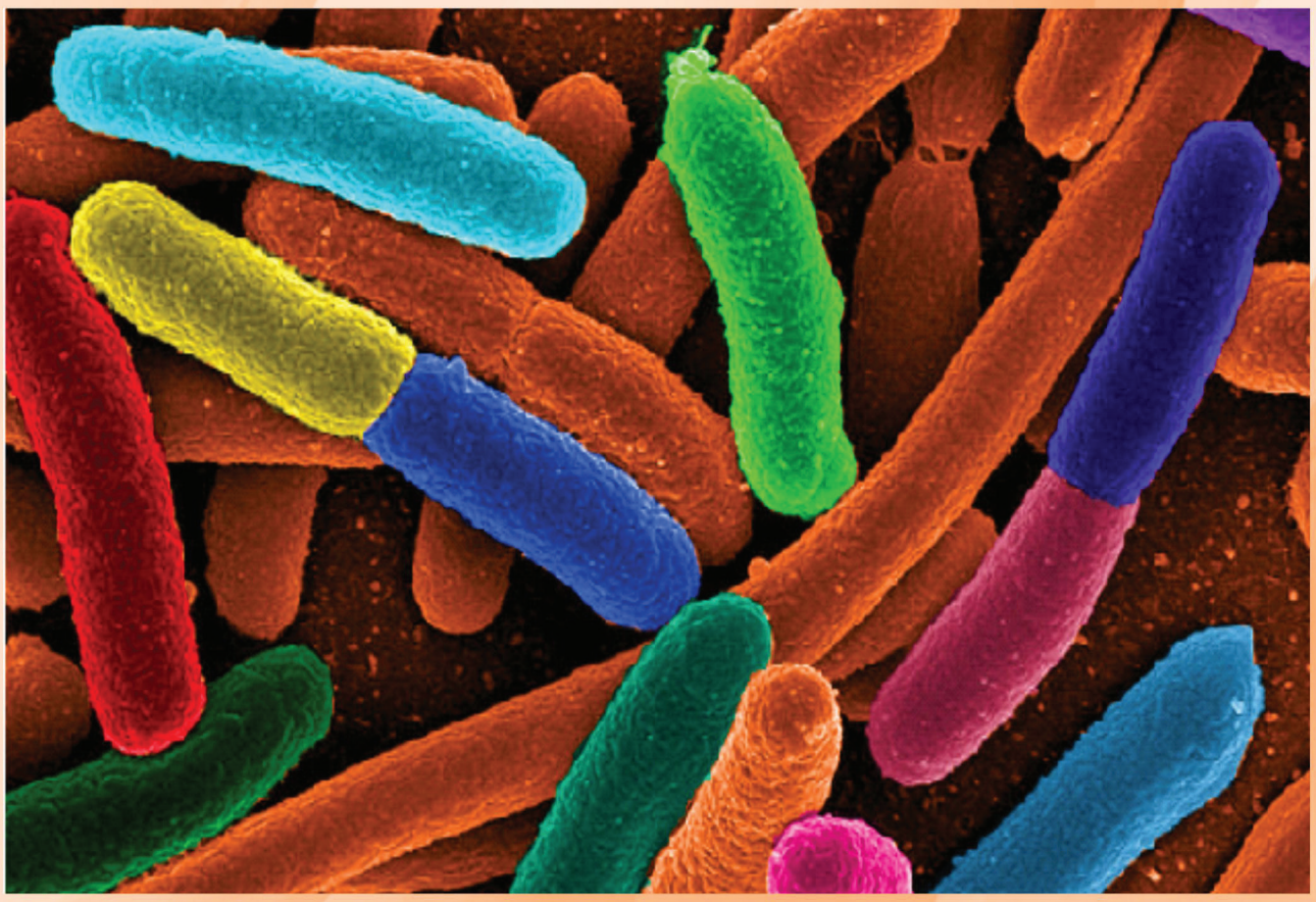
ISSN 1517-6932
ISSN ON LINE 2447-181X

ENDOCRINOLOGIA & DIABETES CLÍNICA E EXPERIMENTAL

HOSPITAL UNIVERSITÁRIO EVANGÉLICO DE CURITIBA
FACULDADE EVANGÉLICA DO PARANÁ

VOL. 16 - NUMBER 1 MAY/JUNE/JULY/AUGUST/2016



The Human Microbiota

EDITORIAL

OUR COVER: THE GUT MICROBIOTA IN HUMAN OBESITY

Researches estimate that about 1014 bacteria live in human gut and that they have 100 times more genes than the human genome. These microbes are called microbiota. Studies has disclosed that human microbiota have influence on metabolism, energy balance and that they are involved in the pathophysiology of human obesity. Human being gut as well as mouse gut have several bacterial phyla called *Bacteroidetes, Firmicutes and Actinobacteria*. Researches on the relation¬ship between the composition of the gut microbiota and obesity have shown that the number of *Firmicutes* is increased whereas the number of *Bacteroidetes* is impaired in obese mice as well as in obese human beings compared with lean individuals. A weight loss diet change this microbiota through increasing *Bacteroidetes*.

Studies have revealed that dissociation in gut micro¬biota is associated with prevalence of several diseases in the 21st century. The reduction of microbial diversity, a sign of a dysfunctional ecosystem, decreases the stability of gut microbiota. The change of the microbiota system has been associated with both inflammatory bowel disease and obesity. Factors like toll-like receptors, age, genetic composition, immune system and intercellular communication between microbes could be involved in the mechanism of human obesity.

The activity of lipopolysaccharide (LPS), an essential component of the cell walls of Gram-negative bacteria can cause inflammation and insulin resistance. LPS induces production of the serum amyloid A (SAA) proteins, with inflammatory activity, which is increased in the serum of obese individuals. A high-fat diet modifies the gut microbiota altering the relation Bacteroides / Firmicuites and reducing the levels of *Bifidobacteria* (Actinobacteria), which will lead to an increase in gut permeability and LPS plasma levels.

Finally there are a lot of publications addressing the potential role of specific nutrients, probiotics and prebiotis used to change the gut microbiota in order to control obesity and its comorbidities.

And what about pre and probiotics?

Probiotic is used to name ingested microorganisms associated with benefits for humans beings and animals. Prebiotics are substances that induce the growth or activity of microorganisms that contribute to the well-being of their host. About them some authors stated:

"The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" said Élie Metchnikoff Nobel winner with his research in 1907 about a possible relationship between yogurt-consuming among Bulgarian people and a longer life.

"A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" said Roberfroid (**Journal of Nutrition**, March 2007).

Will it be pre and pro-biotics consumption a promising treatment for obesity? ... or will they be mere "supporting actors" in this huge puzzle called human obesity?

Mirnaluci Paulino Ribeiro Gama

References

1-Obesity and the microbiota Gastroenterology. 2009 May;136(5):1476-83.

2-Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A 2005; 102:11070–1107

3-Human colonic microbiota associated with diet, obesity and weight loss. Int J Obesity 2008; 32:1720–1724.

4- Prebiotics: The Concept Revisited". J Nutr. 137 (3 Suppl 2): 830S–7S.

Contents

Cover: Human Microbiota
Fonte: Google.com
Microbiota Humana www.oarquivo.com.br

Endocrinol. diabetes clín. exp. - VOL.XVI - NUM. 1

Endocrinology & Diabetes - Clinical and Experimental is a journal of open access that publishes case reports, original article, reviews with new insights in pathogenesis, physiology and metabolism of hormone secretion, cellular mechanisms and tissue action. This journal belongs to the Discipline of Endocrinology and Metabolism of Faculdade Evangélica do Paraná and Service of Endocrinology and Diabetes - Diabetes Unit - Hospital Universitário Evangélico de Curitiba - Paraná - Brazil

Endocrinologia & Diabetes Clínica e Experimental
Disciplina de Endocrinologia e Metabologia da Faculdade Evangélica
do Paraná, Serviço de Endocrinologia e Diabetes do Hospital
Universitário Evangélico de Curitiba. – v.16, nº 1 (Maio/Junho/Julho/Agosto/2016)
– Curitiba:
FEPAR/HUEC, 2000-
p.1861 -1886: il.; 29cm

Quadrimestral
ISSN 1517-6932
ISSN on line 2447-181X

1.Endocrinologia – Periódicos. 2. Saúde – Periódicos. I. Faculdade Evangélica do Paraná. II. Hospital Universitário Evangélico de Curitiba.

CDD 616.4
CDU 612.34

CASE REPORT
PRIMARY HYPOTHYROIDISM SIMULATING THE PRESENTATION OF PITUITARY ADENOMA

HIPOTIREOIDISMO PRIMÁRIO SIMULANDO A APRESENTAÇÃO DE ADENOMA HIPOFISÁRIO

CAROLINE WALGER DA FONSECA*

Keywords: Hypothyroidism, Pituitary adenoma, Hyperprolactinemia
Descritores: Hipotireoidismo, Adenoma hipofisário, Hiperprolactinemia

Abstract

The primary hypothyroidism, a common disease, can rarely be revealed by pituitary hyperplasia. The low levels of thyroid hormones induce an overproduction of TRH which stimulates thyrotroph cells and also lactotroph cells. Such action results in hyperplasia and subsequent pituitary enlargement that can mimic an adenoma. In this article we report a case of a 28-year-old woman with headache, galactorrhea and a pituitary mass suggestive of a macroadenoma. Endocrine workup and follow-up with imaging studies led to the diagnosis of pituitary hyperplasia secondary to primary hypothyroidism. After therapy with levothyroxine there was resolution of the condition. This case highlights the importance of proceeding etiological investigation facing a patient with sellar mass allowing in this way the correct diagnosis and treatment. **Endocrinol diabetes clin exp 2016 1865 -1867.**

Resumo

O hipotireoidismo primário, uma doença comum, pode raramente ser revelado por hiperplasia hipofisária. Os baixos níveis de hormônios tireoidianos levam à hipersecreção do TRH que estimula as células tireotróficas e, também, lactotróficas. Tal ação resulta em hiperplasia e subsequente aumento hipofisário que pode mimetizar um adenoma. Neste artigo nós descrevemos o caso de uma mulher de 28 anos com cefaléia, galactorréia e massa hipofisária sugestiva de macroadenoma. Avaliação endocrinológica e seguimento com exames de imagem levaram ao diagnóstico de hiperplasia hipofisária secundária ao hipotireoidismo primário. Após terapia com levotiroxina houve resolução do quadro. Este caso ressalta a importância de prosseguir investigação etiolológica frente a um paciente com massa selar, permitindo, assim, o diagnóstico e tratamento corretos. **Endocrinol diabetes clin exp 2016 1865 -1867.**

BACKGROUND

The primary hypothyroidism, a common medical condition, can rarely be presented by pituitary hyperplasia (1,2). This association was first described by Niepce in 1985 in autopsy of patients with cretinism (3). Pituitary enlargement secondary to primary hypothyroidism (acquired or congenital) had been previously described in both children and adults (4,5).

The lack of thyroxine (T4) results in loss of negative feedback with overproduction of thyrotropin-releasing hormone (TRH) and thyrotroph hyperplasia with a consequent pituitary enlargement (1,6). This can mimic the appearance of a pituitary adenoma and can be large enough to compress the adjacent structures (2,5,7).

The elevated levels of TRH also stimulate the lactotroph cells increasing the secretion of prolactin (PRL) (1). Hyperprolactinemia causes hypogonadotropic hypogonadism by inhibiting the pulsatile secretion of GnRH (mainly) and direct inhibition of gonadal steroidogenesis (7,8). It may also result in galactorrhea, infertility or remain asymptomatic (9). The prevalence of hyperprolactinemia in overt hypothyroidism is reported in about 40% of patients (10).

We report a case of a young woman presenting headache, galactorrhea, hyperprolactinemia and a pituitary mass suggestive of a macroadenoma. Further investigation revealed primary hypothyroidism as a diagnosis for the clinical presentation.

Table 1. Laboratory evaluation at diagnosis and after treatment

Parameter (units)	Before therapy	12 months later	Normal range
TSH (µIU/mL)	**1080,00**	1,88	0,34-5,60
Free T4 (ng/dL)	**0,41**	0,86	0,70-1,48
PRL (ng/mL)	**32,2** **43,85** (diluted)	8,5	2,8-29,2
IGF-1 (ng/mL)	171	n.a.	117,0-329,0
FSH (mUI/mL)	7,63	n.a.	1,4-9,9
LH (mUI/mL)	2,83	n.a.	2,12-10,89
Estradiol (pg/mL)	46	n.a.	27-122
UFC (ug/24hr)	51,6	n.a.	28,5-213,7
Thyroid peroxidase antibodies (IU/mL)	**> 1000**	n.a.	< 5,61
Thyroglobulin antibodies (IU/mL)	**> 1000**	n.a.	< 4,11

TSH: thyroid-stimulating hormone; T4: thyroxine; PRL: prolactin; IGF-1: insulin-like growth factor I; FSH: follicle-stimulating hormone; LH: luteinizing hormone; UFC: urinary free cortisol; N.a.: not available.

*Service of Endocrinology and Diabetes, Hospital Universitário Evangélico de Curitiba, Paraná, Brazil.
E mail:carolzinhawf@yahoo.com.br

CASE REPORT

History

A 28-year-old woman was referred to our Evangélico University Hospital presenting frontal headache, alternating either throbbing or burning sensation, associated with nausea and vomiting (10 episodes), over the last five days. She had no complaints of visual impairment. During the previous two days she started to show spontaneous bilateral galactorrhea. She denied amenorrhea, the last menstrual period had occurred eleven days before, and informed use copper intrauterine device (IUD) for seven years. The patient did not report tiredness, weight gain, hair loss, constipation or any other symptoms. She had previous history of nephrolithiasis and intermittent smoking. There was neither history of alcohol, drug consumption, nor the use of medication. Her family history was positive for hypertension, type 2 diabetes and thyroid disease (mother).

Investigation

In the emergency service a computed tomography (CT) scan was performed and showed the anterior pituitary with volume increased. Laboratory data were also conducted. After the initial assessment result a pituitary adenoma was suspected hence the patient was referred to our department.

On admission, her heart rate was 84 bpm, arterial blood pressure 120/80 mmHg and temperature 36,6º C. Physical examination revealed myxedema of the face, dry skin and bilateral manually expressed nipple discharge (galactorrhea). On neck palpation, thyroid gland was normal.

Additional laboratory investigation (**Table 1**) revealed slightly elevated PRL of 32,2 ng/mL. Diluted PRL level, performed in sequence, was 43,85 ng/mL. In thyroid function tests, a strongly elevated TSH (>100,00 µIU/mL) was found and confirmed in the second sample (1080,00 µIU/mL) accompanied by low free T4 (FT4) of 0,41 ng/dL. High titer of thyroid peroxidase antibodies of >1000 IU/mL and thyroglobulin antibodies >1000 IU/mL were also found.

Pituitary magnetic resonance imaging (MRI) was performed and it showed a pituitary enlargement, extended on the suprasellar cistern, with a hypointense area on a T1 weighted image of up to 18mm in sellar region, reported to be suggestive of a pituitary macroadenoma by radiologist (**Figure 1**).

Based on clinical history and laboratory data, a diagnosis of primary hypothyroidism resulting from chronic autoimmune thyroiditis was made. We considered that the pituitary mass was hyperplasia secondary to primary hypothyroidism.

Treatment and follow-up

Therapy with levothyroxine was started at 25µg/day and gradually increased to 150µg daily. After thyroxine replacement, thyroid function had returned to the normal range (Table 1) with the serum TSH level 1,88 µIU/mL and FT4 0,86 ng/dL. The PRL level was normalized (8,5 ng/mL). A follow-up MRI scan 12 months after levothyroxine replacement showed complete resolution of the pituitary mass, confirming the diagnosis of pituitary hyperplasia (**Figure 2**).

