MARCLÉBIO MANUEL COELHO DOURADO

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Tese apresentada ao Programa de Pós-Graduação em Neuropsiquiatria e Ciências do Comportamento do Centro de Ciências Médicas da Universidade Federal de Pernambuco para obtenção do título de Doutor.

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“a mente que se abre a uma nova idéia jamais retorna ao seu tamanho original” Albert Einstein

RESUMO

Doença renal crônica (DRC) tem uma prevalência elevada e crescente na população geral, principalmente porque as principais causas de injúria renal são diabetes *mellitus* (DM) e hipertensão (HAS). DRC tem alta morbidade e está associada a aumento de mortalidade cardiovascular, com 5 a 10 milhões de mortes atribuíveis anualmente em todo o mundo. Fatores de risco cardiovascular tradicionais, como DM, HAS, hipertrofia ventricular esquerda e doença vascular periférica, são muito prevalentes nessa população. No entanto, esta população também está exposta a fatores de risco não tradicionais como hiperfosfatemia, hiperparatireoidismo, inflamação e anemia. Prolactina pode entrar neste contexto já que se acumula no sangue com perda da função renal, também atua na cascata de inflamação como citocina inflamatória, e está associada a aumento do risco cardiovascular devido a mecanismos ainda incertos. Objetivo desta tese foi levantar os principais pontos entre prolactinemia e DRC avaliando, de forma inédita, a relação entre hiperprolactinemia e citocinas inflamatórias em pacientes em hemodiálise. Foi realizado um estudo de corte transversal com pacientes em um único centro de hemodiálise, idade superior a 18 anos, mais de 6 meses em hemodiálise intermitente e com acesso vascular definitivo fístula arteriovenosa. Foram excluídos aqueles com infecções virais ou bacterianas ativas; com hipotireoidismo (diagnosticado com base em níveis de levotiroxina ou hormônio da tireóide > 5 mU / L) ou câncer ativo; usuários de medicamentos conhecidos por elevar os níveis de prolactina (por exemplo, amitriptilina, citalopram, escitalopram, fluoxetina, paroxetina, entre outros); gestantes e lactantes; em uso de imunossupressores; e pacientes com doenças hepáticas crônicas. Nos pacientes incluídos foram avaliados parâmetros clínicos e bioquímicos, além da dosagem de prolactina e painel de citocinas inflamatórias [(interleucina (IL) -2, IL-4, IL-6, IL-10, IL-17A, fator de necrose tumoral alfa (TNF- α) e interferon gama (IFN- γ)]. Os dados de pacientes com prolactina elevada foram comparados com os dos pacientes com o hormônio normal. Dos 361 pacientes regulares no serviço, 111 foram incluídos no estudo, com hiperprolactinemia em 61 pacientes (54,95%). Não houve diferença estatística em relação à idade, sexo, índice de massa corporal ou etiologia da DRC em pacientes com hormônio normal e elevado. Em relação ao tempo de hemodiálise - em média, pacientes com prolactina elevada estavam em terapia há 111,4 meses,

enquanto pacientes com hormônio normal em média há 73,65 meses ($p = 0,016$). Parâmetros laboratoriais como hemoglobina, glicemia de jejum, hemoglobina glicada, perfil lipídico, cálcio, fósforo, fosfatase alcalina e paratormônio também foram semelhantes nos 2 grupos. Em relação às citocinas inflamatórias, os pacientes com prolactina elevada apresentaram níveis mais altos de IL-6 e menor IL-10, com significância estatística ($p < 0,001$ e $0,046$, respectivamente). Em relação ao nível sérico de prolactina, houve correlação positiva com os níveis séricos de IL-6 ($R = 0,44$, $p < 0,0001$); enquanto que em relação à IL-10 a correlação foi inversamente proporcional e estatisticamente significativa ($R = 0,2$, $p < 0,046$). Portanto, pacientes em hemodiálise apresentaram alta prevalência de hiperprolactinemia, e esta elevação hormonal mostrou-se associada a aumento da interleucina-6 e redução de interleucina-10.

Palavras-Chave: Prolactina. Hemodiálise. Citocinas. Interleucina-6. Interleucina-10.

ABSTRACT

Chronic kidney disease (CKD) has a high and increasing prevalence in general population, mainly because the main causes of kidney injury are diabetes mellitus (DM) and hypertension. CKD has high morbidity and is associated with increased cardiovascular mortality, with 5 to 10 million deaths attributable annually worldwide. Traditional cardiovascular risk factors, such as DM, hypertension, left ventricular hypertrophy and peripheral vascular disease, are quite prevalent in this population. However, CKD patients are also exposed to non-traditional risk factors such as hyperphosphatemia, hyperparathyroidism, inflammation and anemia. Prolactin can be included in this context as it accumulates in the blood with loss of renal function, also acts on the inflammation cascade as an inflammatory cytokine, and is associated with increased cardiovascular risk due to mechanisms still uncertain. Upon this, the objective of this thesis was to evaluate the main links between prolactinemia and CKD by assessing, for the first time, relationship between hyperprolactinemia and inflammatory cytokines in hemodialysis patients. A cross-sectional study was performed evaluating patients in a single hemodialysis center, over 18 years of age, more than 6 months on intermittent hemodialysis and using definitive vascular access an arteriovenous fistula. Those with active viral or bacterial infections; with hypothyroidism (diagnosed based on levels of levothyroxine or thyroid hormone $> 5 \text{ mU / L}$) or active cancer; drug users known to raise prolactin levels (eg, amitriptyline, citalopram, escitalopram, fluoxetine, paroxetine, among others); pregnant and lactating women; using immunosuppressants; and patients with chronic liver disease were excluded. Clinical and biochemical parameters were evaluated, in addition to the measurement of prolactin and inflammatory cytokine panel [(interleukin (IL) -2, IL-4, IL-6, IL-10, IL-17A, tumor necrosis factor alpha (TNF- α) and gamma interferon (IFN- γ)]. Data from patients with elevated prolactin were compared with those from patients with normal hormone. Of the 361 regular patients, 111 were eligible. Hyperprolactinemia was found in 61 patients (54.95%). There was no statistical difference regarding age, sex, body mass index or etiology of CKD in patients with normal and elevated hormone. Regarding hemodialysis time - on average, patients with elevated prolactin were on therapy 111.4 months ago, while patients with normal hormone averaged 73.65 months ($p = 0.016$). Laboratory parameters such as hemoglobin, fasting glycemia, glycated hemoglobin, lipid profile,

calcium, phosphorus, alkaline phosphatase and parathormone were also similar in the 2 groups. Analysing inflammatory cytokines, patients with high prolactin had higher levels of IL-6 and lower IL-10, with statistical significance ($p < 0.001$ and $p < 0.046$, respectively). Regarding the serum level of prolactin, there was a positive correlation with serum levels of IL-6 ($R = 0.44$, $p < 0.0001$); while in relation to IL-10 the correlation was inversely proportional and statistically significant ($R = 0.2$, $p < 0.046$). In conclusion, hemodialysis patients had a high prevalence of hyperprolactinemia, and this hormonal increase was associated with an increase in interleukin-6 and a reduction in interleukin-10.

Key words: Prolactin. Hemodialysis. Cytokines. Interleukin-6. Interleukin-10.

LISTA DE FIGURAS

Figura 1	Número estimado de pacientes em diálise crônica por ano	19
Figura 2	História da hemodiálise	20
Figura 3	Taxa de mortalidade bruta anual estimada pacientes em diálise	21
Figura 4	Mortalidade por todas as causas (A) e cardiovascular (B) de acordo com TFG estimada e Albuminúria (ACR)	22
Figura 5	Receptores de prolactina em placas ateroscleróticas	29
Figura 6	Disfunção do sistema imune em pacientes com DRC contribuindo para mortalidade cardiovascular, susceptibilidade a infecções e progressão da DRC	31
Figura 7	Papel da Interleucina-6 na imunidade e inflamação	34
Figura 8	Interleucina-10 interligando inflamação em pacientes dialíticos	35
Figura 9	Eventos cardiovasculares e mortalidade cardiovascular em homens e mulheres com doença renal crônica tratamento conservador e hemodiálise	37

APÊNDICE D - ARTIGO ORIGINAL

Figure 1	Comparisons of hemodialysis time, in months, in patients with elevated and normal prolactin (PRL) levels	83
Figure 2	Dispersion graph between prolactin levels and time on hemodialysis, along with the trend line	83
Figure 3	Scatter plot between interleukin-6 (IL-6) (pg/mL) and prolactin (PRL) (3A) and interleukin-10 (IL-10) (pg/mL) and prolactin (3B)	85

LISTA DE TABELAS

Tabela 1	Definição doença renal crônica estabelecida pelo KDIGO 2012	17
Tabela 2	Classificação da doença renal crônica estabelecida pelo KDIGO	18
Tabela 3	Principais causas de Hiperprolactinemia	24
Tabela 4	Causas farmacológicas de hiperprolactinemia	25
Tabela 5	Efeitos dos agonistas dopaminérgicos Bromocriptina e Cabergo lina sobre anormalidades metabólicas em pacientes com hiperprolactinemia	26
Tabela 6	Efeitos da prolactina (PRL) em células do sistema imune	27
Tabela 7	Principais citocinas inflamatórias e suas características	32

APÊNDICE C – ARTIGO DE REVISÃO

Table 1	Main causes of hyperprolactinemia	65
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APÊNDICE D – ARTIGO ORIGINAL

Table 1	Clinical data of the 111 patients included in the study	81
Table 2	Comparison of clinical variables in patients with normal and elevated prolactin levels	82
Table 3	Laboratory parameters of patients with normal and elevated prolactin levels	83
Table 4	Comparison of interleukin levels between patients with normal and elevated prolactin levels	84

LISTA DE ABREVIATURAS E SIGLAS

CCM	Centro de ciências médicas
CEP	Comite de ética e pesquisa
CMP	miocardiopatia periparto
DCV	Doença cardiovascular
DRC	Doença renal crônica
FA	Fosfatase alcalina
HC/UFPE	Hospital das Clínicas da Universidade Federal de Pernambuco
HD	Hemodiálise
HDL	Lipoproteína de alta densidade
HVE	hipertrofia de ventrículo esquerdo
IL	Interleucina
IMC	Índice de massa corpórea
INF-gama	Interferon gama
KDIGO	<i>Kidney Disease Improving Global Outcomes</i>
Kg	Kilogramas
Kg/m ²	Kilograma por metro quadrado
LDL	Lipoproteína de baixa densidade
LIKA	Laboratório de Imunopatologia Keito Asami
mg	Miligramas
mg/dL	Miligramas por decilitro
mL	Mililitro
mL/min/m ²	Mililitro por minuto por metro quadrado
mmHg	Milímetro de mercúrio
PRL	Prolactina
PTH	paratormônio
RHP	Real Hospital Português
TNF- α	Fator de Necrose Tumoral alfa
TRS	Terapia Renal Substitutiva (hemodiálise, diálise peritoneal ou transplante renal)
U/L	Unidades por litro
UFPE	Universidade Federal de Pernambuco
UI/L	Unidades internacionais por litro
VR	Valor de Referência

SUMÁRIO

1	INTRODUÇÃO	14
2	REVISÃO DA LITERATURA	17
2.1	DOENÇA RENAL CRÔNICA	17
2.2	DOENÇA CARDIOVASCULAR NA DOENÇA RENAL CRÔNICA	21
2.3	PROLACTINA	24
2.4	PROLACTINA E O SISTEMA IMUNE	27
2.5	PROLACTINA E DOENÇA CARDIOVASCULAR	28
2.6	DOENÇA RENAL CRÔNICA E INFLAMAÇÃO	30
2.7	PROLACTINA E DOENÇA RENAL CRÔNICA	35
3	JUSTIFICATIVA DA PESQUISA	39
4	HIPÓTESES	40
5	OBJETIVOS	41
5.1	OBJETIVO PRINCIPAL	41
5.2	OBJETIVOS ESPECÍFICOS	41
6.1	MÉTODOS	42
6.2	TIPO DE ESTUDO	42
6.3	LOCAL E POPULAÇÃO	42
6.4	CRITÉRIOS DE INCLUSÃO E EXCLUSÃO	42
6.5	DOSAGENS SANGUÍNEAS E VARIÁVEIS	43
7	ANÁLISE ESTATÍSTICA	47
8	ASPECTOS ÉTICOS	48
9	RESULTADOS	49
10	CONCLUSÕES	50
11	CONSIDERAÇÕES FINAIS	51
	REFERÊNCIAS	52
	APÊNDICE A – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO	58
	APÊNDICE B – FICHA DE COLETA	60
	APÊNDICE C - ARTIGO DE REVISÃO NARRATIVA <i>Relationship between prolactin, chronic kidney disease and cardiovascular risk</i>	61
	APÊNDICE D – ARTIGO ORIGINAL <i>Prolactin and inflammatory cytokines in hemodialysis patients: a cross-sectional study</i>	75

ANEXO A – COMPROVANTE APROVAÇÃO DO COMITÊ DE ÉTICA	94
ANEXO B - COMPROVANTE PUBLICAÇÃO DE ARTIGO DE REVISÃO	96
ANEXO C – MÉTRICAS 2019 <i>INTERNATIONAL JOURNAL OF ENDOCRINOLOGY</i>	97
ANEXO D - CARTA-RESPOSTA DO EDITOR DA <i>INTERNATIONAL JOURNAL OF ENDOCRINOLOGY</i>	98
ANEXO E – COMPROVANTE DE SUBMISSÃO <i>EUROPEAN JOURNAL OF ENDOCRINOLOGY</i>	99
ANEXO F – COMPROVANTE DE ACEITE APRESENTAÇÃO Eposter ASN KIDNEY WEEK 2020	100
ANEXO G – REGRAS PARA PUBLICAÇÃO <i>EUROPEAN JOURNAL OF ENDOCRINOLOGY</i>	101

1 INTRODUÇÃO

Doença renal crônica (DRC) é definida como a perda progressiva e irreversível da função renal. Apresenta prevalência elevada e crescente na população geral, principalmente por quê as principais etiologias de falência renal são diabetes *mellitus* (DM) e hipertensão arterial, doenças bastante comuns. A DRC tem alta morbidade e está associada a aumento de mortalidade cardiovascular, com cerca de 5 a 10 milhões de mortes atribuíveis anualmente no mundo todo (XIE, 2018).

Os fatores de risco cardiovascular ditos tradicionais, como DM, hipertensão arterial sistêmica, idade avançada, hipertrofia do ventrículo esquerdo, dislipidemia e doença vascular periférica são bem prevalentes nesta população (GANSEVOORT *et al*, 2013).

Entretanto, fatores de risco tidos como não-tradicionais, como hiperfosfatemia, hiperparatireoidismo, inflamação e anemia também são prevalentes nesta população de doentes pois, com a perda de função renal, ocorre acúmulo de diversas substâncias, muitas delas tóxicas ao organismo. Esse estado, denominado uremia, ocasiona, em vias finais, aterosclerose acelerada e aumento de mortalidade (YILMAZ *et al*, 2011).

Dentre estas substâncias que se acumulam com a perda progressiva da filtração glomerular temos a prolactina. A elevação deste hormônio é a anormalidade endócrina mais comum do eixo hipotalâmico-hipofisário. Possui várias etiologias, como alterações fisiológicas (gravidez e amamentação), farmacológicas (neurolépticos, estrogênio e procinéticos) e doenças sistêmicas (tumores hipofisários e hipotalâmicos, hipotireoidismo primário e DRC) (MARANO *et al*, 2014). Nos pacientes com DRC, além de uma redução na excreção renal de prolactina, ocorre também um aumento da sua secreção pelos lactotrofos (BERNARD *et al*, 2015).

A prolactina é um hormônio polipeptídico sintetizado e secretado principalmente pelas células lactotróficas da glândula pituitária anterior. O principal papel atribuído à ela é estimular a proliferação e diferenciação das células mamárias necessárias para a lactação, atuando em seu receptor transmembrana (r-PRL) (HORSEMAN *et al*, 2013). Mas nos últimos anos outras ações ditas “expressões não pituitárias da prolactina” vem sendo relatadas, com mais de 300 ações em diferentes

sítios em diferentes espécies. Estudos também demonstram associação da prolactina com eventos cardiovasculares na população geral (HARING *et al*, 2014)

Um número crescente de processos biológicos continua a ser atribuído à prolactina. Isso se deve, em parte, a presença de seu receptor em diferentes tecidos, com múltiplos domínios intracelulares, fornecendo uma base estrutural que pode sinalizar para várias quinases em diferentes sítios. Dentre estes processos biológicos podemos citar modulação da inflamação, haja vista que o receptor da prolactina é um membro da superfamília de citocinas do tipo 1, sendo amplamente expresso no sistema imunológico, incluindo linfócitos, monócitos, macrófagos, células *natural killer* e células epiteliais tímicas. Ela age então como hormônio e também como citocina, participando em diversas atividades imunomodulatórias (IGNACAK *et al*, 2012).

Portanto, hiperprolactinemia em pacientes em hemodiálise torna-se bastante prevalente, e estudo de Carrero *et al* demonstrou que, nestes pacientes, está associada a aumento de mortalidade cardiovascular por motivos ainda desconhecidos (CARRERO *et al*, 2012).

Até o momento existem poucos estudos avaliando prolactinemia em pacientes em hemodiálise. Nosso objetivo com esta tese é levantar os principais pontos que tornariam a prolactina uma toxina urêmica através de revisão de literatura e trabalho original.

Esta tese de doutorado será apresentada em forma de capítulos constando referencial teórico, justificativa, objetivos e hipóteses da pesquisa, metodologia empregada na pesquisa original realizada no centro de hemodiálise do Real Hospital Português e resultados sob a forma de 2 artigos.

Os 2 artigos resultantes foram o de revisão e a pesquisa original. O artigo de revisão narrativa (Apêndice C) avaliando a relação entre prolactina, risco cardiovascular e doença renal crônica foi aceito e publicado na revista *International Journal of Endocrinology* em 22/06/2020 (Fator de impacto 2,23; Qualis B1 2019). O segundo artigo veio da pesquisa original, intitulado “Prolactina e citocinas inflamatórias em pacientes em hemodiálise: estudo de corte transversal” (Apêndice D). Esta pesquisa foi submetida em formato de artigo original à revista *European Journal of Endocrinology* (Fator de impacto 5,107; Qualis A1 2019).

Após os resultados, é feita a conclusão e considerações finais sobre esta tese.

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2 REVISÃO DA LITERATURA

2.1 DOENÇA RENAL CRÔNICA

Doença renal crônica (DRC) é caracterizada por perda progressiva e irreversível da função renal. É definida por pelo menos um dos seguintes aspectos, por mais de 3 meses: taxa de filtração glomerular menor que 60mL/min/1,73m^2 , ou presença de marcadores de dano renal (**Tabela 1**) (EKNOYAN *et al*, 2013)

TABELA 1 - Definição da doença renal crônica estabelecida pelo KDIGO 2012 - critérios 1 ou 2 por > 3 meses, podendo ser concomitantes

Critério 1 Marcadores de dano renal (um ou mais)	- Albuminúria $\geq 30\text{mg/24 horas}$ ou $\text{RAC} \geq 30\text{mg/g}$; - Hematúria glomerular; - Alterações eletrolíticas devido à tubulopatia; - Anormalidades detectadas pela histologia; - Anormalidades estruturais detectadas por exames de imagem; - Passado de transplante renal.
Critério 2 Redução da TFG	$\text{TFG} < 60\text{mL/min/1,73m}^2$

DRC, Doença Renal crônica; KDIGO, *Kidney Disease - Improving Global Outcomes*; RAC, Relação albumina/creatinina urinária; TFG, Taxa de Filtração Glomerular. FONTE: EKNOYAN G. *et al*. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int*, v.3, n.1, 2012

A DRC tem uma prevalência crescente no Brasil e no mundo, estimada em até 700 milhões de pessoas, mais até que diabetes, osteoartrite e asma. Isso se deve principalmente aos principais fatores etiológicos da DRC, doenças crônicas bem comuns na população geral - hipertensão arterial sistêmica (HAS) e diabetes *mellitus* (DM) (COCKWELL *et al*, 2020).

A DRC, independente da etiologia, é classificada em cinco estágios de acordo com a taxa de filtração glomerular (TFG). Esta taxa pode ser estimada na prática clínica por equações matemáticas através de sexo, raça, idade, creatinina e/ou cistatina C séricas (**Tabela 2**) (ANDERSON *et al*, 2012).

TABELA 2 - Classificação da doença renal crônica em estágios estabelecida pelo KDIGO

ESTÁGIO DA DRC	TFG (mL/min/1,73m ²)
Estágio 1	≥ 90
Estágio 2	60 – 89
Estágio 3	30 - 59
3A	45 – 59
3B	30 - 44
Estágio 4	15 - 29
Estágio 5 *	< 15

* dividido em tratamento conservador ou renal substitutivo (hemodiálise, diálise peritoneal ou transplante)

Esta classificação foi estabelecida em 2012 pela *National Kidney Foundation* e publicada em suas diretrizes *Kidney Disease Improving Global Outcomes – KDIGO 2013*. É amplamente utilizada e serve para avaliação, estratificação de risco e acompanhamento dos pacientes com DRC (EKNOYAN *et al*, 2013). A partir da classificação em estágios, pode-se instituir tratamento conservador ou terapia renal substitutiva (TRS) – hemodiálise (HD), diálise peritoneal ou transplante renal (INKER *et al*, 2014).

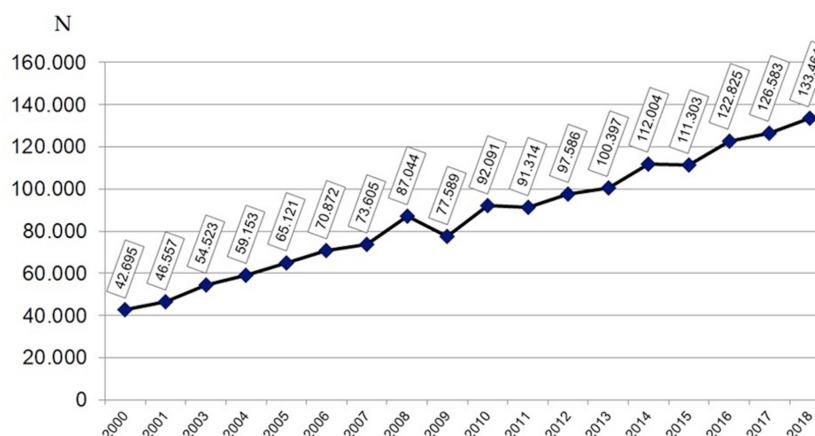
Quanto maior o estágio, pior a função renal do paciente, maior o acúmulo de toxinas urêmicas ocasionando maior prevalência de complicações como anemia, hipertensão arterial, hipertrofia de ventrículo esquerdo, aterosclerose, hiperfosfatemia, inflamação e disfunção endotelial (ASTOR *et al*, 2009).

