ENDOCRINOLOGIA & DIABETES CLÍNICA E EXPERIMENTAL

FEMPAR - HUEM - CURITIBA - BRASIL

SPANISH FLU:
A KNOWLEDGE OF HISTORY

CORONA VIRUS:
A PRESENT THAT WE WILL NEVER FORGET

SPANISH FLU : A KNOWLEDGE OF HISTORY
CORONA VIRUS: A PRESENT THAT WE WILL NEVER FORGET

The influenza pandemic caused by the H1N1 influenza, also called the Spanish flu, lasted from 1918 to 1920. The Spanish Flu did not originate in Spain; during World War I, this country was neutral with a free media, that reported the first cases in Madrid on May, 1918.

The scientists never knew for sure where the Spanish Flu originated.

They believed that infected soldiers who fought in the I World War, spread the disease to other military camps across the European countryside. On March, 1918, more than 200 thousands of American soldiers crossed the ocean taking the virus with them. The first known case in United States was reported at Camp Funston in Fort Riley, Kansas, on March 11, 1918. In the same year, more american soldiers died from Spanish flu than in the battle war fields. About 40% of soldiers from U.S.A Navy and the Army transported back home on trains and ships became ill, so they helped to spread the killer virus.

The pandemic occurred in three waves, all around the world.

The first wave occurred in the spring of 1918 and it was mild. The symptoms were fatigue and mild fever, that got better within a few days with resting and oral hydration. The number of reported deaths was low.

The second and third waves were very contagious; the symptoms were cough, high fever, and difficulty to breath due to severe pneumonia and death occurred in a few days of acute respiratory disease. This virus was not more agressive than other strains that have been found in outbreaks of influenza, but it was much more contagious. The researches believed that the recent war with malnourishment, overcrowded medical camps and hospitals, and poor hygiene, helped to spread the bacterial infection .The Spanish flu had higher than expected mortality rate in younger ages. In September 1918, the Red Cross recommended a two-layer gauze masks to contain the spread of the pandemic. The stricts measures were taken as mandatory use of masks, limiting the number of people in the streets, in their work and in entertainment places. The stores were closed and the govern decreed lockdown. The Spanish Flu pandemic killed more than 50 million people, a quarter of the world population at the time.

Until now, the COVID-19 pandemic killed almost 4,000,000 people in the world. This number could be as higher as deaths caused by Spanish Flu mostly because worldwide vaccination.

LET´S CELEBRATE SCIENCE!

LET´S CELEBRATE LIFE!

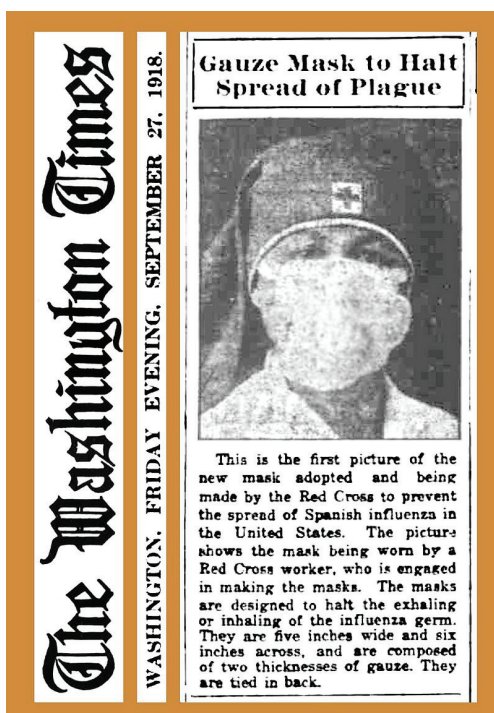**Editors of Revista de Endocrinologia & Diabetes Clínica e Experimental**

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The Guardian

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Our Cover: Spanish Flu -1918-1920
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TOPICS IN MEDICAL CLINIC

ORIGINAL ARTICLES
ANTIMALARIALS AND ELECTROCARDIOGRAPHIC ALTERATIONS: A CROSS SECTIONAL STUDY IN 100 LUPUS PATIENTS

INFLUÊNCIA DOS ANTIMALÁRICOS EM ALTERAÇÕES ELETROCARDIOGRÁFICAS: ESTUDO TRANSVERSAL EM 100 PACIENTES COM LÚPUS.

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Abstract

In this study we report the case of a patient with primary hypobetalipoproteinemia. This is characterized by genetic disorders that imply in very low levels of LDL, total cholesterol and apolipoprotein B. Abetalipoproteinemia (ABL), chylomicron retention disease (CMRD), combined familial hypolipidemia (FCHL) and familial hypobetalipoproteinemia (FHBL) are part of this group of diseases. The affected patients, depending on the genetic disorder, can develop from asymptomatic to severe clinical manifestations, which can be present in the first years of life. Laboratory and imaging tests associated with the patient's clinical manifestations may suggest which of the diseases is responsible for the changes present in the patient, but the definitive diagnosis requires a genetic study. In some cases, there is a need for early and intensive treatment, but in others there is no need for a medical approach. Thus, after contextualizing this group of diseases with a clinical case, we conducted a literature review addressing pathophysiological and clinical aspects, diagnosis and treatment of each of these pathologies. **Endocrinol diabetes clin exp 2021 / 2203 - 2206**

Resumo

Neste estudo relatamos o caso de um paciente com hipobetalipoproteinemia primária. Está é caracterizada por distúrbios genéticos que implicam em níveis muito baixos de LDL, colesterol total e apolipoproteina B. Fazem parte deste grupo de doenças a abetalipoproteinemia (ABL), a doença de retenção dos quilomícrons (CRMD), hipolipidemia familiar combinada (FCHL) e a hipobetalipoproteinemia familiar (FHBL). Os pacientes afetados, dependendo do distúrbio genético, podem ser desde assintomáticos até ter manifestações clínicas graves presentes já nos primeiros anos de vida. Os exames laboratoriais e de imagem associados as manifestações clínicas do paciente podem sugerir qual das doenças é a responsável pelas alterações presentes no paciente, mas o diagnóstico definitivo necessita de estudo genético. Em alguns casos há necessidade de tratamento precoce e intensivo, mas em outros não há necessidade de abordagem médica. Assim, após contextualizar este grupo de doenças com um caso clínico, realizamos uma revisão de literatura abordando aspectos fisiopatológicos e clínicos, diagnóstico e tratamento de cada uma destas patologias. **Endocrinol diabetes clin exp 2021 / 2203 - 2206.**

INTRODUCTION

Primary hypobetalipoproteinemia corresponds to a series of congenital disorders that have a variable incidence from 3.2% to less than 1 in 1 million, depending on the type of genetic mutation (1,2). The diseases that comprise it occur due to different types of mutations in genes that will encode important proteins at different stages of lipid metabolism (3). Individuals with determinant mutations of severe phenotypes (such as ABL, CMRD, homozygous FHBL) may present manifestations even during childhood with a picture of malabsorption of fats with vomiting, steatorrhea and weight-height deficit, and later on, they progress with progressive affections resulting from deficiency of fat-soluble vitamins, such as retinal degenerations and neuropathies (4,5). Heteozygous individuals are often asymptomatic although they may develop fatty liver disease and eventually some vitamin deficiencies (6,7). In addition, these individuals are believed to have cardiovascular protection possibly due to low LDL levels (8) The diagnosis requires clinical and laboratory investigation and genetic testing (4,9). Treatment depends on the severity of the disease and may include a low-fat diet, supplementation with essential fatty acids and fat-soluble vitamins. The prognosis is variable, and depends on the severity of the disease, early diagnosis and adherence to treatment (9,10).

CASE REPORT

Patient AGLT, male, 20 years old, sought endocrinology service due to alteration in the lipidogram. Clinically, the patient had no complaints, except for a history of atopic dermatitis. He reported having a normal diet, without never having been subjected to vegetarian diets or other types of diets. He maintained a preserved intestinal habit, without history of diarrheal conditions. He denied comorbidities, use of narcotics and use of medications, including lipid-lowering drugs. The family history showed no particularities. The physical examination of the patient was normal. He had a weight of 63.1 kg, a height of 170 cm and a BMI of 21.83. Regarding the complementary tests, table 1 shows general biochemical tests and table 2 shows two lipidograms performed with a difference of 4 months between them.

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Table 1. Biochemical tests

Hemoglobin 14,9 g/dl	Hematocrite 44,2 %	Leucocytes 7420 /mm^3	Platelets 270.000 /mm^3
Glicose 94 mg/dl	TSH 2,11 mIU/ml	T4 free 1,02 ng/dl	AST 19,00 U/L
ALT 46,00 U/L	RNI 1,09	Vitamin D 19,2 ng/ml	Vitamin A 0,44 mg/L*
Vitamin E 4,53 mg/L**	Vitamin K 0,3 mcg/L***		

*Reference range: 0,30 a 0,70 mg/L **Reference range: 5,00 a 20,00 mg/L***Reference range: 0,5 a 5,0 mcg/L

Table 2. Lipidogram

First lipidogram	Second lipidogram	Reference range
Total cholesterol 61 mg/dl	Total cholesterol 68 mg/dl	Less than 190 mg/dl
HDL 30,4 mg/dl	HDL 40,3 mg/dl	Higher than 40 mg/dl
LDL 17,4 mg/dl	LDL 22 mg/dl	Less than 130 mg/dl
VLDL 10,6 mg/dl	VLDL 10,6 mg/dl	------------------------
Triglycerides 53 mg/dl	Triglycerides 21 mg/dl	Less than 150 mg/dl

First lipidogram was performed on 05/27/2020. Second lipid profile was performed on 9/24/2020. In addition to these laboratory tests, apolipoprotein B was measured with a result of 24 mg/dl (reference range 55 to 155 mg/dl). The patient also had an abdominal ultrasound showing mild hepatic steatosis.