DISCUSSION

Pituitary hyperplasia corresponds to the enlargement of the pituitary gland due to a non-neoplasic increase in the number of one or more functionally distinct types of pituitary cells (2,5). This can occur as a normal response to physiological stimuli like puberty and pregnancy, or as a pathological condition (2,5,7). These include, for example, primary hypothyroidism (thyrotroph hyperplasia) and more rarely due to gonadotroph, somatotroph or corticotroph hyperplasia (7).

In primary hypothyroidism the low levels of thyroid hormones induce a compensatory increase

in TRH secretion from hypothalamus (1,2). The TRH overproduction results in hyperplasia of the thyrotroph cells with subsequent enlargement of the pituitary gland (6,11). It is important to notice that it can be large enough to mimic a tumor and compress the adjacent structures. Patients with hypothyroidism present an incidence of pituitary hyperplasia that varies from 25% to 81% (6).

Those with TSH levels ≥ 50 µIU/mL a high incidence (70%) is reported (12). The compensatory increase in TRH also results in stimulatory effect on lactotroph cells with PRL secretion (10,13). Mild to moderate hyperprolactinemia (almost always < 100 ng/mL) occur in up to 40-50% of patients (13,14). In fact several other mechanisms have been proposed for the increase in PRL levels in primary hypothyroidism (10). First, pituitary cells have a reduction sensitivity to the inhibitory action of dopamine and dopamine agonists (1,10,13). Secondly, in hypothyroid patients PRL clearance from the circulation is decreased (1,10). Thirdly, reduced thyroid hormone levels result in increased PRL synthesis as demonstrated by Davis et al. that 3,5,3`-triiodothyronine lower PRL messenger RNA levels in rat pituitary cells (1,15). The hyperprolactinemia inhibits the pulsatile secretion of GnRH and gonadal steroidogenesis, resulting in hypogonadotropic hypogonadism (8). In women this can cause menstrual irregularity and amenorrhea. In both genders: infertility, sexual dysfunction and bone loss (8,9). Galactorrhea is the most typical manifestation of hyperprolactinemia, on the other hand it is not regarded as a specific signal because it can be found in individuals with normal PRL levels (8,14,16). Hyperprolactinemia may also remain asymptomatic (9).

Primary hypothyroidism is rarely revealed by galactorrhea and hyperprolactinemia associated with pituitary enlargement (1).

In our patient, the investigation at the emergency service started with cerebral imaging studies due to the main complaint of headache associated with nausea and vomiting. The increase in the pituitary gland demonstrated by CT was confirmed by MRI and suggested the presence of a pituitary macroadenoma. Despite recent advances in imaging techniques, CT and MRI still cannot distinguish pituitary macroadenoma from hyperplasia, even using MRI with gadolinium injection (1,2,5,6). On imaging studies (CT, MRI), the traditional characteristics of a macroadenoma include homogeneous enlargement of the gland to a height of greater than 10mm, with or without perisellar extention and deviation of the pituitary stalk (1,2). Secondary pituitary hyperplasia is often described as a homogeneously enhancing lesion that may grow rapidly following the onset of hypothyroidism (6). These findings overlap considerably with those of macroadenoma like in the case of our patient, where the pituitary mass was reported as a macroadenoma. Thus, the interpretation of a pituitary mass only with imaging studies does not allow a definite diagnosis. Further investigation including endocrinological evaluation is essential and may avoid unnecessary surgery.

At first presentation, in our case, a pituitary macroadenoma was considered. After endocrine workup, it was demonstrated a slight increase in PRL and a strongly elevated TSH. The PRL level measured, even after dilution, was not consistent with macroprolactinoma, which usually presents much higher levels. The differential diagnosis with TSH secreting adenoma can be made by the finding of inappropriate normal or elevated TSH levels with high range values of T3 and T4 (1,6). In our patient FT4 was below normal range and thyroid antibodies were positive. Therefore, thyroid function tests were consistent with primary hypothyroidism resulting from chronic autoimmune thyroiditis. Based on these findings, we considered the diagnosis of thyrotrophic pituitary hyperplasia and a thyroid hormone replacement therapy was initiated. It is described in the literature, on follow up MRI, that thyroxine replacement therapy led to a decrease in the size of the pituitary gland in 85% of patients with pituitary hyperplasia (1,12). The

time of response varies from 1 week to months, being variable (1). Surgery in pituitary hyperplasia should be reserved for the decompression of the optic chiasm when vision is compromised or in cases where a pituitary mass does not respond to, or worsens under, after thyroid hormone replacement (6,17).

On the follow up of our patient, MRI scan, which was done 1 year after initiated levothyroxine therapy, demonstrated complete regression of the pituitary mass. Considering repeat MRI findings, we confirmed the diagnosis of pituitary hyperplasia secondary to primary autoimmune hypothyroidism.

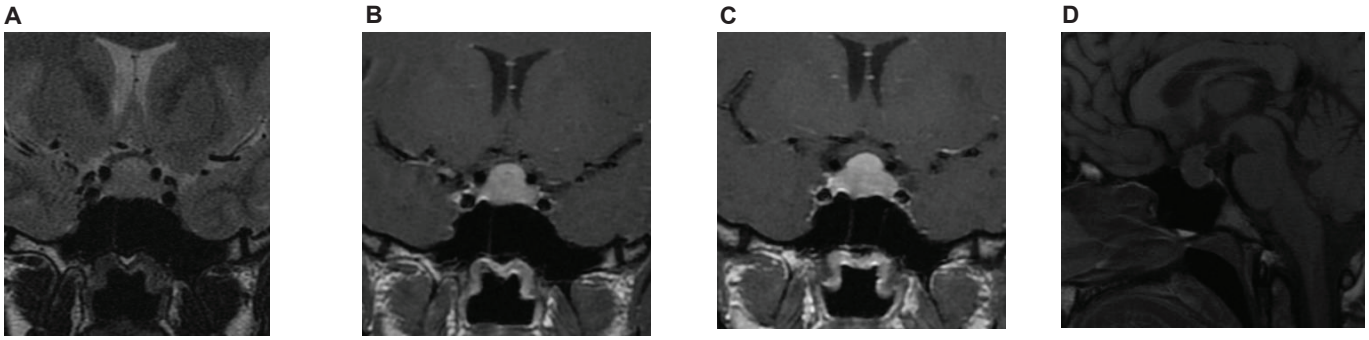


Figure 1. Coronal (A, B and C) and sagital (D) pituitary MRI showing an enlarged pituitary gland with suprasselar extension.

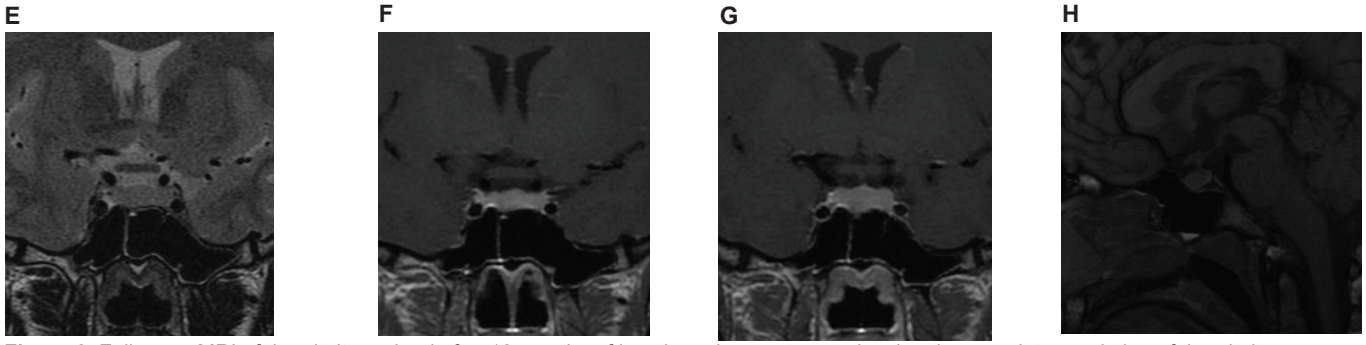


Figure 2. Follow-up MRI of the pituitary gland after 12 months of levothyroxine treatment showing the complete resolution of the pituitary enlargement. Coronal (E, F and G) and sagital (H).

CONCLUSION

In conclusion, facing a patient with sellar mass, primary hypothyroidism should be considered in the differential diagnosis. Further investigation, including endocrine workup, is crucial to the correct diagnosis and treatment, avoiding unnecessary surgery.

References

1. Neves CP, Massolt ET, Peeters RP et al. Pituitary hyperplasia: an uncommon presentation of a common disease. **Endocrinol Diabetes Metab Case Rep**, 2015; 2015:160056.
2. Moumen A, Meftah A, Jadi HE et al. An unusual pituitary mass revealing a primary hypothyroidism. **Clin Pract**, 2015;5(1): 733.
3. Niepce B. Traité du goitre et du crétinisme. **Baillière**, 1851, Paris.
4. Joshi AS, Woolf PD. Pituitary hyperplasia secondary to primary hypothyroidism: a case report and review of the literature. **Pituitary**, 2005;8(2):99-103.
5. Toromanovic A, Tahirovic H. Pituitary hyperplasia mimicking macroadenoma secondary to primary hypothyroidism. **Paediatrics Today**, 2016;12(1):108-112.
6. Franceschi R, Rozzanigo U, Fallo R et al. Pituitary hyperplasia secondary to acquired hypothyroidism: case report. **Ital J Pediatr,** 2011;37:15.
7. Musolino NR, Vilar L, Kodaira S et al. Diagnóstico diferencial das massas selares. In: Vilar, L. **Endocrinologia Clínica**, 5ª edição. Rio de Janeiro. Guanabara Koogan; 2013. p. 3-23.
8. Glezer A, Bronstein MD. Prolactinoma. **Arq Bras Endocrinol Metab**, 2014;58(2).
9. Melmed S, Casanueva FF, Hoffman AR et al. Diagnosis and treatment of hyperprolactinemia: an endocrine society clinical practice guideline. **J Clin Endocrinol Metab**, 2011; 96(2):273-288.
10. Goel P, Kahkasha, Narang S et al. Evaluation of serum prolactin level in patients of subclinical and overt hypothyroidism. **J Clin Diagn Res**, 2015;9(1):BC15-17.
11. Betônico CC, Rodrigues R, Mendonça SC et al. Hipotireoidismo primário simulando volumoso macroadenoma hipofisário. **Arq Bras Endocrinol Metab**, 2004;48/3.
12. Khawaja NM, Taher BM Barham ME et al. Pituitary enlargement in patients with primary hypothyroidism. **Endocr Pract**, 2006;12(1):29–34.
13. Freitas MC, Lima LH. Diagnóstico e tratamento do hipotireoidismo. In: Vilar, L. **Endocrinologia Clínica**, 5ª edição. Rio de Janeiro. Guanabara Koogan; 2013. p. 297-309.
14. Vilar L, Naves LA, Gadelha M. Armadilhas no diagnóstico da hiperprolactinemia. **Arq Bras Endocrinol Metab**, 2003;47(4):347-57.
15. Davis JR, Lynam TC, Franklyn JA et al. Tri-iodothyronine and phenythoin reduce prolactin messenger RNA levels in cultured rat pituitary cells. **J Endocrinol**, 1986;109:359–64.
16. Vilar L, Naves LA. Avaliação diagnóstica da hiperprolactinemia. In: Vilar, L. **Endocrinologia Clínica**, 5ª edição. Rio de Janeiro. Guanabara Koogan; 2013. p. 39-49.
17. Şimşek E, Şimşek T, Savaş-Erdeve S et al. Pituitary hyperplasia mimicking pituitary macroadenoma in two adolescent patients with long-standing primary hypothyroidism: case reports and review of literature. **Turk J Pediatr**, 2009;51(6):624–30.

Received in: 02-06-2016
Accepted in: 30-06-2016
Conflict of interests: none

Address for correspondence:
Caroline Walger da Fonseca
Deputado Heitor Alencar Furtado 2381 ap 601 CEP 81200110
Curitiba – PR - Brazil

ORIGINAL ARTICLE
RELATIONSHIP BETWEEN TYPE 1 DIABETES AND HASHIMOTO'S THYROIDITIS – BIOINFORMATICS STUDY

RELAÇÃO ENTRE A DIABETES TIPO 1 E TIREOIDITE DE HASHIMOTO – ESTUDO COM BIOINFORMÁTICA

LUÍS JESUÍNO DE OLIVEIRA ANDRADE[1]
GABRIELA CORREIA MATOS DE OLIVEIRA[1]
ROBSON DA SILVA ALMEIDA[1]
PAULO ROBERTO SANTANA DE MELO[1]
ALCINA MARIA VINHAES BITTENCOURT[2]

Keywords: Type 1 diabetes; Thyroiditis; Homology; Bioinformatics
Descritores: Diabetes tipo 1; Tireoidite; Homologia; Bioinformática

Abstract

The GAD2 glutamate decarboxylase 2 [Homo sapiens (human)] gene (GAD65) encodes one of several forms of glutamic acid decarboxylase, identified as a major auto-antigen in DM1. The TPO thyroid peroxidase [Homo sapiens (human)] gene (TPO) encodes a membrane-bound glycoprotein, and encoded protein acts as an enzyme and plays a central role in thyroid gland function. **Objective:** Use bioinformatics tool to identify protein homology between GAD65 and TPO, to explain the relationship between DM1 and HTby formation of similar antibodies.**Method:** We performed a comparison between amino acids (AA) sequence of theGAD65 and TPO, available in the database of NationalCenter for Biotechnology Information (NCBI) with the Basic Local Alignment Search Tool (BLAST) for to find regions of local homology between the nucleotide sequences. **Results:** The homologies between the GAD65 and TPO ranged from 25.0 % (8 identical residues out of 32 AA in the sequence) to 50.0% (6 identical residues out of 12 AA in the sequence). **Conclusion:** Bioinformatics data, suggest a possible pathogenic link between DM1 and HT(GAD65 and TPO). Through of molecular mimicry is observed that sequences homologies between glutamate decarboxylase and thyroid peroxidase could be a mechanism of induction of crossover immune response to self-antigens. **Endocrinol diabetes clin exp 2016 1868 -1871.**

Resumo

Estudos mostram a possível associação entre diabetes tipo 1 (DM1) e doenças autoimunes. A tireoidite de Hashimoto (TH) é uma desordem autoimune associada ao DM1 que sugere compartilhar uma homologia genética. O GAD2 2 glutamato descaborxilase 2 [Homo sapiens] gene (GAD65) codifica uma das várias formas do ácido glutâmico descarboxilase, uma importante fonte de auto-antígeno em DM1. A TPO peroxidase da tireóide [Homo sapiens] gene (TPO) codifica uma glicoproteína ligada à membrana e sua atividade, como enzima, desempenha um papel central na função tireoidiana. **Objetivo:** Usar ferramentas de bioinformática para identificar a homologia entre o GAD65 e a TPO, para explicar a relação entre DM1 e TH pela formação de anticorpos semelhantes. **Método:** Foi realizada uma comparação entre a sequência dos aminoácidos (AA) do GAD65 e da TPO, disponíveis no banco de dados do Centro Nacional de Informações sobre Biotecnologia (NCBI) com a ferramenta de alinhamento (BLAST) para encontrar regiões de homologia local entre as sequências de nucleotídeos. **Resultados:** As homologias entre o GAD65 e TPO variou de 25,0% (8 resíduos idênticos de 32 AA na sequência) a 50,0% (6 resíduos idênticos de 12 AA na sequência).
Conclusão: Os dados de Bioinformática sugerem uma possível ligação patogênica entre DM1 e TH(GAD65 e TPO). Através de mimetismo molecular é observado que sequências de homologias entre GAD65 e a TPO pode ser um mecanismo de indução de resposta imune cruzada para auto-antígenos. **Endocrinol diabetes clin exp 2016 1868 -1871.**

INTRODUCTION

Studies show possible association between type 1 diabetes (DM1) and autoimmune disorders. Hashimoto's thyroiditis (HT) is an autoimmune disorders associated with DM1 suggesting to a shared genetic homologies (1).