O tratamento dito conservador na DRC é realizado em todos os seus estágios e de forma precoce. Consiste no manejo clínico das complicações mais prevalentes: controle de pressão arterial, glicose e lipídeos, orientação sobre dieta adequada, tratamento de anemia e hiperfosfatemia, resolução de acidose metabólica, diagnóstico e tratamento do distúrbio mineral/ósseo e estratificação de risco para DCV. Quando o paciente atinge os estágios finais, passa a ter orientação também sobre TRS, para que escolha a modalidade de sua preferência (BASTOS *et al*, 2011).

Apesar de transplante renal ser a TRS de escolha para maioria dos pacientes que atingem DRC estágio 5, a HD é a TRS mais utilizada no Brasil e no mundo devido principalmente a falta de disponibilidade de órgãos (CHAN *et al*, 2019).

Sobre o Brasil, último censo nacional foi divulgado é de 2018, quando 36,6% dos centros de HD responderam questionário solicitado pela Sociedade Brasileira de Nefrologia. Em julho de 2018, no Brasil, o número total estimado de pacientes em diálise foi de 133.464 (FIGURA 1), com uma taxa bruta de mortalidade anual bastante elevada, de 19,5%. Dos pacientes prevalentes em TRS dialítica, 92,3% estavam em HD e 7,7% em diálise peritoneal, com 29.545 (22,1%) na lista de espera para transplante (NEVES *et al*, 2020).

Figura 1. Número estimado de pacientes em diálise crônica por ano



Fonte: Adaptado de NEVES *et al*, 2020

A hemodiálise consiste basicamente em, através de acesso vascular, realizar a depuração de solutos, por gradiente de concentração, e a ultrafiltração de água, por gradiente de pressão hidrostática, através de um dialisador com membrana semipermeável. Com isso ocorre controle dos níveis séricos de substâncias de baixo e médio peso molecular (sódio, potássio, fósforo, uréia, dentre outras toxinas urêmicas) e também da água corporal.

É uma técnica recente na medicina, com o primeiro dialisador descrito em 1943 por Willem Kolff, jovem médico holandês. Desde então esta terapia vem em constante evolução, com melhorias nas máquinas, dialisadores e acesso venoso (Figura 2) (SCRIBNER *et al*, 2004).

Figura 2 - História da hemodiálise (adaptado de Scribner *et al*, 2004)



Clyde Shields, primeiro paciente a utilizar acesso vascular um shunt arteriovenoso. 1960, EUA. Adaptado de Scribner *et al*, 2004



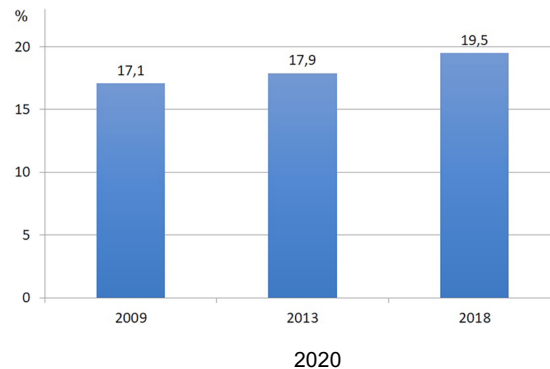
Caroline Helm, primeira paciente de hemodiálise domiciliar, em 1964, EUA. Fonte: Adaptado de Scribner *et al*, 2004

O acesso vascular para HD pode ser temporário ou permanente. Os acessos temporários são os cateteres venosos, geralmente utilizados naqueles pacientes que vão iniciar a TRS, ou naqueles em que há impossibilidade de confecção de fístula arterio-venosa (FAV). Estão mais associados a complicações como trombozes, infecção, inflamação e má eficiência de diálise. Já a FAV é acesso vascular permanente e de escolha nestes pacientes, pois permite uma melhor eficácia da hemodiálise com baixas taxas de complicação (RAVANI *et al*, 2013).

Apesar da HD ter evoluído bastante ao longo dos anos, infelizmente ainda permanece com elevada taxa de mortalidade, particularmente no primeiro ano de tratamento. Por exemplo, as taxas de mortalidade nos primeiros 120 dias após o início da diálise foram de 15% no Japão, 20% na Austrália, 25% nos Estados Unidos (EUA) e 32% na Bélgica. Após o primeiro ano de terapia, as taxas de mortalidade anuais, embora inferiores às do primeiro ano, continuam altas, cerca de 15-20%. Dentro de cinco anos após o início da hemodiálise, apenas 42% dos pacientes em diálise nos EUA e 50% dos pacientes em diálise na Austrália permanecem vivos (SARAN *et al*, 2019).

No Brasil, o último censo sobre mortalidade foi divulgado este ano mas sendo referente a 2018, mostrando uma taxa bruta anual estimada de quase 20%(FIGURA 03).

Figura 3 - Taxa de mortalidade bruta anual estimada de pacientes em diálise



Fonte: Adaptado de NEVES et al,

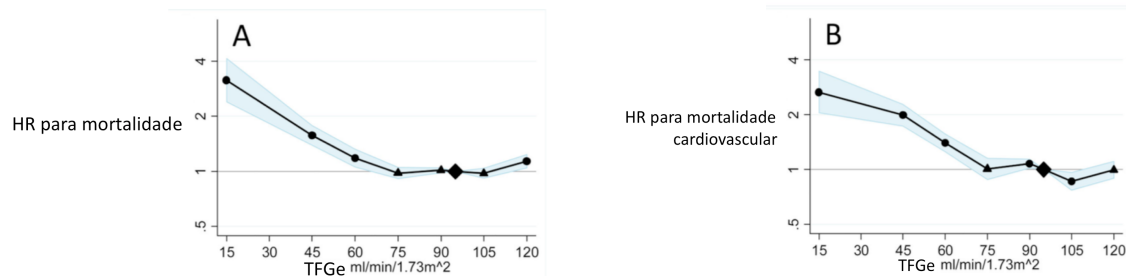
Portanto, os pacientes com DRC, estejam em tratamento conservador ou estejam em tratamento dialítico, apresentam elevadas taxas de mortalidade, principalmente de origem cardiovascular.

2.2 DOENÇA CARDIOVASCULAR NA DOENÇA RENAL CRÔNICA

Em todos os estágios, independentemente do tipo de tratamento e da etiologia da DRC, mortalidade cardiovascular é a principal causa de óbito nestes pacientes (MEGUID EL NAHAS *et al*, 2005). Tanto redução da filtração glomerular (TFG) como aumento da proteinúria, marcadores de DRC, aumentam o risco de doença cardiovascular. Pacientes com DRC nos estágios iniciais são muito mais propensos a morrer de DCV antes de progredir para doença renal estágio 5 (KEITH *et al*, 2004).

Como exemplo podemos citar um grande estudo de meta-análise com 1.234.182 participantes. Comparado com os participantes com TFG estimada normal, redução da TFG estimada para 60, 45 e 15 mL/min/1,73m² tiveram aumento de mortalidade por todas as causas em 18%, 57% e 214% respectivamente (Figura 4) (MATSUSHITA *et al*, 2010).

Figura 4 - Mortalidade (HR e IC 95%) por todas as causas (A) e cardiovascular (B) de acordo com TFG estimada e Albuminúria (ACR).



HR – hazard ratio; TFGe – taxa de filtração glomerular estimada; Pontos representam estatisticamente significantes; triângulos, não significantes (Fonte: adaptado de MATSUSHITA *et al*, 2010)

Remodelamento arterial e miocárdico encontram-se na patogênese central da doença cardíaca dos pacientes com DRC (KOTTGEN *et al*, 2007). Como consequência da sobrecarga de volume, sobrecarga pressórica, retenção de toxinas urêmicas, calcificação vascular e disfunção endotelial, evoluem nestes pacientes aterosclerose acelerada e hipertrofia de ventrículo esquerdo (HVE) (SCHIFFRIN *et al*, 2007).

Por exemplo, estudo de coorte de Zoccali *et al* avaliando sobrecarga hídrica em 39.566 pacientes em hemodiálise durante 1 ano, através de pressão arterial e bioimpedância, chegaram a conclusão que quanto maior a sobrecarga volêmica crônica nestes pacientes, maior a mortalidade. (ZOCCALI *et al*, 2017).

Como mencionado, uma das consequências mais importantes desta sobrecarga volêmica é a HVE. Juntamente com anemia, hiperparatireoidismo secundário e inflamação, sobrecarga pressórica leva ao desenvolvimento de HVE em até 80% dos pacientes em hemodiálise, com boa parte não tendo sintomas clínicos de insuficiência cardíaca (DI LULLO *et al*, 2015).

Diabetes mellitus (DM) é um importante fator de risco para doenças cardiovasculares e, a depender da população estudada, também é bastante prevalente nos pacientes com DRC. Por exemplo, DM é a principal etiologia de DRC nos Estados Unidos e Europa, podendo chegar a 40% dos pacientes, enquanto que glomerulopatias primárias são a principal causa na China e no Japão (HILL *et al*, 2016).

Pacientes diabéticos tem risco de DRC até 10 vezes maior que pacientes

não-diabéticos. Interessante citar que, nos diabéticos sem nefropatia, a mortalidade cardiovascular não difere de forma significativa da população geral (AFKARIAN *et al*, 2013).

Portanto, fatores de risco cardiovascular tradicionais como hipertensão arterial, HVE, DM, dislipidemia e síndrome metabólica são muito prevalentes nos pacientes com DRC, sendo bastante estudados. Contudo, é importante lembrar que nestes pacientes há fatores de risco cardiovascular não tradicionais.

Por exemplo, calcificação vascular é observada mesmo em adultos jovens em diálise que não apresentam hipertensão, tabagismo ou dislipidemia associados (GOODMAN *et al*, 2000). Calcificação vascular nos pacientes com DRC pode ocorrer de forma diferente da clássica deposição de cálcio na camada íntima decorrente da aterosclerose. Nestes pacientes pode haver deposição também na camada média, como resultado de uma mudança fenotípica de células musculares lisas para células semelhantes a osteoblastos, um fenômeno induzido por hiperfosfatemia, hipercalcemia e hiperparatireoidismo (VERVLOET *et al*, 2017).

Outro fator de risco cardiovascular não tradicional comum em pacientes com DRC é anemia. Níveis baixos de hemoglobina e deficiência relativa de eritropoetina levam a um aumento do débito cardíaco, redução da oferta de oxigênio ao miocárdio, estresse oxidativo e apoptose de cardiomiócitos. Por isso anemia é um fator de risco para o desenvolvimento e progressão de HVE, insuficiência cardíaca e mortalidade (CHANG *et al*, 2014).

Albuminúria elevada (acima de 30mg em urina de 24 horas) também é um fator de risco associado a doença cardiovascular (DCV), independente da presença ou não de DM. Apesar do mecanismo pelo qual a albuminúria está associada à DCV não ser bem elucidado, parece ser um sinal de que a vasculatura, particularmente o endotélio, está lesado. Por exemplo, em pacientes diabéticos o grau de disfunção endotelial coronariana parece maior naqueles com albuminúria moderadamente aumentada (BARZILAY *et al*, 2004).

Além dos fatores não tradicionais acima mencionados podemos citar a prolactina. Este hormônio se acumula no sangue com a perda de função renal e está associado a desfechos cardiovasculares por vias ainda desconhecidas, sendo considerado, por alguns autores, uma toxina urêmica (ROS *et al*, 2013).

2.3 PROLACTINA

Prolactina (PRL) é um hormônio polipeptídico sintetizado e secretado principalmente pelas células lactotróficas da glândula pituitária anterior. A primeira função descrita da PRL foi estimular a proliferação e diferenciação das células mamárias necessárias para a lactação, atuando em seu receptor transmembrana (r-PRL) (HORSEMAN *et al*, 2013).

O tamanho da PRL é heterogêneo em termos de formas moleculares circulantes. A forma predominante em indivíduos saudáveis e pacientes com hiperprolactinemia é a monomérica, com peso molecular de 23 kDa. Também pode ser dimérica (*big prolactina*, com 45-60 kDa) ou macro (*big-big prolactina*, com 150-170 kDa), ambas correspondendo a menos de 20% do total de PRL circulante (VILAR *et al*, 2019).

Hiperprolactinemia é a alteração endócrina mais comum do eixo hipotalâmico-hipofisário. Possui várias etiologias (Tabela 3), mas que podem ser subdivididas em três categorias: fisiológicas, farmacológicas e doenças sistêmicas. Entre estas últimas, incluem-se qualquer afecção da região selar, doenças sistêmicas endócrinas e não endócrinas. A causa mais comum dentre as doenças sistêmicas é o prolactinoma, presente em 50% dos pacientes, seguido por drogas (Tabela 4) (VILAR *et al*, 2019).

Tabela 3 - Principais causas de Hiperprolactinemia

CAUSAS DE HIPERPROLACTINEMIA	Exemplos
Fisiológicas	Gravidez Amamentação Estresse Atividade física Estimulação mamária
Doenças da região selar	Adenomas lactotróficos (prolactinomas) Tumores hipotalâmicos Sarcoidose, histiocitose
Doenças sistêmicas endócrinas	Hipotireoidismo Insuficiência adrenal
Doenças sistêmicas não-endócrinas	Doença renal crônica Cirrose hepática
Farmacológicas	Vide tabela 04

Tabela 4 - Causas farmacológicas de hiperprolactinemia

Antipsicóticos Típicos Atípicos	- Haloperidol, clorpromazina, flupentixol - Risperidone, paliperidona, molindona, quetiapine, olanzapina
Antidepressivos Tríciclicos SSRI SNRI MOAi	- Clomipramine, amitriptilina, amoxapina - Fluoxetina, Fluvoxamina, Paroxetina, citalopram, escitalopram, sertralina - Venlafaxina, duloxetina, reboxetina - Pargilina, clorgilina
Antihipertensivos	Verapamil, metildopa, reserpine, labetalol
Procinéticos	Metoclopramide, domperidona
Bloqueadores receptor H2	Cimetidine, ranitidina
Outros	Estrógenos, morfina, alprazolam, heroína, cocaína, marijuana

SSRI – inibidores seletivos da recaptação de serotonina; SNRI – inibidores da recaptação de serotonina e noradrenalina; MOAi – inibidores da monoamina oxidase. Fonte: Adaptado de VILAR *et al*, 2019.

A PRL foi descrita inicialmente como hormônio estimulador da lactação em mamíferos. Mas nos últimos anos “expressões não pituitárias da prolactina” vem sendo relatadas, com mais de 300 ações em diferentes sítios em diferentes espécies. Isso se deve, em parte, a presença de seu receptor em diferentes tecidos, com múltiplos domínios intracelulares, fornecendo uma base estrutural que pode sinalizar para várias quinases em diferentes sítios. Portanto, a ativação destas vias de sinalização podem produzir diferentes respostas celulares. Dentre estes processos biológicos podemos citar resistência insulínica, síndrome metabólica, modulação da inflamação, disfunção endotelial e aterosclerose acelerada (HORSEMAN *et al*, 2013).

Hiperglicemia foi demonstrada em homens e mulheres com hiperprolactinemia, devido aos efeitos diretos da PRL no crescimento de ilhotas de Langerhans e produção de insulina (TUZCU *et al*, 2003). Pacientes com prolactinoma apresentam maior risco de hiperglicemia, acompanhados de obesidade e resistência à insulina (BERINDER *et al*, 2011). Interessante que o excesso de PRL piora o perfil de glicose, por aumento de resistência à insulina, tanto em pacientes obesos como também não obesos, com a dopamina provavelmente desempenhando um papel como modulador das funções da insulina (AURIEMMA *et*

al, 2019). Devido a estes efeitos a bromocriptina, agonista dopaminérgico que inibe a secreção de prolactina, foi aprovada nos Estados Unidos em 2011 para tratamento de *diabetes mellitus* tipo 2 (tabela 5) (DEFRONZO *et al*, 2011).

Tabela 05 - Efeitos dos agonistas dopaminérgicos Bromocriptina e Cabergolina sobre anormalidades metabólicas em pacientes com hiperprolactinemia

	Bromocriptina	Cabergolina
Peso corporal	↓	↓
Índice de Massa Corporal	↓	↓
Glicemia de jejum	↓	↓
Resistência insulínica	↓	↓
Prevalência de Síndrome metabólica	↓	↓

Fonte: Adaptado de AURIEMMA, 2019

Prolactinemia elevada também está associada a efeitos adversos no perfil lipídico, aumentando LDL e triglicerídeos, e reduzindo o HDL (BERNABEU *et al*, 2013). Estudo em 38 pacientes com prolactinomas que foram tratados com cabergolina, agonista do receptor da dopamina, demonstrou melhora no perfil metabólico (CIRESI *et al*, 2013).

Um outro estudo, caso-controle, com 19 pacientes com prolactinoma e 20 controles, demonstrou melhora do perfil metabólico [redução do LDL, triglicerídeos, glicemia de jejum, Índice de Massa Corporal (IMC) e circunferência abdominal] naqueles pacientes com prolactinomas tratados com cabergolina por seis meses (PALA *et al*, 2015).

Recentemente teve publicação de metanálise com 14 estudos observacionais envolvendo 387 participantes com prolactinoma avaliando-se peso, resistência insulínica, proteína C reativa (PCR) e perfil lipídico. Este trabalho concluiu que houve redução de peso, bem como um melhor perfil lipídico, inflamatório e de tolerância à glicose após o tratamento com agonista dopaminérgico nestes pacientes (BYBERG *et al*, 2019).

Além destas alterações metabólicas, a prolactina também está envolvida como mediador da comunicação entre o sistema neuroendócrino e o sistema imune (IGNACAK *et al*, 2012). O receptor de PRL é um membro da superfamília de citocinas do tipo 1, sendo amplamente expresso no sistema imunológico, incluindo linfócitos, monócitos, macrófagos, células *natural killer* e células epiteliais tímicas.

2.4 PROLACTINA E O SISTEMA IMUNE

A prolactina é um hormônio secretado na hipófise, através do eixo hipotálamo-hipófise-adrenal, sob inibição da dopamina. Além dessa regulação dopaminérgica, a secreção de PRL, principalmente em sítios fora deste eixo principal, também é regulada por citocinas inflamatórias como a interleucina (IL)-1, IL-2 e IL-6, estimuladoras da secreção, enquanto que a endotelina-3 e o interferon (IFN)- γ desempenham um papel inibitório (SAVINO *et al*, 2017).

O receptor da PRL é um membro da superfamília de citocina tipo 1/receptor hematopoiético, sendo amplamente expresso através do sistema imunológico como monócitos, linfócitos, macrófagos, células *Natural Killer*, granulócitos e células epiteliais do timo. Portanto, a ligação da PRL ao seu receptor ativa vias de sinalização que influenciam a proliferação, diferenciação, secreção e sobrevivência de células imunes (Tabela 06) (BORBA *et al*, 2018). Essa molécula é um membro integrante de uma rede imuno-neuro-endócrina, com hiperprolactinemia sendo amplamente associada a doenças autoimunes como lupus eritematoso sistêmico, esclerose múltipla, tireoidite de Hashimoto, artrite reumatoide e síndrome do anticorpo antifosfolípide (ORBACH *et al*, 2007).

Tabela 6 - Efeitos da prolactina (PRL) em células do sistema imune

Célula Imune	Secreção de PRL	Expressão do receptor de PRL	Efeito imunológico da prolactina
Timócitos	sim	sim	Diferenciação de timócitos CD4- e CD8 em células CD4+ e CD8+
Células T	sim	sim	Efeito imunomodulatório nos estágios iniciais da maturação; aumenta secreção de TNF- α , IFN- γ e IL-2; proliferação de IL-2; aumenta adesão a células endoteliais
Células B	sim	sim	Influencia na maturação de células B
Células NK	?	sim	Estimula diferenciação e modula citotoxicidade das células NK
Monócitos	sim	sim	Aumenta expressão de TNF
Macrófagos	sim	sim	Secreção de quimiocinas e citocinas (IL1-beta, IL-12, IFN- γ , TNF)

NK – *natural killer*; Fonte: Adaptado de BORBA *et al* 2018

A prolactina tem papel nas respostas imunes inatas e adaptativas, gerenciando por exemplo, a maturação dos timócitos CD4- e CD8- em células T CD4+ e CD8+, através da modulação da expressão do receptor de IL-2 (PEREIRA-SUAREZ *et al*, 2015). Com relação aos linfócitos B, foi relatada uma correlação direta entre o número destas células e os níveis séricos de PRL (BRAND *et al*, 2004). Hiperprolactinemia pode ainda prejudicar a exclusão clonal das células B e diminuir seu limiar de ativação, promovendo assim auto-imunidade. Por exemplo, pacientes com hiperprolactinemia apresentam uma prevalência aumentada em relação à população geral de autoanticorpos como anti-cardiolipina, anti-La e anti-Ro (SAHA *et al*, 2009).

Outra ação promovida pela PRL é a secreção de IL-6 e INF- γ , através de um papel regulador nos níveis de IL-2. Além disso, a PRL aumenta a produção de imunoglobulinas e estimula o desenvolvimento de células apresentadoras de antígenos, potencializando a resposta inflamatória (CEJKOVA *et al*, 2009).

Portanto a PRL age como hormônio e também como citocina, participando em diversas atividades imunomodulatórias (COSTANZA *et al*, 2015). Do ponto de vista de patogenia, aterosclerose e aterogênese envolve processos inflamatórios em todas as suas etapas (WONG *et al*, 2012), com a prolactina podendo ter algum papel nessa cascata.

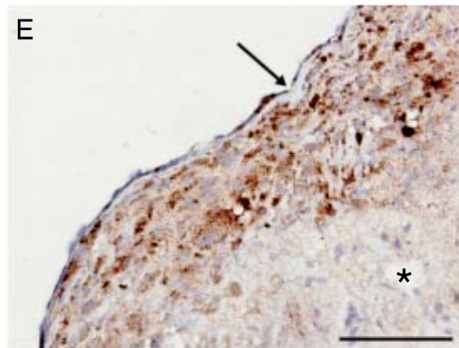
2.5 PROLACTINA E DOENÇA CARDIOVASCULAR

Vários estudos demonstram associação de prolactinemia a diversos desfechos cardiovasculares.