LITERATURE REVIEW

1.1 Diseases: pathophysiology and clinical manifestations

Primary hypobetalipoproteinemia corresponds to a group of diseases who characteristics are LDL cholesterol, apolipoprotein--B and total cholesterol below the 5th percentile of the population, for age and sex. The group's diseases include abetalipoproteinemia (ABL), chylomicron retention disease (CMRD), combined familial hypolipidemia (FCHL) and familial hypobetalipoproteinemia (FHBL) (4,11,12,13). Of these, the first three have recessive inheritance and the last has dominant inheritance. (5,14). ABL, also known as *Bassen-Kornzweig* syndrome, is a rare autosomal recessive disorder (incidence less than 1: 1000000) often associated with consanguinity (1,15). It occurs due to a mutation in the MTTP gene, responsible for the formation of the microsomal triglyceride transfer protein, which, when functional, is essential for the assembly and secretion of lipoproteins that contain apo B in the liver and intestine (1, 9). Therefore, in the presence of a mutation in the gene, defective proteins will form and, as a result, patients will have low plasma levels of apo B-dependent lipoproteins associated with lipid retention in the cytoplasm of hepatocytes and enterocytes (9). Clinically, the patient may present, in childhood, with growth failure, oral fat intolerance, diarrhea, steatorrhea and abnormalities related to the deficiency of fat-soluble vitamins (for example: neuromuscular manifestations, coagulopathy, myopathy, anemia and pigmentous atypical retinitis) (16). In these individuals, complementary exams usually show low levels of fat-soluble vitamins (vitamins A, D, E, K), acanthocytosis, fatty liver and accumulation of fat in the enterocytes (16,17.) In addition, the levels of LDL, VLDL, and total cholesterol, in general, are extremely low and there is almost complete absence of apo B100 and apo B48 (16,17).

In turn, CMRD (also known as Anderson's disease) is also a very rare autosomal recessive disorder with a prevalence of less than 1 in 1 million (18). It occurs due to a mutation in the SAR1B gene (formerly called SARA 2) that will encode the Sar1b protein, necessary - in the intestine - to transport pre-chylomicron from the endoplasmic reticulum to the Golgi apparatus (18,19,20). Thus, the individual does not secrete chylomicrons and fat from the diet accumulates in the enterocytes in the form of droplets (9,19,20). The main manifestation resulting from this disease is diarrhea, which appears early in life. Other manifestations are vomiting, abdominal distention, growth retardation and deficiencies of fat-soluble vitamins (18,19,20). In the laboratory, triglyceride levels are normal, while total cholesterol, LDL, HDL are less than 50% of control levels (18).

FCHL has a prevalence of about 15% among individuals with hypobetalipoproteinemia (LDL less than the 5th percentile) (21). It occurs due to the loss of function of the ANGPTL3 protein due to a mutation present in the ANGPTL3 gene (5,22). Physiologically, this protein acts by inhibiting the action of lipoprotein lipase (responsible for the hydrolysis of chylomicrons and VLDL) and endothelial lipase (responsible for the hydrolysis, mainly, of HDL) (12,14,23). Thus, individuals who have this alteration have a low expression of the ANGPTL3 protein and, therefore, have a higher expression of lipoprotein lipase and endothelial lipase (3). This generates a biochemical phenotype with markedly low levels of LDL, VLDL and HDL (10), however, there is no typical clinical phenotype (5).

FHBL, on the other hand, is a dominant inheritance disorder that can be diagnosed in heterozygous and, rarely, in compound heterozygotes and homozygotes individuals. (13,22) The mutations that lead to this condition occur in the APOB gene and proprotein convertase sublisin / kexin-type 9 (PCSK9) (4,5,9,15). In addition, there are cases in which the genetic alteration responsible for the pathology is still unknown - called primary orphan familial HBL (9).

Regarding the APOB gene, it will encode apoprotein B (apo--B), which, in a physiological way, is involved in the transport and metabolism of cholesterol and triglycerides, and can be found in plasma in the form of both apoB48 and apoB100 (24,25). The first, produced in the intestine, comprises 48% of the length of an apoB, being responsible for the formation of chylomicrons (QM) and transportation of lipids from the diet (24,25). The second, produced by the liver, is composed of 100% of the extension of an apo B and will participate in the formation of very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low lipoprotein density (LDL) (4,24,25). The mutation in the APOB gene leads to the formation of defective apo-B of various sizes or, less frequently, to intracellular changes that impact the shape

of Apo B (9,15). In heterozygous individuals who have truncated apo-B production, the production of apo-B-dependent lipoproteins is reduced in the liver, with less VLDL formation, and, in certain cases, in the intestine, with reduced QM (9,15). As the formation of LDL occurs from the lipolysis of the IDL (product of the lipolysis of the VLDL), the plasma levels of apo-B and LDL will be low, being less than 30% of the population of the same age and sex in heterozygotes (13,25). In the case of homozygous or compound heterozygous individuals, the presence of LDL and apo-B is null or about 10 - 20% of controls (9).

The prevalence of the heterozygous mutation of APOB is about 1 in 3000 individuals, who are normally asymptomatic, with the suspected diagnosis proposed after lipidogram (10,26). However, some manifestations such as diarrhea, due to malabsorption of fat, and hepatic steatosis can occur (5,13,24). In this context, a study by *Schonfeld et al*. (2004) showed an average percentage of liver fat three times higher in these individuals compared to the control group (7). This occurs, possibly, due to the failure in the adequate production of apo-B100, with consequent alteration of the assembly of VLDL with lipids in the liver (2). Therefore, in view of the association of FHBL with liver disease (including the possibility of cirrhosis and hepatocellular carcinoma), it is recommended that regular liver monitoring be performed by biochemical and imaging tests (5,10). The homozygous and compound heterozygous forms, on the other hand, are extremely rare and are clinically indistinguishable from ABL, thus affected individuals can have severe manifestations such as failure to grow, intestinal malabsorption and fatty liver (15,27). Regarding cardiovascular risk, it is interesting to observe data obtained by the study by Peloso and Nomura (2019), which showed a 72% lower risk of cardiovascular disease in heterozygous individuals for hypobetalipoproteinemia with APOB gene mutation (8). In the same study, LDL was, on average, 55 mg / dL lower than in the control group, while triglycerides were 53% lower (8).

In addition to those mentioned above, the PCSK9 gene, responsible for the formation of the proprotein convertase subtilisin–kexin type 9, also undergoes mutations that can cause familial hypobetalipoproteinemia. In the absence of mutations, this protein interferes with the recycling of LDL by binding to LDL receptors present in the liver and signaling their destruction by lysosomes, thus preventing the return of these receptors to the cell surface and the endocytosis and degradation of new particles of that lipoprotein. (5,15,28) Thus, the mutation of the PCSK9 gene leads to loss of function of its corresponding protein, promoting an increase in the amount of LDL receptors on the cell surface and a consequent reduction in plasma LDL levels due to its greater uptake by liver (5). The prevalence of mutations associated with this gene is up to 3.2% (2). Individuals with the PCSK9 gene mutation have an average LDL reduction of about 20 - 40% and a cardiovascular risk reduction of up to 88%, depending on the type of mutation (2,29,30). Despite the laboratory alteration, individuals with this mutation do not appear to have other manifestations (5,9).

1.2 Diagnosis

For diagnosis, other conditions that lead to a clinical and / or laboratory alterations of hypobetalipoproteinemia should first be excluded. This can occur due to a strict vegetarian diet, intestinal malabsorption of fats, chronic pancreatitis, severe liver disease, malnutrition, hyperthyroidism, beta-thalassemia, and sickle cell anemia. Once these pathologies are ruled out, genetic sequencing should be performed according to the patient's clinical condition. Mild phenotypes usually occur due to a heterozygous mutation of the APOB or PCSK9 gene or a compound heterozygous or homozygous mutation of the ANGPTL3 gene. Severe phenotypes occur due to homozygous mutation of the APOB gene or due to mutation of the Sar1b or MTP gene. If the mutation is not found, the diagnosis will be primary orphan

familial hypobetalipoproteinemia (4,9).

1.3 Treatment

Treatment for mild phenotypes is generally not necessary, except in cases of hypovitaminosis, where supplementation should be performed (6). In severe phenotypes, treatment is based on a low-fat diet, eventually supplemented with medium-chain triglycerides and fat-soluble vitamin (9.10).