DM1 it's an autoimmune disease characterized by selective destruction of pancreatic-islet beta cells of genetically susceptible individuals in combination with environmental factors (2).

The enzyme glutamic acid decarboxylase encoded is responsible for catalyzing the production of gamma-aminobutyric acid from L-glutamic acid. Glutamic acid decarboxylase 65 kDa isoform (GAD65) is an important immunological marker in individuals with DM1. High title of autoantibodies against GAD65 has been detected in DM1 and some other neural autoimmune diseases (3).

The thyroid peroxidase gene provides instructions for making an enzyme called thyroid peroxidase (TPO) that plays a central role in the function of the thyroid gland. The TPO assists the chemical reaction that adds iodine to a thyroglobulin, fundamental in thyroid hormones production. The TPO was first identified as being the thyroid microsomal antigen involved in autoimmune thyroid disease (4).The TPO is an important autoantigen in autoimmune thyroid disease, and TPO [Homo sapiens] species coding for a 933-amino acid protein (termed TPO-1) and a second in which exon 10 is deleted and which is 57 residues shorter (termed TPO-2) (5).

The prevalence of autoimmune thyroiditis in individuals with DM1 varies between 3 and 50% in different countries (6).

Bioinformatics program currently available to construct the structure analyses of TPO component provides an essential framework for TPO assembly, and understanding of your molecular mechanisms. Similarly, molecular modeling has facilitated the understanding of molecular mimicry as a result of cross-immune response to similar GAD65 antigen with TPO human.

Until recently, little structure information about GAD65 and TPO human were available and molecular model has been built on partial structures, with assembly guided by biochemical data. The purpose of this study is to explore the possible sequence

[1]Health Department, Post-Graduation Program in Health Sciences, UniversidadeEstadual de Santa Cruz, Ilhéus – Bahia – Brazil.
[2]MedicalSchool, Universidade Federal da Bahia – Salvador – Bahia – Brazil.
E-mail: luis_jesuino@yahoo.com.br

homology between the amino acids (AA) sequences of TPO, which are potential B- and T-cell epitopes of these antigens and GAD65, using databanks of proteins and immunogenic peptides to explain the relationship between DM1 and HT by formation of similar antibodies.

MATERIAL AND METHODS

Were performed the comparison between the AA sequence of the GAD65 and TPO human, available in the database of National Center for Biotechnology Information (NCBI) on Basic Local Alignment Search Tool (BLAST2p) (7).In bioinformatics, BLAST is an algorithm of NCBI for comparing biological sequence information, such as the amino-acid sequences of different proteins or nucleotides of DNA sequences. A BLAST search compares a query sequence with a database of sequences, and identify homology or similarities between protein sequences. The BLAST was introduced as a sequence alignment heuristic that was an order of magnitude faster than earlier approaches for analyzing biological information. Very quickly, this software became a landmark enabling technique for bioinformatics. Thus, the BLAST2p refers to a program used to generate alignments between a nucleotide or protein sequence, referred to as a query and nucleotide sequences against other database of nucleotide, referred to as subject sequences.

The BLAST2p program was used for performing the comparisons. The expect value is a parameter that describes the number of hits one can expect to see by chance when searching a database of a particular size (expect value considered as statistical significance <0.05). It decreases exponentially as the score of the match increases. The low expect value, or the closer it is to zero, the more significant the match. However, identical short alignments have relatively high E values. This is because the calculation of the E value takes into account the length of the query sequence. These high expect value make sense because shorter sequences have a higher probability of occurring in the database purely by chance. The expect value can also be used as a convenient way to create a significance threshold for reporting results.

Sequence Homology searches - Protein database search and analysis

One protein-protein sequence alignment method, BLAST2p program, was used to search for sequence homology between GAD65 and TPO human. This method is limited to searching for linear epitope homologies, which will miss three-dimensional conformational homologies and possible cross-reactivity between protein and non-protein epitopes, since most of molecular mimicry is likely to involve T-cell mediation, and T cells generally recognize linear peptides 8–20 AA in length.

TPO and GAD65 examined

Were evaluated the following TPO nucleotides, with the respective NCBI sequence identification number (GI):thyroid peroxidase [Homo sapiens]GI:4680721, thyroid peroxidase [Homo sapiens]GI: 18539488, thyroid peroxidase [Homo sapiens] GI: 339871, thyroid peroxidase [Homo sapiens]GI:339867, thyroid peroxidase [Homo sapiens]339865, Thyroid peroxidase [Homo sapiens]GI: 63100775, and thyroid peroxidase [Homo sapiens]GI: 62865489. The GAD65 nucleotides examined were: glutamate decarboxylase 2 [Homo sapiens]GI:197276620, glutamate decarboxylase 2 [Homo sapiens]GI:4503875, and RecName: Full=Glutamate decarboxylase 2; AltName: Full=65 kDa glutamic acid decarboxylase; Short=GAD-65; AltName: Full=Glutamate decarboxylase 65 kDa isoformGI:1352216.

Exposed Protein Databases

• TPO:

Regarding thyroid peroxidase [Homo sapiens] Accession: AAA61217.2, the ID contained 922 AA protein sequences (8). In the case of thyroid peroxidase [Homo sapiens] Accession:AAA61216.1, the ID had 876 AA protein sequences (9). The thyroid peroxidase [Homo sapiens] Accession:AAA61215.1 study allowed us to obtain an ID that comprised 933AA protein sequences (9). Concerning thyroid peroxidase [Homo sapiens] Accession:AAA97517.1 we were able to assemble an ID contained 933 AA protein sequences (10). The Thyroid peroxidase [Homo sapiens] Accession:AAH95448.1 study allowed us to obtain an ID that comprised 933 AA protein sequences (11). In the case of thyroid peroxidase [Homo sapiens]Accession:AAY16985.1, the ID had 933 AA protein sequences (12).

The structure of TPO was built from SWISS-MODEL that is a fully automated protein structure homology-modelling server, accessible via the ExPASy web server, or from the program DeepView (**Figure 1**).

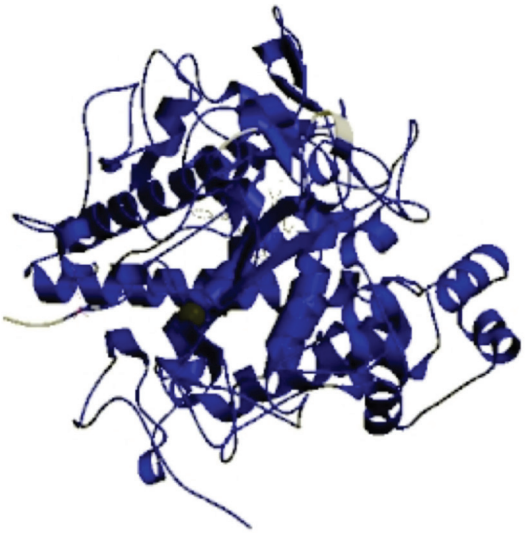


Figure 1. The Structure of the Thyroid Peroxidase (TPO)
Source http://swissmodel.expasy.org/interactive/jhZ9D8/templates/

• GAD65:

Regarding glutamate decarboxylase 2 [Homo sapiens] Accession:NP_001127838.1, the ID contained 585 AA protein sequences (13). In the case of glutamate decarboxylase 2 [Homo sapiens]Accession:NP_000809.1, the ID had 585 AA protein sequences (13). The RecName: Full=Glutamate decarboxylase 2; AltName: Full=65 kDa glutamic acid decarboxylase; Short=GAD-65; AltName: Full=Glutamate decarboxylase 65 kDa isoformAccession:Q05329.1, the ID contained 585 AA protein sequences (14).

The crystal structure of GAD65 was determined in 2007 (**Figure 2**), providing an atomic positioning of the epitope-mapping data, as well as insights into the molecular determinants of antigenicity (15).

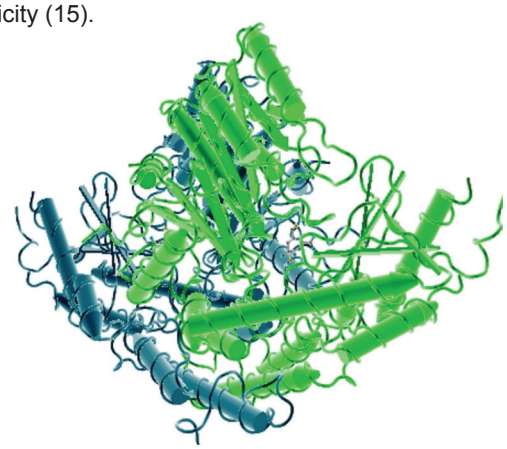


Figure 2. The X-ray Crystal Structure of the 65kda Isoform of Glutamic Acid Decarboxylase (GAD65). Source http://www.ncbi.nlm.nih.gov/protein

RESULTS

The sequence homology was related each TPO to each GAD65 (Figure 3).The homologies between the GAD65 and TPO ranged from 25.0 % (8 identical residues out of 32 AA in the sequence) to 50.0% (6 identical residues out of 12 AA in the sequence) (**Table 1)**.

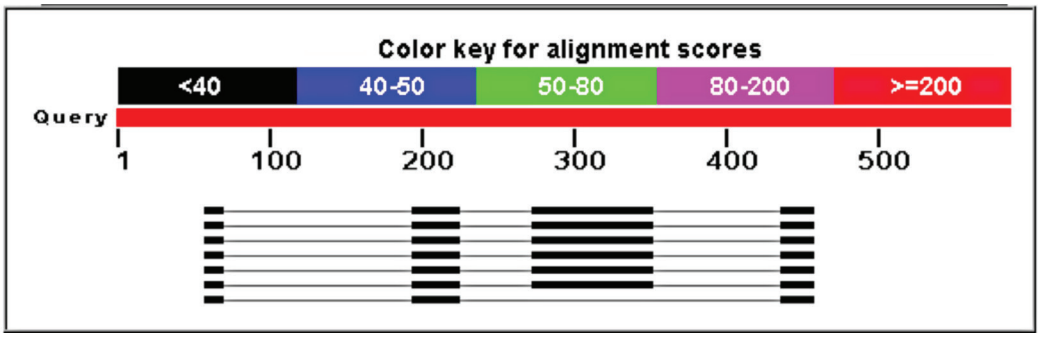


Figure 3. Distribution of 5 Blast Hits on the Query Sequence

Range 1: 241 to 312

Score	Expect	Method	Identities	Positives	Gaps
23.5 bits(49)	0.037	Compositional matrix adjust.	23/80(29%)	33/80(41%)	9/80(11%)

```
Query  274  IAFTSEHSHFSLKKGAAALGIGTDSVILIKCDERGKMIPSDLERRILEAKQKGFVPFLVS  333
            IAFT + +          AA G G+D     + C+ +     P  L       A      +PF  S
Sbjct  241  IAFTPQST------SKAAFGGGSD--CQMTCENQNPCFPIQLPEEARPAAGTACLPFYRS  292

Query  334  ATA-GITVYGAFDPLLAVAD  352
            + A GT    GA     L+ A+
Sbjct  293  SAACGIGDQGALFGNLSTAN  312
```

Range 2: 615 to 636

Score	Expect	Method	Identities	Positives	Gaps
19.2 bits(38)	0.80	Compositional matrix adjust.	8/22(36%)	10/22(45%)	0/22(0%)

```
Query  436  SYDTGDKALQCGRHVDVFKLWL  457
            S    DK L    +H D    +WL
Sbjct  615  SRSVADKILDLYKHPDNIDVWL  636
```

Range 3: 635 to 666

Score	Expect	Method	Identities	Positives	Gaps
17.7 bits(34)	2.4	Compositional matrix adjust.	8/32(25%)	13/32(40%)	0/32(0%)

```
Query  195  WLISTANTNMFTYEIAPVFVLLEYVTLKKMRE  226
            WL   A    +       P+F   L    +K +R+
Sbjct  635  WLGGLAENFLPRARTGPLFACLIGKQMKALRD  666
```

Range 4: 890 to 902

Score	Expect	Method	Identities	Positives	Gaps
17.3 bits(33)	2.7	Compositional matrix adjust.	6/13(46%)	8/13(61%)	0/13(0%)

```
Query  440  GDKALQCGRHVDV  452
            G   L+CG+H  V
Sbjct  890  GTPELRCGKHQAV  902
```

Range 5: 903 to 914

Score	Expect	Method	Identities	Positives	Gaps
16.2 bits(30)	6.6	Compositional matrix adjust.	6/12(50%)	9/12(75%)	0/12(0%)

```
Query  60   GSQPPRAAARKA  71
            G+ P RAAA+ +
Sbjct  903  GTSPQRAAAQDS  914
```

Table 1. Blast alignments

DISCUSSION

This study suggests the possible role of molecular mimicry between the AA sequences of TPO and GAD65 to explain the relationship between DM1 and HT by formation of similar antibodies.

GAD65 is an important autoantigen in DM1 and latent autoimmune diabetes of adults, and patients positive for GAD65 showed an increased risk for thyroid autoimmunity, revealed by the presence of thyroid peroxidase autoantibodies (16,17). It has been reported that the prevalence of autoantibodies to thyroid peroxidase and/or thyroglobulin are 15%-30% in patients with DM1 at the time of diagnosis of the diabetes, this suggests that both diseases share etiopathogenic mechanisms (18,19).

Studies suggesting the existence in humans of polarized T

helper (Th) cell subsets, coded as Th1 and Th2, with defined cytokine secretion profiles. The Th1 cells seem to be involved in organ-specific autoimmunity, such as DM1 and HT (20). The triggering of autoimmunity secondary to infection or immunization is often related to antigenic mimicry because only five to six AA are necessary to induce an immune response (21).

The molecular mimicry could explain the development of autoimmune diseases, through of cross reactions between epitopes and antigens present in the body that promote an adverse autoimmune response (22).

In this work, we analyzed the sequence homology between the AA sequences of the seven TPO human three GAD65. We found that TPO and GAD65 share AA sequence homology, wherein some similar regions contain epitopes of both TPO and GAD65 very high.No have studies been done in medical literature to correlate the protein homology between TPO and GAD65.

Despite the limitations posed by the unavailability of complete proteome data for GAD65 proteins, losing three-dimensional conformational homologies and possible cross-reactivity between protein and non-protein epitopes, the homologies between the AA sequences of TPO, which are potential B- and T-cell epitopes of these antigens and proteins of GAD65, were successfully identified.

Thus, the observed homologies could be functionally important in molecular mimicry, and receptor binding and cell signaling will be involved in autoimmunity, with important implications for the understanding of the relationship between DM1 and HT by formation of similar antibodies.

CONCLUSION

In conclusion, it is important to draw attention that bioinformatics data suggest a possible pathogenic link between DM1 and thyroid disorders. Therefore, through of molecular mimicry is observed that sequences homologies between GAD65 and self-proteins thyroid that could be a mechanism of induction crusade immune response to self-antigens resulting in autoimmune disease thyroid.