Foi visto, em estudo com ratos, que diferentes níveis plasmáticos de PRL têm efeitos opostos: PRL pouco elevada causa diminuição na pressão arterial (PA) causada pelo aumento da produção de óxido nítrico (NO), enquanto que elevações hormonais maiores levam a um aumento na PA associado a insuficiência cardíaca devido a diminuição da produção de NO (GONZALEZ *et al*, 2015) (CHANG *et al*, 2016).

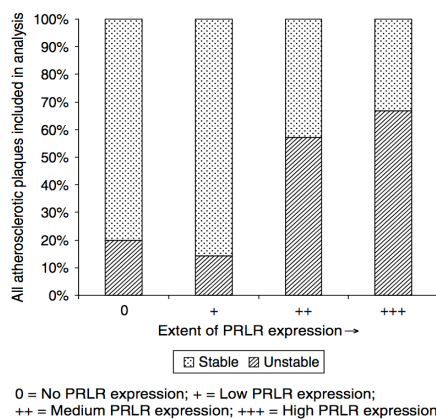
Estudo de Reuwer *et al* com pacientes submetidos à endarterectomia identificou nos macrófagos das placas ateroscleróticas receptores para prolactina, o que pode demonstrar um possível efeito modulatório na aterogênese (Figura 5) (REUWER *et al*, 2011).

Figura 5 - Receptores de prolactina em placas ateroscleróticas



Coloração imuno-histoquímica para receptores de prolactina.

Seta indicando capa fibrótica e receptores para prolactina em macrófagos da placa.
* indicando núcleo necrótico.



Cada barra representa uma intensidade específica de coloração do receptor de Prolactina.

A expressão do receptor de prolactina foi associada com o estágio da lesão aterosclerótica: coloração muito positiva ocorreu em placas mais instáveis.

Fonte: Adaptado de REUWER *et al*, 2011

Estudo de 2009 com mulheres na menopausa precoce foi visto que PRL, mesmo em níveis normais, se correlacionou com o *HeartScore* da *European Society of Cardiology*, índice composto que prediz mortalidade cardiovascular em 10 anos, aventando a hipótese de que a PRL pode desempenhar um papel na arteriosclerose acelerada, ao elevar pressão e rigidez arterial (GEORGIOPOULOS *et al*, 2009).

Estudo de Arslam *et al* que incluiu 35 pacientes hiperprolactinêmicos com adenomas hipofisários não tratados e 36 controles saudáveis foi visto que espessura média da camada íntima da carótida, glicemia capilar, resistência insulínica (HOMA-IR) e proteína C-reativa ultrasensível foram significativamente maiores nos pacientes com PRL elevada, demonstrando que hiperprolactinemia está associada a aterosclerose pré-clínica e também a anormalidades metabólicas (ARSLAM *et al*, 2014). Outro estudo semelhante de caso-controle, desta vez com 31 pacientes com

prolactinoma e 60 controles, demonstrou aumento da espessura da camada íntima-média carotídea, com aumento de resistência insulínica, inflamação e disfunção endotelial naqueles com prolactina elevada (JIANG *et al*, 2014).

Outro interessante efeito cardiovascular descrito em associação com hiperprolactinemia é a cardiomiopatia periparto (CMP). CMP é uma doença rara associada ao final da gravidez ou período peri-parto, marcada por disfunção sistólica grave levando a redução da fração de ejeção e sintomas de insuficiência cardíaca (GIVERTZ *et al*, 2013). Foi demonstrado que, nestas pacientes, por motivos ainda desconhecidos, ocorre a clivagem da PRL a partir da sua forma de 23 kDa a uma forma de 16 kDa pela catepsina-D. Esta PRL de 16 kDa induz a apoptose de células endoteliais, vasoconstrição, redução do metabolismo e da função de cardiomiócitos, levando a CMP (HONIGBERG *et al*, 2019). Em 2010, estudo piloto utilizando tratamento padrão associado a bromocriptina em mulheres com CMP demonstrou que o grupo que recebeu a bromocriptina apresentou melhora da fração de ejeção em 6 meses quando comparadas ao grupo de terapia padrão (SLIWA K *et al*, 2010).

O primeiro estudo a relatar associação positiva de níveis séricos de PRL com mortalidade cardiovascular foi o de Haring *et al*. Este foi um estudo de coorte avaliando 3.929 indivíduos (1.946 homens e 1.983 mulheres) entre 20 e 81 anos durante 10,1 anos (com um total de 38.231 pessoas-ano) que, após análise multivariada, observou uma associação positiva da PRL com mortalidade por todas as causas em homens e mulheres (HARING *et al*, 2014).

Outro estudo de coorte mais recente avaliando prolactinemia e incidência de riscos cardiovasculares não achou associação entre eles, mas convém ressaltar que pacientes com prolactina elevada (acima de 30mg/dL) foram excluídos [(níveis de prolactina médio foram normais em mulheres (11.9 mg/dL) e homens (8.0 mg/dL)] (THERKELSON *et al*, 2016). Neste mesmo estudo foi visto que nos homens um aumento de 5 mg/dL na PRL, mesmo em níveis normais, foi associado com aumento significativo de hipertensão e DM.

2.6 DOENÇA RENAL CRÔNICA E INFLAMAÇÃO

Cada vez mais a DRC tem sido reconhecida como uma condição de inflamação crônica, havendo alterações na imunidade inata e adquirida, tendo relação estreita com DCV e infecções (Figura 6). Esta disfunção imunológica em

imunoativação, resultando em inflamação e aumento de citocinas pró-inflamatórias (ZOCCALI *et al*, 2006).

Citocinas inflamatórias são pequenas proteínas (<40 kDa) produzidas por quase todas as células com a função de regular a ativação e produção de células imunes, bem como a liberação de outras citocinas. Podem ser pró-inflamatórias, perpetuando a cascata de reação inflamatória, ou anti-inflamatórias, contrabalanceando uma resposta imune exagerada. A liberação simultânea de citocinas pró e anti-inflamatórias ocorre em qualquer processo inflamatório (AKDIS *et al*, 2016).

Cada citocina pode ser produzida por diferentes tipos de células e pode agir também em diferentes células. Diferentes citocinas podem ter ação semelhante, algo até redundante, mas quando em conjunto serem sinérgicas (Tabela 7).

Tabela 7 - Principais citocinas inflamatórias e suas características

Citocina	Estrutura	Peso molecular	Receptor	Produção	Alvo	Funções
IL-2	monômero	15,5 kDa	IL2-r	Células T CD4 e CD8, DC, células NK	Células T CD4 e CD8, células NK, células B	Proliferação de células T e células B; desenvolvimento de células TReg; diferenciação e proliferação de células NK; estímulo para síntese de anticorpos
IL-4	monômero	15 kDa	IL-4r tipo I e tipo II	Células Th2, eosinófilos, basófilos, mastócitos, células T NK	Células T e células B	Indução de diferenciação Th2; produção de IgE; <i>upregulation</i> de expressão MHC classe II; proliferação de células T e B; papel em adesão celular
IL-6	monômero	19-26 kDa	IL-6r	Células endoteliais, fibroblastos, monócitos, macrófagos, células T, células B, músculo liso, condrócitos,	Hepatócitos, leucócitos, células B, células T, células hematopoiéticas	Indução de proteínas de fase aguda nos hepatócitos; ativação de leucócitos; ativação e diferenciação de células T; diferenciação de

				osteoblastos		células B, com produção de IgG, IgM e IgA; hematopoiese; neoangiogênese; sobrevivência de neurônios colinérgicos
IL-10	monômero	20,5 kDa	IL10r1 e IL10r2	Células T; células B; monócitos; macrófagos	Macrófagos; monócitos; células T; células B; células NK; mastócitos; granulócitos	Efeito anti-inflamatório direto em células T; supressão de IgE e indução de IgG pelas células B
IL 17A	Monômero e heterodímero	35 kDa	IL-17Ar	Células Th17; células T CD8; células NK; neutrófilos	Células epiteliais e endoteliais; fibroblastos; osteoblastos; monócitos; macrófagos; linfócitos T e B; células estromais da medula óssea	Indução de citocinas pró-inflamatórias, quimiocinas e metaloproteinases; recrutamento e ativação de neutrófilos
Interferon γ	monômero	40-60 kDa	IFN γ r1 e IFN γ r2	Células NK; macrófagos; células Th1; células T citotóxicas; células B	Células epiteliais; macrófagos; células dendríticas; células NK; células T e células B	Propriedades antivirais; promoção de atividade citotóxica e diferenciação Th1; <i>upregulation</i> de MHC classe I e classe II; efeitos pro-apoptóticos
TNF-alfa	trímero	26 kDa	TNFr1 e TNFr2	Macrófagos ativados; células T CD4; células B; neutrófilos; células NK; fibroblastos; células endoteliais; adipócitos	Células nucleadas	Defesa do hospedeiro; caráter duplo como mediador pro-inflamatório iniciando intensa cascata de inflamação, mas também mediador imunossupressivo limitando a inflamação; papel em doenças autoimunes e gênese de câncer

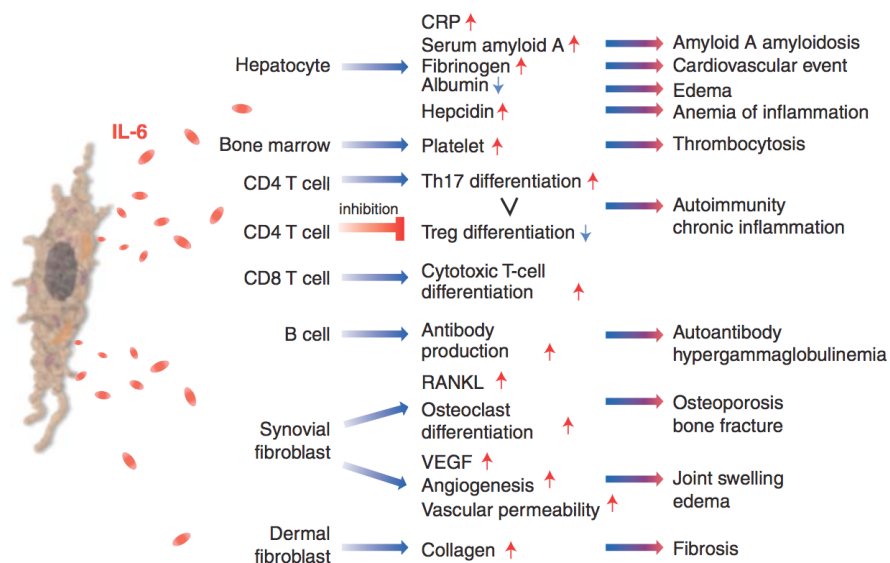
IL - interleucina; kDa – kilodaltons; NK – *natural killer*

Fonte: Adaptado de AKDIS *et al*, 2016

Dentre as citocinas inflamatórias destaca-se na DRC a interleucina (IL)-6. A IL-6 é um polipeptídeo secretado a partir de monócitos ativados, macrófagos, fibroblastos, adipócitos e células endoteliais em resposta a diversos estímulos. Esta potente citocina pró-inflamatória foi descrita inicialmente como fator de diferenciação

de células T citotóxicas e estimulação de células B, posteriormente sendo vista com papel central tanto em inflamação local quanto sistêmica (TANAKA *et al*, 2014). É uma citocina pleiotrópica envolvida também em respostas de fase aguda e hematopoiese (figura 7).

Figura 7 - Papel da Interleucina-6 na imunidade e inflamação



Fonte: Adaptado de TANAKA *et al*, 2014

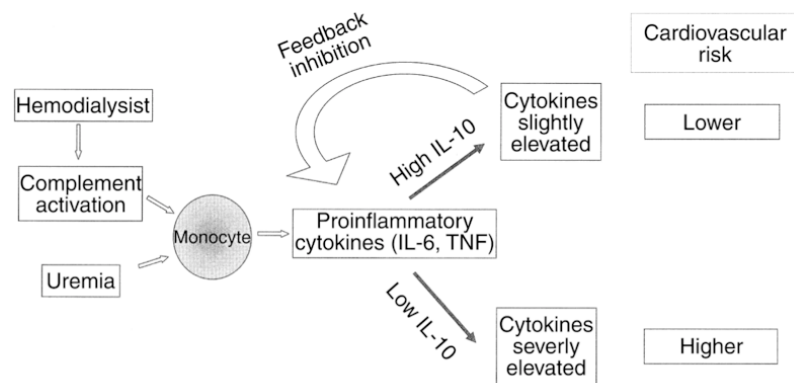
Metanálise com 82 estudos avaliando relação entre IL-6 e doenças coronarianas com amostras genéticas e biomarcadores, em pacientes com função renal normal, com mais de 200.000 participantes, chegou a conclusão que existe associação causal entre níveis séricos de IL-6 e doença coronariana (SARWAR *et al*, 2012).

Não há muitos estudos avaliando esta citocina em pacientes com doença renal, mas podemos citar estudo de coorte de Barreto *et al* que avaliou associação entre níveis de IL-6, calcificação da aorta e mortalidade em 125 pacientes com DRC em tratamento conservador e em hemodiálise, demonstrando que a IL-6 foi preditor independente de mortalidade geral e cardiovascular (BARRETO *et al*, 2010). Outro estudo de coorte, de Pecoits-Filho *et al*, acompanhando por uma média 3,1 anos 173 pacientes com DRC logo antes de iniciarem diálise observou forte valor preditivo dos níveis de IL-6 para mortalidade (PECOITS *et al*, 2002).

A IL-10 é uma potente citocina anti-inflamatória sintetizada por linfócitos T, monócitos, células B e macrófagos. Ela afeta diretamente as funções das células apresentadoras de antígenos ao regular negativamente a expressão de MHC classe II e também de moléculas co-estimulatórias nas superfícies de macrófagos e monócitos. A IL-10 inibe a expressão de muitas citocinas pró-inflamatórias, quimiocinas e receptores de quimiocinas, fisiologicamente limitando e reduzindo inflamação sistêmica (SABAT *et al*, 2010).

Nos pacientes com DRC avançada é esperado níveis mais elevados dessa citocina, já que ela normalmente é filtrada e depois metabolizada em túbulos renais, ficando então com a meia-vida aumentada (MORITA *et al*, 1997). Além disso, esta citocina é contra-reguladora da inflamação excessiva existente nos pacientes em diálise, sendo encontrada elevada em até 2/3 dos pacientes. Inclusive nos pacientes em HD com IL-10 em níveis reduzidos ocorre aterosclerose acelerada e aumento de mortalidade cardiovascular (GIRNDT *et al*, 2002).

Figura 8 - Interleucina-10 interligando a inflamação em pacientes dialíticos



Fonte: Adaptado de GIRNDT *et al*, 2002

2.7 PROLACTINA E DOENÇA RENAL CRÔNICA

Os rins desempenham papel importante na regulação endócrina do organismo, não apenas produzindo alguns hormônios como eritropoetina e renina, mas também atuando na metabolização de outros, como insulina, cortisol e prolactina. Portanto, pacientes com DRC apresentam inúmeras disfunções endócrinas, com alterações em alças de *feedback*, redução no transporte de

hormônios ligados à proteínas, além de redução da metabolização e eliminação hormonal (NIEMCZYK *et al*, 2012).

Em relação à PRL nos pacientes com disfunção renal ocorre sua acumulação por inúmeros mecanismos. Um dos principais é uma redução em sua metabolização (SIEVERTSEN *et al*, 1980). Ocorre também aumento da secreção pelos lactotrofos, já que o estado urêmico, por reduzir a disponibilidade de dopamina no cérebro, estimula diretamente sua secreção (ADACHI *et al*, 2001).

Com isso, hiperprolactinemia nestes pacientes torna-se bastante prevalente, podendo variar de 30% nos estágios precoces a 65% naqueles em hemodiálise (CARRERO *et al*, 2012). Em estudo com 31 pacientes em hemodiálise, 30 em diálise peritoneal, 30 transplantados renais e 72 controles foi visto que a prolactina estava significativamente mais elevada naqueles em diálise, com diferença estatística em relação aos transplantados e controles, que apresentaram prolactina em níveis normais. Importante ressaltar que a hiperprolactinemia observada nos pacientes com DRC não está associada à presença de isoformas de macroprolactina (YAVUZ *et al*, 2005).

Mesmo com uma prevalência bastante aumentada na DRC, o diagnóstico clínico de hiperprolactinemia nesta população é difícil. Os sinais e sintomas de PRL elevada se confundem com algumas manifestações da própria DRC como oligomenorreia, amenorreia, diminuição da libido, disfunção erétil, infertilidade e osteoporose. Galactorréia e ginecomastia são sinais bastante sugestivos, mas apresentam limitada sensibilidade para o diagnóstico (NIEMCZYK *et al*, 2012).

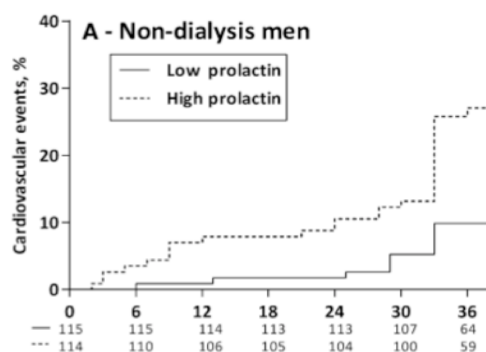
Terapias dialíticas, como hemodiálise convencional e diálise peritoneal, não normalizam a prolactinemia (CARRERO *et al*, 2012). Isso ocorre por quê as terapias dialíticas no geral não promovem a remoção eficaz de moléculas de tamanho médio, como a prolactina. Nem na hemodiálise frequente, 6 ou mais vezes por semana, foi vista redução da prolactinemia, como demonstrado em estudo com 177 pacientes em diálise diária e 60 em hemodiálise noturna (LO *et al*, 2017). Já a hemodiafiltração (HDF), com capilar de alto fluxo, melhora a depuração de moléculas até 25 kDa, às vezes até 50 kDa, ocorrendo redução de prolactinemia. Apesar disto foi evidenciado que algumas horas após a sessão de HDF os níveis de PRL retornam aos valores pré-hemodiálise (WOLLEY *et al*, 2018).

Com o transplante renal e consequente melhora da filtração glomerular ocorre normalização dos níveis séricos de PRL (BRY-GAULLARD *et al*, 1999). Em estudo

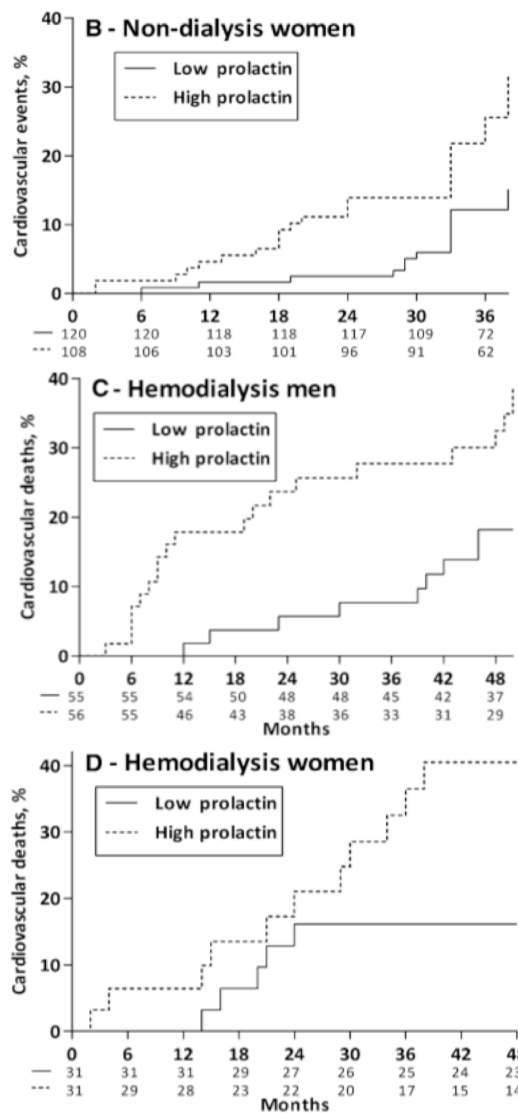
com 14 homens e 7 mulheres avaliando PRL em 3 momentos (imediatamente antes do transplante, 8 dias e 6 meses após) foi visto que houve uma normalização sustentada do nível sérico do hormônio (SAHA *et al*, 2002). Estudos com uso de agonistas dopaminérgicos (cabergolina e bromocriptina) são escassos na população com DRC, mas naqueles com indicação clínica (galactorréia ou hipogonadismo) o seu uso parece seguro (SERRI *et al*, 2006; ESPOSTI *et al*, 1985).

Há poucos estudos avaliando prolactinemia em DRC. Um dos principais a avaliar esta relação foi de Carrero *et al*. Este estudo foi composto de 2 coortes, uma com pacientes DRC em tratamento conservador e outra com pacientes em hemodiálise, durante 3 e 4 anos respectivamente. A coorte com pacientes DRC em conservador constituiu 457 pacientes, com idade média de 52 anos, sendo 229 homens, avaliando-se dilatação fluxo-mediada (FMD) e espessura de íntima-média carotídea. Na coorte com pacientes em hemodiálise foram incluídos 173 pacientes, idade média de 65 anos, com 111 homens, avaliando-se velocidade de onda de pulso (PWV). Como resultado o trabalho evidenciou que os níveis de prolactina aumentaram com a redução da função renal, estando associada a aumento de FMD nos pacientes DRC conservador e da PWV nos dialíticos. Também viram que a cada aumento de 10ng/mL de prolactina ocorria aumento de 27% no risco cardiovascular de pacientes não-dialíticos e aumento de 12% em mortalidade cardiovascular nos dialíticos. Concluíram que os níveis séricos de prolactina estão diretamente associados a disfunção endotelial e aumento de risco e mortalidade cardiovascular em pacientes com DRC (Figura 9) (CARRERO *et al*, 2012).

Figura 9 - Eventos cardiovasculares e mortalidade cardiovascular em homens e mulheres com doença renal crônica tratamento conservador e hemodiálise



A - Eventos cardiovasculares em homens com DRC em tratamento conservador. Tempo em meses



B - Eventos cardiovasculares em mulheres com DRC em tratamento conservador. Tempo em meses

C - Mortalidade cardiovascular em homens em hemodiálise

D - Mortalidade cardiovascular em mulheres em hemodiálise

Fonte: Adaptado de CARRERO *et al*, 2012

3 JUSTIFICATIVA DA PESQUISA

A doença renal crônica (DRC) é bastante prevalente na população geral. Tem elevada morbidade e está associada a aumento de mortalidade cardiovascular, até mesmo naqueles pacientes com terapia renal substitutiva a hemodiálise (COCKWELL *et al*, 2020).