DISCUSSION

The reported patient did not present any clinical evidence of secondary causes of hypocholesterolemia, thus indicating the presence of a possible genetic cause as the basis for his laboratory alterations. Since he did not have a severe phenotype and the LDL was greater than 10-20% of the general population average for age and sex (about 100 mg / dl) (31), the possibility of mutating the Sar1b gene or of the MTP was low. In addition, HDL in the first dosage was slightly low and in the second test it was normal, making the probability of FCHL also low. Among the remaining mutations - mutation of the APOB gene and mutation of the PCSK9 gene - the most likely is the heterozygous mutation of the APOB gene. This is because the patient had mild clinical manifestations – hepatic steatosis and mild vitamin D, E and K deficiency – ruling out the possibility PCSK9 gene mutation. Although LDL is around 20 mg / dl (which would be 20% of the general population average for age and sex corresponding to that of the patient), it is not as low as in other case reports (11,32,33) of homozygous or compound heterozygous mutation of the APOB gene. However, despite the proposed hypothesis, to have a definitive diagnosis it is necessary to perform a genetic research on the genes mentioned above.

Finally, the conduct chosen for the patient of this study was a normocaloric and hypolipidic diet with supplementation of vitamin D (800 - 1200 IU / day), vitamin E (100 - 300 IU / kg / day) and vitamin K (5 - 35 mg/week). The minimum outpatient follow-up interval recommended for the patient was every 6 - 12 months. There is a plan annual follow up with general biochemical, fat-soluble vitamins, liver function and liver enzymes tests. In addition, we advise the performance of bone densitometry, abdominal ultrasound, ophthalmological and neurological examinations due to possible complications of the disease.

CONCLUSION

Hypobetaliproteinemia corresponds to a heterogeneous group of diseases. Most of them do not cause serious manifestations and go unnoticed. However, even these can lead to late repercussions such as liver disease and hypovitaminosis. Therefore, it is important to be attentive to patients with low levels of LDL and total cholesterol, avoiding dismissing these findings as "benign" changes. Despite this, it must be recognized that, possibly, these patients have some cardiovascular protection due to low LDL titers. The severe forms, in spite of being very rare, lead to evident clinical manifestations in the first years of life and can be life threatening if not treated.

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ORIGINAL ARTICLES
INFLUENCE OF FAT AND LEAN MASS ON BONE MINERAL DENSITY IN WOMEN DIVIDED BY AGE-BASED GROUPS

INFLUÊNCIA DAS MASSAS GORDA E MAGRA NA DENSIDADE MINERAL ÓSSEA EM MULHERES DIVIDIDAS POR GRUPOS DE IDADE

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Abstract

Aim: The aim of this study was to analyse the influence of lean (LM) and fat mass (FM) on bone mineral density (BMD) in women, in order to identify which of these components exerts the greatest influence on bone density. **Material and Methods:** Participants were divided in two groups: women under 50 years of age (W<50) and ≥ 50 years (W≥50). Body composition assessments and BMD were obtained by dual-energy x-ray absorptiometry and included: total body weight, body mass index (BMI), LM, FM, fat percentage (FP) and BMD at lumbar (lBMD) and femoral neck (fBMD). **Results:** The results showed that LM is the most important component to influence BMD of adult women, mainly in the hip area. Additionally, BMI, FM and LM seem to have a similar effect in the fBMD, although being significantly lower in the lBMD in the W<50 group. Similar values were found between fBMD and lBMD in the W≥50 group, with LM having greater influence than FM. **Conclusion:** Despite the fact that women under 50 years of age showed FM and LM with proportional influences on BMD; in the older group BMD suffered greater influence of LM, thus demonstrating the importance of stimulating and maintaining muscle mass throughout life in order to assist bone health . **Endocrinol diabetes clin exp 2021 / 2207 - 2210.**

Resumo

Objetivo: O objetivo deste estudo foi analisar a influência da massa magra (LM) e da massa gorda (FM) na densidade mineral óssea (DMO) em mulheres, a fim de identificar qual desses componentes exerce maior influência na densidade óssea. **Material e Métodos:** Os participantes foram divididos em dois grupos: mulheres com menos de 50 anos (W <50; (N = 72; média 31,80 ± 12,60) e com 50 anos ou mais (W≥50; N = 217; média; 66,30 ± 13,50). As avaliações da composição corporal e DMO foram obtidas por absorsiometria de raios-X de dupla energia e incluíram: massa corporal total, índice de massa corporal (IMC), LM, FM, percentual de gordura (FP) e DMO lombar (IMC) e colo femoral (fBMD). **Resultados:** Neste estudo, a LM representou o componente mais importante para influenciar a DMO de mulheres adultas, principalmente na região do quadril. Além disso, IMC, FM e LM parecem ter um efeito semelhante no fBMD, embora sejam significativamente menores no lBMD no grupo W <50. Valores semelhantes foram encontrados entre fBMD e lBMD no grupo W≥50, com LM tendo maior influência do que FM. **Conclusão:** Apesar de mulheres mais jovens apresentarem FM e ML com influências proporcionais na DMO; no grupo ≥50 anos a DMO sofreu maior influência da ML, demonstrando assim a importância de estimular e manter a massa muscular ao longo da vida para auxiliar na saúde óssea. **Endocrinol diabetes clin exp 2021 / 2207 - 2210.**

INTRODUCTION

The treatment of bone fractures, especially those caused by osteoporosis, requires a huge expenditure of money from health systems worldwide. Hip fractures are commonly found in people with osteoporosis and are associated with high rates of morbidity and mortality (1).

Many studies have demonstrated the effect of body mass on BMD and, when comparing the incidence of fractures in obese and non-obese people, obesity seems to protect some areas but it does not have the same protective role in other places (2). These findings suggest that different approaches are needed to reduce the risk of fractures, especially the most limiting ones such as those suffered in the hip (3).

About body components, studies have shown that fat mass (FM) is not directly associated with total bone mineral density (BMD) or osteoporosis, suggesting that muscle mass may be the more important intervening variable associated with bone health (4).

An important study describing the relationship between bone and muscle mass concluded that it occurs during childhood as well as during the aging process. There seems to be one or more factors linked to the decline in bone and muscle tissues in the elderly, thus preventive interventions such as exercise prescription can assist both of them (5).

Furthermore, also the effects of the menopause can be noticed a period of time after its occurrence, thus research on the topic is required to define the cut-off age. An important study about menopause stages involving 16,251 American women with reproductive life expectancy and longevity, showed associations of age at menarche and menopause, where the odds ratio for 45-49 years of age was 1.22 (1.05-1.41) and for 50-54 years of age was 1.31 (1.14-1.50) (6). According to a study that analysed 456 Brazilian women, in the age group of 45-60 years old the minimum age of occurrence of natural menopause was 28 and the maximum was 58, with an average of 51.2 years.

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Approximately 60% of all women were 51 years old or younger and had reached the menopause by then. Thus, 50 years of age seems to be an ideal average to qualify physiological and bone changes caused by menopause (7).

Therefore, the aim of this study was to perform a descriptive analysis of body composition and to verify the influence of fat (FM) and lean mass (LM) on BMD in women divided into two age-based groups: under 50 years of age (W<50) and 50 years old or older (W≥50).

Table 1 shows the body composition of women according to their age group. The W<50 group showed mean age of 31.8 years, moderate obesity, normal BMD, excess of FM and normal LM; while the W≥50 group showed mean age of 66.3 years, overweight, diminished BMD, high fat percentage (FP) but normal FM and lower LM. BMD values, divided in low and normal BMD in both groups, are also described.

MATERIAL AND METHODS
Sample
289 women, aged between 22 and 92 years old, had their body composition and BMD measured in the Laboratory for Studies in Biomedical Engineering and Health – PEBS, at the Federal University of Technology in Curitiba, Brazil, between 2013 and 2019. The BMD cut-off point was determined for normal and low values, where a Z-score ≤ -2.0 was used for the W <50 group and the T- score <-1.0 for the W≥50 group.

Inclusion and exclusion criteria
From the database of 1042 women initially available, 849 were within the required age group, 289 had both bone mineral density and body composition tests performed at PEBS. Exclusion criteria determined that individuals who did not have their data collected and stored at PEBS during the period between 2013 and 2019 would be excluded from the study, as well as those who showed any discrepancy on BMD results that prevented its diagnostic analysis.

Measurements
After collecting personal and clinical data from the participants, height was obtained with a stadiometer (Wiso 210, Santa Catarina, Brazil) and a digital scale was used for total body mass (Wiso W801, Santa Catarina, Brazil). Body composition (BMI, LM, FM, FP and BMD) was obtained through Dxa Hologic Discovery A (Mississauga, Canada) scan. BMD of hip (fBMD) and lumbar spine (lBMD) L1 to L4 were evaluated, where any vertebrae with morphological alterations of any kind were excluded for the average diagnosis. When any these two parts was impossible to verify, the 33% radius (also called 1/3 radius) was used.

STATISTICAL ANALYSIS
All statistical procedures were performed using SPSS (Version 17.0, Chicago, United States) for minimum, maximum, average and standard deviation of variables related to body composition and BMD, where descriptive analysis was used and the sample classified by age group. Pearson's correlation was used to determine univariate correlations and linear regression was used to predict the BMD of fBMD and lBMD based on BMI, FM, FP and LM. The p<.05 value was considered statistically significant.