References

1. Tomer Y, Dolan LM, Kahaly G, Divers J, D'Agostino RB Jr, Imperatore G, et al. Genome wide identification of new genes and pathways in patients with both autoimmune thyroiditis and type 1 diabetes. **J Autoimmun**. 2015;60:32-9.
2. Atkinson MA, Eisenbarth GS.Type 1 diabetes: new perspectives on disease pathogenesis and treatment. **Lancet**. 2001;358:221-9.
3. Khan MW, Sherwani S, Khan WA, Ali R.Characterization of hydroxyl radical modified GAD65: a potential autoantigen in type 1 diabetes. **Autoimmunity**. 2009;42:150-8.
4. Ruf J, Carayon P.Structural and functional aspects of thyroid peroxidase. **Arch Biochem Biophys**. 2006;445:269-77.
5. Gardas A, Lewartowska A, Sutton BJ, Pasieka Z, McGregor AM, Banga JP.Human thyroid peroxidase (TPO) isoforms, TPO-1 and TPO-2: analysis of protein expression in Graves' thyroid tissue. **J Clin Endocrinol Metab**. 1997;82:3752-7.
6. Kordonouri O, Klinghammer A, Lang EB, Grüters-Kieslich A, Grabert M, Holl RW.Thyroid autoimmunity in children and adolescents with type 1 diabetes: a multicenter survey. **Diabetes Care**. 2002;25:1346-50.
7. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. **J Mol Biol**. 1990;215:403-10.
8. Le Fourn V, Ferrand M, Franc JL. Differential expression of thyroperoxidase mRNA splice variants in human thyroid tumors. **Biochim Biophys Acta**. 2004 28;1689:134-41.
9. Kimura S, Kotani T, McBride OW, Umeki K, Hirai K, Nakayama T, et al.Human thyroid peroxidase: complete cDNA and protein sequence, chromosome mapping, and identification of two alternately spliced mRNAs. **Proc Natl Acad Sci U S A**. 1987;84:5555-9.
10. Kimura S, Hong YS, Kotani T, Ohtaki S, Kikkawa F.Structure of the human thyroid peroxidase gene: comparison and relationship to the human myeloperoxidase gene. **Biochemistry**. 1989;28:4481-9.
11. Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS, et al. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. **Proc Natl Acad Sci U S A**. 2002;99:16899-903.
12. Abramowicz MJ, Targovnik HM, Varela V, Cochaux P, Krawiec L, Pisarev MA, et al.Identification of a mutation in the coding sequence of the human thyroid peroxidase gene causing congenital goiter. **J Clin Invest**. 1992;90:1200-4.
13. Ling Z, De Pauw P, Jacobs-Tulleneers-Thevissen D, Mao R, Gillard P, Hampe CS, et al.Plasma GAD65, a Marker for Early β-Cell Loss After Intraportal Islet Cell Transplantation in Diabetic Patients. **J Clin Endocrinol Metab**. 2015;100:2314-21.
14. Karlsen AE, Hagopian WA, Grubin CE, Dube S, Disteche CM, Adler DA, et al.Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10. **Proc Natl Acad Sci U S A**. 1991;88:8337-41.
15. Fenalti G, Buckle AM.Structural biology of the GAD autoantigen. **Autoimmun Rev**. 2010;9:148-52.
16. Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, et al. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. **Nature**. 1990;347:151-6.
17. Falorni A, Gambelunghe G, Forini F, Kassi G, Cosentino A, Candeloro P, et al. Autoantibody recognition of COOH-terminal epitopes of GAD65 marks the risk for insulin requirement in adult-onset diabetes mellitus. **J Clin Endocrinol Metab**. 2000;85:309-16.
18. Kawasaki E, Yasui J, Tsurumaru M, Takashima H, Ikeoka T, Mori F, et al. Sequential elevation of autoantibodies to thyroglobulin and glutamic acid decarboxylase in type 1 diabetes. **World J Diabetes**. 2013;4:227-30.
19. Araujo DB, Barone B, Melleti NF, Dantas JR, Oliveira MM, Zajdenverg L, et al. Thyroid disorders are common in first-degree relatives of individuals with type 1 diabetes mellitus. **Arch Endocrinol Metab**. 2015;59:112-5.
20. De Carli M, D'Elios MM, Zancuoghi G, Romagnani S, Del Prete G. Human Th1 and Th2 cells: functional properties, regulation of development and role in autoimmunity. **Autoimmunity**. 1994;18:301-8.
21. Oldstone MB.Molecular mimicry and immune-mediated diseases. FASEB J. 1998;12:1255-65.
22. Cusick MF, Libbey JE, Fujinami RS. Molecular mimicry as a mechanism of autoimmune disease. **Clin Rev Allergy Immunol**. 2012;42:102-11.

Received in: 14-06-2016
Accepted in: 21-06-2016
Funding: None
Conflicts of interest: The authors declare no conflicts of interest.

Address for correspondence:
Dr. Luís Jesuíno de Oliveira Andrade
Nações Unidas, 511 – Centro– Itabuna (BA), Brazil
CEP 45600-673

ORIGINAL ARTICLE
TOPICS IN MEDICAL CLINIC
UPPER LIMB DYSFUNCTION IN PATIENTS TREATED FOR BREAST CANCER

DISFUNÇÃO DO MEMBRO SUPERIOR EM PACIENTES TRATADAS PARA CÂNCER DE MAMA

PAUL JOSEPH JAKOBI*
PEDRO GIANELLO LABATUT*
JEAN ALEXANDRE FURTADO CORREIA FRANCISCO*
THELMA L SKARE*

Key words: Breast cancer, Shoulder, Pain, Dysfunction, Lymphedema
Descritores: Câncer de mama, Ombro, Dor, Disfunção, Linfedema

Abstract

Background: Breast cancer treatment has reached high rates of success concerning patient's survival. However it can lead to impairment of shoulder function.
Objective: To study the function of the upper limb in patients undergoing breast cancer treatment in our region.
Methods: Epidemiological and treatment data were obtained in 58 patients that underwent treatment for breast cancer. These patients were evaluated for range of shoulder motion, grip strength, degree of pain, presence of lymphedema and upper limb function by DASH (Disability of the Arm, Shoulder and Hand Questionnaire) questionnaire.
Results: Of the 58 patients analyzed, 29.3% had mastectomy and 70,6% quadrantectomy; 82.7% had axillary dissection and 17.2% sentinel lymph node biopsy. The mean time post-surgery was of 2.8±1.8 years and radiotherapy was done in 91.2% of the sample.
In this group of patients, 43.1% had loss of mobility of the shoulder; 60.3% had loss of grip strength on the surgical side in relation to the non-operated and 56.8% of patients met criteria for lymphedema. The results of the hand function questionnaire were positively associated with loss of shoulder flexion (p=0.004) and pain (p<0.0001) and negatively correlated with patient age in univariate analysis (p=0.007). In multivariate analysis, only pain corresponded with loss of limb function independently. It was not possible to associate the loss of shoulder flexion or pain with treatment variables. Only the loss of shoulder flexion showed a trend (p=0.08) to be more common in patients submitted to radical surgery.
Conclusion: There is high prevalence of morbidity for upper limb in patients undergoing treatment for breast cancer. Pain is the variable that correlates independently with arm loss of function. **Endocrinol diabetes clin exp 2016 1872 -1876.**

Resumo

Justificativa: O tratamento do câncer de mama tem alcançado altas taxas de sucesso em termos de sobrevida. Todavia pode prejuízos À articulação do ombro.
Objetivo: Estudar a função do membro superior de pacientes submetidas a tratamento de câncer de mama em nossa região.
Métodos: Obtiveram-se dados epidemiológicos e quanto ao tratamento realizado de 58 pacientes submetidas a tratamento de câncer de mama. Estas pacientes também foram avaliadas quanto à amplitude de movimentos do ombro, força de preensão palmar, grau de dor, presença de linfedema e função do membro superior pelo questionário DASH (*Disability of the Arm, Shoulder and Hand Questionnaire*).
Resultados: Das 58 pacientes analisadas, 29,3% foram submetidas à mastectomia e 70,6% à quadrantectomia; 82,7% ao esvaziamento axilar níveis 1 e 2 e 17,2% à biópsia de linfonodo sentinela. O tempo médio após a cirurgia foi de 2,8± 1,8 anos e radioterapia foi realizada em 91,2% da amostra.
Neste grupo de pacientes, 43,1% apresentavam perda de mobilidade do ombro; 60,3% tinham prejuízo da força de preensão palmar do lado operado em relação ao não operado e 56,8% das pacientes preenchiam critérios para linfedema. Os resultados do questionário de função da mão se associaram positivamente com perda da flexão do ombro (p=0,004) e dor (p <0,0001) e negativamente com idade da paciente em análise univariada (p=0.007). Na análise multivariada apenas a dor se correspondeu com perda de função do membro de maneira independente. Não foi possível associar a perda de flexão do ombro, nem a dor com as variáveis de tratamento estudadas. Apenas a perda de flexão mostrou uma tendência (p=0,08) para ser mais comum em pacientes submetidas à cirurgia radical.
Conclusão: Existe alta prevalência de morbidade em membro superior de pacientes submetidas a tratamento para câncer de mama. Dor é a variável que se correlaciona de maneira independente com perda de função do braço. **Endocrinol diabetes clin exp 2016 1872 -1876.**

INTRODUCTION

One of the complications of breast cancer treatment is the upper limb dysfunction, ipsilateral to surgery (1). Complaints of stiffness and shoulder pain, arm swelling and difficulty to move are not rare in this context and these symptoms may affect adversely the limb function bringing physical, social and psychological consequences for the affected women (1). An Australian study showed that approximately 60% of patients surgically treated for breast cancer had at least one symptom related to upper limb six years after the intervention (2).

Breast cancer is common worldwide and it is the most frequent cancer in women (3). In Brazil, its incidence is exceeded only by skin cancer; according to the INCA (National Cancer Institute José Alencar Gomes da Silva), 57.120 new cases occurred in 2015 (4). Currently, patients with this form of cancer have enjoyed a long and disease-free survival after treatment thanks to the new therapeutic strategies (3). According to Rietman et al, the average survival after surgery is at least 79% in five years (3). So, it is important that all efforts should be made to minimize the complications arising from treatment aiming to improve the quality of life in this population.

The causes of upper limb dysfunction after breast cancer

*Rheumatology Unit of Evangelic University Hospital. Curitiba, PR, Brazil
E-mail: pauljakobi86@yahoo.com.br

treatment are not fully known, but it is observed that more aggressive forms of treatment (mastectomy versus breast-conserving surgery, sentinel node vs. axillary dissection, magnitude of radiation etc.) seem to influence its occurrence (1,5).

This research was conducted to study the prevalence and degree of upper limb dysfunction in patients undergoing treatment for breast cancer in our region and to analyze the variables that may influence in their appearance.

MATERIAL AND METHODS

This is an observational cross-sectional study approved by the local Ethics Committee in Research. All participants signed consent. All women with breast cancer treated surgically in a single Unit from a tertiary hospital during the period from September 2014 to January 2016 were invited to participate. The patients were included according to the order of arrival for consultation and willingness to participate in the study. They should have had at least 6 months from surgery. Women with bilateral breast cancer, patients with intellectual incapacity to understand the consent form, patients with rheumatic, orthopedic and neurological disorders involving the joints, muscles and nervous network from upper limb were excluded.

Data collection included epidemiological information (age, ethnicity, dominant side, smoking habits and type of work) and information on cancer treatment: chemotherapy and/or hormonotherapy, radiotherapy, type of surgical treatment (mastectomy vs quadrantectomy, axillary dissection vs lymph node sentinel lymphadenectomy) and time after surgery.

The study of the upper limb involvement was performed using the following variables:

a) *Mobility of shoulders:* measurement of flexion, extension, abduction and horizontal adduction bilaterally, done with a two arms goniometer. Loss of mobility was considered if the patient had a difference ≥ 20° between the two sides in either of studied movements. O (3,6).

b) *Hand grip strength:* measured in both hands with a Jamar® dynamometer. This measurement was made in triplicate with the patients sitting with the forearm lying flat positioned at 90o and the handle at 30o. For statistical purposes the highest value was used. A difference of strength between members ≥10% was considered significant (3,7).

c) *Edema:* Arm diameter measurements were done in four points of each limb (two points 10 and 20 cm below the olecranon and two points 10 and 20 cm above the olecranon) using an inelastic tape. When a difference ≥2 cm between members at any level was found, lymphedema was considered to exist (8,9).

d) *Upper limb function:* It was evaluated through DASH questionnaire (Disability of the Arm, Shoulder and Hand Questionnaire), which consists of 30 questions designed to measure upper limb symptoms and physical function. The DASH questionnaire assesses the degree of difficulty in performing daily activities; pain intensity, weakness, stiffness and paresthesias, social activities impairment; sleep difficulty and psychological impairment as well as the confidence degree of the patient in carrying out daily activities with the operated arm. Each of these items was graduated from one and five and the total score ranges from 0 (no impairment) to 100 (severe dysfunction) (9).

e) *Pain:* It was measured through a visual analogue scale (VAS) going from zero to 10 where zero means no pain and 10 the maximum value assigned to it.

The statistical analysis was performed by calculating percentages to determine frequency. Measures of central tendency were expressed as means and standard deviations when the sample was Gaussian and median and interquartile range (IQR) if the sample was not Gaussian. Data normality determination was done using the Kolmogorov-Smirnov test. Association studies were done by Fisher and chi-squared tests when the data were nominal and Mann Whitney when numerical. The correlation study of DASH values with age, hand strength, degrees of shoulder mobility and VAS of pain were made by the Spearman test. All data that showed an association or correlation with the DASH in the univariate analysis with p> 0.1 were studied by multiple regression to test the independence of the variables. The tests were performed with the software MEDCALC® version 10.0 and the adopted significance was of 5%.

RESULTS

a) Description of the sample:

We included 58 women aged 30-85 years (mean 56.5 ± 12.6 years). In this sample 52/58 (89.6%) were self-reported Caucasian; 50/58 (86.2%) were right-handed; 45/58 (77.5%) were manual workers and 17/58 (29.3%), smokers. Breast surgery was done in the right side in 36/58 (62.0%); 22/58 (37.9%) in the left and in 31/58 (53.4%) the operated side was coincident with side of the dominant hand.

Regarding surgical treatment: 17/58 (29.3%) had undergone mastectomy; 41/58 (70.6%) quadrantectomy; 48/58 (82.7%) were submitted to axillary dissection level I and II and 10/58 (17.2%) to examination of sentinel lymph node. As adjuvant treatment, 24/58 (41.3%) received doxorubicin, cyclophosphamide and taxane; 11/58 (18.9%) received cyclophosphamide, doxorubicin, 5-fluorouracil; 43/58 (74.1%) received tamoxifen; 1/58 (1.7%) trastuzumab and 1/58 (1.7%) anastrozole. About 52/57 (91.2%) were submitted to radiotherapy. The time between data collection and surgery ranged from 0.8 to 9.5 years (mean 2.8 ± 1.8 years).

In this sample the pain VAS ranged from zero to 10 (median of 0; IQR 0-6). The difference between arms flexion went from 0 to 700 (median 10o; IQR=0-100); extension from 0 to 30º (median 10; IQR=0-10º), abduction from 0 to 50º (median 0°; IQR=0-20º) and horizontal adduction from 0 to20º (median 20; IQR=0 to 100). Criteria for shoulder mobility impairment was fulfilled in 25/58 (43.1%) of them. The prevalence of the different forms of shoulder mobility defects are seen in **Figure 1**.