Fatores de risco cardiovascular tradicionais como diabetes *mellitus*, hipertensão arterial sistêmica, idade avançada, hipertrofia do ventrículo esquerdo e doença vascular periférica são bem estabelecidos nesta população (GANSEVOORT *et al*, 2013).

Mas os fatores de risco tidos como não-tradicionais, como hiperfosfatemia, hiperparatireoidismo, inflamação e anemia também são de relevância nesta população de doentes. Pacientes com DRC em hemodiálise apresentam prevalência elevada de hiperprolactinemia, estando associada a aumento de mortalidade cardiovascular ainda por mecanismos desconhecidos (CARRERO *et al*, 2012).

Considerando o relatado, e considerando ainda as ações inflamatórias da prolactina, nosso estudo se propõe a avaliar, de forma inédita, relação entre citocinas inflamatórias e prolactinemia em pacientes com DRC em hemodiálise.

4 HIPÓTESES

H0

Pacientes em hemodiálise com prolactina elevada não apresentam diferença nos níveis de citocinas inflamatórias em relação a pacientes com o hormônio em níveis na faixa de normalidade;

H1

Pacientes em hemodiálise com prolactina elevada apresentam diferença nos níveis de citocinas inflamatórias em relação a pacientes com o hormônio em níveis na faixa de normalidade.

5 OBJETIVOS

5.1 GERAL:

- Avaliar níveis séricos de prolactina e citocinas inflamatórias de pacientes em hemodiálise em centro de referência em Pernambuco;

5.2 ESPECIFICOS:

- Correlacionar os níveis séricos de prolactina com citocinas inflamatórias [interleucina (IL)-2, IL-4, IL-6, IL-10, IL-17A, TNF- α e IFN- γ] em pacientes em hemodiálise;
- avaliar perfil clínico e epidemiológico de pacientes com prolactina normal e elevada em hemodiálise;
- correlacionar níveis séricos de prolactina com tempo em hemodiálise, índice de massa corporal, perfil lipídico, glicemia de jejum, hemoglobina glicada, cálcio, fósforo, fosfatase alcalina e PTH em pacientes em hemodiálise.

6 MÉTODOS

6.1 TIPO DE ESTUDO

Foi realizada um estudo de corte transversal, observacional, em centro de referência em hemodiálise no estado de Pernambuco.

6.2 LOCAL E POPULAÇÃO DO ESTUDO

O projeto de pesquisa foi elaborado no programa de Pós-Graduação em Neuropsiquiatria e Ciências do Comportamento da Universidade Federal de Pernambuco (UFPE) e realizado no serviço de hemodiálise do Real Hospital Português, com 360 pacientes regulares do programa.

A população estudada foi composta por pacientes em terapia renal substitutiva da modalidade hemodiálise do Real Hospital Português, provenientes do Sistema Único de Saúde e também do Sistema Complementar de Saúde, compreendendo 361 pacientes.

As amostras de sangue foram analisadas no REALLAB, laboratório referência do Real Hospital Português. Os pacientes da hemodiálise realizam regularmente exames de sangue como hemoglobina, albumina, colesterol total e frações, cálcio, fósforo, magnésio, fosfatase alcalina, PTH. A estes exames de rotina foi adicionado a dosagem de prolactina. A dosagem de citocinas inflamatórias foi realizada no Laboratório de Imunopatologia Keito Asami (LIKA) da UFPE.

Naqueles incluídos foram avaliados parâmetros clínicos (sexo, idade, tempo de hemodiálise, índice de massa corporal, medicações em uso), bioquímicos [hemoglobina, glicose em jejum, hemoglobina glicada, colesterol total e frações, albumina, cálcio, fósforo, fosfatase alcalina, hormônio paratireoidiano (PTH)] e citocinas inflamatórias [interleucina (IL)-2, IL-4, IL-6, IL-10, IL-17A, fator de necrose tumoral alfa (TNF- α) e interferon gama (IFN- γ)].

6.3 CRITÉRIOS DE INCLUSÃO E EXCLUSÃO

Foram Incluídos:

- Pacientes acima de 18 anos;
- Pacientes com mais de 6 meses em hemodiálise;
- Utilização de acesso vascular fistula arterio-venosa.

Foram excluídos os pacientes com:

- Utilização de prótese vascular ou catéter (de curta ou longa permanência) como acesso vascular;
- infecções virais (HIV, hepatite C, hepatite B) ou bacterianas ativas;
- em uso de antibiótico;
- hipotireoidismo (TSH acima de 5mU/L) ou uso de levotiroxina;
- uso de medicação que sabidamente eleve a prolactina:

Antipsicóticos Típicos Atípicos	- Haloperidol, clorpromazina, flupentixol - Risperidona, paliperidona, molindona, olanzapina
Antidepressivos Tricíclicos SSRI SNRI MOAi	- Clomipramine, amitriptilina, amoxapina - Fluoxetina, Fluvoxamina, Paroxetina, citalopram, escitalopram, sertraline - Venlafaxina, duloxetina, reboxetina - Pargilina, clorgilina
Antihipertensivos	Verapamil, metildopa, reserpina, labetalol
Procinéticos	Metoclopramide, domperidona
Bloqueadores receptor H2	Cimetidine, ranitidina
Outros	Estrógenos, morfina, alprazolam, heroína, cocaína, marijuana

- gestantes e lactantes;
- uso regular de imunossupressores (passado de transplante renal ou doenças autoimunes ativas);
- doença hepática crônica;
- neoplasia ativa em quimioterapia;

6.4 DOSAGENS SANGUÍNEAS E VARIÁVEIS

Todas as dosagens sanguíneas foram realizadas com o paciente em jejum, logo ao início da primeira sessão de hemodiálise da semana, com a amostra sendo retirada da linha venosa. O capilar utilizado na hemodiálise foi o HF80, e todos os pacientes apresentaram boa adequação da hemodiálise, com Kt/V semanal > 1.2 (K,

clearance de uréia pelo dialisador; t, tempo da diálise; V, volume de distribuição de uréia).

A dosagem de prolactina foi realizada uma única vez pelo método de eletroquimioluminescência (Ensaio ADVIA Centaur® Prolactin, da SIEMENS ®). Hiperprolactinemia foi considerada naqueles pacientes com valores de prolactina sérica acima dos limites superiores da normalidade do método: normal até 17,7 ng/mL em homens e até 20,3 ng/mL em mulheres.

As amostras de sangue para dosagem de citocinas inflamatórias foram coletadas em tubos Vacutainer® com EDTA. Para a obtenção do soro os tubos foram centrifugados a 3000 RPM durante 10 min a 4°C e, em seguida, congelado a -80°C, por um período de 10 dias até a análise. O kit CBA (*Cytometric Bead Assay*, Th1, Th2 e Th17, # 560484, BD Biosciences) foi usado para análise das citocinas: IL-2, IL-4, IL-6, IL-10, IL-17A, TNF- α e IFN- γ . Após seguir as instruções do kit para a reação, o ensaio foi adquirido por citometria de fluxo (FACSCalibur™, BD Biosciences©) e os dados obtidos foram analisados com software FCAP Array 3.0 (BD Biosciences©). A quantificação das citocinas foi apresentado em pg/ml. Quantificações que não ficaram dentro do limite de detecção foram consideradas zero ou não detectado (ND) durante a análise por FCAP Array.

MEDIDAS CLÍNICAS E EPIDEMIOLÓGICAS:

Variável Biológica	Definição	Categorização
IDADE	Variável continua definida pela data de nascimento que consta no registro geral	
SEXO		1 – masculino 2 – feminino
ETIOLOGIA DA DOENÇA RENAL CRÔNICA		1 – Diabetes 2 – Hipertensão 3 – Glomerulopatias 4 – Doença Renal Policística Autossômica Dominante 5 – Pielonefrite crônica 6 - Indeterminada
TEMPO DE	Variável continua	

HEMODIÁLISE (em meses)	definida de acordo com mês de início da hemodiálise	
ÍNDICE DE MASSA CORPORAL (IMC) (kg/m ²)	Variável contínua resultado da razão entre o peso e a altura ²	

MEDIDAS LABORATORIAIS:

Variável Biológica	Definição	Categorização
PROLACTINA (ng/mL)		NORMAL Homens ≤ 17,7ng/mL Mulheres ≤ 20,3 ng/mL ELEVADA Homens > 17,7 ng/mL Mulheres > 20,3 ng/mL
CITOCINAS (INTERLEUCINA-2, -4, -6, -10, 17A, TNF- α, IFN- γ) (pg/mL)	Variável contínua	
HEMOGLOBINA (mg/dL)	Variável contínua	
GLICEMIA DE JEJUM (mg/dL)	Variável contínua	
HEMOGLOBINA GLICADA (%)	Variável contínua	
COLESTEROL TOTAL (mg/dL)	Variável contínua	
LDL (mg/dL)	Variável contínua	
HDL (mg/dL)	Variável contínua	
TRIGLICERÍDEOS (mg/dL)	Variável contínua	
ALBUMINA (mg/dL)	Variável contínua	
CÁLCIO (mg/dL)	Variável contínua	
FÓSFORO (mg/dL)	Variável contínua	
FOSFATASE ALCALINA (U/L)	Variável contínua	
PARATORMÔNIO (pg/mL)	Variável contínua	

- a. **PROLACTINA:** dosada pelo método de eletroquimioluminescência
(Ensaio ADVIA Centaur® Prolactin, da SIEMENS ®); VR: 4,04–17,7

ng/mL em homens e 4,79–20,3 ng/mL em mulheres. Dosagem realizada no Real Hospital Português;

- b. **INTERLEUCINA-2, -4, -6, -10, 17A, TNF- α , IFN- γ** : kit CBA (*Cytometric Bead Assay*, Th1, Th2 e Th17, #560484, BD Biosciences); dosagem realizada no Laboratório de Imunopatologia Keito Asami (LIKA) da Universidade Federal de Pernambuco (UFPE).

ETIOLOGIA DA DOENÇA RENAL CRÔNICA

- i. **DIABETES:** nefropatia em diabético tipo I ou tipo II com albuminúria de 24 horas maior que 30 mg, associada à lesão em outro órgão alvo (retina, nervo periférico, coronária, cerebrovascular ou vascular periférico).
- ii. **HIPERTENSÃO:** presença de níveis tensionais > 140 x 90 mmHg associado à lesão de órgão-alvo também de característica hipertensiva.
- iii. **GLOMERULOPATIA:** presença de diagnóstico de glomerulopatia em biópsia renal.
- iv. **DOENÇA RENAL POLICÍSTICA:** presença de exame de imagem (ultrassonografia, tomografia computadorizada ou ressonância nuclear magnética) compatível com o diagnóstico.
- v. **PIELONEFRITE CRÔNICA:** presença de cálculos obstrutivos ou coraliformes com cicatrizes renais avaliados por cintilografia ou piodenografia estabelecida após nefrectomia.
- vi. **INDETERMINADA:** não definida por uma das etiologias descritas acima ou de etiologia multifatorial.

7 ANÁLISE ESTATÍSTICA

Os dados foram armazenados utilizando-se planilha do *Microsoft Office Excel for MAC, versão 15.14 2015*®. Para a obtenção dos resultados estatísticos foi realizado inicialmente um estudo descritivo com o objetivo de discriminar possíveis tendências nas variáveis do estudo. Foi realizado o teste de normalidade e por ocorrer violação da suposição de normalidade, fez-se uso do teste não-paramétrico Kruskal-Wallis. Para os casos onde ocorreram diferenças significativas entre os estágios, seguiu-se com o teste par-a-par não-paramétrico Mann-Whitney para verificar onde ocorria tal diferença. Para as relações entre o estágio da doença e as demais variáveis qualitativas nominais, fez-se uso do teste não paramétrico Qui-quadrado. Para as correlações entre o estágio da doença e as variáveis numéricas utilizou-se, considerando os diferentes estágios, o Coeficiente de *Spearman*. Além disso, para cada estágio foi verificado e testado a existência de possíveis diferenças nas variáveis quando se comparassem os pacientes de prolactina elevada e os pacientes de prolactina normal. Para todos os testes realizados considerou-se o nível de significância de 5%.

No painel de citocinas inflamatórias foi utilizado o teste não paramétrico de Mann-Whitney para fazer as comparações entre os dois grupos. O nível de significância de 0.05 foi considerado, sendo as variáveis que apresentaram p-valor menor que 0.05 estatisticamente significativas.

8 ASPECTOS ÉTICOS

Este estudo foi aprovado pelo Comitê de Ética em Pesquisa envolvendo seres humanos do Centro de Ciências da Saúde da Universidade Federal de Pernambuco (CEP/CCS/UFPE) sob o registro número CAAE: 74031617.0.0000.5208, de acordo com a Resolução nº 466/12 do Conselho Nacional de Saúde (ANEXO A).

Não houve qualquer tipo de remuneração ou privilégio para aqueles que optaram em participar da pesquisa. Os pacientes que apresentaram alterações hormonais significativas (prolactina acima de 100pg/mL) foram encaminhados ao ambulatório de endocrinologia para avaliação e seguimento.

9 RESULTADOS

Desta tese resultaram 2 artigos.

O primeiro artigo, uma revisão narrativa avaliando a relação entre prolactina, risco cardiovascular e doença renal crônica, foi aceito e publicado na revista *International Journal of Endocrinology* em 22/06/2020 (Fator de impacto 2,23; Qualis B1 2019) (**APÊNDICE C, página 61**).

O segundo artigo veio da pesquisa original, intitulado “Prolactina e citocinas inflamatórias em pacientes em hemodiálise: estudo de corte transversal”. Esta pesquisa foi submetida em formato de artigo original à revista *EUROPEAN JOURNAL OF ENDOCRINOLOGY* (Fator de impacto 5,107; Qualis A1 2019)(**APÊNDICE D, página 75**).

10 CONCLUSÕES

Pacientes em hemodiálise apresentam prevalência elevada de hiperprolactinemia, estando essa elevação hormonal associada a maiores níveis de interleucina-6 e menores níveis de interleucina-10.

Pacientes com mais tempo em terapia substitutiva renal a hemodiálise apresentaram maiores níveis séricos de prolactina em comparação a pacientes com menos tempo.

Não foi vista correlação entre níveis séricos de prolactina com níveis de outras citocinas inflamatórias (IL-2, IL-4, TNF-alfa, INF-gama), bem como também com idade, sexo, etiologia da DRC, glicemia de jejum, hemoglobina glicada, IMC, albumina doença mineral óssea (cálcio, fósforo, fosfatase alcalina, paratormônio).

11 CONSIDERAÇÕES FINAIS

Doença renal crônica (DRC) apresenta prevalência elevada no Brasil e no mundo, acometendo 1 de cada 10 pessoas. Apresenta também aumento de novos casos ano após ano devido principalmente a elevada prevalência de suas principais etiologias: diabetes *mellitus* (DM) e hipertensão arterial. Além de elevada prevalência a DRC ainda é fator de risco independente para mortalidade cardiovascular, com cerca de 5 a 10 milhões de mortes atribuíveis anualmente no mundo todo.

Portanto, risco e mortalidade cardiovascular devem ser um dos principais focos dos estudos na nefrologia. Além destes pacientes terem maior prevalência de fatores de risco cardiovascular ditos tradicionais, ainda são acometidos por fatores não comumente encontrados na população geral.

Consideramos estudar a prolactina haja vista escassez de estudos com este hormônio na população com DRC. Este hormônio é considerado por alguns autores uma toxina urêmica - acumula-se com a perda da função renal e está associada ao aumento dos resultados cardiovasculares. Após extensa revisão da literatura vimos que esta substância, também considerada uma citocina inflamatória, pode contribuir para um maior desequilíbrio em uma já afetada homeostase dos pacientes com deficit de filtração renal.

Nesta pesquisa avaliamos pela primeira vez a relação entre hiperprolactinemia e citocinas inflamatórias em pacientes em hemodiálise. O resultado mais significativo foi a evidência de que aqueles com hiperprolactinemia apresentaram níveis séricos mais elevados de interleucina-6 e menores de interleucina-10. Apesar do objetivo inicial da tese ter sido avaliar este perfil antes e depois de terapia com cabergolina, por inúmeros motivos não pode ser conduzida. Apesar disso, após a conclusão desta tese pretendemos continuar esta linha de pesquisa pois, em nossa opinião, muitos pontos permanecem em aberto: por que alguns pacientes apresentam elevação hormonal e outros não; associação de prolactinemia com marcadores de dano cardiovascular nestes pacientes; relação de prolactinemia com desfechos renais em pacientes transplantados e, o principal, se hiperprolactinemia não poderia ser um alvo terapêutico como inúmeros outros nessa população (PTH, hemoglobina, fósforo e albuminúria, por exemplo).

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APÊNDICE A - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

UNIVERSIDADE FEDERAL DE PERNAMBUCO

Avenida Prof. Moraes Rego, s/n
Cidade Universitária, CEP 50740-900, Recife – PE

Convidamos o(a) Sr.(a) para participar, como voluntário(a), da pesquisa intitulada “*ESTUDO SOBRE O EFEITO DA TERAPIA COM CABERGOLINA SOBRE PROLACTINA, PARÂMETROS METABÓLICOS, MARCADORES INFLAMATÓRIOS E ESPESSURA ÍNTIMA-MÉDIA CAROTÍDEA EM PACIENTES EM HEMODIÁLISE COM HIPERPROLACTINEMIA*”, que está sob a responsabilidade do pesquisador Dr. Marclébio Manuel Coêlho Dourado, o qual pode ser localizado em Avenida General MacArthur, 303, Imbiribeira, Recife-PE, CEP 51150-400. Telefone (s): 81-991157540 ou 81-21263537; E-mail – marclebio@yahoo.com.br.

Após ser esclarecido(a) sobre as informações a seguir, no caso de aceitar a fazer parte do estudo, rubrique as folhas e assine ao final deste documento, que está em duas vias. Uma delas é sua e a outra é do pesquisador responsável. Em caso de recusa o(a) Sr.(a) não será penalizado(a) de forma alguma. O(a) senhor(a) tem o direito de retirar o consentimento a qualquer tempo, sem qualquer penalidade.

INFORMAÇÕES SOBRE A PESQUISA:

O presente estudo tem como objetivos definir as características bioquímicas e hormonais nos pacientes com doença renal crônica em hemodiálise e, principalmente, determinar se o tratamento com cabergolina (remédio em comprimido a ser oferecido na pesquisa) em pacientes com prolactina (hormônio) elevada resulta em redução de marcadores de resistência insulínica e inflamatórios, bem como redução da espessura da íntima-média carotídea (lesão em artéria do pescoço).

Participando deste estudo, o(a) Sr.(a) deverá comparecer no próprio centro de hemodiálise onde realiza as sessões. Em uma dessas visitas, serão realizados procedimentos médicos aos quais o(a) Sr.(a) já está habituado (você conversará com seu médico durante uma consulta médica, ele lhe examinará e pedirá alguns exames).

No caso dos exames de sangue, estes serão coletados junto com a rotina mensal, retirada da linha arterial da hemodiálise logo no seu início, sem prejuízo a sua sessão. O ultrassom do pescoço será realizado por radiologista com treinamento na área e é um exame sem radiação ou uso de contraste.

Os desconfortos e riscos poderão ser os do uso da medicação. Os principais efeitos colaterais, quando aparecem, surgem logo no início do tratamento, geralmente desaparecendo depois de duas semanas de tratamento, e são dose-dependentes, ou seja, mais comuns em doses maiores do que as usadas no estudo. Os principais efeitos adversos que podem acontecer são: dor de cabeça, tontura, sonolência, depressão, diarreia, constipação, náuseas, vômitos. Caso o paciente apresente sintomas leves, poderão ser administradas medicações sintomáticas (para dor de cabeça, náusea, vômito, constipação ou diarreia); caso os sintomas sejam persistentes ou mais severos (como sonolência ou depressão), a droga será suspensa e o paciente retirado do estudo.

Como benefício direto o(a) senhor(a) receberá o eventual diagnóstico de hiperprolactinemia (hormônio elevado) e de lesão em artéria carótida. A redução deste hormônio com a medicação pode trazer benefícios como melhor controle de peso, de glicemia, de colesterol e de inflamação, o que acarretará em menor risco cardíaco e melhor qualidade de vida. Como benefício indireto o estudo possibilitará determinar se a

cabergolina pode ser usada para melhora de parâmetros bioquímicos e inflamatórios em pacientes com prolactina elevada em hemodiálise.

As informações desta pesquisa serão confidenciais e serão divulgadas apenas em eventos ou publicações científicas, não havendo identificação dos voluntários, a não ser entre os responsáveis pelo estudo, sendo assegurado o sigilo sobre a sua participação.

Os dados coletados serão guardados no endereço acima informado, no computador pessoal do pesquisador principal, por um período de 5 anos. Não haverá retenção de amostras para armazenamento em banco de sangue.

O(a) Sr.(a) não pagará nada para participar desta pesquisa. Se houver necessidade, as despesas para a sua participação serão assumidos pelos pesquisadores (ressarcimento de despesas). Fica também garantida indenização em casos de danos, comprovadamente decorrentes da participação na pesquisa, conforme decisão judicial ou extrajudicial.

Em caso de dúvidas relacionadas aos aspectos éticos deste estudo, você poderá consultar o Comitê de Ética em Pesquisa Envolvendo Seres Humanos da UFPE no endereço: (Avenida da Engenharia s/n – 1º Andar, sala 4 - Cidade Universitária, Recife-PE, CEP: 50740-600, Tel.: (81) 2126.8588 – e-mail: cepccs@ufpe.br).

(assinatura do pesquisador)

CONSENTIMENTO DA PARTICIPAÇÃO DA PESSOA COMO VOLUNTÁRIO (A)

Eu, _____, CPF ou IDENTIDADE _____, abaixo assinado, após a leitura (ou a escuta da leitura) deste documento e ter tido a oportunidade de conversar e ter esclarecido as minhas dúvidas com o pesquisador responsável, concordo em participar do estudo *“ESTUDO SOBRE O EFEITO DA TERAPIA COM CABERGOLINA SOBRE PROLACTINA, PARÂMETROS METABÓLICOS, MARCADORES INFLAMATÓRIOS E ESPESSURA ÍNTIMA-MÉDIA CAROTÍDEA EM PACIENTES EM HEMODIÁLISE COM HIPERPROLACTINEMIA”*, como voluntário(a).