RESULTS
The BMI result was of 33.8 Kg/m2 for the W<50 group, with its correlation between BMI and fBMD being 0.735** (p=0.000) while BMI and lBMD was of 0.262* (p=0.018), showing a strong correlation with FM and LM. The W≥50 group showed BMI of 28.0 Kg/m2, with the same group presenting correlation of 0.431**(p=0.000) between BMI and fBMD and 0.353** (p=0.000) between BMI and lBMD, showing a stronger correlation of 0.904** (p=0.000) and 0.756** (p=0.000) for FM

and LM, respectively.

The correlation between variables and fBMD showed that for the W<50 group similar values with strong and moderate correlation were found at FP, total FM and total LM (0.604**, 0.710**, 0.707**, respectively), while in the W≥50 group these correlations did not present similar strength. The lBMD correlation between the same variables was (0.293**, 0.282*, 0.187) for the W<50 group and (0.157*, 0.341**, 0.490**) for the W≥50 group, thus showing a higher influence of LM in the latter.

The regression analysis in the W<50 group shows 57% of total body composition influence on BMD. Table 2 shows that in the same group LM and FM had similar proportion of influence on fBMD with 50% for each component, however their effect on lBMD was 7% and 3%, respectively. Significant statistical differences were found in all analysis.

Table 3 shows the regression analysis of the group of women belonging to the W≥50 group. The influence of total body composition on BMD was 23%, where LM had more influence on fBMD than FM with 22% and 13%, respectively. On lBMD the influence was 24% and 11% for LM and FM, respectively. In all components significant statistical differences were found.

DISCUSSION
Many studies have demonstrated the importance of body components in bone density and its health. In a systematic review, abdominal obesity was adversely associated with the risk of hip fracture in 295,674 individuals. In addition, a positive association between abdominal obesity and the risk of hip fracture was demonstrated (8). In the present study, we found the effect of BMI on BMD in both fBMD and lBMD mainly in the W<50 group, where fBMD received its greater influence.

BMI is considered an important variable as part of the fracture risk assessment tool – FRAX, where a higher BMI is associated with a lower risk of future fracture. However, this claim has been recently challenged, with studies showing that obesity is associated with a lower risk of certain fractures and a greater risk of other types (9).

An investigation about body composition in bariatric surgery aiming to verify the effect of overweight and weight loss on BMD found that BMI had 26% of effect on fBMD before surgery (10). Other risk factors for women are age, previous fractures due to bone frailty, and BMI – which proved to be a relevant fracture risk factor, especially in the hip (11).

A prospective study with 2,968 young adults emphasizes that the components of body composition have different effects on BMD, mainly those caused by FM in the youth, bearing in mind that the longer period of obesity, the greater the damage to bone health (12). In our study we found that fBMD had correlation between variables, with the W<50 group presenting similar values with strong and moderate correlation in FP, FM and LM, while the W≥50 group had the same analysis showing less significant influence in the same variables.

Despite the fact that falls have greater potential detrimental effects for the obese, there seems to be the same risk factors between obese and non-obese individuals. Evidence shows that morbidity, when associated with fractures in obese individuals, is higher than in non-obese individuals; however, a recent study indicates that fracture-related mortality is lower in obese and overweight people than in people with normal weight (13).

BMI is a well-defined risk factor for low BMD and fracture. The risk is greater for individuals with a BMI of <20kg/m2, while higher numbers have lower protective effects. The association of fracture risk with bone thinness also is largely dependent on BMD (14). In our research, we found that lBMD showed correlation with FP, FM and LM (0.293**, 0.282*, 0.187, respectively) in the W<50 group while in the W≥50 group the same variables showed LM as the most significant variable (0.157*, 0.341**, 0.490**, respectively).

In a study with 982 adolescents aged 12 to 19 years old,

overweight and obesity had a positive effect on BMD, where LM was had more significant association with higher BMD. These results suggest the importance of interventions to increase LM as a source to improve bone health in overweight and obese adolescents (15). About different body components, our investigation showed that both FM and LM had similar effect on fBMD in the W<50 group, while a much smaller proportion for lBMD was found.

A meta-analysis showed relationship between BMD and LM during childhood and the aging process, suggesting that one or more factors are linked to the decline in bone and muscle, leading to interventions on the protective action to both elements (5). Another study, evaluating the relationship between obesity and bone health, showed that mechanical load and greater LM are associated with better results in BMD, while the systemic inflammation observed especially through abdominal obesity can have negative effects (9). On the mechanical effect on bones, research presented by Matos et al. concluded that weight bearing has a significant influence on BMD before and after six months of bariatric surgery, especially in fBMD (10).

In the present study, the W<50 group showed mean BMI of 33.8 and mean FP of 40.8, while the W≥50 group had mean BMI of 28.0 and mean FP of 39.4. We concluded that, despite similar values of FP in both groups, their difference in BMI is due to the loss of LM. In our descriptive analysis, we demonstrated that the W<50 group had LM mean of 52,283g while the W≥50 group had 41,898g.

For this purpose, at US National Health and Nutrition Examination Survey 2005–2006 developed a study involving 5287 men and women aged 8 to 69 years, all measures of obesity were positively associated with BMD of femoral neck, but did not have the same effects on BMD of the lumbar spine (16). In our investigation, using linear regression analysis, we found that in the W<50 group there was a similar influence between all components on fBMD (FM 50%, FP 36% and LM 50%), while on lBMD it was less expressive (7%, 4% and 3%, respectively). However, in the W≥50 group there was a smaller influence of the same components on fBMD (FM 13%, FP 2% and LM 22%), but similar values (11%, 2% and 24%) for lBMD.

Many studies have investigated the effects of body composition components on BMD. This paper showed that LM is the most important component to influence BMD, mainly in fBMD. Our findings have also demonstrated that FP, FM and LM equally affect the fBMD but have less action on lBMD in adult women <50 yo. Although presenting similar values between fBMD and lBMD, in W≥50 group, LM had bigger influence on BMD than FM.

CONCLUSION

Therefore, we conclude that regardless of age or weight bearing, the hip region is more affected than the spine and we should place more importance on maintaining muscle mass from younger ages to assist in bone health.

Table 1 – Body composition of 289 women, divided in under 50 years old and 50 years or older.

	W<50			W≥50		
	N	Mean	SD	N	Mean	SD
Age	72	31.8	12.6	217	66.3	13.5
Total mass (Kg)	72	89.14	24.61	217	69.11	14.28
Height (m)	72	1.60	0.186	217	1.57	0.651
BMI (Kg/m^2)	72	33.82	8.84	217	28.00	5.47
Hip BMD (g/cm^2)	72	0.962	0.112	217	0.714	0.140
Spine BMD (g/cm^2)	72	1.057	0.120	217	0.881	0.153
Fat percentage	72	40.8	7.2	217	39.4	5.5
Total Fat mass (g)	72	38,463	15,685	217	28,235	9,229
Total Lean mass (g)	72	52,283	10,899	217	41,898	6,181
Normal BMD (age)	71	34.5	8.8	54	64.5	9.3
Low BMD (age)	01	48.0	-	163	69.0	7.3

W<50 = women under 50 years old; W≥50 = women 50 years of age or older.

Table 2. Regression analysis for women under 50 years of age.

Independent variables	R2	R2 Adjusted	Percentage influence	*P*
FEMORAL NECK				
Total fat mass	0.504	0.498	50%	0.000*
Total lean mass	0.500	0.493	50%	0.000*
Fat mass percentage	0.365	0.356	36%	0.000*
SPINE				
Total fat mass	0.079	0.068	7%	0.011*
Total lean mass	0.035	0.023	3%	0.095
Fat mass percentage	0.045	0.042	4%	0.000*

*p<.05 statistically significant.

Table 3. Regression analysis for women with 50 years of age or older

Independent variables	R2	R2 Adjusted	Percentage influence	*P*
FEMORAL NECK				
Total fat mass	0.130	0.126	13%	0.000*
Total lean mass	0.228	0.225	22%	0.000*
Fat mass percentage	0.026	0.021	2%	0.000*
SPINE				
Total fat mass	0.117	0.113	11%	0.000*
Total lean mass	0.241	0.237	24%	0.000*
Fat mass percentage	0.025	0.020	2%	0.019*

*p<.05- statistically significant.