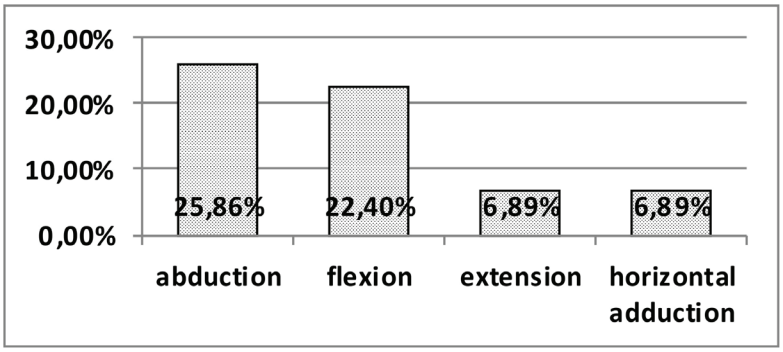


Figure 1- Prevalence of movement impairment in the shoulder ipsilateral to surgery in 58 patients with breast cancer.

The variance in grip strength between the two sides ranged from 0.0 to 22.0 mm Hg (median 2.0; IQR=0 to 4.5 mm Hg). In 35/58 (60.3%) of them it was considered to exist weakness in the operated side compared to the non-operated; 33/58 (56.8%) patients met the criteria for lymphedema. In this sample the values of the DASH ranged from 0 to 84 (median 5.85; IQR=0 to 23.5).

b) Association study of DASH values with clinical, epidemiological and treatment:

The analysis of the DASH values in relation to clinical, epidemiological and treatment variables are on Table 1, where it is observed that arm dysfunction is associated with the abnormal flexion movement.

Table 1- Association studies of DASH (*Disability of the Arm, Shoulder and Hand Questionnaire*) with epidemiological, clinical and treatment variables in breast cancer patients.

	DASH With the variable	DASH Without the variable	P (*)
Caucasian ethnic background	0-84.0 Median 4.1(0-25.0)	0-45.8 Median 19.1 (0-43.3)	0.27
Manual work	1-84.0 Median 8.3(0.4-25.8)	0-65.8 Median 2.9(0-27.7)	0.56
Smokers	0 -58.3 Median 2.5 (0 -18.7)	0.0-84 Median 10.8 (0-27.7)	0.12
Impairment of any type of shoulder movement	0-84 Median 15.8 (4.1-42.0)	0.0-38.0 Median de 3.3 (0-19.5)	**0.004**
Flexion	0-84.0 Median 39.3(3.3-43.3)	0-58.5 Median 4.1(0-20.0)	**0.004**
Extension	2.5-43.3 Median 10.0 (2.9-36.4)	0-84.0 Median de 5.4(0.8-25.8)	0.68
Abduction	0-23.3 Median 16.2 (3.3-22.3)	0-84.0 Median 5.0(0.8-25.0)	0.68
Adduction	0-23.4 Median 16.2(3.32-22.4)	0-84.0 Median 4.5(0-26.0)	0.88
Palmar strength	0-84.0 Median 6.67 (0-25.8)	0-58.3 Median 4.1 (0-25.8)	0.55
Lymphedema	0-84.0 Median 6.6 (0-31.6)	0-65.8 Median 4.1 (0.8-21.6)	0.51
Quadrantectomy	0-84.0 Median 5.8(0.4-25.4)	0-65.8 Median de 10.8(1.2-28.3)	0.96
Surgery in the dominant side	0-65 Median 6.6 (1.6-25.0)	0.0-84.0 Median 4.1 (0-25.8)	0.35
Axillary dissection	0-84.9 Median 8.3(0-25.6)	0-38.3 Median 4.5 (3.9-31.8)	0.70
Radiotherapy	0-84.0 Median 6.6 (0.4 -26.2)	0 -24.1 Median 0.8 (0-15.0)	0.23
Chemotherapy/hormone therapy			
ACT	0-65.8 Median de 5.8 (0-23.3)	0-84.0 Median 5.0 (0.8-30.8)	0.77
CAF	0-84.0 Median 13 (0-36.6)	0-65.8 Median 5.0 (0.4-25.0)	0.76
Tamoxifen	0-84 Median 4.1(0-26.6)	0-65.8 Median 13.3(1.6-24.1)	0.53

(*)Mann Whitney test. ACT=doxorubicin, cyclophosphamide, taxane; CAF = cyclophosphamide, doxorubicin, 5 fluorouracil. Values between brackets = interquartile intervals.

We found a negative correlation between arm dysfunction measured by the DASH and age (Spearman's Rho =-0.34; 95% CI=-0.56 to -0.09; p=0.007) and a positive correlation with pain (Spearman's Rho 0.53, 95% CI=0.31 to 0.69; p<0.0001). There was no correlation between DASH and the time interval after surgery (Spearman's Rho=-0.15; 95% CI =-0.40- 0.11; p=0.24).

Studying the variables that showed an association/correlation with DASH in univariate analysis (shoulder flexion defect, age and pain) by multiple regression to test their independence, it was found that only pain maintained its association with upper limb dysfunction (p=0.0008).

c) Study of arm flexion dysfunction according to the treatment variables:

As the loss in the shoulder flexion was the movement alteration that affected the upper limb function, this variable was studied regarding the received treatment, as seen on Table 2. It was observed a trend to greater loss of flexion in those who underwent mastectomy.

Table 2- Comparison of the patients with and without impairment shoulder flexion regarding treatment variables.

	With impairment of shoulder flexion (N=13)	Without impairment of shoulder flexion (N=45)	P
Mastectomy	6/13 (46.1%)	10/45 (22.2%)	0.08 (*)
Axillary dissection	10/13 (76.9%)	38/45 (84.4%)	0.67(**)
Radiotherapy	11/12 (91.6%)	41/45 (91.1%)	1.00(**)
Chemotherapy/Hormone therapy			
ACT	3/13 (23.0%)	19/45 (42.2%)	0.33(**)
FAC	2/13 (15.3%)	8/45 (17.7%)	1.00(**)
Tamoxifen	9/13 (69.2%)	35/45 (77.7%)	0.71(**)

(*)chi squared test; (**)Fisher test; ACT=doxorubicin, cyclophosphamide, taxane; FAC=.cyclophosphamide, doxorubicin, 5 fluorouracil.

d) Study of the pain in relation to treatment variables:

As pain was the independent variable associated with loss of upper limb function, we sought to identify whether the established forms of treatment were associated or not with their occurrence. No association could be identified as seen in Table 3.

Table 3- Association studies of pain and treatment variables.

	Pain VAS with the variable	Pain VAS without the variable	P (*)
Mastectomy	0-10 Median 0 (0-3.7)	0-10 Median 0 (0-4.0)	0.86
Axillary dissection	0-10 Median 0 (0-2.2)	0-10 Median 5 (0-7.0)	0.20
Radiotherapy	0-10 Median 0 (0-5.5)	0-8.0 Median 3.5(0.5-7.2)	0.33
Chemotherapy/hormone therapy			
ACT	0-10 Median 0 (0-8.0)	0-8 Median 0 (0-2.2)	0.51
FAC	0-8 Median 0 (0-1.7)	0-10 Median 0 (0-4.5)	0.82
Tamoxifen	0-10 Median 0 (0-2.2)	0-10 Median 1.0 (0-8.0)	0.15

(*)Mann Whitney test; VAS=visual analogic scale; ACT=doxorubicina, ciclofosfamida, taxane; FAC =.ciclofosfamida, doxorubicina, 5 fluorouracil.
Values between brakets= interquartile range.

DISCUSSION

Nowadays surgical treatment of breast cancer has become less invasive; pharmacotherapy and radiotherapy techniques have improved the results significantly (10). However, subjective and objective complications of this type of treatment are still present. In this study there was a high prevalence of shoulder movement derangements, loss of hand strength and lymphedema and these complications enclosed almost half of the sample.

Functional impairment was observed in the four studied movements (flexion, extension, abduction and adduction). However, only the flexion damage was associated with the loss of arm function measured by the DASH questionnaire. The shoulder flexion is made by raising the arm in front of the body and above the head; it occurs in the glenohumeral joint and it is complemented by movements in the sternoclavicular, acromioclavicular and scapulo-thoracic joints (11). Changes in the other movements (extension, abduction and adduction) showed no influence in shoulder function in significant ways.

The joint mobility alterations found in this study are very close to those detected by Lauridesen et al (10) that have found loss of mobility in 35% of their patients in one or more directions, particularly flexion and lateral extension. However these authors did not study the individual contribution of each movement to the member function. On the other hand, they did observe an association of loss of joint mobility with local pain.

According to Rietman et al, (5) axillary irradiation affects importantly the shoulder mobility. It is considered that irradiation promotes fibrosis and inflammatory changes in the soft tissue as well as neurological damage to the exposed nerve roots (1). In general, fibrosis is light, but when it is important it contributes to mobility loss of the glenohumeral joint and scapula (1). In the present study we could not demonstrate functional impairment in irradiated patients; however only a small percentage of our sample (8.8%) did not use of this form of treatment, and this may have precluded the comparison. Regarding surgical management, we found only a tendency for mastectomy patients to have higher prevalence of flexion defects than those treated with quadrantectomy. Studies with larger samples are needed to better analyze this variable.

The presence of lymphedema, though common, was not associated with loss of upper limb function in this study. Lymphedema was found in 56% of this sample. The prevalence of this form of complication depends largely on the methodology used to access its presence that is extremely diverse (11,12). Some studies indicate that 45-60% of patients have lymphedema six months after the surgery (1,12,13) while others point a prevalence of 70-80% after 12 months postoperatively (11,14). The sample presently studied had an average of 2.8 years of postoperative follow-up. Lymphedema occurs due to impaired lymphatic system drainage; the long term accumulation of interstitial fluid may favor local fibrosis (15) and thus contribute to loss of joint motion.

In the present findings it was observed in the univariate

analysis that the patients age correlated negatively with the arm function. A similar observation was made by Koostra et al (16) that noted greater dysfunction in younger patients and Yap et al (17) and Husen et al (18) who found that older women have a lower prevalence of symptoms.

However, the most striking findings of this study was the association of loss of upper limb function with pain. Pain is considered one of the main complaints of patients after treatment, with a prevalence of 12-51% (1,19,20). Immobilization is a mechanism of protection against pain and the disuse promotes loss of muscle mass and strength, and allows the development of periarticular fibrosis. Fibrosis, in turn, can lead to loss of mobility and, in extreme cases, to total stiffness by adhesive capsulitis (or frozen shoulder) (1,10). The multivariate analysis suggests that the observed flexion impairment was secondary to the occurrence of pain. However, it is interesting to note that the degree of pain presented by these patients was not very high when measured by EVA. An explanation that can be offered for this finding is that a less acute pain is considered secondary in this context, not properly valued and treated. The patient accommodates with the situation not using the shoulder in all its potential, which gradually leads to loss of function. It is therefore up to the doctor to value any complaint of pain in these patients and treat it properly to prevent shoulder immobilization and impairment of its performance. Analgesia and physiotherapy are of extreme value in these situations.

CONCLUSION

In conclusion, it can be said that there is a high prevalence of shoulder mobility defects, lymphedema and loss of grip strength in patients undergoing treatment for breast cancer in our population. Loss of shoulder flexion and pain were associated with loss of upper limb function and should be actively treated to preserve the quality of life of these patients.

References

1- Hayes S, Johansson K, Sout NL, Prosnitz R Armer JM, Gabram S et al. Upper-body mordibity after breast cancer. **Cancer** 2012; 118 (S8): 2237-49.

2- Schmitz K, Speck RM, Rye SA, DiSipio T, Hayes S. Prevalence of breast cancer treatment sequelae over 6 years of follow-up. The pulling through study. **Cancer** 2012; 118 (suppl 8): 2217-25.

3- Rietman JS, PU Dijkstra, Debreczeni R, Geertzen JH Robinson DP, De Vries J. Impairments, disabilities and health related quality of life after treatment for breast cancer: a follow-up study 2.7 years after surgery. **Disabil Rehabil**. 2004; 26 (2): 78-84.

4- Estimate 2014: Cancer Incidence in Brazil / **National Cancer Institute José Alencar Gomes da Silva**, captured in http://www.inca.gov.br/wcm/outubro-rosa/2015/cancer-de-mama.asp. Accessed February 2015.

5- Rietman JS, PU Dijkstra, Geertzen JH, Baas P, de Vries J, Dolsma WV et al . Treatment-related upper limb morbidity 1 year after sentinel lymph node biopsy or axillary lymph node dissection for stage I or II breast cancer. **Ann Surg Oncol**. 2004; 11 (11): 1018-24.

6- Segerström K, Bjerle P Nyström A. Importance of time in assessing arm and hand function after treatment of breast cancer. **Scand J Plast Surg Hand Surg** 1991; 25 (3): 241-4.

7- Maunsell E, Brisson J, L. Deschênes Arm problems and psychological distress after surgery for breast cancer. **Can J Surg**. 1993; 36 (4): 315-20.

8- Brazil. **Ministry of Health. Breast Cancer Control - Consensus Document, 2004**. Available at: http://www.inca.gov.br/publicacoes/Consensointegra.pdf. Accessed: 06/2014.

9- Cheng HMS. Disabilities of the arm, shoulder and hand-Dash: analysis of the adapted version for the Portuguese [Master degree thesis]. Belo Horizonte: Universidade Federal de Minas Gerais; 2006.

10- Lauridsen MC, Overgaard M, Overgaard J, Hessov IB Cristiansen P. Shoulder disability and late Symptoms Following surgery for early breast cancer. **Acta Oncol**. 2008; 47 (4): 569-75.

11- Caillet R. pain syndromes: **Neck and arm**. Editora Manole Ltda, São Paulo, 1976, p.133.

12- Hayes S, M Janda, Cornish B, Battistutta D, Newman B. Lymphedema secondary to breast cancer: how choice of measure influences diagnosis, prevalence, and identifiable risk factors. **Lymphology**. 2008; 41 (1): 18-28.

13- B Clark, Sitzia J, Harlow W. Incidence and risk of arm edema Following treatment for breast cancer: a three-year follow-up study. **QJM**. 2005; 98 (5): 343-8.

14- Hayes SC, Janda M, Cornish B, Battistutta D, Newman B. Lymphedema after breast cancer: incidence, risk factors, and effect on upper body function. **J Clin Oncol**. 2008; 26 (21): 3536-42.

15- Casley-Smith JR. Alterations of untreated lymphedema and it's over time grids. **Lymphology**. 1995; 28 (4): 174-85.

16- Kootstra JJ, Dijkstra PU, Rietman H, de Vries J, P Baas, Geertzen H, et al. A longitudinal study of shoulder and arm morbidity in breast cancer survivors 7 years after sentinel lymph node biopsy or axillary lymph node dissection. **Breast Cancer Res Treat**. 2013; 139 (1): 125-34.

17- Yap KP, McCready DR, Narod S, Manchul LA, Trudeau M, Fyles A. Factors Influencing arm and axillary symptoms after treatment for node negative breast carcinoma. **Cancer**. 2003; 97 (6): 1369-75.

18- M Husen, Paaschburg B, Flyger HL. Two-step axillary operation Increases risk of arm morbidity in breast cancer patients. **Breast**. 2006; 15 (5): 620-8.