Fui devidamente informado (a) e esclarecido(a) pelo pesquisador(a) sobre a pesquisa, os procedimentos nela envolvidos, assim como os possíveis riscos e benefícios decorrentes de minha participação. Foi-me garantido que posso retirar meu consentimento a qualquer momento, sem que isto leve a qualquer penalidade (ou interrupção de meu acompanhamento/ assistência/tratamento).

Recife, em _____

Assinatura do participante: _____

Presenciamos a solicitação de consentimento, esclarecimentos sobre a pesquisa e aceite do voluntário em participar. (02 testemunhas não ligadas à equipe de pesquisadores):

Nome: _____ Data ____/____/____

Data ____/____/____

Nome

APÊNDICE B – FICHA DE COLETA

FICHA AVALIAÇÃO PACIENTES INCLUÍDOS - DUPLO PREENCHIMENTO EM TABELA DE EXCEL

NOME _____ Sexo ☐ M ☐ F

DN ____/____/____ Registro: _____

IDADE _____

IMC _____

Início de HD _____ Tempo de HD (meses): _____

ETIOLOGIA DA DRC

☐ DM ☐ HAS ☐ Glomerulopatia (_____) ☐ DRPAD ☐ PNC ☐ indeterminado.

☐ PRL normal ☐ PRL elevada

EXAMES LABORATORIAIS:

<u>PRL</u>	<u>Hb</u>	<u>HbA1c</u>	<u>GJ</u>
<u>Ca</u>	<u>P</u>	<u>FA</u>	<u>PTH</u>
<u>CT</u>	<u>LDL</u>	<u>HDL</u>	<u>TG</u>
<u>IL-2</u>	<u>IL-4</u>	<u>IL-6</u>	<u>TNF-a</u>
<u>IFN-gama</u>	<u>IL-10</u>	<u>IL-17A</u>	

APÊNDICE C: ARTIGO DE REVISÃO NARRATIVA

Relationship between prolactin, chronic kidney disease and cardiovascular risk

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Abstract

Chronic Kidney Disease (CKD) has a high prevalence worldwide, mainly due to its main etiologies - diabetes and hypertension. It has high cardiovascular morbidity and mortality, with traditional risk factors such as atherosclerosis, hypertension, diabetes, smoking and left ventricular hypertrophy being common. Non-traditional cardiovascular risk factors such as anemia, hyperparathyroidism, chronic inflammation and microalbuminuria, are also well studied. Prolactin is a hormone not only related to lactation, being considered by some authors a uremic toxin. It accumulates with loss of renal function and it is associated with cardiovascular outcomes in both normal renal function population and CKD population. The purpose of this narrative review is to raise the main common aspects of CKD, prolactinemia and cardiovascular risk.

Introduction

Chronic kidney disease (CKD) has high and increasing prevalence in the general population, mainly because the main causes of kidney failure are diabetes mellitus (DM) and hypertension, very common diseases. CKD has high morbidity and is associated with increased cardiovascular mortality, with 5 to 10 million annual deaths worldwide (XIE, 2018).

The so-called traditional cardiovascular risk factors such as DM, hypertension, smoking, left ventricular hypertrophy (LVH) and peripheral vascular disease are very prevalent in this population (GANSEVOORT, 2013). However, nontraditional risk factors such as hyperphosphatemia, hyperparathyroidism, inflammation, and anemia are also prevalent in this population. Among these, hyperprolactinemia has received increasing attention in recent years (HARING, 2014).

CKD patients have elevated prolactinemia when compared to the general population and those with high hormone have higher cardiovascular mortality compared to those with normal prolactinemia (CARRERO, 2012). Furthermore, a growing number of biological processes continue to be attributed to prolactin. This is partly due to the presence of its receptor in different tissues at different sites, presenting many cellular responses. These biological processes include insulin resistance, metabolic syndrome, inflammation modulation, endothelial dysfunction, and accelerated atherosclerosis (HORSEMAN, 2013).

The aim of this review was to raise the main points regarding prolactin, cardiovascular risks and CKD.

Chronic Kidney Disease

Chronic kidney disease (CKD) is characterized by progressive and irreversible loss of renal function. It is defined by at least one of the following for more than 3 months: glomerular filtration rate less than $60\text{mL} / \text{min} / 1.73\text{m}^2$, with or without renal damage markers (albuminuria $\geq 30\text{mg} / 24 \text{ hours}$ or urinary albuminuria / creatinine ratio $\geq 30\text{mg} / \text{g}$; persistent glomerular hematuria; persistent electrolyte changes due to tubulopathy; structural abnormalities detected by imaging; previous kidney transplant) (EKNOYAN, 2013).

CKD has increasing prevalence worldwide, estimated at 10% of population, being more common in women when dysfunction is mild, and more common in men when dysfunction is severe (CARRERO, 2018). Its main etiological factors are diabetes mellitus (DM), systemic arterial hypertension, glomerulopathy and autosomal dominant polycystic kidney disease (ADPKD), varying depending on the region evaluated. For example, DM is the most prevalent cause of CKD in the United States and Europe, while glomerulopathy are the leading cause in China and Japan (HILL, 2016).

The CKD classification was established in 2012 by the National Kidney Foundation and published in the Kidney Disease Improving Global Outcomes - KDIGO 2013 guidelines. It is widely used for assessment, risk stratification and follow-up of CKD patients (EKNOYAN, 2013). For example, the more severe renal dysfunction, the higher the mortality. Five-year survival for a dialysis patient in the United States is approximately 35%, and only 25% if the patient is diabetic (XIE, 2018).

CKD is a progressive and silent disease, presenting signs and symptoms only in advanced stages. Therefore, its early diagnosis is very important because, in addition to avoiding a rapid progression to final stages, it is possible to reduce its morbidity and mortality (HAMER, 2006).

Besides high morbidity, CKD also has high mortality. Even those patients in renal replacement therapy (RRT) also have high death rates, much higher than certain types of cancer (TONELLI, 2006). Due to fluid accumulation, cardiac remodeling, uremic cardiomyopathy and advanced atherosclerosis, the main cause of mortality in patients with renal failure is cardiovascular (DE JAGER, 2009).

Chronic Kidney Disease and Cardiovascular disease

At all stages, regardless of type of treatment or CKD etiology, cardiovascular disease is the leading cause of death in these patients (MEGUID EL NAHAS, 2005). Both reduced glomerular filtration (GFR) and increased proteinuria, markers of CKD, increase the risk of cardiovascular disease.

In a large meta-analysis study in which 1.234.182 CKD patients were compared with controls with normal estimated GFR, having estimated GFR reduction to 60, 45 and 15 mL / min / 1.73m² increased mortality due to all causes in 18%, 57% and 214%, respectively (MATSUSHITA, 2010).

In the central pathogenesis of heart disease in CKD patients are arterial and myocardial remodeling (KOTTGEN, 2007). As a consequence of volume overload, pressure overload, retention of uremic toxins, vascular calcification and endothelial dysfunction, these patients present with accelerated atherosclerosis and left ventricular hypertrophy (SCHIFFRIN, 2007).

Therefore, traditional cardiovascular risk factors such as hypertension, LVH, DM, smoking, dyslipidemia and metabolic syndrome are very prevalent in this population, and are widely studied (HAMER, 2006; GARG, 2002). However, it is important to remind that in CKD patients there are non-traditional cardiovascular risk factors.

For example, vascular calcification is observed even in young adults on dialysis, who do not have associated hypertension, smoking or dyslipidemia (GOODMAN, 2000). It is also important to mention that vascular calcification in CKD patients may occur differently from the classical intima layer calcium deposition due to atherosclerosis. In these patients, there may also be deposition in the middle layer as a result of a phenotypic shift from smooth muscle cells to osteoblast-like cells, a phenomenon induced by hyperphosphatemia, hypercalcemia, and hyperparathyroidism (VERVLOET, 2017).

Another nontraditional cardiovascular risk factor common in CKD patients is anemia. Low hemoglobin levels and relative erythropoietin deficiency lead to increased cardiac output, reduced myocardial oxygen supply, oxidative stress, and cardiomyocyte apoptosis. Therefore, anemia is a risk factor for the development and progression of LVH, heart failure and mortality (CHANG, 2014).

Elevated albuminuria (above 30 mg in 24-hour urine) is also a risk factor associated with cardiovascular disease, regardless of presence or absence of diabetes. Although the mechanism by which albuminuria is associated with cardiovascular disease is not well understood, it appears to be a sign that vasculature, particularly the endothelium, is damaged. For example, in diabetic patients, the degree of coronary endothelial dysfunction appears to be higher in those with moderately increased albuminuria (BARZILAY, 2004).

In addition to the nontraditional factors mentioned above, prolactin (PRL) also stands out. This hormone accumulates in the blood with loss of renal function and is associated with cardiovascular outcomes, being considered a uremic toxin (ROS, 2013).

Prolactin

Prolactin (PRL) is a polypeptide hormone synthesized and secreted by lactotrophic cells of the anterior pituitary gland. The main role assigned to PRL is to stimulate proliferation and differentiation of breast cells needed for lactation by acting on its transmembrane receptor (HORSEMAN, 2013).

The size of PRL is heterogeneous in terms of circulating molecular forms. The predominant form in healthy individuals and patients with hyperprolactinemia is monomeric, with molecular weight of 23 kDa. It can also be dimeric (*big* prolactin, 45-60 kDa) or macro (*big-big* prolactin, 150-170 kDa), both corresponding to less than 20% of the total circulating PRL (SINHA, 1995).

Hyperprolactinemia is the most common hypothalamic-pituitary-adrenal axis endocrine alteration. It has several etiologies (Table 1), which can be subdivided into three categories: physiological, pharmacological and systemic diseases. These include any pathology of the selar region, endocrine and non-endocrine systemic diseases. The most common cause among systemic diseases is prolactinoma, present in 50% of patients (VILAR, 2014).

Table 1. Main causes of hyperprolactinemia

CAUSES OF HYPERPROLACTINEMIA	Examples
Physiological	Pregnancy Breast-feeding Stress Physical activity Breast stimulation
Pharmacological (main drugs)	Risperidone, Quetiapine, Olanzapine, Haloperidol, Loxapine, Olanzapine, Clomipramine, Amitriptyline, Fluoxetine, Citalopram, Paroxetine, Phenytoin, Metoclopramide, Domperidone, Ranitidine, Methyldopa, Verapamil, Labetalocaine, Morphine, Cocaine, Sibutramine
Diseases of the selar region	Lactotrophic Adenomas (Prolactinomas) Hypothalamic Tumors Sarcoidosis, histiocytosis
Endocrine systemic diseases	Hypothyroidism Adrenal insufficiency

Non-endocrine systemic diseases	Chronic kidney disease Hepatic cirrhosis
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PRL was initially described as a lactation stimulating hormone in mammals. But in recent years other actions called "non-pituitary prolactin expressions" have been reported, with over 300 actions at different sites in different species.

A growing number of biological processes continue to be attributed to prolactin. This is partly due to the presence of its receptor in different tissues with multiple intracellular domains, providing a structural basis that can signal to multiple kinases at different sites. Therefore, activation of these signaling pathways may produce different cellular responses.

These biological processes include insulin resistance, metabolic syndrome, inflammation modulation, endothelial dysfunction, and accelerated atherosclerosis (HORSEMAN, 2013).

Hyperglycemia has been demonstrated in men and women with hyperprolactinemia due to the direct effects of prolactin on Langerhans islet growth and insulin production (TUZCU, 2003). Elevated PRL also has an effect on blood glucose, increasing peripheral insulin resistance (BEN-JONATHAN, 2006). Prolactinoma patients are at higher risk for hyperglycemia, accompanied by obesity and insulin resistance (BERINDER 2011). Due to these effects, bromocriptine, a dopaminergic agonist that inhibits prolactin secretion, was approved in the United States in 2011 for the treatment of type-2 diabetes mellitus (DEFRONZO, 2011).

Another metabolic effect that elevated PRL causes is lipid profile, increasing LDL cholesterol and triglycerides, while reducing HDL (BERNABEU, 2013). A clinical study showed improvement in the metabolic profile of 38 patients with prolactinomas treated with cabergoline, a dopamine receptor agonist that normalizes serum prolactin levels (CIRESI, 2013). Another study showed as secondary outcome improved metabolic profile [reduction in LDL, triglycerides, fasting glucose, Body Mass Index (BMI) and waist circumference] in patients with prolactinomas treated with cabergoline for six months (PALA, 2015).

Prolactin is also described as one of the numerous mediators of communication between the neuroendocrine system and the immune system (IGNACAK, 2012). The PRL receptor is a member of the type 1 cytokine superfamily

and is widely expressed in the immune system, including lymphocytes, monocytes, macrophages, natural killer cells and thymic epithelial cells. Therefore, PRL acts as a hormone and also as a cytokine, participating in several immunomodulatory activities. At endothelial level, PRL stimulates the adhesion of mononuclear cells to endothelial cells in response to inflammatory cytokines (MONTES, 2005). In a recent study, it was found that hyperprolactinemia is associated with activity in autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, scleroderma, and multiple sclerosis (BORBA, 2018).

Prolactin and Cardiovascular events

In addition to effects described above, serum PRL levels demonstrate an association with several cardiovascular effects. In a study of early menopausal women, PRL, even at normal levels, was found to correlate with the Heart Score of the European Society of Cardiology, a composite index that predicts mortality within 10 years, raising the hypothesis that prolactin may play a role in accelerated arteriosclerosis, affecting blood pressure and stiffness (GEORGIOPOULOS, 2009).

A study that included 35 hyperprolactinemic patients with untreated pituitary adenomas and 36 healthy controls found that mean carotid intima layer thickness, capillary blood glucose, insulin resistance (HOMA-IR), and ultra-sensitive C-reactive protein were significantly higher in patients with elevated PRL, demonstrating that hyperprolactinemia is associated with preclinical atherosclerosis and metabolic abnormalities (ASLAM, 2014). Another similar case-control study, this time with 31 prolactinoma patients and 60 controls, demonstrated increased carotid intima-media thickness, with increased insulin resistance, inflammation, and endothelial dysfunction in those with elevated prolactin (JIANG, 2014).

In a study with rats, it was demonstrated that different plasma PRL levels have opposite effects: slightly high PRL causes decrease in blood pressure (BP) caused by increased nitric oxide (NO) production, whereas higher hormonal elevations lead to increased in BP associated with heart failure due to decreased NO production (GONZALEZ, 2015) (CHANG, 2016).

Another interesting cardiovascular effect described in association with hyperprolactinemia is peripartum cardiomyopathy (PPCM). PPCM is a rare disease associated with late pregnancy or the postpartum period, marked by severe systolic dysfunction leading to reduced ejection fraction and symptoms of heart failure

(GIVERTZ, 2013). It has been shown that in these patients, for reasons yet unknown, PRL cleavage occurs from its 23kDa form to a 16kDa form by cathepsin-D. This 16 kDa PRL induces endothelial cell apoptosis, vasoconstriction, reduced metabolism and cardiomyocyte function, leading to PPCM (HONIGBERG, 2019). In 2010, a pilot study using standard bromocriptine-associated treatment in women with PPCM demonstrated that the group receiving bromocriptine had an improvement in the ejection fraction at 6 months compared to the standard therapy group (SLIWA K, 2010).

An interesting study in patients undergoing endarterectomy found that both fibrotic layer and atherosclerotic plaques macrophages had PRL receptors. In the same study, it was also shown that PRL receptor expression was associated with the atherosclerotic lesion stage - more unstable plaques showed higher receptor expression. Thus, the study raised the hypothesis that PRL has a modulatory effect on atherogenesis (REUWER, 2011).

Cohort study evaluating 3,929 individuals (1,946 men and 1,983 women) aged 20-81 years for 10.1 years (with a total of 38,231 person-years) showed, after multivariate analysis, positive association of serum PRL levels with cardiovascular mortality, being the first study to report this positive association (HARING, 2014).

Another more recent cohort study evaluating prolactinemia and incidence of cardiovascular risk found no association between them, but it should be noted that patients with elevated prolactin (above 30mg / dL) were excluded [(average prolactin levels were normal in women (11.9 mg / dL) and men (8.0 mg / dL)] (THERKELSON, 2016). In this study, each 5 mg / dL PRL increase in men, even at normal levels, was associated with significant increase in hypertension and DM.

Prolactin and Chronic Kidney Disease

Kidneys play an important role in endocrine regulation, not only producing hormones such as erythropoietin and renin, but also acting on the metabolism of others such as insulin, cortisol and prolactin. Therefore, CKD patients have numerous endocrine dysfunctions, with changes in feedback loops, reduced transport of protein-bound hormones, and reduced metabolism and hormone elimination (NIEMCZYK, 2012).

Regarding to PRL in patients with renal dysfunction, its accumulation occurs by several mechanisms. One of the main mechanism is reduction in its metabolism

(SIEVERTSEN 1980). Another important one is increased PRL secretion by lactotrophs in uremic state - reduced availability of dopamine in the brain directly stimulates PRL secretion (ADACHI, 2001).

Thus, hyperprolactinemia in these patients becomes very prevalent, ranging from 30% in CKD early stages to 65% in those on hemodialysis (CARRERO, 2012). In a study of 31 hemodialysis patients, 30 peritoneal dialysis patients, 30 renal transplant recipients, and 72 controls, prolactin was significantly higher in those on dialysis, with statistically significant difference compared to those transplanted and controls who had prolactin at normal levels. Importantly, hyperprolactinemia observed in CKD patients is not associated with the presence of macroprolactin isoforms (YAVUZ, 2005).

Even with a greatly increased prevalence of CKD, the clinical diagnosis of hyperprolactinemia in this population is difficult. Signs and symptoms of elevated PRL are confused with some manifestations of CKD itself such as oligomenorrhea, amenorrhea, decreased libido, erectile dysfunction, infertility, and osteoporosis. Galactorrhea and gynecomastia are suggestive signs, but have limited sensitivity for diagnosis in this population (NIEMCZYK, 2012).

Dialysis therapies, such as conventional hemodialysis and peritoneal dialysis, do not normalize prolactinemia levels (CARRERO, 2012). This is because dialytic therapies generally do not promote effective removal of medium-sized molecules, such as prolactin. Even in frequent hemodialysis, 6 or more times a week, prolactinemia reduction was not observed, as demonstrated in a study with 177 patients on daily dialysis and 60 on nocturnal hemodialysis (LO, 2017). High flow capillary hemodiafiltration (HDF) improves the clearance of molecules up to 25 kDa, sometimes up to 50 kDa, resulting in a reduction in prolactinemia. However, it was evidenced that a few hours after HDF session, PRL levels returned to pre-hemodialysis values (WOLLEY, 2018).

An interesting cohort evaluating 457 non-dialysis CKD patients and 173 hemodialysis patients assessed endothelial function, arterial stiffness, cardiovascular outcomes and prolactinemia. In non-dialysis patients it was observed a 27% increased risk of cardiovascular events for each 10ng / mL PRL elevation, as well as a correlation with arterial stiffness. In those on hemodialysis, cardiovascular and all-cause mortality increased by 12% and 15% respectively with each 10ng / mL PRL

increase, as well as correlation of prolactinemia with pulse wave velocity (CARRERO, 2012).

Renal transplantation and consequent improvement in glomerular filtration result in normalization of serum PRL levels (BRY-GAUILARD, 1999). In a study of 14 men and 7 women evaluating PRL at 3 moments (immediately before transplantation, 8 days and 6 months after), sustained normalization of serum hormone levels was observed (SAHA, 2002). Studies using dopaminergic agonists (cabergoline and bromocriptine) are scarce in CKD population, but in those with clinical indication (like galactorrhea or hypogonadism), their use seems to be safe (SERRI, 2006; ESPOSTI, 1985).

Conclusions

Chronic kidney disease is quite prevalent worldwide, with high cardiovascular mortality. Despite the high prevalence of hyperprolactinemia in this population, there is uncertainty about implications of this condition in patients with CKD, especially with regard to cardiovascular effects. Some authors suggested that prolactin could be a uremic toxin but until now it cannot be concluded whether hyperprolactinemia is a cardiovascular risk factor or only an intermediate in a major pathophysiological pathway. Further studies are needed because if causality between hyperprolactinemia and cardiovascular mortality is demonstrated in CKD patients, this could be a potential therapeutic target.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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APÊNDICE D: ARTIGO ORIGINAL

Prolactin and inflammatory cytokines in hemodialysis patients: a cross-sectional study

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1. ABSTRACT

INTRODUCTION: Prolactin is a hormone that accumulates in the blood with loss of kidney function, acts in the inflammation cascade as a cytokine, and is associated with increased cardiovascular risk due to mechanisms that are still uncertain. The aim of this study was to evaluate, in hemodialysis patients, the relationship between hyperprolactinemia and inflammatory cytokines. **METHODS:** A cross-sectional study was performed evaluating patients with more than 6 months on hemodialysis and arteriovenous fistula as a vascular access. Those with active viral or bacterial infections; having hypothyroidism or active cancer; using medications known to elevate prolactin levels; pregnant women and lactating women; those using immunosuppressants; and patients with chronic liver diseases were excluded. Clinical and biochemical parameters were evaluated, in addition to measurement of prolactin and inflammatory cytokine panel [(interleukin (IL) -2, IL-4, IL-6, IL-10, IL-17A, TNF- α and IFN- γ]. Patients with elevated prolactin were compared to those with normal hormone levels. **RESULTS:** Of 361 regular patients in hemodialysis center, 111 were included. Hyperprolactinemia was found in 61 patients (54.95%). There was no statistical difference regarding age, sex, or kidney disease etiology in patients with normal and elevated hormone. Regarding inflammatory cytokines, patients with elevated prolactin had higher levels of IL-6 and lower IL-10, with statistical significance ($p < 0.001$ and $p = 0.046$, respectively). Regarding the serum prolactin, there was a positive correlation with serum levels of IL-6 ($R = 0.44$, $p < 0.0001$) and negative correlation of IL-10 levels ($R = 0.2$; $p < 0.046$). **CONCLUSION:** hemodialysis patients have a high prevalence of hyperprolactinemia, and this hormonal elevation has been shown to be associated with increased interleukin-6 and reduced interleukin-10

KEYWORDS: Prolactin; Hemodialysis; Inflammation; Interleukin-6; Interleukin-10

2. INTRODUCTION

Chronic kidney disease (CKD) is a serious global health problem, with an estimated prevalence of up to 700 million people, which is even higher than those of diabetes, osteoarthritis, and asthma (1). This high prevalence of CKD is due to its primary etiologies, diabetes mellitus (DM) and hypertension, which are chronic noncommunicable diseases that are quite common. The primary cause of mortality in patients with CKD is related to a cardiovascular origin, and even those on hemodialysis have a higher mortality rate than patients with certain types of neoplasms (2).