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ORIGINAL ARTICLES
EXPRESSION OF PHOSPHATIDYLINOSITOL-3-KINASE MEDIATED BY THE AKT SIGNALING PATHWAY IN A HYPERGLYCEMIC MODEL

EXPRESSÃO DE FOSFATIDILINOSITOL-3-QUINASE MEDIADA PELA VIA DE SINALIZAÇÃO AKT EM UM MODELO HIPERGLICÊMICO

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Abstract

Introduction: Akt pathway when linked to growth factors like insulin activates phosphatidylinositol-3-kinase (PI3K), an enzyme that acts on growth processes, cell proliferation and protection against apoptosis. **Objective:** The aims of this study was to induce sustained hyperglycemia in Wistar rats and evaluate Akt expression in renal tissue and changes in cardiovascular, renal and muscular systems. **Methods:** 30 male Wistar rats were divided into two groups: Control (CO) and STZ. STZ group received Streptozotocin (STZ) in order to induce diabetes mellitus. After 62 days of experiment, the animals were euthanized and the kidney, heart, aorta, gastrocnemius muscle and mesenteric fat were removed for further analysis. **Results:** STZ group showed significantly higher glucose and triglyceride and lower fructosamine values compared to the CO group. Regarding immunohistochemical analysis, the animals in the STZ group showed a reduction in the expression of the Akt pathway in renal tissue, compared to the CO group. Regarding histological analysis, there was a predominance of glomerular hypertrophy, mesangial expansion and capillary congestion in the kidneys of animals in the STZ group, in addition to a significant increase in renal weight compared to CO. Comparing with the CO group, pathological changes were found in the myocardium, aorta and gastrocnemius of diabetic rats, as well as a significant reduction in the diameter of the mesenteric adipocytes of these animals. **Conclusion:** Deleterious effects on tissue integrity and function were caused by the diabetic metabolic environment, causing significant changes both in PI3K / Akt signal transduction and in cell growth. **Endocrinol diabetes clin exp 2021 / 2211 - 2217.**

Resumo

Introdução: A via de sinalização intracelular Akt quando ligada a fatores de crescimento, como à insulina, ativa fosfatidilinositol 3-quinase (PI3K), enzima que age nos processos de crescimento, proliferação celular e proteção contra apoptose. **Objetivo:** O objetivo deste estudo foi induzir hiperglicemia sustentada em ratos Wistar e avaliar a expressão de Akt nos sistemas cardiovascular, renal e muscular. Análises antropométricas e sorológicas também foram consideradas. Metodologia: 30 ratos machos Wistar foram divididos em dois grupos: Controle (CO) e STZ. O grupo STZ recebeu Estreptozotocina (STZ) com a finalidade de induzir o processo de diabetes mellitus. Após 62 dias de experimento, foram realizadas a eutanásia dos animais e a retirada de rim, coração, aorta, músculo gastrocnêmio e gordura mesentérica para posterior análise. **Resultados:** O grupo STZ apresentou valores de glicemia e de triglicerídeos significativamente maiores e valores de frutosamina significativamente menores em relação ao grupo CO. Em relação à análise imunohistoquímica, os animais do grupo STZ apresentaram uma redução na expressão da via Akt em tecido renal, comparado ao grupo CO. Em relação à análise histológica, houve um predomínio de hipertrofia glomerular, expansão mesangial e congestão capilar nos rins dos animais do grupo STZ, além de um aumento significativo no peso renal em relação ao CO. Comparando com o grupo CO, alterações patológicas foram encontradas em miocárdio, aorta e gastrocnêmio de ratos diabéticos, assim como uma redução significativa no diâmetro dos adipócitos mesentéricos desses animais. **Conclusão:** Efeitos deletérios sobre integridade e função teciduais foram causados pelo meio metabólico diabético, ocasionando alterações expressivas tanto na transdução de sinal da PI3K/Akt quanto no crescimento celular.. **Endocrinol diabetes clin exp 2021 / 2211 - 2217.**

INTRODUCTION

Diabetes mellitus today is one of the main public health problems in many countries. It is estimated that around 143 million people worldwide suffer from diabetes and it is believed that this number could double by the year 2030, so that some authors consider a diabetic pandemic. Brazil would be among the 10 countries (of the 32 countries studied) with the highest number of diabetic people (1).

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The World Health Organization (2) defines Diabetes mellitus as a metabolic disorder of multiple etiology, characterized by a chronic hyperglycemia that causes disturbances in the metabolism of carbohydrates, fats and proteins resulting from defects in the secretion and / or action of insulin (1).

Insulin is the main regulator and key hormone of glucose balance. It increases the absorption of glucose in muscles and fat tissue and decreases the synthesis of glucose in the liver. In addition, cell growth and differentiation are stimulated by this hormone, which promotes lipogenesis, protein and glycogen synthesis and inhibits lipolysis and proteolysis. Insulin promotes the absorption of glucose in cells, stimulating the glucose transporter GLUT4 transcription and translocation from the intracellular part to the cell membrane (3).

Insulin resistance causes the deregulation of some intracellular signaling pathways, while skeletal muscle and adipose tissue do not respond adequately to insulin, causing an increase in the concentration of glucose in the blood and lipids and an increase in the secretion of insulin by the pancreas. Clinically, insulin resistance refers to higher than normal levels of insulin to keep blood sugar at normal levels, whereas at the cellular level, insulin resistance refers to defects in the signal transduction pathway insulin (3).

In symptomatic patients, polyuria, polyphagia, polydipsia, weight loss and visual changes are common. In addition, patients may experience chronic complications such as atherosclerosis, myocardial infarction and become more susceptible to infections such as carbuncles and generalized furunculosis. In these patients, insulin administration is necessary to prevent the development of ketoacidosis, coma and death (4).

The hereditary factor and obesity are of great importance although these patients produce insulin normally their cells are unable to use all this insulin secreted by the pancreas, causing their levels to remain high in the blood, which is known as insulin resistance (4).

Type 2 individuals have a two to three times higher incidence of cardiovascular disease, since macrovascular complications in these patients are equivalent to twice microvascular complications (4).

With the increasing prevalence of diabetes mellitus worldwide, coupled with the increased survival of diabetic patients, it is expected that the incidence of complications associated with this epidemic will also increase. This reality takes on greater gravity due to the evidence of the relationship between diabetic nephropathy and the increased risk of cardiovascular morbidity and mortality in these patients, making it essential to know the diagnostic, preventive and therapeutic measures necessary for a correct approach and care provided (4).

A major research focus is the understanding of the signaling pathways that affect this pathology, such as the PI3K / AKT pathway. Insulin signaling regulates glucose, lipids and energy homeostasis, predominantly through action on the liver, skeletal muscle and adipose tissue. Accurate modulation of this pathway is essential for adaptation as the individual changes the fed state to the fasting state. Modulators that act in different stages of the signaling pathway, as well as the diversity of protein interaction, guarantee an adequate and coordinated biological response to insulin in different tissues (5).

Considering that genetic mutations are causes of rare and severe insulin resistance, obesity can lead to insulin resistance through a variety of mechanisms, understanding these pathways is essential for the development of new drugs for the treatment of diabetes, metabolic syndrome and your complications (5).

Considering the intense participation of Akt in mechanisms of survival, proliferation and cell growth, a study on the expression of this pathway through PI3K may provide relevant information on the process of diabetes mellitus in target organs, considering the clinical effects that the imbalance of this pathway can entail. Therefore, this study aimed to evaluate PI3K / Akt immunohistochemical expression in target tissue of diabetic rats.

MATERIAL AND METHODS

This study is in accordance with the recommendations of the Ethics Committee on the Use of Animals of Faculdade Evangélica Mackenzie do Paraná - CEUA / FEMPAR, as well as with the Declarations of Helsinki (1964, 1975, 1981 and 1989) and with the International Protection Standards to the Animals.

Experimental model of Diabetes Mellitus

Thirty male Wistar rats were used, aged approximately 2 to 3 months, weighing an average of 250 grams. The animals were kept with free access to water and food, in a light / dark environment of 12 / 12h and a constant temperature of 23ºC. The animals were obtained by the Vivarium of the *Pontifícia Universidade Católica do Paraná* and acclimated in the Vivarium of the *Instituto de Pesquisas Médicas* (IPEM) for 7 days, before the beginning of the experiment.

The animals were divided into two experimental groups: control group, formed by 10 healthy rats and STZ group (Streptozotocin), formed by 20 rats with pharmacologically induced diabetes.

The STZ group rats, after a 12h fasting period, had streptozotocin-induced diabetes (diluted to 2% in 10 mmol sodium citrate solution, pH 6.0 and administered into the penile vein in a single dose of 30mg / kg per kilogram of body weight (6). The animals in the control group received similar treatment, but with only the same proportion of NaCl.

After induction, the diet was again provided to avoid a fatal hypoglycemic condition, due to the massive release of insulin that occurs after the destruction of ß cells (7).

Evidence of experimental diabetes was performed 7 days after drug administration, by determining the capillary glucose concentration from the rat's tail, after a minimum fasting period of 12h. The day the conformation of blood glucose was considered as day 01 of the experiment. Animals were considered diabetic if fasting blood glucose above 200mg / dl (8).

Inclusion and exclusion criteria

10 animals from the Control Group and 14 animals from the STZ Group remained alive after the induction stage, with the STZ Group obtaining blood glucose values above 200mg / dL and, therefore, they were all included in the study analysis. The six animals that died after induction were excluded from the analyzes.

Streptozotocin

Streptozotocin was initially isolated and characterized as a broad spectrum antimicrobial from colonies of Streptomyces achromogenes. Based on preclinical studies of streptozotocin, they revealed that its intravenous or intraperitoneal administration in high doses produces diabetes mellitus in rats and dogs, and that the islets of Langerhans of diabetic animals are ruptured and with a significant decrease or absence of cell granules (7). *Simon and West*, studying the intraperitoneal administration of STZ to Wistar rats, found diabetes at a dose of 30mg / kg lastingly (7).

The STZ-induced model of Diabetes Mellitus in rats has many advantages over other models, such as low cost, ease in inducing disease and the ability to keep animals in proper conditions. Thus, it is considered an appropriate experimental model of DM, being used in many experiments as a pathology induction protocol (6).