19- Gärtner R, Jensen MB, Nielsen J, Ewertz M, N Kroman, Kehlet H. Prevalence of and factors associated with persistent pain following breast cancer surgery. **JAMA**. 2009; 302 (18): 1985-92

20- Torres-Lacomba M, Mayoral del Moral O, Coperias-Zazo JL, Gerwin RD, Goni AZ. Incidence of myofascial pain syndrome in breast cancer surgery: a prospective study. **Clin J Pain**. 2010; 26 (4): 320-5.

Received in: 24-06-2016
Accepted in: 28-06-2016
Funding: none
Conflict of interests: none

Author for correspondence:
Paul J. Jakobi
Augusto Stelfeld 1908 - Bigorrilho, Curitiba – PR – Brazil-
CEP 80730-150

ORIGINAL ARTICLE
TOPICS IN MEDICAL CLINIC
SECONDARY SJÖGREN SYNDROME AND DISEASE ACTIVITY OF RHEUMATOID ARTHRITIS

INFLUÊNCIA DA ATIVIDADE DA ARTRITE REUMATÓIDE NA SÍNDROME DE SJÖGREN SECUNDÁRIA

DANIEL C. ANTERO[1]
MARCELO GEHLEN[2]
ANDREO G. M. PARRA[2]
FERNANDO H. MIYAZAK[2]
MARÍLIA BARRETO SILVA[1]
THELMA L. SKARE [1]

Key words: Rheumatoid arthritis, inflammation, Sjögren's syndrome
Descritores: Artrite reumatóide, inflamação, Síndrome de Sjögren

Abstract

Background: Secondary Sjögren syndrome (SS) is considered an extra articular manifestation of rheumatoid arthritis (RA). Objective: To study the relationship of the presence of secondary SS with disease activity, duration and functional class in RA.
Methods: Eighty two patients with RA were submitted to Schirmer test, minor salivary gland biopsy, questionnaire on sicca symptoms, DAS-28 4v determination and functional class classification.
Results: In this population, 20 (24.3 %) patients fulfilled the American-European classification criteria for secondary SS. No relationship could be found between the presence of secondary SS and disease activity (p= 0.31), patient's functional class (p=0.61) and RA duration (p=0.95).
Conclusion: The presence of secondary SS has no relationship with disease activity and duration of underlying diseases nor with patient's functional class. **Endocrinol diabetes clin exp 2016 1877 -1879.**

Resumo
Justificativa: A síndrome de Sjögren secundária (SS) é considerada uma manifestação articular extra de artrite reumatóide (AR).
Objetivo: Estudar a relação da presença de SS secundária com a atividade da doença, duração e classe funcional na AR. Métodos: Oitenta e dois pacientes com RA foram submetidos ao teste de Schirmer, menor biópsia de glândula salivar, questionário sobre sintomas de secura, DAS-28 4v determinação e classe funcional classificação.
Resultados: Nesta população, 20 (24,3%) pacientes preencheram os Critérios de Classificação Americano-Europeus para SS secundário. Nenhuma relação pode ser encontrado entre a presença de SS secundário e a atividade da doença (p = 0,31), classe funcional do paciente (p = 0,61) e a duração RA (p = 0,95).
Conclusão: A presença de SS secundário tem nenhuma relação com a atividade e com a duração da doença subjacentes. A classe funcional do paciente também não influi na SS. **Endocrinol diabetes clin exp 2016 1877 -1879.**

INTRODUCTION

Sjögren's syndrome (SS) is a chronic autoimmune inflammatory disease that affects lachrymal and salivary glands causing mucous dryness that affects mainly middle aged women (1). SS can be a primary disease that currently is considered more as a subtype of systemic lupus or as secondary form when it is associated with other autoimmune disorders such as scleroderma and rheumatoid arthritis (RA) (2). Superimposed SS in RA is an interesting finding. There is an important role of B cells and type I interferon in primary SS (2,3) in contrast to the predominance of Th17 cytokines in RA (4). As the physiopathology of these two diseases is distinct it is possible to suggest that patients with RA and secondary SS have two different diseases or that RA secondary SS has a different physiopathology than the primary form.

The prevalence of SS in patients with RA varies according to the geographical area and ethnic group showing the importance of genetic background in their appearance. In a study of an Italian population secondary SS was detected in 17.5% of RA patients (5), while in a Spanish study it was found in 55% (6).

In the present study we investigated the relationship of RA disease activity, functional index and disease duration in patients with secondary SS.

MATERIAL AND METHODS

This study was approved by the Committee for Ethics in Research of our institution and all participants signed consent. To participate in the study patients had to have at least four criteria of the American College of Rheumatic Diseases for RA (7). The included patients were from a single Rheumatology Clinic (from Evangelic University Hospital) chosen according to appointment order and willingness to participate in the study. We excluded patients with ophthalmologic complications such as scleritis, episcleritis, scleromalacia, prior eye surgery, contact lenses users or those taking medications such as antidepressants, anticholinergics, antihistamine, diuretics, etc, with hepatitis C or HIV infection or prior irradiation of the neck. Patients were selected.

All included patients had Schirmer test done according to standard recommendations and we considered a patient with definitive dry eye when values were equal or under 5 mm in at least one eye (8). Biopsy of minor salivary gland was done in all included patients and all sections were stained by hematoxylin-eosin and considered positive when a focus of 50 lymphocytes/4mm2 was found (9). Salivary gland biopsy was read by a pathologist unaware of other results. Simultaneously

[1]Rheumatology Service – Evangelic University Hospital Curitiba PR -Brazil
[2]Ophthalmology Service- Evangelic University Hospital Curitiba PR - Brazil
Email: tskare@onda.com.br

with eye tests and minor salivary gland biopsy, patients had DAS-28 4v (10, 11) (using sedimentation rate) and the health assessment questionnaire (HAQ) (12) determined and answered a questionnaire on sicca symptoms (oral and ocular). The patient's joint count were done by just one rheumatologist. The charts were reviewed for demographic data and autoantibody profile (latex, anti CCP, anti Ro, Anti La).

To consider a RA patient as having secondary SS he had to fulfill the American European Criteria for secondary Sjögren syndrome (13,14) .

Data were grouped in contingency and frequency table. For association studies we used chi-squared test for nominal data and unpaired t, Mann-Whitney tests for numeric data. The significance adopted was 5%.

RESULTS

Eighty two patients were included; 72 women and 10 men with a mean age of 51.8±10.0 years and a mean disease duration of 10.2±7.0 years. In this sample 50/82 (75%) had rheumatoid factor; 25/35 (71.4%) had CCP positive; 25/82 (30.4%) were ANA positive; 6/82 (7.3%) had anti-Ro and 1/82 (1.2%) had anti-La. Dry eye complaints were found in 47/82 (57.3%) and dry mouth in 29/82 (35.3%); 46/82 (56.0%) had positive minor salivary gland biopsy. The Schirmer test varied between 0 and 35 mm (mean 14.3±10.1 mm) and under 5 mm in 34/82 (41.4%). Five (6%) patients had also ceratitis. DAS-28 varied from 0.6 to 6.99 (mean 3.22±1.41). In this sample 39/82 (47.5%) had functional class 1; 40/82 (48.7%) had class 2; 2/82 (2.4%) had class 3 and 1/82 (1.2%) class 4. Twenty patients (24.39%) fulfilled the American-European criteria for Secondary SS.

Studying DAS-28 according to the presence of secondary SS, we found a mean value of 2.81±1.14 in those with SS and 3.35±1.47 in those without it (p=0.13). The mean value of DAS-28 in patients with Schirmer test under 5 mm was 3.1±1.3 and in those with values higher than 5 mm it was 3.3±1.4 (p=0.50). Presence of Secondary SS according to DAS-28 is shown in **figure 1**. Mean DAS-28 value in patients with subjective symptoms for dry eyes was 3.35±1.58 and in those without 3.04±1.13 (p= 0.31).

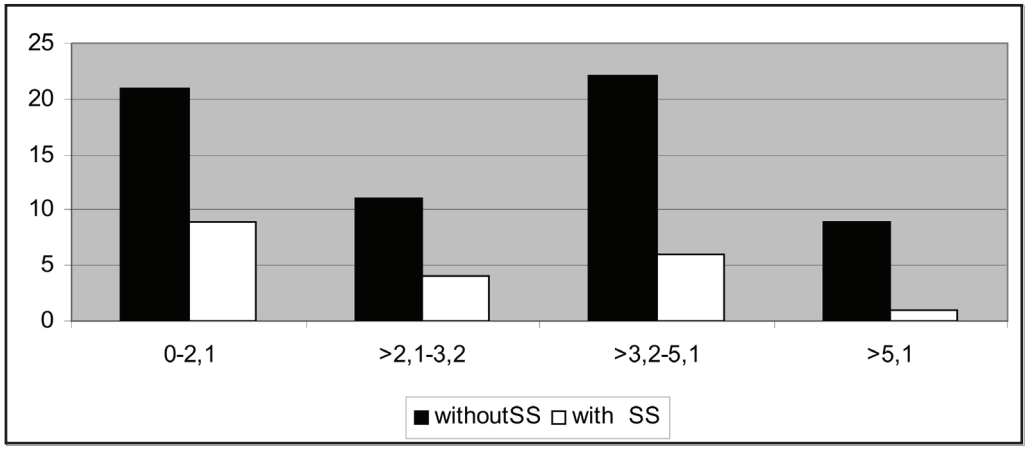


FIGURE 1: Presence of secondary Sjögren syndrome according to DAS-28 in 82 patients with rheumatoid arthritis (p=0.61).

Table 1 shows the study of Secondary Sjogren's syndrome presence according. RA variables.

Table 1 Data in 82 rheumatoid arthritis patients with and without secondary Sjögren syndrome

	With SS N=20	Without SS N=62	p
Mean disease duration (years)	9.9 ±6.3	10.4 ±7.2	0.95
Gender (female/male)	18/2	54/8	1.00
Rheumatoid factor	14/20 (70%)	36/62 (58%)	0.24
Anti CCP	6/8 (75%)	19/27 (70.3%)	1.00
Antinuclear antibody	6/20 (30%)	19/62 (30.6%)	0.95
Mean HAQ			

SS= Sjögren's syndrome; CCP= Cyclic citrullinated antibody; HAQ= Health Assessment Questionnaire.

DISCUSSION

The presence of sicca symptoms was high in the studied population but only 24% of patients fulfilled criteria for secondary SS. The high prevalence of dry eyes in RA patients without fulfilling the diagnostic criteria for secondary SS has been notice by Fujita et al that found it in 90% of their non-SS RA patients. In their study of 72 RA Japanese patients, 10% of them had Secondary SS. The presence of secondary SS in RA has been found to be higher in other studies. Cimmino et al (5) found it in 17.5% of Italian RA patients and Martinez Castro et al, in 55% of Spanish RA population. This high variability may be due to the genetic background of the studied population and methods chosen to evaluate glandular dysfunction.

Secondary SS is usually included as an extra-articular manifestation of rheumatoid arthritis (15). According to Fox et al (16), SS associated with RA occurs in a different genetic background than the primary disease (HLA DR4) and this author suggests that SS associated with RA has a different

pathogenetic process than that associated with lupus and with scleroderma. As we found in the present work, this later author noticed that ocular symptoms of dryness are more common that oral ones in RA patients.

Our results show also that neither secondary SS occurrence nor eye sicca subjective and objective findings have any relationship to disease duration. A study done in Spain found that patients with RA duration up to 10 years had a prevalence of secondary SS of 17% and after 30 years it was as much as 25% (17). This relationship with disease duration was not confirmed by Uhlig et al (18) but was present in a Study done in the United Kingdom (19).

The association of secondary SS with disease activity was also studied by Fujita et al (20) that found that RA activity had no significant correlation with the presence of dry eye although it had some relationship in those patients that fulfilled the diagnosis of secondary SS. Although we did not graduate severity of sicca findings, we could not find a higher RA activity measured by DAS-28 in patients with secondary SS when compared to those without it in the present study. No relationship could also be established with functional index. Wolfe et al (21) , although they did not study Secondary Sjögren syndrome, found that sicca symptoms are more common in patients with RA with increased HAQ scores, pain and global severity as well as total joint replacement and work disability.

According to the present findings rheumatologists should be aware of high indices of sicca symptoms in RA and seek for secondary SS independently of the severity, activity and duration of rheumatoid arthritis.

CONCLUSION

In the present study the inflammatory activity of RA do not affect severity of sicca symptoms. Also, no influence of RA duration neither of patient's functional class on secondary SS was noted.

References

1- Pillimer S R Sjogren's syndrome. In Klippel JH, Crofford LJ, Stone JH, Weyand CM.(eds). **Primer on the Rheumatic Diseases, Arthritis Foundation**. Atlanta, 2001, p.377-84.

2- Fox RI, Liu AY. Sjogren's syndrome in dermatology. **Clin Dermatol** 2006; 24:393-413.

3- Dorner T. Crossroads of B cell activation in autoimmunity: rationale of targeting B cells. **J Rheumatol Suppl** 2006; 77-3-11.

4- Kvien TK, Scherer HU, Burmester GR. Rheumatoid arthritis. In Bijlsma JWJ (ed). Eular Compendium on Rheumatic Diseases.2009, **BMJ Publishing Group London** ,p.61-80.

5- Cimmino MA, Salvarani C, Macchioni P, Montecucco C, Fossaluzza V, Mascia MT. Extra-articular manifestations in 587 Italian patients with rheumatoid arthritis. **Rheumatol Int** 2000;19:213-7

6- Martinéz-Castro E, Olivé Marqués A, Bonet Llorach M, Carbonell Abeló J, Cobo Valeri E, Juncà Valdor S. Rheumatoid aarthritis and Sjögren syndrome: special reference to the course time of rheumatoid arthritis. **Med Clin** (Barc) 1990; 94: 655-9.

7- Arnett FC, Edworthy SM, Bloch DA et al. The American Rheumatology Association 1987 revised criteria for the classification of rheumatoid arthritis. **Arthritis Rheum** 1988; 31:315-24

8- Afonso AA,Monroy D, Stern ME et al. Correlation of tear fluorescein clearance and Schirmer test scores with ocular irritation symptoms. **Ophtaolmology** 1999;106:803-10.

9- Daniels TE, Whitcher JP. Association of patterns of labial salivary gland inflammation with kerotoconjuctivitis sicca. Analysis of 618 patients with suspected Sjögren's syndrome. **Arthritis Rheum** 1994;37:869-77.

10- Mäkinen H, Kautiainen H, Hannonen P, Sokka T. Is DAS-28 an appropriate tool to assess remission in rheumatoid arthritis? **Annals Rheum Dis** 2005; 64:1410-1413.

11- Fransen J, van Riel PLCM. DAS remission cut points. **Clin Exp Rheumatol** 2006; 24 (S-43):S29-S32.

12- Ferraz MB. **Tradução para o português e validação do questionário para avaliar a capacidade funcional "Stanford Health Assessment Questionnaire"** [Thesis]. São Paulo: Universidade Federal de São Paulo; Escola Paulista de Medicina, 1990

13- Vitali C, Bomardieri S, Jonsson R et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American European consensus group. **Ann Rheum Dis** 2002; 61:554-8.

15- Theander E, Jacobsson LTH. Relationship of Sjogren's syndrome to other connective tissue and autoimmune disorders. **Rheum Dis Clin N Am** 2008; 34: 935-47.

16- Fox R. Sjögren's syndrome. **Lancet** 2005; 366:321-30.

17- Carmona L, Gozalez-Alvaro I, Balsa A et al. Rheumatoid arthritis in Spain: occurrence of extra-articular manifestations and estimates of disease severity. **Ann Rheum Dis** 2003; 62:897-900.