CKD is an independent risk factor for cardiovascular mortality, and it is also associated with several other traditional factors such as hypertension, left ventricular hypertrophy, DM, smoking, dyslipidemia; and advanced age (3). However, nontraditional risk factors such as hyperphosphatemia, anemia, inflammation, and microalbuminuria are common in these patients and are also being investigated (4). Hyperprolactinemia could be considered in this context, as it accumulates in the blood with the loss of renal function and is associated with increased mortality in this patient population; its underlying mechanisms are still unknown (5).

Hyperprolactinemia is the most common endocrine disorder of the hypothalamic–pituitary axis (6). It has several etiologies such as physiological changes (e.g., pregnancy and breastfeeding), pharmacological changes (e.g., use of neuroleptics, estrogen, and prokinetics), and systemic diseases (e.g., pituitary and hypothalamic tumors, primary hypothyroidism, and CKD). In patients with CKD, there is an accumulation of prolactin in the blood due to decreased excretion and also increased central secretion (7).

Prolactin was initially described as a lactation hormone, and currently, it is known to have several actions, ranging from insulin resistance and proinflammatory effect to accelerated atherosclerosis (8). Evidence shows that prolactin levels in a population with normal renal function are directly associated with endothelial dysfunction, metabolic syndrome, increased levels of inflammatory markers, and increased cardiovascular risk (9); moreover, in patients with CKD, prolactin levels are related to increased mortality (5). Prolactin also acts as an inflammatory cytokine as its receptor is a member of the superfamily of type 1 cytokines, which are extensively

expressed through the immune system, whose increased expression increases the production of proinflammatory cytokines such as IL-6, TNF- α , and IL-1 β (10).

Based on the above-described evidence, we conducted this study to determine the association between serum levels of proinflammatory and anti-inflammatory cytokines in hemodialysis patients and normal and elevated prolactin levels.

3. METHODS

We conducted a single-center cross-sectional study to evaluate all patients regularly enrolled in hemodialysis (HD) program of the Real Hospital Português (RHP) in August and September 2019. All patients who agreed to participate in the study were informed of the study details, after which they willingly signed a consent form (approval by the ethics committee CAAE: 74031617.0.0000.5208).

We included both men and women aged >18 years who were on hemodialysis for at least 6 months through an arteriovenous fistula made for dialysis. Those on hemodialysis with nonpermanent vascular access (short- or long-term catheter) or vascular prosthesis; with active viral or bacterial infections; patients using antibiotics, having hypothyroidism (diagnosed based on levothyroxine or thyroid hormone levels >5 mU/L), and using medications known to elevate prolactin levels (e.g., amitriptyline, citalopram, escitalopram, fluoxetine, paroxetine, risperidone, metoclopramide, domperidone, alfamethyldopa, and verapamil); pregnant women and lactating women; those using immunosuppressants (in case of transplantation or autoimmune diseases); and patients with chronic liver diseases were excluded.

Clinical parameters (gender, age, time on hemodialysis, weight, height, body mass index, and medications in use), biochemical parameters [hemoglobin, fasting glucose, glycated hemoglobin, total cholesterol and fractions, albumin, calcium, phosphorus, alkaline phosphatase and parathyroid hormone (PTH) levels], and inflammatory cytokines [interleukin (IL)-2, IL-4, IL-6, IL-10, and IL-17A, tumor necrosis factor alpha (TNF- α), and interferon gamma (IFN- γ)] were evaluated.

All blood measurements were performed with the patient fasting, at the beginning of the first hemodialysis session of the week, with the blood sample being collected from the venous line. The capillary used in hemodialysis was HF80, and all patients showed good adequacy of hemodialysis, with weekly Kt/V > 1.2 (K, dialysis

urea clearance; t, dialysis time; V, volume of urea distribution).

Prolactin measurement was performed only once using the electrochemiluminescence method (ADVIA Centaur® Prolactin test, from SIEMENS®). Hyperprolactinemia was considered in those patients with serum prolactin levels above the upper limits of normality of the method as follows: normal up to 17.7 ng/mL in men and 20.3 ng/mL in women.

Blood samples for evaluating inflammatory cytokine levels were collected in EDTA-containing Vacutainer® tubes. To obtain serum, the tubes were centrifuged at 3000 rpm for 10 min at 4°C and then frozen at -80°C for a period of 10 days until analysis. The CBA kit (Cytometric Bead Assay, Th1, Th2, and Th17, #560484, BD Biosciences) was used for analyzing the following cytokines: IL-2, IL-4, IL-6, IL-10, IL-17A, TNF- α , and IFN- γ . The measurements were done according to the manufacturer's instructions, and the assay was acquired by flow cytometry (FACSCalibur™, BD Biosciences®). The obtained data were analyzed using the FCAP Array 3.0 software (BD Biosciences®). The quantification of cytokine levels was presented in pg/ml. Quantifications that did not fall within the detection limit were considered as zero or not detected during the analysis by the FCAP Array.

The data were stored using the spreadsheet of *Microsoft Office Excel for MAC, versão 15.14 2015*®. To obtain statistical results, a descriptive analysis was initially conducted with the objective of discriminating possible trends in the study variables. The normality test was performed and as there was a violation of the assumption of normality, the nonparametric Kruskal–Wallis test was used. For cases showing significant differences between the stages, the nonparametric Mann–Whitney peer-to-peer test was conducted to confirm the origin of this difference. The nonparametric Chi-square test was used to assess the relationships between the stage of the disease and the other nominal qualitative variables. For assessing the correlations between the disease stage and the numerical variables, Spearman's coefficient was used, considering the different stages. Furthermore, for each stage, the possible differences in variables were verified and tested when comparing patients with high prolactin levels and patients with normal prolactin levels. A significance level of 5% was considered for all statistical tests.

In the panel of inflammatory cytokines, the nonparametric Mann–Whitney test was used to make comparisons between two groups. A level of significance of 0.05

was considered, and variables with $p < 0.05$ were considered to be statistically significant.

4. RESULTS

We included 111 patients of the 361 regular patients in the RHP hemodialysis service. The primary reasons for patient exclusion were: 110 patients using access to hemodialysis through a long-term or short-term catheter, 71 patients with active viral infection (60-virus C, 7-HIV, 4-virus B) and 23 patients who were on regular use of medications known to elevate prolactin levels (6-fluoxetine, 5-escitalopram, 5-paroxetine, 4-amitriptyline, 2-domperidone, and 1-methyldopa). Other excluded patients were: 18 regularly using immunosuppressants (10 previous kidney transplant, 6 active autoimmune disease, 2 heart transplant); 8 patients with active bacterial infection; 5 patients with cancer undergoing chemotherapy; 5 patients with hypothyroidism; 5 patients less than 6 months on hemodialysis; 3 patients with cirrhosis; 2 patients with pituitary adenoma.

Table 1 summarizes the clinical characteristics of the patients included in the study. The mean age was 52.31 years (range 26–84 years), with females being slightly more prevalent than males (53% vs 47%). Hyperprolactinemia was found in 61 patients (54.95%).

Table 1. Clinical data of the 111 patients included in the study

Variables	
Age (years)	52,31 ± 14,01(26–84)
Sex	
Male	74 (66,67 %)
Female	37 (33,33 %)
Prolactin *	
Normal	50 (45,05%)
Elevated	61 (54,95%)
CKD etiology	
Diabetes mellitus	29 (26,13%)
Hypertension	24 (21,62)
Glomerulopathies	15 (13,51)
ADPKD	8 (7,21)
Chronic pyelonephritis	9 (8,11)
Undetermined	26 (23,42)

* Normal prolactin - up to 17.7 ng/mL in men and 20.3 ng/mL in women; CKD-chronic kidney disease; ADPKD-Autosomal dominant polycystic kidney disease

The clinical data of patients with elevated and normal prolactin levels are presented in Table 2. There was no statistical difference in terms of age, sex, BMI, or etiology of CKD. Patients with hyperprolactinemia had a statistically significantly longer time on hemodialysis ($p = 0.016$) (Figure 1). There was also a significant correlation between prolactin levels and time on hemodialysis ($p = 0.004369$, $S = 139668$, Spearman rank correlation test); however, the correlation coefficient revealed that it was a weak association ($\rho = 0.2760299$) (Figure 2).

Table 2. Comparison of clinical variables in patients with normal and elevated prolactin levels

Variables	Normal Prolactin	Elevated Prolactin	<i>p</i>
	≤17.7 ng/mL in men ≤20.3 ng/mL in women	>17.7 ng/mL in men >20.3 ng/mL in women	
Age (years)	53.2 (26–80)	51.57 (26–84)	0.55
HD (months)	73.65 (6–211)	111.4 (6–341)	0.016
BMI (kg/m ²)	24.81 (16.23–34.57)	25.82 (16.17–40.62)	0.45
Sex			
Male	34 (68%)	40 (65.57%)	0.95
Female	16 (32%)	21 (34.43%)	
CKD etiology			
Diabetes mellitus	12 (24%)	17 (27.87%)	0.81
Hypertension	11 (22%)	13 (21.31%)	
Glomerulopathies	8 (16%)	7 (11.48%)	
ADPKD	2 (4%)	6 (9.84%)	
Chronic pyelonephritis	5 (10%)	4 (6.56%)	
Undetermined	12 (24%)	14 (22.95%)	

HD-hemodialysis; BMI-body mass index; CKD-chronic kidney disease; ADPKD-Autosomal dominant polycystic kidney disease. Continuous values are shown in average and minimum–maximum.

Figure 1. Comparisons of hemodialysis time, in months, in patients with elevated and normal prolactin (PRL) levels.

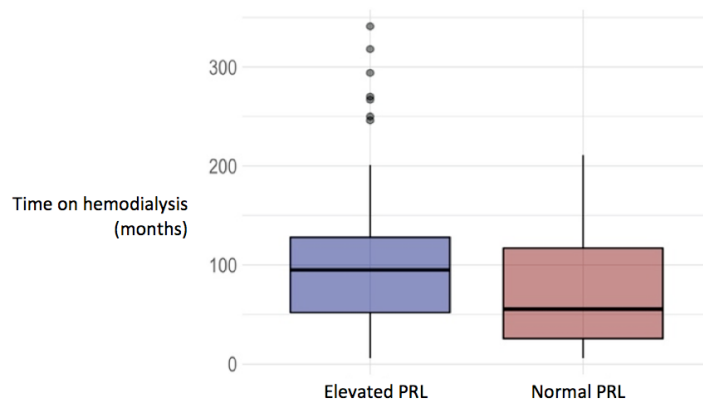
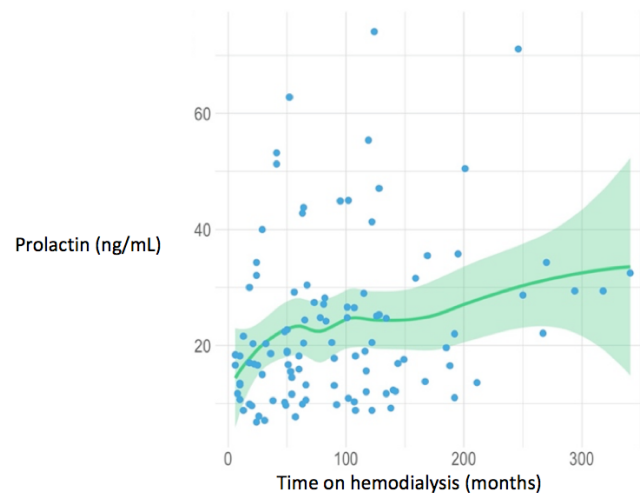


Figure 2. Dispersion graph between prolactin levels and time on hemodialysis, along with trend line. Shaded region is the confidence interval associated with the trend line.



The 111 patients included in the study were divided into two groups on the basis of serum prolactin levels. Blood count and biochemical data of these two groups were compared (Table 3).

Table 3. Laboratory parameters of patients with normal and elevated prolactin levels

Variables	Normal Prolactin (N = 50)	Elevated Prolactin (N = 61)	<i>p</i>
Prolactin (ng/mL)	12.64 (6.8–19)	31.88 (17.8–74.1)	0.008
Albumin (mg/dL)	3.87 ± 0.4	3.82 ± 0.4	0.56
HbA1c (%)	5.64 ± 1.05	6.18 ± 1.82	0.1
Glucose fasting (mg/dL)	96.67 ± 30.21	125.12 ± 94.36	0.41
TC (mg/dL)	145.21 ± 37.11	138.84 ± 39.25	0.6
LDL (mg/dL)	77.13 ± 32.75	71.33 ± 34.1	0.5

HDL (mg/dL)	40.36 ± 13.54	40.11 ± 14.68	0.82
Triglycerides (mg/dL)	136.62 ± 69.94	147.71 ± 99.2	0.9
Hemoglobin (mg/dL)	10.86 ± 1.47	10.96 ± 1.56	0.72
Calcium (mg/dL)	8.7 ± 0.67	8.7 ± 1.01	0.86
Phosphorus (mg/dL)	4.81 ± 1.28	5.21 ± 1.53	0.24
Alkaline phosphatase (U/L)	191.64 ± 208.5	206.97 ± 248.02	0.65
PTH (pg/mL)	706.6 (4.2–2400)	718.04 (3–2439)	0.84

HbA1c-glycated hemoglobin; TC-total cholesterol; PTH-parathyroid hormone. Continuous values are shown as mean ± standard deviation. Prolactin and PTH levels are shown as mean and minimum–maximum.

Results of the inflammatory cytokine panel are shown in Table 4, showing the comparison of normal prolactin and high prolactin levels in patient serum. It was observed that patients with hyperprolactinemia had statistically significantly higher levels of IL-6 and lower levels of IL-10 ($p < 0.001$ and $p = 0.046$, respectively). TNF- α and IL-4 levels were not adequately measured in both patient groups.

Table 4. Comparison of interleukin levels between patients with normal and elevated prolactin levels. Values are presented as mean ± standard deviation (minimum–maximum)

Variables	Normal Prolactin	Elevated Prolactin	p^*
IL-6 (pg/mL)	3.23 ± 4.9 (0–28.46)	8.18 ± 7.09 (0.22–35.84)	< 0.001
IL-17A (pg/mL)	1.71 ± 5.64 (0–34.87)	3.12 ± 10.01 (0–64.08)	0.71
IFN- γ (pg/mL)	0 ± 0.02 (0–0.17)	0.05 ± 0.24 (0–1.49)	0.25
IL-10 (pg/mL)	2.78 ± 18.04 (0–126.45)	1.4 ± 5.74 (0–43.66)	0.046
IL-2 (pg/mL)	0.01 ± 0.08 (0–0.56)	0.22 ± 1.26 (0–9.25)	0.41

IL-interleukin; IFN- γ -interferon gamma. Continuous values are shown as mean ± standard deviation (min–max).

Regarding the serum prolactin level, a positive correlation was observed with serum levels of IL-6 ($R = 0.44$, $p < 0.0001$) (Figure 3A). The correlation between prolactin and IL-10 levels was statistically significant ($p < 0.046$), with the Spearman's correlation coefficient being 0.2 (Figure 3B).

Figure 3 - Scatter plot between interleukin-6 (IL-6) (pg/mL) and prolactin (PRL) (3A), and interleukin-10 (IL-10) (pg/mL) and prolactin (3B), together with adjusted linear regression line. Gray shaded area represents the prediction confidence interval.

FIGURE 3A

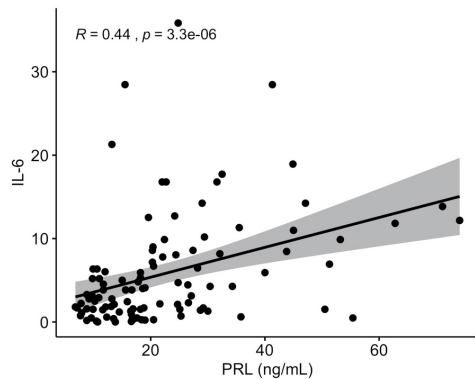
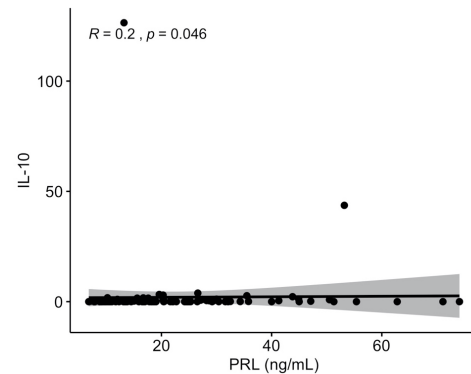


FIGURE 3B



5. DISCUSSION

To our knowledge, this is the first study conducted on patients with CKD on hemodialysis assessing serum prolactin levels and their correlation with inflammatory cytokines. We observed that patients with hyperprolactinemia had significantly higher interleukin-6 (IL-6) levels and lower IL-10 levels ($p < 0.001$ and $p = 0.046$, respectively).

The loss of renal function causes an increase in the prevalence of hyperprolactinemia. This prevalence can vary from 30% in patients with mild renal dysfunction to >65% in patients on HD (5). Prolactin is a hormone with different isoforms and a molecular size ranging from 23 to 150 kDa. The 23-kDa monomeric isoform is the most biologically active and accumulates in the blood resulting in loss of renal function (11).

In patients with CKD, serum prolactin levels are increased in the blood due to both reduced excretion and increased secretion by lactotrophs, cells of the pituitary gland that produce the hormone (12). Compared with normal individuals, the rate of prolactin secretion increases by approximately three to four times in patients with CKD (13), probably due to the increased resistance of prolactin secretion to dopaminergic suppression.

In our study, we found a prevalence of hyperprolactinemia of 54.95% in patients on HD. Although there are only a few studies, those who have evaluated these data have reported higher prevalences. This difference in results could perhaps be because we excluded more patients due to the unprecedented analysis

of the inflammatory aspect. In fact, the presence of a long-term catheter changes the inflammatory profile even in the absence of infection (14), as well as infections or use of immunosuppressants.

For example, Lo et al evaluated 117 patients on intermittent daytime HD and 60 patients on nocturnal hemodialysis and observed that 81% of them had elevated prolactin levels, considered as >30 ng/mL. They also observed that more frequent hemodialysis sessions did not normalize the hormone levels (15). Yavuz et al compared 31 patients on HD, 30 patients on peritoneal dialysis, 30 kidney transplant patients, and 72 healthy controls and observed that serum prolactin levels of patients on HD and peritoneal dialysis were similar but significantly higher than those of renal transplant patients and healthy controls ($p < 0.001$), with the prolactin levels of transplant patients and healthy controls being similar, suggesting that improvement of renal function after transplantation normalizes prolactinemia (11). The presence of macroprolactinemia was also evaluated, which was not significant in any group, that is, the prolactin isoform >100 kDa was not common.

The prevalence of hyperprolactinemia in the general population has been estimated at 0.4%, which was found to increase with age and reach 17% in women with secondary amenorrhea (16). In our study, there was no difference between age and sex in HD patients with normal and elevated prolactinemia. Perhaps, in patients with advanced CKD, the prevalent time on dialysis is more important, as our study revealed that those with the highest hormone level had been on hemodialysis for more months ($p = 0.016$). Longer the time since replacement therapy starts, greater the loss of residual renal function and inflammation, leading to a higher likelihood of complications from long-standing uremia (17).

We also evaluated the correlation between hyperprolactinemia and body mass index (BMI) but did not detect any statistical difference between the groups. Adipose tissue is recognized as an endocrine organ due to the production of adipokines, substances that function as hormones. Patients with CKD, as well as obese patients, have higher levels of adipokines such as adiponectin, leptin, and resistin, all of which are related to increased inflammation, insulin resistance, and endothelial damage (18). Prolactin has an effect on glucose metabolism by reducing the production of insulin in pancreatic beta cells, in addition to increasing peripheral insulin resistance (19). In view of this evidence since 2011, bromocriptine, a dopaminergic agonist that inhibits prolactin secretion, has been a component of the therapeutic arsenal for the

treatment of diabetes in the United States (20). It has been reported that in patients with normal renal function, hyperprolactinemia is associated with insulin resistance and alteration of the lipid profile (21). A systematic review reported in 2019 revealed that therapy with dopaminergic agonists leads to weight reduction and improved lipid and glycemic profile in patients with prolactinoma (22). In our study, we did not assess insulin resistance, but no difference was detected between groups in terms of the number of diabetic patients, glycated hemoglobin, and fasting blood glucose levels. It has been demonstrated that hyperprolactinemia in patients with normal renal function is associated with reduced bone mineral density and increased risk of vertebral fractures, including dopaminergic agonist therapy improving bone mineral density (23). Our study did not evaluate bone densitometry or fractures, but in the biochemical assessment of bone mineral disease (calcium, phosphorus, alkaline phosphatase, and PTH levels), we found no difference in serum levels between patients with normal prolactin and those with elevated prolactin levels.

Uremia is characterized by the retention of more than 150 substances that are generally excreted or metabolized by the kidney, including numerous inflammatory cytokines, leading to a state of dysregulation and chronic inflammation (24). This proinflammatory status causes increased morbidity and mortality in these patients through several mechanisms such as malnutrition, sarcopenia, oxidative stress, accelerated atherosclerosis, anemia, endothelial dysfunction, and immune dysfunction (25).

One of the most important findings of our study was the association between hyperprolactinemia and increased serum levels of interleukin-6 ($p < 0.001$). IL-6 is a 22.2-kDa polypeptide secreted from activated monocytes, macrophages, fibroblasts, adipocytes, and endothelial cells in response to various stimuli (26). This potent proinflammatory cytokine was initially described as a differentiating factor for cytotoxic T cells and for stimulation of B cells, and later, it was reported to play a central role in both local and systemic inflammation (27). Patients with CKD generally have increased levels of this cytokine due to the loss of renal function, oxidative stress, and also factors related to hemodialysis itself. Even before the start of dialysis, patients with CKD already have elevated inflammatory markers, including IL-6 (28).

A meta-analysis of 82 studies evaluating the relationship between IL-6 and

coronary heart disease, with more than 200,000 participants, concluded that there is a causal association between serum levels of IL-6 and coronary heart disease (29). There are not many studies evaluating this cytokine in patients with kidney disease, but a cohort study reported by Barreto et al evaluated an association between IL-6 levels, aortic calcification, and mortality in 125 patients with CKD before and after hemodialysis initiation, demonstrating that IL-6 was an independent predictor of general and cardiovascular mortality (30). Another cohort study conducted by Pecoits-Filho et al, following for an average of 3.1 years with 173 patients with CKD just before starting hemodialysis and peritoneal dialysis, reported a strong predictive value of IL-6 levels for mortality (31).