Euthanasia

At the end of 62 days of the experiment (considering day 01 as the confirmation of hyperglycemia after induction), the rats were anesthetized with intraperitoneal application of ketamine hydrochloride 50 mg / kg and xilasine hydrochloride 10 mg / kg. For this procedure, the animals were fasted for 12h. After complete anesthesia, the animals were submitted to a median ventral incision, allowing blood collection by direct cardiac puncture in the left ventricle, with blood stored in anticoagulated tubes for subsequent biochemical analysis. After this procedure, heart, thoracic aorta, gastrocnemius muscle were removed (right leg

dissection - Silvado), white adipose tissue and kidney for further histopathological and immunohistochemical analysis.

Anthropometric Assessment

The animals were weighed at four moments of the experiment (the first on the 1st of the experiment, the second on the 30th, the third on the 45th and the last on the 62nd - day of euthanasia), using a digital electronic and technical scale standardized, the values being recorded for later analysis. Weights were also collected, after euthanasia, of the heart and kidney, for purposes of comparative analysis.

Serological Evaluation

Blood samples were collected from the animals' tails at three moments of the experiment (day 01, day 45, day 62), being analyzed instantly through a glucometer by strips. Through serological analysis, using a colorimetric method, the analysis of fructosamine and lipid profile (total cholesterol, HDL and triglycerides) was performed.

Histopathological Evaluation

Histological changes in the kidney (through hematoxylin Eosin - HE staining) were evaluated according to the methodology described by *Melorose*(9), which consists of observing the presence of glomerular hypertrophy, mesangial expansion, fibrosis (collagen deposition) and glomerular atrophy (deletion of epithelial cells).

Myocardial changes, in turn, were evaluated in HE through the presence or absence of areas of coagulation necrosis, vacuolar degeneration, edema, pycnosis and loss of nuclei and the presence of lymphocytes between myocardial fibers and / or necrotic cells (10).

The thoracic aorta was analyzed by HE, according to definitions proposed by American Heart Association (11). The lesion of the artery was classified in 6 degrees, through the observation of thickening of the intima, foam cells, extracellular lipid deposits, extracellular lipid nucleus, thickening of the intima with or without calcifications and thrombosis or rupture of atheromatous plaque.

The white adipose tissue, with HE staining, was evaluated through a 400x magnification in the optical microscope, the area with the largest number of adipocytes being photographed and the diameter of the adipocytes analyzed using the Image J program (12).

The fragments of the right gastrocnemius muscle (standardization) were stained with HE, being classified according to the severity of the histological changes proposed by *Silvado* (13), in increased endomysial connective tissue, infiltration by adipose tissue, vessels (thickened wall), fiber morphology (atrophic or hypertrophic, central or pycnotic nuclei) and internal fiber structure (necrosis or inflammatory infiltrate).

Immunohistochemical Evaluation

Renal tissue was evaluated considering the presence or absence of angiogenesis, with increased or reduced expression of PI3K / Akt.

Primary and secondary antibodies

Primary Anti-Akt antibody: this polyclonal antibody detected endogenous levels of the total Akt protein. The incubation with the secondary antibody occurred for 1 hour, at room temperature. The sections were washed with a buffer to remove excess secondary antibody that did not bind to the tissue.

Analysis of Immunohistochemistry Slides

During the quantification of the marked area for slides submitted to immunohistochemistry, the uniformity of the signal of digital images, taken by a high resolution camera installed in an Olympus optical microscope, was considered, with a 40-fold increase for all regions. The counting of enzymatic expression was performed using the Image J program, in 4 squares of 50x50 per region, where the squares considered in the photos were the places with the greatest accumulation of cells.

Statistical Analysis

The results of quantitative variables were described by means, medians, minimum values, maximum values and standard deviations and qualitative variables by frequencies and percentages. For comparing two groups in relation to quantitative variables, the Mann-Whitney non-parametric test was considered. More than two groups were compared using the Kruskal-Wallis non-parametric test. Fisher's exact test was considered to assess the association between two qualitative dichotomous variables. The correlation between two quantitative variables was assessed by estimating Spearman's correlation coefficient. Values of $p < 0.05$ indicated statistical significance. The data were analyzed with the computer program GraphPad Prism 6.0.

RESULTS
Serological Analysis
Glucose

Regarding the first glycemic measurement, the control group had lower values of fasting capillary glucose when compared to the STZ group (79.2mg / dL vs 398.1mg / dL, with $p < 0.0001$). Likewise, in the second measurement, the control group had a lower mean capillary blood glucose, while the STZ group had 6x higher values, with significant p (84.8mg / dL vs 576mg / dL, $p < 0.0001$). The third measurement, performed on the day of euthanasia, also showed a significant difference between the groups, with the STZ group having an average of 655.7mg / dL and the control group an average of 96mg / dL of capillary blood glucose, with $p < 0, 0001$.

Fructosamine

The dosage of fructosamine, at the end of the experiment, showed a significant difference between the groups analyzed, and the STZ group had higher values of fructosamine compared to the control group (averages of 0.9300mmol / L vs 0.6173mmol / L), with $p < 0.0001$ (Fig. 1).

Fig. 1. Comparison between groups of final capillary blood glucose and fructosamine values, both with $p < 0.0001$.
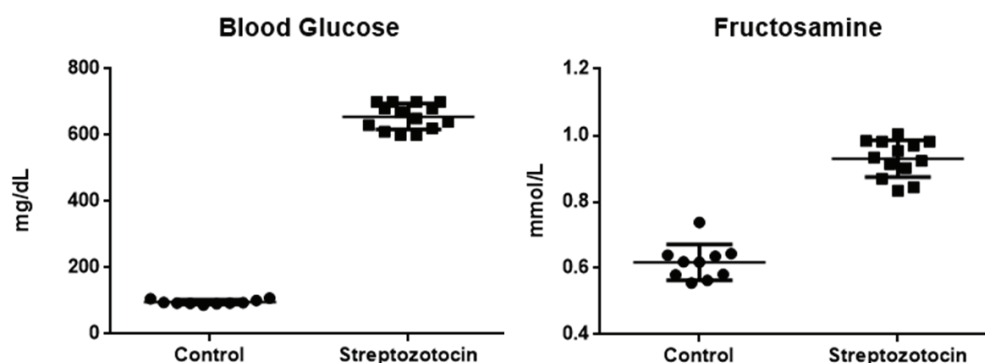
Triglycerides

At the end of the experiment, the mean triglyceride values for the STZ group were significantly higher than the control group (243.3 mg / dL x 136.2 mg / dL), with p = 0.0004 (Fig. 2) .

Fig. 2. Analysis of triglycerides in the control and STZ groups, with significant p.
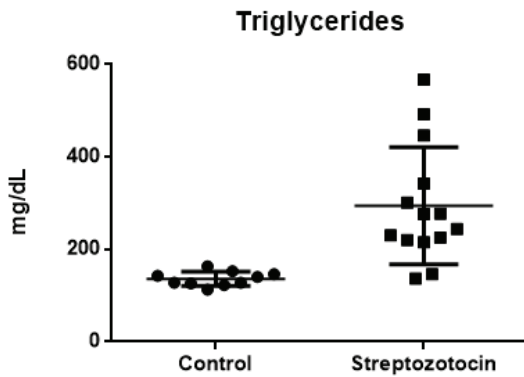
Kidney
Kidney Weight

As for kidney weight, there was a significant difference between the control and STZ groups, with the control group having lower values when compared to the STZ group (1.427g vs 1.701g, p = 0.0012). Regarding the relative weight of the organ, there was a significant difference between the means of each group, with lower values being observed in the control group compared to the STZ group (p <0.0001) (Fig. 3).

Fig. 3. Comparative analysis of kidney weight, in grams, between the control and STZ groups (p <0.0001).

Histology of Renal Tissue by Hematoxylin-Eosin (HE)

From the histopathological analysis of the kidney, at the end of the experiment, 50% of the animals in the STZ group had glomerular hypertrophy. In 65% of the animals in the same 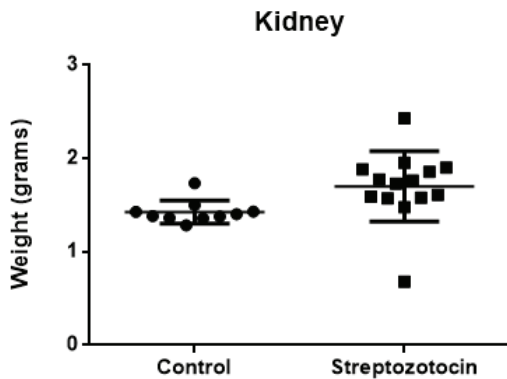group there was mesangial expansion in up to 25% of the glomerulus. Interstitial changes (edema, capillary congestion, inflammatory cells) affected the kidneys of 36% of diabetic animals (STZ).

The animals in the control group did not present glomerular changes in the majority (70%), while 30% presented glomerular hypertrophy, showing no other changes.

Immunohistochemistry in Renal Tissue

The rats in the STZ group showed, by immunohistochemical analysis in renal tissue, a reduction of 20.24% in the expression of the PI3K enzyme when compared to the control group (Fig. 4).