18- Uhlig T, Kvien TK, Jensen JL et al. Sicca symptons, saliva and tear production and disease variables in 636 patients with rheumatoid arthritis. **Ann Rheum Dis** 1999;58: 415-22.

19- Young A, Koduri G. Extra-articular manifestations and complications of rheumatoid arthritis. **Best Pract Res Clin Rheumatol** 2007; 21:909-27.

20- Fujita N, Igarashi T,Kurai T, Sakane N, Yoshino S, Takahasi H. Correlation between dry eye and rheumatoid arthritis activity. **Ophthalmol** 2005;140:808-13

21- Wolfe F, Michaud K. Prevalence, risk and risk factors for oral and ocular dryness with particular emphasis on Rheumatoid arthritis. **J Rheumatol** 2008; 35:1023-30.

Received in: 01-07-2016
Reviewed in: 11-07-2016
Accepted in: 25-07-2016
Conflict of interests: none
Financial source: none

Adress for correspondence:
Thelma L Skare
João Alencar Guimarães, 796
80310 420 Curitiba PR

ORIGINAL CONTRIBUTION
USE OF STATINS IN HIV-INFECTED PATIENTS

USO DE ESTATINAS EM PACIENTES PORTADORES DE HIV

CARINE PSENDZIUK*

Keywords: HIV, Hypertriglyceridemia, Protease inhibitors, Statins
Descritores: HIV, Hipertrigliceridemia, Agentes antiretrovirais, Estatinas

Abstract

People who are infected by the HIV present a high risk of developing dyslipidemia. This is due to the advances in the treatment of Acquired Immune Deficiency Syndrome (AIDS), which brought about an increase in patients' life expectancy and so a higher incidence of cardiovascular disease (CVD). Apart from that, dyslipidemic effects of some antiretroviral agents and chronic inflammation resulting from HIV infection may also contribute to it. Statins are agents of choice in the treatment of hypercholesterolemia, but most of these agents are metabolized by the cytochrome P450, which also metabolizes protease inhibitors (PIs). Thus, there may be an increase in the level of plasma statins, causing muscle, skeleton and hepatic toxicity, as well as other adverse effects. Therefore, statins that act in distinct metabolism sites should be preferred, such as pravastatin, fluvastatin and rosuvastatin, and those that are metabolized exclusively by the P450, such as simvastatin, should be avoided and atorvastatin may be used with caution. **Endocrinol diabetes clin exp 2016 1880 -1883.**

Resumo

A população infectada pelo vírus do HIV apresenta- se com alto risco de desenvolver dislipidemia. Isto devido aos avanços no tratamento da síndrome de imunodeficiência adquirida (SIDA) o qual proporcionou aumento da expectativa de vida e assim maior incidência de doença cardiovascular (DCV). Além disso, os efeitos dislipidêmicos de alguns agentes anti-retrovirais e a inflamação crônica resultante da infecção pelo HIV também podem contribuir. As estatinas são agentes de escolha no tratamento da hipercolesterolemia, mas a maioria desses agentes é metabolizada pelo citocromo P450, o qual também metaboliza os inibidores da protease (IP). Dessa forma, pode ocorrer aumento dos níveis das estatinas plasmáticas, ocasionando toxicidade muscular, esquelética, hepática e outros efeitos adversos. Portanto,deve ser dada preferência para estatinas que atuem em sítios de metabolização distintos, como a pravastatina, a fluvastatina e a rosuvastatina,evitando-se aquelas com metabolização exclusiva pelo citocromo P450, como a sinvastatina e a atorvastatina pode ser usada com cautela. **Endocrinol diabetes clin exp 2016 1880 -1883.**

INTRODUCTION

The treatment of AIDS had important advances after the introduction of combined antiretroviral therapy (highly active antiretroviral therapy – HAART). The use of PI in these patients' treatment has allowed for a more effective control of the infection, the restoration of immunity, and a significant reduction of the morbidity and mortality of the disease. However, the benefits of the use of PIs are accompanied by numerous side effects caused by these medicines, mainly metabolic alterations (1). Among the metabolic alterations reportedly present in these patients, we may highlight insulin resistance, glucose intolerance, lipodystrophy characterized by an abnormal distribution of body fat, and dyslipidemias, which are recognized risk factors for CVD (1). Apart from these traditional risk factors, chronic inflammation caused by HIV infection can also contribute to CDV risk (2). Accordingly, HIV treatment has improved these patients' life expectancy while also increasing the risk of cardiovascular complications associated with HIV infection and related to the adverse effects of antiretroviral agents.

Cardiovascular risk

Several studies have verified the association of antiretroviral therapy with the risk of cardiovascular problems. Baum et al (3) found a risk of cardiovascular problems of 4.8 ± 1.7 in 10 years by Framingham score in HIV-positive patients undergoing antiretroviral therapy. Bergensenet al. (4) found a prevalence of 11.9% of cardiovascular risk above 20% (high risk) among patients undergoing HAART, whereas in the control group the prevalence was of 5.3%. Also, in the same group there was an increase of chest angina in the patients undergoing HAART of 5.2% in relation to the control group (4). The DAD (Data collection on Adverse events of Anti-HIV Drugs) study showed an increase of the incidence of acute myocardial infarction after having used antiretroviral medication for a long period, demonstrating an increase in the risk of heart attacks of 0.3% before HAART in relation to 1.07% in patients undergoing HAART. Furthermore, they also identified an increase of cerebrovascular disease of 1.26% after using HAART (5).

STATINS

Statins are agents of choice in the treatment of hypercholesterolemia and hypertriglyceridemia, as they reduce serum concentrations of LDL by 18 to 55%, and triglycerides concentrations by 7 to 30%, while also increasing HDL by 5 to 10% (6). Most of the agents in this group are metabolized by cytochrome P450, which also metabolizes PIs. Consequently, there may be an increase in the level of plasma statins, generating muscle, skeleton and hepatic toxicity, as well as other adverse effects. Thus, statins that act in distinct metabolism sites should be preferred, such as pravastatin, lovastatin and rosuvastatin, and those that are metabolized exclusively by the P450, such as simvastatin, should be avoided. Atorvastatin may be used with caution, and there is favorable but limited data related to rosuvastatin (6,7).

Rosuvastatin

In relation to rosuvastatin, the study done by Domingos et al. (6) demonstrated the fact that ciprofibrate, rosuvastatin or a combination of both may be considered an effective and safe lipid-lowering treatment, being well tolerated by AIDS patients undergoing potent antiretroviral therapy. When different regimes of HAART were taken into consideration, there were no significant differences in the lipid-lowering responses to the agents evaluated. In that study, 346 HIV patients undergoing HAART presented refractory hyperlipidemia to diet and exercice were

*Serviço de Endocrinologia e Diabetes do Hospital Universitário Evangélico de Curitiba PR-Brazil
E-mail: carinepsendziuk@yahoo.com.br

divided into 3 groups. Group I was composed by 200 patients who had hypertriglyceridemia and were treated with ciprofibrate. Group II was composed by 79 patients who had hypercholesterolemia and were treated with rosuvastatin; and Group III was composed by 67 patients who had both hypertriglyceridemia and hypercholesterolemia, and were treated with ciprofibrate associated with rosuvastatin.

As for tolerance to the agents used in all three groups studied, there were isolated instances of myalgia (three cases), all in patients from Group II (taking rosuvastatin), but none of them was associated to an increase in creatine phosphokinase and therefore without characterizing rhabdomyolysis; dyspepsia was cited by only five patients, three from Group I (taking ciprofibrate) and two from Group III (taking both ciprofibrate and rosuvastatin). In relation to potential muscle and hepatic toxicity, no increase was observed in a significant number of cases. When such increases did occur, both in transaminases and creatine phosphokinase, they did not exceed the maximum tolerance limits recommended by the National Cholesterol Education Program (NCEP/ATP) as suspension criteria for such agents (6).

A randomized study (8) compared pravastatin 40 mg/d (n = 42) with rosuvastatin 10 mg/d (n = 42) in patients that had dyslipidemia and HIV. Rosuvastatin reduced LDL by 47% in comparison with a reduction of 19% by pravastatin (P <0.001). Triglyceride (TG) levels were reduced by 19% with rosuvastatin and 7% with pravastatin (P = 0.035). And HDL levels did not differ among the groups. This study suggests that rosuvastatin may be superior to pravastatin for high LDL in HIV-infected people who take PIs (8).

Therefore, rosuvastatin, the newest and most potent agent among statins, having no known interaction with PIs (which, as pravastatin and fluvastatin, does not use cytochrome P450), seems promising for the treatment of dyslipidemia in HIV-infected patients (9). It is important to note that many PIs interact with the cytochrome P450 system and may affect the toxicity potential of other medicines (10). Thus, besides being less harmful, rosuvastatin demonstrated a higher probability of reaching the targets established by NCEP for non-HDL levels (11).

Simvastatin

Simvastatin and lovastatin which are highly metabolized by CYP3A4 and are not recommended for those undergoing antiretroviral therapy (12,13). After all, it was shown that there may be significant interactions and 30-fold increases in the area under the curve (AUC) of simvastatin, which occurred when taken with ritonavir boosted with saquinavir (14). Such interactions suggest that simvastatin is not recommended with PIs, especially because the excessively high levels should expose patients to a higher risk of rhabdomyolysis (15).

Atorvastatin

In the presence of PIs, there may be moderate increases in levels of atorvastatin, which may be used, but at lower doses than in the general population (14). On the other hand, efavirenz has been shown to decrease the AUC of atorvastatin by 43% and simvastatin by 58%, suggesting that higher doses of atorvastatin and simvastatin may be required to reduce LDL effectively in patients who take efavirenz (16).

Pravastatin

Because of the safety of pravastatin with most PIs and non--nucleoside reverse transcriptase inhibitors (NNRTIs), pravastatin has been the most studied statin in clinical essays (13). However, not all statin drugs can be safely used in the treatment of the population infected with HIV on antiretroviral therapy. As pravastatin is eliminated by multiple pathways that do not include CYP3A4, it can be used safely in patients receiving PIs, different from darunavir. In the presence of darunavir, the pravastatin levels increase up to five times through a mechanism that has not yet been described (14,17,18).

Ezetimibe: Why not?

Negredo et al. (19) evaluated the addition of ezetimibe 10 mg/day in 19 HIV-infected individuals who had LDL levels above 130 mg/dL and who were taking pravastatin 20 mg/day. On week 24, 61.5% of the patients had levels of LDL <130 mg/dL. They observed significant decreases in LDL levels between initial levels and weeks 6, 12 and 24, independently from the type of antiretroviral agent (PIs or NNRTIs).

Bennett did a retrospective analysis of the lipid parameters of 33 HIV-infected patients who took ezetimibe 10 mg/day (20). The average total cholesterol was reduced from 269 mg/dL (6.95 mmol/L) to 213 mg/dL (5.51 mmol/L), a reduction of 21% (P <0.001). Average level of LDL was reduced from 157 mg/dL (4.05 mmol/L) to 102 mg/dL (2.63 mmol/L), a reduction of 35% (P <0.001), and the average level of TG was reduced from 551 mg/dL (6.22 mmol/L) to 341 mg/dL (3.85 mmol/L), a reduction of 34% (P = 0.006). Average HDL increased from 41 mg/dL (1.07 mmol/L) to 45 mg/dL (1.16 mmol/L), an increase of 8% (P = 0.038) (20).

Furthermore, there was another study with ezetimibe 10 mg/day in 48 HIV-infected individuals, without any other therapy for lipid-lowering treatments.21 The authors reported a small but significant change in the level of LDL: 35% of the individuals had a reduction of at least 17% in LDL (21).

DISCUSSION

Apart from traditional cardiovascular risk factors, the dyslipidemic effects of some antiretroviral agents and the chronic inflammation that results from HIV infection may also contribute to the CVD risk (2). Hence, a growing number of HIV-infected patients are being treated with statins (2).

Current guidelines recommend dyslipidemia treatment in HIV-infected people as well as in the population in general, according to NCEP, and recommend that HIV-infected patients who present two or more traditional cardiovascular risk factors have their cardiovascular risk score calculated with Framingham's equation, and that their lipid targets be assessed individually (13). Changes in lifestyle, such as quitting smoking, diet changes and exercising should be prescribed. If another intervention is necessary in order to reach lipid targets, lipid-lowering medication should be taken, or the antiretroviral therapy should be interrupted (13).

Numerous studies have demonstrated that there are many interactions between PIs and statins (13). Pravastatin and rosuvastatin have been shown to be safe and effective.

On the other hand, hypertriglyceridemia remains the most common lipid abnormality among HIV patients (13). Although there is still certain controversy, many specialists believe that hypertriglyceridemia is an independent risk for heart diseases (22,23,24). Despite statins having light or moderate effects on TG, first-class therapy for hypertriglyceridemia is fibrate followed by fish oil (13). Some studies have shown that it is unlikely that HIV-infected patients who have hypertriglyceridemia will reach NCEP's targets with fibrate treatment exclusively, and it is necessary to use another strategy. Fish oil is an interesting supplement due to its anti-inflammatory properties, the reduction of cardiovascular atherogenic effects, and absence of drug interaction with antiretroviral therapy. Diet guidelines from the American Heart Association recommend that healthy adults should eat at least two portions of fish every week, and that people who have high levels of TG need from 2 to 4 g/day of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids as dietary supplements (25).

Therefore, statins should be prescribed due to their great benefit, but care should be taken in relation to drug interactions with antiretroviral therapy; moreover, fibrates may be added, if

necessary. Beyond cardiovascular benefits of statins, a study showed that the use of statins is associated with a reduction of 55% in the occurrence of cancer among HIV-infected patients; such effect seems to be even stronger in relation to AIDS-related pathologies which were not observed in individuals who take statins. This study evaluated the effect of statins in both AIDS and non-AIDS-defining malignancies in a large number of individuals undergoing treatment for a period of over 10 years (26).

The exact mechanisms through which statins may reduce cancer probability have not been completely elucidated yet. However, hypothetically statins have an antineoplastic effect (27,28,29) as well as anti-inflammatory properties (30,31,32). Statins seem to interact with essential cell functions, such as cell proliferation and differentiation (27,28) so that their antineoplastic effects include inhibiting growth of tumor cells, inhibiting angiogenesis and inducing apoptosis of tumor cells (29).

On the other hand, the use of statins has been associated with a moderately higher risk of diabetes mellitus (DM) in HIV--infected people, similarly to existing data for the population in general (2). HIV-infected patients must be monitored for glucose intolerance, but statin use should not be interrupted if it was prescribed for reducing cardiovascular disease risk (2).

The association of the use of statins with a greater risk of developing DM has been reported recently (33,34,35,36). The JUPITER study found an increase of 25% in the incidence of DM from the use of rosuvastatin (37). In a meta-analysis of 13 assays, Sattar (38) found an increased risk of DM of 9% in patients who were treated with statins. Also, CVD occurs more often in HIV-infected people when compared to control groups of non-HIV patients (39). This is also due to ageing of the population, which increases the risk of developing cardiovascular diseases (40).

CONCLUSION

From all the above, we may conclude that HIV-infected people present an increased risk of developing cardiovascular disease because of the chronic inflammatory state associated to the virus itself and the side effects of antiretroviral therapies apart from known genetic and traditional risk factors. Thus, they often need to take statins for controlling the lipid profile. The benefits of statins therapy for primary and secondary prevention of myocardial infarction have been demonstrated by several large-scope studies (2). Additionally, studies indicate a potential protective effect from the use of statins in relation to the incidence of cancer among patients infected with HIV-1, especially with regard to AIDS-associated pathologies (26). Nevertheless, there should be a preference towards statins that act in distinct metabolizing sites, such as pravastatin, fluvastatin and rosuvastatin, thus avoiding those that are metabolized exclusively by cytochrome P450, such as simvastatin. Also, atorvastatin may be used with caution and there is favorable data related to rosuvastatin (6,7).