In addition to being a hormone, prolactin acts as an inflammatory cytokine, as its receptor is a member of the type 1 cytokine superfamily, which is widely expressed through the immune system, including monocytes, lymphocytes, macrophages, Natural Killer cells, granulocytes, and epithelial cells of the thymus. Increased serum prolactin levels cause increased proliferation of immune cells, thereby increasing the production of proinflammatory cytokines such as IL-6, TNF- α , and IL-1 β (10,32).

In our study, we also found that increased serum prolactin levels correlated significantly with reduced levels of IL-10 ($p = 0.046$). IL-10 is a potent anti-inflammatory cytokine synthesized by T lymphocytes and macrophages, physiologically limiting and reducing systemic inflammation (33). In patients with advanced CKD, higher levels of this cytokine would be expected, since it is normally filtered and then metabolized in renal tubules, thus having an increased half-life (34). In addition, this cytokine is a counter-regulator of excessive inflammation in patients on dialysis, being found to be elevated in up to two-thirds of patients. Even those with low levels of IL-10 experience accelerated atherosclerosis and increased cardiovascular mortality (35). INF- γ is known as a potent inhibitor of IL-10 secretion (36). Prolactin increases the secretion of INF- γ by T cells, macrophages, and NK cells; perhaps in this manner, it causes a reduction in serum levels of IL-10 (37). However, our study revealed only a slight tendency toward high levels of INF- γ in patients with high prolactin levels, with no statistical difference ($p = 0.25$).

Although not an objective of our study, it is necessary to mention that hyperprolactinemia is associated with several cardiovascular outcomes in both patients with normal renal function and those with CKD, for reasons still unknown. In

patients with normal renal function, an increase in the thickness of the intima-media carotid layer has been demonstrated in cases with untreated prolactinomas (38), including increased aortic stiffness and pulse wave velocity in postmenopausal women with elevated prolactin levels (39). In patients undergoing endarterectomy, receptors for prolactin were identified on macrophages of the atherosclerotic plaques, and in the more unstable plaques, there was a higher density of these receptors, suggesting a possible direct modulatory effect of this hormone on atherogenesis (40). A cohort study evaluating the clinical outcomes of 3929 individuals followed up for 10.1 years (38,231 person-years) reported an association between serum prolactin concentrations and general and cardiovascular mortality (9).

There are few studies evaluating prolactinemia in patients with CKD. One of the primary studies to evaluate this relationship was conducted by Carrero et al. consisting of two cohorts, one with CKD patients under conservative treatment and the other with hemodialysis patients. The cohort with conservative CKD patients consisted of 457 patients with a mean age of 52 years, including 229 men, evaluating flow-mediated dilation (FMD) and carotid intimal-media thickness. In the cohort with hemodialysis patients, there were 173 patients with a mean age 65 years, including 111 men, evaluating pulse wave velocity (PWV). Their results demonstrated that prolactin levels increased with reduced renal function, showing an association with increased FMD in conservative CKD patients and PWV in dialysis patients. The authors also observed that with each 10 ng/mL increase in prolactin level, there was a 27% increase in cardiovascular risk in nondialysis patients and a 12% increase in cardiovascular mortality in dialysis patients. They concluded that serum prolactin levels are directly associated with endothelial dysfunction and increased cardiovascular risk and mortality in patients with CKD (5).

Some limitations in our study can be pointed out. It is a cross-sectional research and therefore of an observational design, conducted in a single hemodialysis center. After applying the exclusion criteria, we ended up with a small number of participants in each group. However, we detected a high prevalence of hyperprolactinemia, and these patients exhibited a significant increase in IL-6 levels and a reduction in IL-10 levels compared to those in patients with normal prolactin levels. In fact, additional studies are required to evaluate the relationship between prolactinemia and renal function, as this hormone at normal levels is a permissive molecule, adjuvant to homeostasis, but at high levels, it can cause an imbalance in

this balance in several manners. As CKD is a complex disease, with numerous factors influencing cardiovascular mortality, the function of prolactin in this network needs to be further investigated.

In conclusion, patients on hemodialysis have a high prevalence of hyperprolactinemia, with the increase in prevalence being significantly associated with an elevation of IL-6 levels and a reduction of IL-10 levels. Such a finding in these patients may constitute a more marked deregulation of inflammatory homeostasis. Prospective studies are warranted to evaluate the relationship between prolactin levels and inflammation in patients with CKD.

6. DISCLOSURE

All the authors declared no competing interests.

8. ACKNOWLEDGEMENTS

We acknowledge Immunopathology Laboratory Keito Asami staff, as well as Siemens Healthineers.

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ANEXO A: COMPROVANTE APROVAÇÃO COMITÊ DE ÉTICA

Plataforma Brasil

27/04/19 19:29

Saúde

 principal
  sair

Marcílio Manuel Coêlho Dourado - Pesquisador | V3.2

Cadastros

Sua sessão expira em: 38min 48

DETALHAR PROJETO DE PESQUISA

DADOS DA VERSÃO DO PROJETO DE PESQUISA

Título da Pesquisa: ESTUDO SOBRE O EFEITO DA TERAPIA COM CABERGOLINA SOBRE PROLACTINA, PARÂMETROS METABÓLICOS, MARCADORES INFLAMATÓRIOS E ESPESSURA ÍNTIMA-MÉDIA CAROTÍDEA EM PACIENTES EM HEMODIÁLISE COM HIPERPROLACTINEMIA

Pesquisador Responsável: Marcílio Manuel Coêlho Dourado

Área Temática:

Versão: 1

CAAE: 74031617.0.0000.5208

Submetido em: 22/08/2017

Instituição Proponente: CENTRO DE CIÊNCIAS DA SAÚDE

Situação da Versão do Projeto: Aprovado

Localização atual da Versão do Projeto: Pesquisador Responsável

Patrocinador Principal: Financiamento Próprio

Comprovante de Recepção:  PB_COMPROVANTE_RECEPCAO_953041

DOCUMENTOS DO PROJETO DE PESQUISA

- Versão Atual Aprovada (PO) - Versão 1
 - Pendência Documental (PO) - Versão 1
 - Documentos do Projeto
 - Comprovante de Recepção - Submissão 4
 - Cronograma - Submissão 4
 - Declaração de Instituição e Infraestrutura - Submissão 4
 - Declaração de Pesquisadores - Submissão 4
 - Folha de Rosto - Submissão 4
 - Informações Básicas do Projeto - Submissão 4
 - Orçamento - Submissão 4
 - Outros - Submissão 4
 - Projeto Detalhado / Brochura Investigação - Submissão 4
 - TCLE / Termos de Assentimento / Justificativa - Submissão 4
 - Apreciação 4 - UFPE - Universidade Federal de Pernambuco
 - Projeto Completo

Tipo de Documento	Situação	Arquivo	Postagem	Ações

LISTA DE APECIAÇÕES DO PROJETO

Apreciação *	Pesquisador Responsável *	Versão *	Submissão *	Modificação *	Situação *	Exclusiva do Centro Coord. *	Ações
PO	Marcílio Manuel Coêlho Dourado	1	22/08/2017	05/10/2017	Aprovado	Não	   

HISTÓRICO DE TRÂMITES

Apreciação	Data/Hora	Tipo Trâmite	Versão	Perfil	Origem	Destino	Informações
PO	05/10/2017 09:35:50	Parecer liberado	1	Coordenador	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	PESQUISADOR	
PO	05/10/2017 09:08:28	Parecer do colegiado emitido	1	Coordenador	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	
PO	03/10/2017 20:47:53	Parecer do relator emitido	1	Membro do CEP	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	
PO	01/10/2017 00:11:03	Aceitação de Elaboração de Relatoria	1	Membro do CEP	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	
PO	22/09/2017 09:07:01	Confirmação de Indicação de Relatoria	1	Coordenador	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	
PO	22/09/2017 08:49:33	Indicação de Relatoria	1	Coordenador	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	
PO	22/08/2017 08:31:56	Aceitação do PP	1	Secretária	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	
PO	22/08/2017	Submetido para	1	Pesquisador	PESQUISADOR	Universidade Federal de Pernambuco	

	08:30:44	avaliação do CEP		Principal		Centro de Ciências da Saúde / UFPE-CCS	
PO	22/08/2017 08:27:35	Rejeição do PP	1	Secretária	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	PESQUISADOR	devolvido para alterações
PO	21/08/2017 16:36:09	Submetido para avaliação do CEP	1	Pesquisador Principal	PESQUISADOR	Universidade Federal de Pernambuco Centro de Ciências da Saúde / UFPE-CCS	

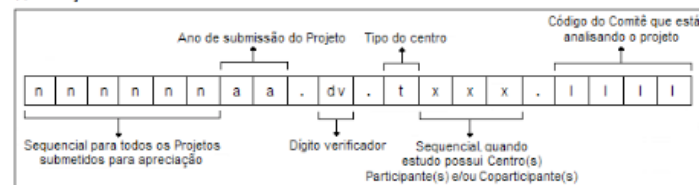
« « « « Ocorrência 1 a 10 de 14 registro(s) » » » »

LEGENDA:

(*) Apreciação

PO = Projeto Original de Centro Coordenador	POp = Projeto Original de Centro Participante	POc = Projeto Original de Centro Coparticipante
E = Emenda de Centro Coordenador	Ep = Emenda de Centro Participante	Ec = Emenda de Centro Coparticipante
N = Notificação de Centro Coordenador	Np = Notificação de Centro Participante	Nc = Notificação de Centro Coparticipante

(*) Formação do CAAE



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(versão 9 ou superior)

ANEXO B: COMPROVANTE PUBLICAÇÃO DE ARTIGO DE REVISÃO

ON-LINE: <https://www.hindawi.com/journals/ije/2020/9524839/>

Hindawi
International Journal of Endocrinology
Volume 2020, Article ID 9524839, 6 pages
<https://doi.org/10.1155/2020/9524839>



Review Article

Relationship between Prolactin, Chronic Kidney Disease, and Cardiovascular Risk

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CKD has a high prevalence worldwide, mainly due to its main etiologies—diabetes and hypertension. It has high cardiovascular morbidity and mortality, with traditional risk factors such as atherosclerosis, hypertension, diabetes, smoking, and left ventricular hypertrophy being common. Nontraditional cardiovascular risk factors, such as anemia, hyperparathyroidism, chronic inflammation, and microalbuminuria, are also well studied. Prolactin is a hormone not only related to lactation but also being considered a uremic toxin by some authors. It accumulates with loss of renal function, and it is associated with cardiovascular outcomes in both normal renal function population and CKD population. The purpose of this narrative review is to raise the main common aspects of CKD, prolactinemia, and cardiovascular risk.

1. Introduction

Chronic kidney disease (CKD) has high and increasing prevalence in the general population, mainly because the main causes of kidney failure are diabetes mellitus (DM) and hypertension, very common diseases. CKD has high morbidity and is associated with increased cardiovascular mortality, with 5 to 10 million annual deaths worldwide [1].

The so-called traditional cardiovascular risk factors such as DM, hypertension, smoking, left ventricular hypertrophy (LVH), and peripheral vascular disease are very prevalent in this population [2]. However, nontraditional risk factors such as hyperphosphatemia, hyperparathyroidism, inflammation, and anemia are also prevalent in this population. Among these, hyperprolactinemia has received increasing attention in recent years [3].

CKD patients have elevated prolactinemia when compared to the general population, and those with high hormone have higher cardiovascular mortality compared to those with normal prolactinemia [4]. Furthermore, a growing number of biological processes continue to be attributed to prolactin. This

is partly due to the presence of its receptor in different tissues at different sites, presenting many cellular responses. These biological processes include insulin resistance, metabolic syndrome, inflammation modulation, endothelial dysfunction, and accelerated atherosclerosis [5].

The aim of this review was to raise the main points regarding prolactin, cardiovascular risks, and CKD.

2. Chronic Kidney Disease (CKD)

Chronic kidney disease (CKD) is characterized by progressive and irreversible loss of renal function. It is defined by at least one of the following for more than 3 months: glomerular filtration rate less than 60 mL/min/1.73 m², with or without renal damage markers (albuminuria $\geq$ 30 mg/24 hours or urinary albuminuria/creatinine ratio $\geq$ 30 mg/g; persistent glomerular hematuria; persistent electrolyte changes due to tubulopathy; structural abnormalities detected by imaging; previous kidney transplant) [6].

CKD has increasing prevalence worldwide, estimated at 10% of population, being more common in women when

ANEXO C: MÉTRICAS 2019 *INTERNATIONAL JOURNAL OF ENDOCRINOLOGY***Journal metrics**

Acceptance rate	27%
Submission to final decision	50 days
Acceptance to publication	47 days
CiteScore	3.500
Impact Factor	2.299

ANEXO D: CARTA-RESPOSTA DO EDITOR DA *INTERNATIONAL JOURNAL OF ENDOCRINOLOGY*



Manuscript Tracking System

Marclebio Dourado [Update Account](#) [Logout](#)

[Submit a Manuscript](#) [Author Activities](#)

9524839.v1 Review Report

Subject appropriateness of the manuscript

The topic of this manuscript falls within the scope of International Journal of Endocrinology

Recommendation

Publish unaltered

Comments for the author

The narrative review focuses on detailing the main aspects that link hyperprolactinemia with chronic kidney disease and cardiovascular disease. The paper provides an updated overview of the current evidence and that is why I consider it is of interest for the readers of International Journal of Endocrinology. Given the high prevalence of CKD and the fact that cardiovascular disease is the main cause of death in these patients, it is of interest to detail any potential contributors to the cardiovascular risk in this special population - like hyperprolactinemia in this case.

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ANEXO E: Comprovante de submissão *EUROPEAN JOURNAL OF ENDOCRINOLOGY*

Manuscript submitted for review to European Journal of Endocrinology



Prolactin and inflammatory cytokines in hemodialysis patients

Journal:	<i>European Journal of Endocrinology</i>
Manuscript ID	EJE-20-0943
mstype:	Clinical Study
Date Submitted by the Author:	19-Aug-2020
Complete List of Authors:	Dourado, Marclébio; Federal University of Pernambuco, Nephrology Branco, Frederico Castelo; Real Hospital Português (RHP), Nephrology Souto, Fabricio; Federal University of Pernambuco, LIKA Vilar, Lucio; Federal University of Pernambuco, Department of Endocrinology Cantilino, Amaury; Federal University of Pernambuco, Neuropsychiatry post-graduation program
Keywords:	Prolactin, hemodialysis, Cytokines, interleukin-6, interleukin-10

SCHOLARONE™
Manuscripts

ANEXO F: Comprovante de aceite apresentação ePoster ASN KIDNEY WEEK 2020

Yahoo Mail - Abstract Notification 3445416 - Kidney Week

19/08/20 18:38

Abstract Notification 3445416 - Kidney Week

De: ASN Kidney Week 2020 (onbehalf@abstractcentral.com)
 Para: marclebio@yahoo.com.br
 Data: sexta-feira, 14 de agosto de 2020 10:53 BRT



1401 H Street, NW • Suite 900 • Wa
 202.640.4660 • v

Friday, 14-Aug-2020

Dear Dr. Marclebio Dourado:

On behalf of the American Society of Nephrology (ASN) and the Kidney Week Education Committee, thank you for submitting an abstract for Kidney Week, which will be a fully digital meetin your abstract **3445416 "Prolactin and inflammatory cytokines in hemodialysis patients:cross-sectional study"** has been selected by the Committee for an ePoster presentation. The de below:

ePoster Session

Session Title: Hemodialysis and Frequent Dialysis - 3
 Session Release Date, Time: Thursday, October 22, 10:00 a.m. EDT
 Your ePoster #: **PO1159**

Please note that any information **beyond what is contained in the four corners of the abstract** may not be released until ASN lifts the embargo. [Click here](#) for the full embargo policy.

As the first author, you have the primary responsibility for communications with ASN, you will be the first author listed in the abstract publication, and your abstract's ePoster credentials will author will receive a separate email from ASN's ePoster partner in September with instructions for preparing and uploading your ePoster.

Your poster number will correspond with the number printed in ASN's 2020 Abstract Supplement. Posters will be presented digitally, as an ePoster. All ePosters will be available for attendee Thursday, October 22. A discussion board will be available on each ePoster's presentation page for Kidney Week participants to ask questions and for you to respond. Instructions on how to provided prior to the meeting.

Program and Abstracts

The Kidney Week program is available on the [ASN website](#). Abstracts will be available on the ASN website by October 9, 2020. The abstracts will remain online for approximately one year. A changes to your abstract title.

Registration

Presenting authors of abstracts accepted for oral or ePoster presentations must register for the Annual Meeting ([fees apply](#)) to access the digital meeting platform.

Congratulations on having your abstract selected for ePoster presentation. We appreciate your willingness to participate and look forward to seeing you online. Please direct any questions to

Sincerely,

Linda F. Fried, MD, MPH, FASN, Kidney Week Committee Co-Chair
 Jon B. Klein, MD, PhD, FASN, Kidney Week Committee Co-Chair

ANEXO G: Regras para publicação na *EUROPEAN JOURNAL OF ENDOCRINOLOGY*

- Author guidelines
-

Author guidelines

Ready to submit? Go straight to [submission](#).

The *European Journal of Endocrinology* (EJE) acknowledges the unprecedented challenge posed by the global COVID-19 pandemic, demanding a global medical response we could never have anticipated facing in our lifetime. EJE is committed to support the global endocrine community in this time of crisis.

We ask our authors to continue submitting manuscripts and revising them in line with the reviewers' comments. Likewise, we ask our reviewers to still commit to reviewing manuscripts submitted to EJE. However, we will be very accommodating to authors and reviewers with regards to the urgent time constraints placed upon them, and provide you with more time for your tasks if you need it. Simply contact the [Editorial Office](#) and we will grant extensions to deadlines as required.

In turn we would ask you to be lenient with us if the turnaround time of EJE papers becomes slightly longer than you are used to.

[Editorial Process](#)

[Submission Checklist](#)

[Preparation of Manuscripts](#)

[Manuscript Categories](#)

[Supplementary Data](#)

[Editorial Policies](#)

Editorial Process

Peer review

Submissions are assessed by the [Editorial Board](#) and are subject to external peer review using the single blind method whereby the authors are blinded to the identity of the reviewers and editors.

Due to the high volume of submissions received, the Editor-in-Chief and the Editorial Board operate a rapid triage system and only the top 40% of papers undergo full external peer review. This workflow is in place to allow authors to submit to a more appropriate journal with minimal delay.

The journal aims to return a decision on a peer-reviewed paper in less than a month.

Manuscript Transfer

If your paper is found to be outside the scope of *European Journal of Endocrinology* you may be offered the opportunity to transfer it to *Endocrine Connections* or *Endocrinology, Diabetes & Metabolism Case Reports* for rapid consideration. The offer to transfer is made at the discretion of the Editor-in-Chief, and may be made prior to or after full peer review. Authors may accept or decline the offer to transfer. If the offer is accepted after peer review, then the paper will be transferred along with the reviewer reports. This will assist the Editor-in-Chief of *Endocrine Connections* or *Endocrinology, Diabetes & Metabolism Case Reports* to assess the suitability of the article for the journal, meaning that it may be possible for your paper to be accepted and

published rapidly without further peer review, although acceptance is not guaranteed. All decisions made by an Editor-in-Chief of either journal are final.

Appeals

Authors who feel they have grounds to appeal a rejection decision should send a rebuttal letter to the [editorial office](#), detailing the reasons for the appeal. Rebuttals will be considered by the Editor-in-Chief, often in consultation with the Editorial Board Member who handled the paper. Decisions on appeals are final.

Submission Checklist

Your article

- Structure – Ensure the submission is structured as requested by the journal, and contains all relevant sections. See 'Preparation of Manuscripts' for further details.
- Title page – All submissions must have a title page stating all of the relevant information. See 'Preparation of Manuscripts' for further details.
- Format – All submissions should follow the journal guidelines for word count, page margins and line numbering. See 'Preparation of Manuscripts' for further details.
- English language – Non-native English speakers are encouraged to have their manuscript professionally edited before submission. See Bioscientifica's recommended [English language editing services](#).
- Reported data – Data accuracy is crucial. Authors are strongly encouraged to double-check all reported data for accuracy and to confirm that all units of measurement are correct and consistent.
- References – Please see 'References' for full details of the journal's required style.
- Graphics – All figures and tables should be presented in a clear and informative manner with accompanying legends.
- Ethical compliance – All articles are required to meet the requirements outlined in our [ethical policy](#). Ensure you have included all relevant ethical approval statements.

Before submitting

- Approval – Ensure all authors have seen and approved the final version of the article prior to submission. All authors must also approve the journal you are submitting to.
- Open access – The appropriate open-access option must be selected on submission. Authors are responsible for ensuring any funder mandates are followed. For further details, please see the [open-access policy](#).
- Charges – *European Journal of Endocrinology* is committed to keeping costs to authors to a minimum, however some charges may apply. Authors are responsible for familiarising themselves with these prior to submission. Full details are available on our [publication charges](#) page.

Uploading your submission

- Author list – All authors must be listed on the title page and entered on the ScholarOne Manuscripts submission in the correct order. Ensure all author email addresses provided are valid. Author information entered into ScholarOne Manuscripts will be used to generate PubMed listings for published papers.
- Cover Letter – This letter should introduce your paper and outline why your work is important and suitable publication at this time.
- File formats – Ensure all files are in the correct format for revised submissions. See 'Preparation of Manuscripts' for further instructions.

- Figures and tables – Ensure all figures and table files are present and correct, and that they display clearly in the pdf proof.

Preparation of Manuscripts

Manuscripts should:

- Be concise and clear.
- Be limited to 3500 words for clinical studies. For information on other manuscript types please see the relevant section below.
- Display the word count on the title page.
- Contain no more than 10 figures and 60 references as recommended by the journal.
- Use double line spacing throughout (including reference list and figure legends), and contain continuous line numbering down the left-side of each page.
- Define all abbreviations when first mentioned.
- Be submitted in the correct file type, i.e. main document in an editable Word format.
- Be written in either UK or US English.
- Contain a title page.

Accepted file types:

- Please be aware that the combined size of your files should not exceed **40 MB**.
- For article text: txt, doc, docx, tex. We are unable to accept pdf files for article text for revised manuscripts but can do so for first submissions.
- For figures: eps, tiff, jpg, pdf.