Fig. 4. Immunohistochemistry of kidney tissue in a 40x magnification. On the left, tissue from the control group; on the right, tissue from the STZ group with decreased Akt expression.
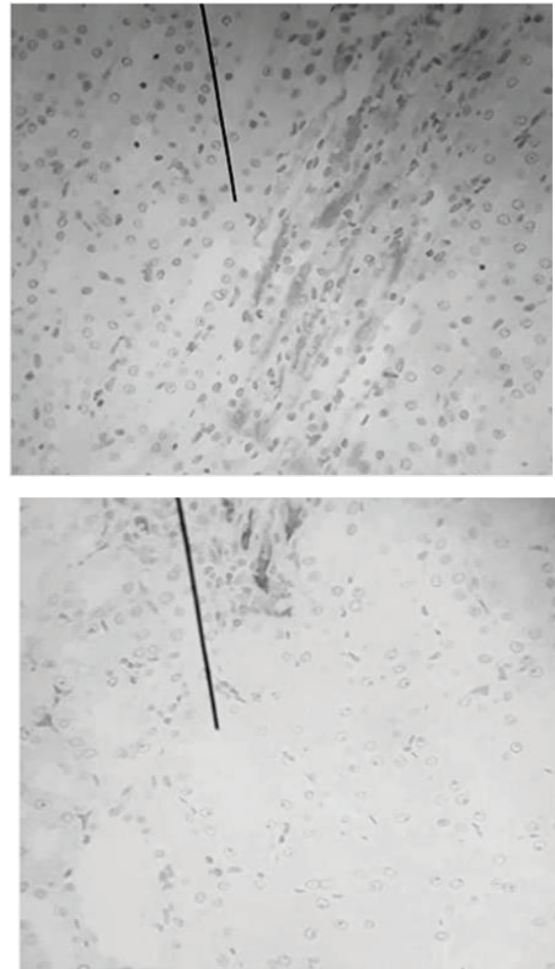
Aorta
Hematoxylin-Eosin Histology

As for the aortic artery, type 1 lesions were found in animals in the STZ group, according to definitions proposed by the American Heart Association (11) with thickening of the intimate layer in 57% of diabetic animals.

Heart
Heart Weight

Regarding the absolute weight of the heart, there was a significant difference between the control and STZ groups, with the control group having greater weight than the STZ group (1.525g vs 1.093g, p <0.0001). Regarding the relative weight, there was also a significant difference between the control and STZ groups, with the control group having lower relative values than the STZ group (p = 0.0002).

Histology of Cardiac Tissue by Hematoxylin-Eosin

Regarding the myocardial, degenerative changes were observed in 14% of the animals in the STZ group, with the presence of coagulation necrosis, pycnosis and vacuolar degeneration.

Muscle
Histology of the Gastrocnemius Muscle by Hematoxylin-Eosin

Through the analysis of muscle fibers in HE staining, it is possible to observe a series of changes highlighted in the diabetic group. In 72% of the animals in the STZ group, atrophic muscle fibers were found, while only 30% of the animals in the control group showed the same change (Fig. 5).

Fig. 5. Histology of gastrocnemius muscle tissue in a 100x magnification. On the left, tissue from the control group without changes; on the right, STZ group tissue with atrophic fibers.
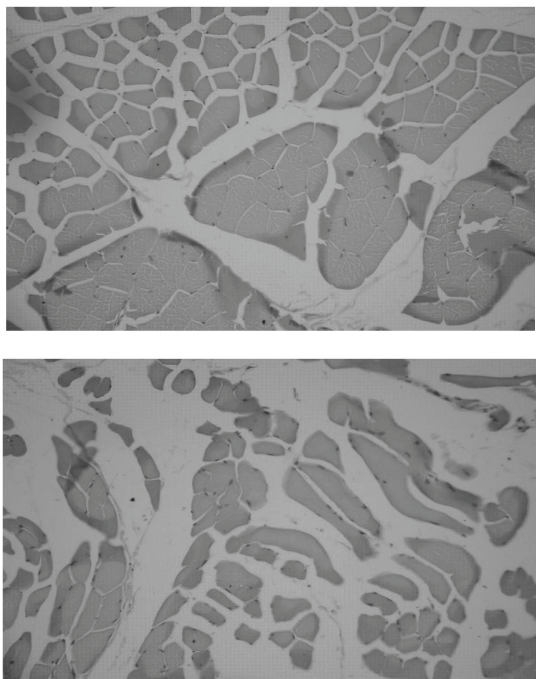
43% of the animals in the STZ group showed signs of vascular congestion, with thickened wall vessels, against 20% in the control group. Likewise, muscle fibers with the presence of necrosis were mostly observed in diabetic animals (STZ) in relation to the control group.

White Adipose Tissue
Histological Analysis by Hematoxylin-Eosin

After pathological analysis, the white adipose tissue with HE staining was found to be altered in almost 60% of the animals in the STZ group, with macrophage infiltration and tissue inflammation. In the animals of the control group, there were no changes in this tissue (Fig. 6).

Fig. 6. Histology of mesenteric fat in 400x magnification. On the left, Control group; on the right, STZ group showing alteration in adipocyte architecture and local inflammation.
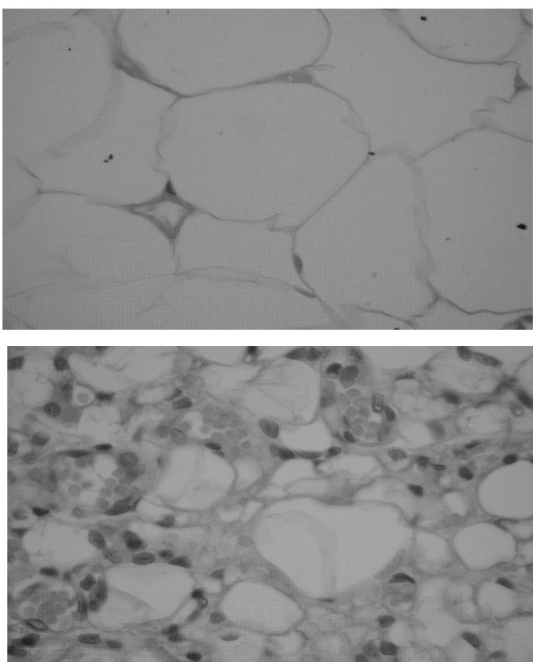
As for the diameter of the adipocytes, the STZ group showed lower values compared to the control group (198.3mm x 522.1mm), with p <0.0001 (Fig. 7).

Fig. 7. Adipocyte diameter values in the control and STZ groups.
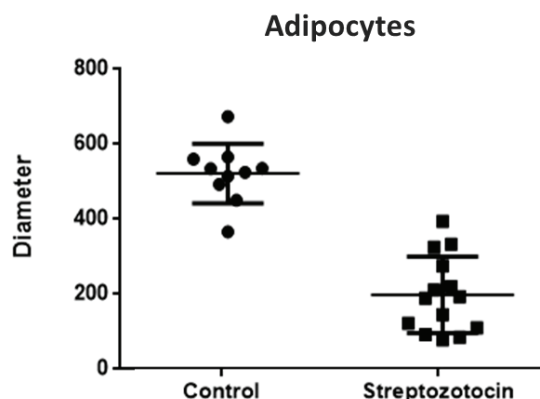
DISCUSSION

The symptoms of type 1 diabetes and decompensated type 2 diabetes mellitus are similar, according to World Health Organization.(14) the main difference being the speed and intensity with which they occur, which may never occur in type 2 DM.(15)

In general, when compared to the Control Group, the rats in the STZ Group showed a large intake of liquids and elimination of urine, a wet specimen daily and with a foul odor. The polydipsia present in diabetic animals occurs due to blood hyperosmolarity, due to high levels of circulating glucose, which makes the water pass from the intracellular to the extracellular medium in order to maintain the osmotic balance. Intracellular dehydration is recognized by brain osmoreceptors that generate a response triggering the intense thirst characteristic of diabetes (16).

From the middle of the experiment, some rats from the STZ Group manifested ocular changes, macroscopically seen as thick, white areas. Results obtained by *Oliveira*, using a similar methodology, they demonstrate that the increase in blood glucose is associated with an increase in the total thickness of the cornea and stroma, which can be explained by the change in the corneal hydration mechanisms, promoting its edema.(17)

According to the American Diabetes Association, diabetes currently imposes a significant burden on society in the form of higher medical costs, loss of productivity, premature mortality and a great reduction in quality of life. In this context, STZ is considered, today, the most used diabetogenic drug to induce diabetes in experimental animal models to deepen the theme in question. It is a pancreatic β-selective cell toxin that induces random and rapid and irreversible necrosis of β cells. Such effects are generally sufficient to induce non-insulin dependence on diabetes mellitus (type 2) in animals. STZ is able to pass through any cell membrane that contains glucose transporter (GLUT-2) (18).

Quinna and Badwan indicated that STZ is capable of producing mild to severe types of diabetes that vary according to the selected dose, strain and age of the animals, in addition to the nutritional status and route of administration. The drug administration protocol in the present study was effective and corroborates with such information from the literature, since it provided significant hyperglycemia and nutritional changes in the animals under study (19).