In the absence of data about drug interactions between lipid-lowering medicines and the new classes of antiretroviral therapy, we must be cautious, and patients must be monitored in relation to any signs or symptoms of toxicity or responses that are lower than expected (13).

Current guidelines support the treatment of dyslipidemia in HIV-infected people as in the population in general; however, drug interactions between lipid-lowering medicines and antiretroviral agents may influence the choice of therapy (13). There have been no studies evaluating long-term heart results of different statins prescribed for HIV-infected patients, and so the statin preference is usually based on safety and tolerability, as well as inferences from data about the general population. Similarly, the use of ezetimibe, either isolated or in combination, is largely based on its reported benefits to the population in general (13).

References

1. Yu, Pai Ching et al. Terapia hipolipemiante em situações especiais: Síndrome da imunodeficiência adquirida. **Arq Bras Cardiol**, v. 85, n. supl5, p. 58-61, 2005.
2. Lichtenstein, Kenneth A. et al. Statin use is associated with incident diabetes mellitus among patients in the HIV outpatient study. **JAIDS Journal of Acquired Immune Deficiency Syndromes**, v. 69, n. 3, p. 306-311, 2015.
3. Baum MK, Rafie C, Lai S, Xue L, Sales S, Page JB, et al. Coronary heart disease (CHD) risk factors and metabolic syndrome in HIV-positive drug users in Miami. **Am J Infect Dis**. 2006;2(3):173-9.
4. Bergensen BM, Sandvik L, Bruun JN, Tonstad S. Elevated Framingham risk score in HIV-positive patients on highly active antiretroviral therapy: results from a Norwegian study of 721 subjects. **Eur J Clin Microbiol Infect Dis**. 2004;23(8):625-30.
5. Mehta N, Reilly M. Atherosclerotic cardiovascular disease risk in the HAART-treated HIV-1 population. **HIV Clin Trials**. 2005;6(1):5-24.
6. Domingos, Hamilton et al . Rosuvastatina e ciprofibrato no tratamento da dislipidemia em pacientes com HIV. **Arq. Bras. Cardiol.**, São Paulo , v. 99, n. 5, p. 997-1007, Nov. 2012 .
7. Sposito AC, Caramelli B, Fonseca FA, Bertolami MC, Afiune Neto A, Souza AD, et al.; Sociedade Brasileira de Cardiologia. IV Diretriz brasileira sobre dislipidemias e prevenção da aterosclerose. **Arq Bras Cardiol**. 2007;88(supl 1):1-18.
8. Aslangul, E.; Assoumou, L.; Bittar, R., et al. ANRS 126, a prospective, randomized, open label trial comparing the efficacy and safety of rosuvastatin versus pravastatin in HIV-infected subjects receiving ritonavir boosted PI with lipid abnormalities [abstract LBPS7/2]. **Eleventh European AIDS Conference**; October 24–27; Madrid. 2007.
9. Calza L, Colangeli V, Manfredi R, Legnani G, Tampellini L, Pocaterra D, et al. Rosuvastatin for the treatment of hyperlipidaemia in HIV-infected patients receiving protease inhibitors: a pilot study. **AIDS**. 2005;19(10):1103-5.
10. Stein JH. Managing cardiovascular risk in patients with HIV infection. **J Acquir Immun Defic Syndr**. 2005;38(2):115-23.
11. Singh, Sudershan, et al. "Comparative effectiveness and toxicity of statins among HIV-infected patients." **Clinical Infectious Diseases** (2010): ciq111.
12. Sprinz, E, Lazzaretti, R K, Kuhmmer, R, & Ribeiro, J P. (2010). Dyslipidemia in HIV-infected individuals. **Brazilian Journal of Infectious Diseases**, 14(6), 575-588.
13. Aberg, Judith A. "Lipid management in patients who have HIV and are receiving HIV therapy." **Endocrinology and metabolism clinics of North America** 38.1 (2009): 207-222.
14. Fichtenbaum CJ, Gerber JG, Rosenkranz SL, et al. Pharmacokinetic interactions between protease inhibitors and statins in HIV seronegative volunteers: ACTG study A5047. **AIDS**. 2002;16:569–577.
15. Aboulafia DM, Johnston R. Simvastatin-induced rhabdomyolysis in an HIV-infected patient with coronary artery disease. **AIDS Patient Care STDS**. 2000;14:13–18
16. Gerber JG, Rosenkranz S, Fichtenbaum CJ, et al. The effect of efavirenz on the pharmacokinetics of simvastatin, atorvastatin, and pravastatin: results of ACTG 5108 study. **J Acquir Immune Defic Syndr**. 2005;39:307–312.
17. Aberg JA, Rosenkranz S, Fichtenbaum CJ, et al. Pharmacokinetic interaction between nelfinavir and pravastatin in HIV-seronegative volunteers: ACTG study A5108. **AIDS**. 2006;20:725–729.
18. Raritan NJ. Prezista (Darunavir) package insert. **Tibotec Therapuetics**. 2008 February; [Accessed April 27, 2008]; Available at:http://www.tibotectherapeutics.com/tibotectherapeutics/documents/us_package_insert.pdf.
19. Negredo E, Molto J, Puig J, et al. Ezetimibe, a promising lipid-lowering agent for the treatment of dyslipidaemia in HIV-infected patients with poor response to statins. **AIDS** 2006;20:2159–2164.
20. Bennett MT, Johns KW, Bondy GP. Ezetimibe is effective when added to maximally tolerated lipid lowering therapy in patients with HIV.**Lipids Health Dis** 2007;6:15.
21. Wohl, D.; Hsue, P.; Richard, S., et al. Ezetimibe' effects on the LDL cholesterol levels of HIV-infectedpatients receiving HAART [abstract 39]. **14th Conference on Retroviruses and Opportunistic Infections**; February 25–28; Los Angeles. 2007.
22. Calza L, Manfredi R, Chiodo F. Dyslipidaemia associated with

antiretroviral therapy in HIV-infected patients. **J Antimicrob Chemother**. 2004;53:10-14.
23. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density cholesterol level: a meta-analysis of population-based prospective studies. **J Cardiovasc Risk** 1996;3:213–219.
24. McBride PE. Triglycerides and risk factors for coronary heart disease: editorial. **JAMA** 2007;298:236–238.
25. Kris-Etherton PM, Harris WS, Appel LJ. for the AHA Nutrition Committee. Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association. **Arterioscler Thromb Vasc Biol** 2003;23:151–152.
26. Galli L, Spagnuolo V, Poli A. ; et al. Use of statins and risk of AIDS-defining and non-AIDS-defining malignancies among HIV-1 infected patients on antiretroviral therapy. **AIDS**. 2014 Oct 23;28(16):2407-15.
27. Casey PJ. Protein lipidation in cell signaling. **Science** 1995; 268:221–225.
28. Boss JL. Ras oncogenes in human cancer: a review. **Cancer Res** 1989; 49:4682–4689.
29. Hindler K, Cleeland C, Rivera E, Collard C. The role of statins in cancer therapy. **Oncologist** 2006; 11:306–315.
30. Deeks SG. Immune dysfunction, inflammation, and accelerated aging in patients on antiretroviral therapy. **Top HIV Med** 2009; 17:118–123.
31. Niessner A, Steiner S, Speidl WS, Pleiner J, Seidinger D, Maurer G, et al. Simvastatin suppresses endotoxin-induced upregulation of toll-like receptors 4 and 2 in vivo. **Atherosclerosis** 2006; 189:408–413.
32. Iwasaki A. Innate immune recognition of HIV-1. **Immunity** 2012; 37:389–398.
33. Carter AA, Gomes T, Camacho X, et al. Risk of incident diabetes among patients treated with statins: population based study. **BMJ**. May 23, 2013;346:f2610. doi: 10.1136/bmj.f2610.
34. Coleman CI, Reinhart K, Kluger J, et al. The effect of statins on the development of new-onset type 2 diabetes: a meta-analysis of randomized controlled trials. **Curr Med Res Opin**. 2008;24:1359–1362.
35. Mills EJ, Wu P, Chong G, et al. Efficacy and safety of statin treatment for cardiovascular disease: a network meta-analysis of 170255 patients from 76 randomized trials. **QJM**. 2011;104:109–124.
36. Shah RV, Goldfine AB. Statins and risk of new-onset diabetes mellitus. **Circulation**. 2012;126:E282–E284.
37. Ridker PM, Pradhan A, MacFadyen JG, et al. Cardiovascular benefits and diabetes risks of statin therapy in primary prevention: an analysis from the JUPITER trial. **Lancet**. 2012;380:565–571.
38. Sattar N, Preiss D, Murray HM, et al. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. **Lancet**. 2010;375:735–742.
39. Triant VA, Lee H, Hadigan C, et al. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. **J Clin Endocrinol Metab**. 2007;92:2506–2512.
40. Brooks JT, Buchacz K, Gebo KA, et al. The public health perspective. **Am J Public Health**. 2012;102:1516–1526.

Received in: 10-05-2016
Reviewed in: 23-05-2016
Accepted in: 31-05-2016
Funding: none
Conflict of interests: none
Address for correspondence:
Carine Psendziuk
Cândido Freire Cesar Leão 112 ap 201 – Tubarão SC- Brazil
CEP 88705-040

Instructions for the publication of the Journal Endocrinology & Diabetes Clinical and Experimental

The journal follows the International Committee of Medical Journal Editors

01 All the manuscripts will be published in English. The journal accepts original articles, preliminary notes, case reports, review articles, updates and letters to editor. There a topic dedicate to internal medicine linking endocrinology and medical clinic. The journal strongly encourages on line submissions of manuscripts. Those should be accompanied by a title, keywords and an abstract in English for the purposes of international registration. Abstracts in other languages may also be attached.

02 The articles received by the Editor will be analyzed with the Assistance of the Editorial Board. Minor changes to "copy desk" can be effective with the purpose of standardizing the articles, without substantial changes in original text.

03 Manuscripts can be sent on CD or via on line to publicacao@revistaendocrino.com. The text should be typed on pages containing 20 to 24 rows and rows with 70 to 75 spaces, with the objective of enabling the diagramming the calculation of space required for each article. The word processor used must be either Microsoft Windows compatible program (Word, Write etc.).

05 The article must have title, full name of the authors; quote from site (full address) where out performed the work; full titles of authors, key words (or "keywords") without exceeding a limit of 250 words; introduction; material or material and methods or description of the case; results; discussion and/or comments (when applicable); conclusions (when applicable); summary (summary in English), consisting in the correct version of the summary, not exceeding 250 words; references (as quoted below in item 08) in alphabetical order; the accompanying illustrations must follow appropriate rules, described in item 07.

06 Illustrations are of figures and graphs referred to in Arabic numerals (example: fig. 3, graph 7), in the form of ink drawings photographs ECG EEG etc. When possible must be submitted in original form. The illustrations will be accepted only allow good reproduction. Should not be glued in the middle of the article text and it must be attached with the respective legends typed on the bottom of the same (one sheet for each illustration). Must take care to number each illustration on the back of the same and indicate the correct place where should be introduced. Tables and frames are specified in Arabic numerals, consisting always the respective title, accurately. Tables and frames without its description in the text and are intended to summarize the article. The units used to express the results (m, g, g/100 ml, etc.) will appear at the top of each column. It will be up to the Editor to judge excessive illustrations (figures, tables, graphs, tables etc.), deleting the redundant.

07 The references must follow the alphabetical order or the order of appearance in the text. Showing them all authors cited in the text. It must be contain: name of author, name of the journal abbreviated in accordance with the criteria used in the Index Medicus (www.nlm.nih.gov/tsd/serials/lji.html). Papers accepted but not yet published may be included in the references. You should avoid using as reference poster or free themes from conferences unless they are of high relevance. Articles published online may be cited in the references and should bear the name of the site as well as the date of access.Chapter of Book: Ruch, TC.Somatic Sensation. In Ruch T C et al. Neurophysiology. Philadelphia Saunders 1963; 330-332 Journal article: R.W.G Gruessner, Sutherland D.E.R, Najarian j. S, et al. Solitary pancreas transplantation for non uremic patients with insulin-dependent diabetes mellitus labile. Transplantation 1997; 64: 1572-77.

08 The names of drugs cited in the text (names of fantasy, officers, patented, and acronyms of chemical research) shall comply with corresponding regulations of the World Health Organization, according to rules summarised by KOROLKOVAS, a.-Regulatory Editorial Nomenclature-Names of drugs (Drug Nomen--clature). Rev. Bras. Clin. Terap. 5: 1976 (February).

09 The authors will receive ten copies of the issue in which their work was published (for reprints), which will be sent directly to the place where the work performed. Reprints must be ordered and previously combined with the Commercial Direction.

10 The manuscripts that don't fit the standards or that does not suit the needs of the journal editorials may be forwarded to the authors to carry out the necessary adjustments that will be indicated in the personal letter from the Editor. Will be mentioned the dates of receipt and approval of work for publication, in order to safeguard the interests of the author's priority. In the case of re-routing of work to adapt to our rules for publication, the date cited is always receive the first forwarding of work. The content of the articles is the responsibility of the authors. The link between the author (s) and pharmaceutical laboratories, as well as another source that is generating resources must always be quoted by author (s). The copyright of the manuscripts are of the magazine in question.

11 Will be given top priority in the publication of articles and/or notes that they concerned about matters directly or indirectly related to the basic purpose of the journal Endocrinology & Diabetes Clinical and Experimental

12 Studies that involve animals, research or human, should obey the rules of the Helsinki Declaration of 1979 and revised in 2000. The authors is entitled to explanation, if your search is not in accordance with the rules of the Declaration of Helsinki. In addition, when the study involves humans must be approved by the Ethics Committee of your institution.

13 Mailing address of the main author must appear at the end of the article. Your article is your own responsibility, and the same answer for your account both within the medical ethics as in legal proceedings.

14 Structural definition of the main types of articles: Original articles: Articles Are produced through scientific research, presenting original data scientific findings with respect to experimental or observational aspects of Medical Biochemistry and social feature. Includes descriptive analysis and data inferences or own. In its structure should contain the following items: Introduction, Material and methods, results obtained and studied by an appropriate statistical method discussion and conclusion. Review articles: Are articles that seek to summarize, analyze, evaluate or synthesize research already published in scientific journals. The revisions are expected to be commissioned by the editors, except in case of scientific relevance for the medical class. Articles from Update or disclosure: These report updated information of interest to the magazine or a new technique or laboratory research. This topic is distinct in its account of the review article. Case report: Present descriptive data about a pathology with academic relevance in relation to the disease, treatment, laboratory or association with another pathology

15 The Journal of Endocrinology & Diabete clinical and Experimental use the peer review form of review the manuscripts Peer review is an important process for all authors to understand. Ultimately, peer review was created to protect scientific integrity and promote the sharing of research with other colleagues. It can help authors discover problems and helps to strengthen the credibility of their research. The extensive amount of published material relating to peer review can be overwhelming for readers to sort through, and this paper provides a relevant guide for authors regarding the peer-review process. The necessity of having quality control measures for published work is important to the scientific community, and without such measures, the quality of published work would not be what it is today. Peer review is necessary to identify scientific manuscripts worthy of publication and to improve the quality of published research


ENDOCRINOLOGIA & DIABETES CLÍNICA E EXPERIMENTAL
FEPAR - HUEC - CURITIBA - BRASIL