Changes within revised manuscripts should be highlighted using the highlighter function or coloured text, and should be accompanied by a full response letter to editor and reviewer comments.

Manuscript Categories

Clinical Studies

Authors are encouraged to follow the relevant reporting guidelines available at <http://www.equator-network.org/>. The EQUATOR network provides a database of reporting guidelines, aiming to improve the reliability of published health research literature by promoting transparent and accurate reporting.

Authors are encouraged to refer to the checklists provided for the following study designs:

[STROBE](#) reporting guidelines for observational studies

[PRISMA](#) reporting guidelines for systematic reviews

[STARD](#) reporting guidelines for diagnostic/prognostic studies.

Clinical studies should be formatted with the following sections:

1. Title page

Include a separate title page with:

- Title (maximum 85 characters)
- All authors names and full addresses
- Corresponding author's postal and email address
- A short title (maximum 46 characters, including spaces)
- A minimum of four keywords describing the manuscript
- Word count of the full article, excluding references and figure legends

2. Abstract

The abstract should be no more than 250 words in length. Please divide up your abstract using the headings Objective (giving the context of the study), Design, Methods, Results and Conclusions. Avoid abbreviations and references in this section.

3. Introduction

The introduction should set the study in context by briefly reviewing relevant knowledge of the subject; follow this with a concise statement of the objectives of the study.

4. Materials and methods

Provide sufficient information for other workers to repeat the study. If well-established methods are used give a reference to the technique and provide full details of any modifications.

- Include the source of chemicals, reagents and hormones and give the manufacturer's name and location (town, country) in parentheses.
- Give the generic name, dose and route of administration for drugs.
- Specify the composition of buffers, solutions and culture media.
- Use SI symbols, give concentrations in mol/L and define the term % as w/v or v/v for all solutions. For international units use IU (U should be used for enzyme activity).
- Specify the type of equipment (microscopes/objective lenses, cameras, detectors) used to obtain images.
- Specify any image acquisition software used, and give a description of specialized techniques requiring large amounts of processing, such as confocal, deconvolution, 3D reconstructions, or surface and volume rendering.

5. Results

The results should read as a narrative leading the reader through the experiments and investigations performed. Referencing and mention of others studies is permitted in the Results section where necessary or helpful.

6. Discussion

Should not simply re-state results, but should put them in the broader context and highlight the importance and novelty of the work.

7. Declaration of interest, Funding and Acknowledgements

Declaration of interest

Actual or perceived conflicts of interest for all authors must be declared in full.

Please either (a) declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported; or (b) fully declare any financial or other potential conflict of interest.

Conflicts of interest include, but are not limited to:

- Employment and consultancies
- Grants, fees and honoraria
- Ownership of stock or shares
- Royalties
- Patents (pending and actual)
- Board membership

Funding

Please detail all of the sources of funding relevant to the research reported in the following format:

This work was supported by the Medical Research Council (grant numbers xxxx, yyyy); the Wellcome Trust (grant number xxxx); and Tommy's Baby charity (grant number xxxx).

Where research has not been funded please state the following:

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Author contribution statement (optional)

Please include a statement specifying the contribution of each co-author.

Acknowledgements

Please be as brief as possible.

8. References

All references cited in the text must be included in the reference list and *vice versa*. However, if a reference consists of only a web address do not include it in the reference list but cite it in the text, giving the date the page was accessed.

Unpublished work

Any unpublished work (personal communications, manuscripts in preparation and manuscripts submitted but not yet accepted for publication) must be referred to in the text and not listed in the references.

Give the full list of authors, including their initials. For example:

(A Stone, J Brown & M R Smith, unpublished observations)

(J Brown, personal communication)

Articles accepted for publication but not yet published may be listed as 'in press' in the reference list, using the current year as the publication year. If an 'in press' article is included in the Accepted Preprint service or a similar scheme, then the Digital Object Identifier (DOI) should be included; otherwise, provide a copy of the article as a supplementary file for reviewing purposes.

In the text

Cite references in the text in numerical order.

In the reference list

List references in the order they are cited in the text.

List all authors.

Reference in the following format:

1. Inkster S, Yue W & Brodie A. Human testicular aromatase: immunocytochemical and biochemical studies. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 1941–1947.
2. Matthews CH, Borgato S, Beck-Peccoz P, Adams M, Tone Y, Gambino G, Casagrande S, Tedeschini G, Benedetti A & Chatterjee VK. Primary amenorrhea and infertility due to a mutation in the β -subunit of follicle-stimulating hormone. *Nature Genetics* **5** 83–86.
3. Royer P. Hormonal regulation of calcium metabolism: biology and pathology. In *Pediatric Endocrinology*, edn 2, ch. 6, pp 477–543. Eds J-C Job & M Pierson. New York: John Wiley & Sons, 1981.

List a maximum of ten authors. Where there are more than ten authors, list the first ten and then use *et al*.

EndNote

Please use Vancouver style.

9. Tables

Tables should be concise. Tables too large for print publication should be submitted as supplementary data.

- Number tables in the order they are cited in the text
- Include a title – a single sentence at the head of the table that includes the name of the organism studied
- Use footnotes to provide any additional explanatory material, cross-referenced to the column entries
- Give a short heading for each column
- Do not use internal horizontal or vertical lines, colour or shading

- Explain all abbreviations used in the table in the footnotes

Please note that the option to print large tables in a final article is subject to editorial approval. If the tables are deemed too large for the final article, you will be asked to publish your tables as supplementary data and [charges](#) will apply.

10. Figures

The journal has produced digital image guidelines in order to clarify the standards expected by the journal. All submitted digital images must adhere to these guidelines.

Colour figures will be published online at no charge to the author. Publication of colour figures in the print version will incur a charge that must be paid before publication. Please note the option to print in greyscale is subject to editorial approval, if the meaning of your image is unclear you will be asked to print in colour and a charge will apply.

- Number figures in the order they are cited in the text
- Include legends to all figures, giving the figure number, keys to any symbols used, the name of the organism studied, the names of any statistical tests used and the probability levels used for comparisons
- Label figure sections as A, B etc in the top left-hand corner
- Use Arial or a similar sans-serif font for text labels
- Do not enclose figures in boxes
- Indicate magnification by a scale bar in the bottom right-hand corner of the image and give the measurement in the legend
- Use the preferred symbols of closed and open circles, squares and triangles. Ensure that symbols are large enough to be read clearly when the figure is reduced for publication
- Use Courier or a similar non-proportional font for amino acid, DNA, RNA and PCR primer sequences and highlight sections of homology between sequences with grey shading

File types and resolution

European Journal of Endocrinology is committed to publishing high quality figures.

EPS or TIFF files are preferred. Files should be exported in Illustrator compatible format, avoiding PowerPoint or Word files:

- Line images/graphs: EPS, TIFF, high-resolution PDF, AI (Adobe Illustrator). Resolution at final published size: 1200 dpi.
- Half-tone (greyscale) images: TIFF, high-resolution PDF, JPEG. Resolution at final published size: 600 dpi.
- Colour images: TIFF, high-resolution PDF, JPEG. EPS or AI files can be used for graphical data and illustrations that don't include photographs. Resolution at final published size: 300 dpi. Colour format: CMYK (not RGB).

European Journal of Endocrinology also accepts the following manuscript types.

Reviews

The format of review articles is more fluid but should include the following:

1. Title page
2. Abstract
3. Conclusions
4. Declaration of interest, Funding, Author contributions statements (where appropriate)
5. References
6. Figure legends
7. Figures/ tables

Review submissions should be limited to 5000 words. We recommend a maximum of 60 references for review articles, with 2–6 figures and tables. Original summary diagrams and illustrations of proposed models (in colour where appropriate) are encouraged. Line drawings

may be redrawn. Boxes can be used to separate detailed explanations and background information from the main part of the text.

Review articles are normally by invitation and undergo peer review by experts in the field. Authors intending to submit unsolicited reviews should send an outline of the proposed article to the [editorial office](#).

Please note that systematic reviews, meta-analyses and literature reviews are classified as clinical studies.

Brief Reports

These are reports, with up to 1500 words and up to 2 tables or figures, and which report an important message or data that is very interesting but does not yet fulfil the rigorous criteria that we apply to clinical research studies.

Debates

Debate articles aim to cover the latest controversies in clinical endocrinology. Debates take the format of a general introduction written jointly by the proposer and opposer, followed by two distinct sections presenting the evidence for each point of view, followed by concluding remarks. Debates are published as single articles, providing readers with an excellent overview resource for topics around which there is no current consensus. Debate articles are commissioned by the editorial board and undergo peer review by experts in the field. If you would like to suggest a debate topic please submit a brief outline to the editorial office.

Clinical Practice Guidelines

Clinical practice guidelines are produced by the [European Society of Endocrinology](#), either in collaboration with other Societies or independently, and are aimed at providing recommendations for patient care for specified conditions. The [ESE Clinical Committee](#) play a leading role in the development of guidelines.

The European Society of Endocrinology's clinical guidelines are reviewed by our members and relevant interested parties (such as patient support groups) before publication and are presented during the annual European Congress of Endocrinology. Where it is considered beneficial, patient support literature will be prepared alongside the guidelines to provide patients with assistance in understanding and managing their condition.

Position Statements

Position statements are submitted by professional task forces, networks or society working groups, detailing an official view on a specific issue. Statements present all relevant background information and the rationale behind any recommendations set out.

Editorials

All Editorials must be a maximum 1500 words (including references, legends and tables) and 10 references.

Commentaries

Commentaries are typically invited by the Editor-in-Chief, although unsolicited commentaries will be considered. Commentaries are opinion articles that will examine novel concepts and findings introduced into the scientific record. They are typically no more than 2500 words in length, should have no more than eight references, and have no figures or tables.

Letters to the Editor

Letters to the Editor have a flexible format and may be published on occasion, where comments on a paper published in *European Journal of Endocrinology* or a topical issue in the field are of broad interest to the readership. Authors should submit letters through the journal's [submission system](#) for consideration by the Editor-in-Chief.

Supplementary Data

Supplementary data too large for print publication or exceeding the bounds of the manuscript may be submitted for online publication.

Supplementary data files intended for online publication should be submitted online via ScholarOne Manuscripts as 'Supplemental File for Review', and referred to as supplementary data in the text:

(Supplementary Table 1)

(Supplementary Figures 1 and 2)

Supplementary information will be reviewed as part of the manuscript, evaluated for its importance and relevance and, if accepted, will be referenced in the text of the article, directing readers to the website. There is a [charge](#) for publication of supplementary data. Should authors not wish to publish their supplementary data, they must notify the editorial office prior to acceptance.

Editorial Policies

Human subjects research

Authors must ensure research involving human subjects complies with the [Declaration of Helsinki](#).

Authors must include a statement that consent has been obtained from each patient after full explanation of the purpose and nature of all procedures used. For research requiring ethics committee approval, please include a statement to this effect in the manuscript. Also indicate whether patient consent was obtained in line with the below policy. We will be unable to accept research papers without this statement.

Patient consent

Where possible, identifying information, including names, initials, or hospital numbers, should not be published in written descriptions, photographs, or pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives written informed consent for publication. Any identifiable patient must be shown the manuscript to be published before being asked to give consent. Authors should disclose to these patients whether any potential identifiable material might be available online or in print after publication. Informed consent should be obtained if there is any doubt that anonymity can be maintained. We no longer publish pictures with black bands across the eyes without a signed consent form, because bands fail to mask someone's identity effectively.

Authors submitting case reports are required to state that they have obtained informed consent from the patient or the patient's guardian for publication of the submitted article and accompanying images. Authors should obtain written consent from the patient for use of potential identifiable material including photographs.

The patient (or parent or guardian) must give written informed consent for publication by signing our [consent form](#). Signed consent forms should then be retained in the patient's clinical notes for future reference, and a copy should be made available for review by the Editor on request.

The manuscript reporting this patient's details should state that 'Written informed consent for publication of their clinical details and/or clinical images was obtained from the patient/parent/guardian/ relative of the patient'. If the patient is deceased the authors should seek permission from a relative and include a statement to this fact. If neither the patient or a relative can be traced, we can only publish if we are satisfied the information has been sufficiently anonymised, making it impossible to identify the patient with any certainty.

Permission is not required to publish the 'recordings' listed below, provided that, the recordings are effectively anonymised by the removal of any identifying marks, and patient details (i.e. patient name, date of birth, name of hospital) from images before submission:

- Images taken from pathology slides
- X-rays
- Laparoscopic images
- Images of internal organs

- Ultrasound images

When such an image is accompanied by text that could reveal the patient's identity through clinical or personal detail, however, a signed consent form and declaration as listed above, will be required before publication.

Clinical trials

Papers reporting clinical trials will only be considered if the trials have been pre-registered according to the guidelines set out in [The Lancet 364 \(9438\) 911--912](#).

Authors should also refer to the [CONSORT 2010 Statement](#), and in particular the checklist within it, when preparing manuscripts detailing clinical trials.

Authors must state the clinical trial registration number within their article.

Animal studies

Experiments with animals must be performed in accordance with international, national and institutional requirements. Include a statement that investigations have been approved by the local ethical committee, along with the following:

- Give the full binomial Latin names for all experimental animals other than common laboratory animals
- State the breed or strain and source of animals, and give details of age, weight, sex and housing
- Detail the procedures and anaesthetics used, including doses given

Articles will only be considered if the procedures used are clearly described and conformed with the international and national legal and ethical requirements, as well as the requirements outlined by the institution in which the work took place. A statement identifying the committee approving the study must also be included in the Methods section.

Authors are encouraged to refer to the [ARRIVE guidelines](#), and in particular the checklist within them, when preparing manuscripts detailing animal experiments.

Editors reserve the right to request further information on the exact procedures and ethical approval obtained as part of the review process. Papers may be rejected on ethical grounds should the editors feel the study does not adequately meet current international guidelines for humane research.

Experiments with genetically engineered mice

In inbred mice, genetic strain effects can have significant effects on phenotype. Because of this the following controls for experiments with genetically-manipulated mice should be used: parental inbred strain, or wild-type littermates.

Human genotype–phenotype association studies

Until recently almost all genotype–phenotype observations were done using candidate gene approaches. The sequencing of the human genome and the comprehensive mapping of haplotypes of human SNPs have revolutionized gene association studies, which can now be conducted through genome-wide approaches. Unfortunately, many of the reported genotype–phenotype associations are questionable, and have not been replicated. *European Journal of Endocrinology* recognizes that genotype–phenotype association studies, performed by either genome-wide or candidate gene approaches, are of potential interest as a first step in the discovery process, although subsequent validation will be needed to confirm or refute the observation. However, these initial association reports must be methodologically sound. To ensure this the journal has adopted the recommendations made by the NCI-NHGRI Working Group on Replication in Association Studies ([Nature 447 \(7145\) 655--660](#)), and authors should adhere to these criteria as listed below.

These criteria are intended for studies of genotype–phenotype associations assessed by genome-wide or candidate-gene approaches:

- Statistical analyses demonstrating the level of statistical significance of a finding should be published or at least available so that others can attempt to reproduce the reported results
- Explicit information should be provided about the study's power to detect a range of effects
- The study should be epidemiologically sound, with careful accounting for potential biases in selection of subjects, characterization of phenotypes, comparability of environmental exposures (when possible) and underlying population structure in cases and controls
- Phenotypes should be assessed according to standard definitions provided in the report
- Associations should be consistent (within the range of expected statistical fluctuation) and reported for the same phenotypes across study subgroups or across similar phenotypes in the entire study group
- Significance should not depend on altering the quality control methods beyond standard approaches that could change inclusion or exclusion of large numbers of samples or loci
- Measures to assess the quality of genotype data should include results of known study sample duplicates or publicly available samples
- The results for concordance between duplicate samples (if applicable) as well as completion and call rates per SNP and per subject should be disclosed, along with rates of missing data
- A subset of notable SNPs should be evaluated with a second technology that verifies the same result with excellent concordance, because no technology is error-free
- Associations with nearby SNPs in strong linkage disequilibrium with the putatively associated SNP should be reported (and should be similar)
- The results of replication studies of previous findings should be reported even if the results are not significant
- Testing for differences in underlying population structure in case and control groups should be performed and reported
- Appropriate correction for multiple comparisons across all statistical tests examined should be reported. Comparison to genome-wide thresholds should be described. Similarly, for bayesian approaches, the choice of prior probabilities should be described

Gene and protein nomenclature

Wherever possible, manuscripts must be prepared in accordance with approved gene nomenclature.

- In gene and protein symbols, substitute Greek letters with the corresponding roman letter, eg TGFBR2 not TGFβR2
- Avoid hyphens unless they are part of the approved symbol, eg IGF1 not IGF-1
- Use arabic rather than roman numerals, eg BMPR2 not BMPRII

Follow species-specific formatting standards as follows:

Humans, non-human primates and domestic species

- Gene symbols should be in italics with all letters capitalised, eg *SOX2*
- Protein designations should be the same as the gene symbols but not italicised, eg *SOX2*
- Please use symbols approved by the [HUGO Gene Nomenclature Committee \(HGNC\)](#)

Mice and rats

- Gene symbols should be in italics with only the first letter capitalised, eg *Sox2*

- Protein designations should be the same as the gene symbols except that all letters should be capitalised and in roman (ie not italicised), eg SOX2
- Please use symbols approved by the International Committee on Standardized Genetic Nomenclature for Mice and the Rat Genome and Nomenclature Committee, which can be queried at the [MGI website](#)

Digital image integrity

No specific feature within an image may be enhanced, obscured, moved, removed, or introduced. The groupings of images from different parts of the same gel, or from different gels, fields or exposures must be made explicit by the arrangement of the figure (eg using dividing lines) and in the text of the figure legend. Adjustments of brightness, contrast, or colour balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (eg changes to gamma settings) must be disclosed in the figure legend. Adjustments should be applied to the entire image. Threshold manipulation, expansion or contraction of signal ranges and the altering of high signals should be avoided.

Microscopy

Microscope images should be made available to referees in images that are at least 300 dpi at the size which they will be published. 'Pseudo-colouring' and nonlinear adjustment (for example 'gamma changes') are only allowed if unavoidable and must be disclosed.

Digital image guidelines

Recognizing that the inappropriate use of computer software for digital image analysis and processing can raise concerns, the journal has produced the following requirements for the representation of research data:

- No specific feature within an image may be enhanced, obscured, moved, removed, or introduced
- Adjustments of brightness, contrast, or colour balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Any such adjustments should be applied to the entire image
- Threshold manipulation, expansion or contraction of signal ranges and the altering of high signals should be avoided
- The legend to a digital image should state if and what digital modifications were applied

The following guidelines apply to digital images that result from gel electrophoresis and blotting procedures:

- Band intensity should be quantified from several independent experiments. If only a "typical" experiment is shown in the figure, authors should be prepared to provide unprocessed images of gels or blots at the request of the Editor-in-Chief.
- Extensively cropped images are not acceptable. Images can be cropped to enhance clarity of presentation, but should always contain at least two markers (one with a smaller, one with a larger molecular size than the band of interest) with their molecular sizes indicated.
- Producing spliced images by combining lanes from gels or blots from different experimental runs should be avoided. A lane containing markers should be on the same gel for each run. If spliced images are presented, the vertical lanes obtained from gels or blots from different experimental runs should be clearly demarcated with lines.
- As the validity of immunoblots relies heavily on antibody specificity, an appropriate control (tissue from knockout mice or protein knockdown in cell lines) must be included, or alternatively a reference should be given in the methods section referring to such a control (Saper 2009; Burry 2011).

- The reuse of images of loading controls from other experiments or previous publications is unacceptable.

References

Burphy RW 2011 Controls for immunocytochemistry: An update. *Journal of Histochemistry & Cytochemistry* **59** 6–12. (doi:10.1369/jhc.2010.956920)
 Saper CB 2009 A guide to the perplexed on the specificity of antibodies. *Journal of Histochemistry & Cytochemistry* **57** 1–5. (doi:10.1369/jhc.2008.952770)

Statistical analysis

It is the author's responsibility to document that the results are reproducible and that the differences found are not due to random variation. No absolute rules can be applied but, in general, quantitative data should be from no fewer than three replicate experiments. Appropriate statistical methods should be used to test the significance of differences in results. The term 'significant' should not be used unless statistical analysis was performed, and the probability value used to identify significance (eg $P < 0.05$) should be specified.

When several t-tests are employed, authors should be aware that nominal probability levels no longer apply. Accordingly, the multiple t-test, multiple range test, or similar techniques to permit simultaneous comparisons should be employed. Also, in lieu of using several t-tests, it is often more appropriate to utilize an analysis of variance (ANOVA) to permit pooling of data, increase the number of degrees of freedom, and improve reliability of results. Authors should use appropriate nonparametric tests when the data depart substantially from a normal distribution.

In presenting results of linear regression analyses, it is desirable to show 95% confidence limits.

When data points are fitted with lines, specify the method used for fitting (graphical, least squares, computer program). If differences in slopes and/or axis intercepts are claimed for plotted lines, these should be supported by statistical analysis.

Give sufficient details of the experimental design and analysis so that the reader can assess their adequacy and validity for testing the hypotheses of interest. In particular:

- Describe the numbers of experimental units used and the way in which they have been allocated to treatments
- Justify the omission of any observations from the analysis
- Describe methods of analysis precisely and state any necessary assumptions, as these may affect the conclusions that can be drawn from the experiment

Your article may be sent to the Statistical Advisor for comments.

Preprint and data repositories

Preprint repositories

A preprint is a version of the article prior to submission to the journal for peer review, and has not been copyedited or typeset.

Bioscientifica allows deposition of preprints to recognized repositories, such as bioRxiv, provided that Bioscientifica is informed of this at the time of submission and it does not infringe any subsequent copyright or licence agreement.

Upon final publication, authors are required to add a link from the preprint to the published article (version of record).

Depositing data in public databases

Authors are strongly encouraged to deposit data sets in appropriate public databases. Authors should include the relevant database identifiers and accession numbers for deposited sequences within the manuscript using the following format: Database: xxxx, e.g: GEO: GSE6364. Authors are also required to provide the URL for the sequence(s).

Below is a list of public databases:

- [Addgene](#)
- [ClinVar](#)
- [dbGaP](#)
- [dbSNP](#)
- [ENA's Sequence Read Archive](#)
- [GenBank](#)
- [PeptideAtlas](#)
- [Gene Expression Omnibus \(GEO\)](#)

This list is not exhaustive. Please contact the [editorial office](#) if you have a query about relevant databases.

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