Regarding the laboratory control of the disease, in acute glycation studies, the dosage of fructosamine is the best parameter to be evaluated for demonstrating the average concentration of glycation between albumin and glucose in the last two or three weeks. Fructosamine is a product proportional to the concentration of glucose and correlates with fasting glycemia and glycated hemoglobin in a shorter period. Induction studies using

a methodology similar to that demonstrated in this work, using STZ, but lasting ten days, did not show significant difference in the value of fructosamine between the groups, due to the shorter exposure time.(20)

Due to the randomness of the deterioration of different organs involved in glucose homeostasis by STZ, some target organ tissues may not show pathological changes, while others, clearly the organs involved in insulin secretion, metabolism and elimination, become more affected by the drug, albeit at random (19).

Most studies of diabetes induction through STZ do not show changes in organ weight. *Silva et al* (21) found no significant difference regarding the weight of the liver, but the weight of the kidneys was 1.5x higher in the diabetic group. Like wise noted *Dantas et al* (22). The present study is in agreement with these data, since the relative weight of the kidney and heart were significantly higher in diabetic animals (20).

Immunological and inflammatory mechanisms play an important role in the progression of diabetic nephropathy with activation of innate immune cells and pro-inflammatory cytokines. Macrophages and T lymphocytes (which exist in large numbers in diabetic glomeruli) as well as different molecules (such as chemokines, adhesion molecules, growth factors, nuclear factors and cytokines) have been implicated in several pathogenic pathways related to diabetic nephropathy. Late complications are mainly caused by damage to the vessels that end up compromising the feeding of tissues and organs, with serious consequences (15).

Changes in the large and medium vessels (macrovascular disease) have repercussions for the brain, heart and feet. Lesions in the small vessels (microvascular disease) are responsible for changes in the fundus (retina), kidneys and peripheral nerves. More than half of the diabetic animals in the SZT group showed changes in large vessels, with thickening of the intimate layer. However, the vessels that initially suffer injuries are those of a smaller caliber, where the vessels that irrigate the kidneys are included, thus promoting diabetic nephropathy. Glomerular lesions were observed significantly more in diabetic animals, which corroborates with data from the current literature (15).

It is well established that diabetes causes disorders in several organs and systems, including the musculoskeletal system. Through the histopathological analysis of the animals in the SZT group, it was possible to observe the presence of muscle atrophy, as well as local vascular congestion in more than 70% of the animals (23).

Changes in the musculoskeletal system, when they exist, worsen the general state of health and lead to a worsening in the quality of life of the affected individuals. The current literature shows concern with the changes in this system caused by diabetes mellitus and characterizes them as "diabetic myopathy" (23).

As for the appearance of white adipose tissue in diabetic animals, lipid overload at the level of central adipocytes initiates an inflammatory response and dysfunction at that level. Pro-inflammatory cytokines, which accumulate in the liver and muscle tissue, activate the mechanism of insulin resistance, the same mechanism that is activated in the case of an infection or stress. Since insulin resistance was a physiological process used by man to survive in periods of hunger, the same chronic mechanism is activated inappropriately, leading to the appearance of such changes, making up the metabolic syndrome (24).

The main risk factors for the development of DM2 are low levels of insulin or the inability of tissues directed to insulin to respond adequately to this hormone. Insulin resistance can occur as a result of the insulin signaling pathway at several levels, including reduced insulin receptor concentration, as well as defects in concentration, phosphorylation, activity of the intracellular enzymes involved in the insulin signaling pathway and GLUT4 translocation. *Second Khorami et al* (3), insufficiency in the PI3K / Akt intracellular pathway is the main defect found, in this respect, in diabetes (3).

Chen et al (25) similarly and corroborating with our study, it demonstrated in a model with mice the occurrence of symptoms of insulin resistance appearing concomitantly with the infra-regulation of the PI3K / Akt pathway.

CONCLUSION

The diabetogenic process caused deleterious effects on the integrity of the renal tissue, causing a reduction in immunohistochemical expression in PI3K / Akt signal transduction and histopathological changes in target organs. As diabetes mellitus is a chronic metabolic disease, the inflammatory process triggered by the hyperglycemic state has caused potentially irreversible tissue, organ and vasculature injuries. In this sense, having the flaws in PI3K / Akt signaling as the main responsible for metabolic changes in diabetes mellitus, studies based on the improvement of the function of the intracellular pathway become increasingly necessary, with the objective of increasing the peripheral sensitivity to insulin.

New and important observations should be emphasized. A redundant description of the results is not acceptable. The significance and limitation of the observed findings should be described. There should be a link between the conclusions and the goals of the study.

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TOPICS IN MEDICAL CLINIC

ORIGINAL ARTICLES
ASSOCIATION OF INFLAMMATORY PARAMETERS WITH CLINICAL PROFILE IN SPONDYLOARTHRITIS PATIENTS.

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Key words: Espondiloartrite, Atividade de doença, Perfil clínico, Anti-TNF alfa.
Descritores: Spondyloarthritis; Disease activity; Clinical profile, Anti TNF-alpha.

Abstract

Background: Spondyloarthritis (SpA) are heterogeneous diseases with several forms of musculoskeletal involvement as well as extra articular manifestations. **Aim:** To study if the SpA inflammatory activity is linked to a particular disease phenotype. **Material and Methods:** One hundred and forty one patients with axial SpA (pure or mixed) with and without the main clinical manifestations (low back pain, buttock pain, cervicalgia, enthesitis, dactylitis, peripheral arthritis, uveitis and skin involvement) had their inflammatory activity [measured by ASDAS (Ankylosing Spondylitis disease Activity Score)-CRP (C reactive protein), ASDAS-ESR (erythrocyte sedimentation rate) and global judgment of the physician] as well as treatment requirement compared. The comparison group of patients (without the symptom) were tailored to pair the studied sample in gender, age and disease duration. **Results:** Patients with upper limb arthritis and skin psoriasis had higher ASDAS-CRP (p=0.01 and 0.04 respectively) and ASDAS-ESR (p=0.01 and 0.03 respectively) than those without it. Those with lower limb arthritis were judged to be worse by doctors' global evaluation (p=0.01). Patients with dactylitis required more biologic (p=0.02) and non-biologic treatment (p=0.02) and those with uveitis received more anti TNF-α drugs (p=0.006).
Conclusion: Knowing a SpA patients' clinical profile may help the clinician to judge disease activity and/or treatment requirement. **Endocrinol diabetes clin exp 2021 / 2218 - 2221.**

Resumo
Justificativa: As espondiloartrites (SpA) são doenças heterogêneas com várias formas de envolvimento musculoesquelético, bem como manifestações extra-articulares. **Objetivo:** Estudar se a atividade inflamatória da SpA está ligada a um fenótipo da doença em particular. **Material e Métodos:** Cento e quarenta e um pacientes com Axial SpA tiveram sua atividade inflamatória [medida pelo ASDAS (Ankylosing Spondilitis Disease Activity Score)-PCR (proteína reativa C), ASDAS-VHS (taxa de sedimentação eritrócito) e julgamento global do médico, bem como a necessidade de tratamento comparados de acordo com a presença das principais manifestações clínicas (dor lombar, dor na nádega, cervicalgia, entesite, dactilite, artrite periférica, uveíte e envolvimento com a pele). O grupo de comparação dos pacientes (sem o sintoma) foi recortado para parear a amostra estudada em sexo, idade e duração da doença. **Resultados:** Pacientes com artrite do membro superior e psoríase da pele apresentaram maior ASDAS-CRP (p=0,01 e 0,04, respectivamente) e ASDAS-ESR (p=0,01 e 0,03, respectivamente) do que aqueles sem ele. Aqueles com artrite de membros inferiores foram julgados como piores pela avaliação global dos médicos (p=0,01). Pacientes com dactilite necessitaram de mais tratamento seja com biológicos (p=0,02) e não biológicos (p=0,02). Aqueles com uveíte receberam mais anti-TNF-α (p=0,006). **Conclusão:** Conhecer o perfil clínico de um paciente de SpA pode ajudar o médico a julgar a atividade da doença e/ou a exigência de tratamento. . **Endocrinol diabetes clin exp 2021 / 2118 - 2221.**

INTRODUCTION

SpA (spondyloarthritis) are a group of rheumatic diseases that can be quite heterogeneous from the clinical point of view (1). Regarding musculoskeletal manifestations, they can be divided into disease with axial or with peripheral involvement or with a mixed pattern (axial and peripheral). In addition, extra articular features such as ocular, skin and gastrointestinal involvement may modulate their clinical expression (1).

It is well known that there are common pathophysiologic mechanism under musculoskeletal and extra-articular manifestations in SpA (2) but little is known about the influence of the clinical variability or presence of extra-articular features in the disease activity.

Judging disease activity is fundamental to tailor the treatment of a patient with rheumatic disease as inflammatory disease activity is responsible for the patients' future functional impair (3). Therefore, knowing if the presence of a determinate clinical profile is associated with more inflammation could help the clinician to choose therapeutic strategies.

In the present study we analyzed if a special musculoskeletal complain as well as the presence of extra articular symptoms could help predict which SpA patient will have more disease activity.

MATERIAL AND METHODS

This is a cross sectional study approved by the Committee of Ethics in Research of the three participant centers and all included individuals signed consent. To be included the patients must fulfill the ASAS classification criteria for SpA diagnosis and to have axial involvement (pure axial or axial mixed with peripheral arthritis) (4). This was a convenience sample of SpA patients that come for regular consultation in a tertiary center and that agreed to participate in the study during the period of one year. Epidemiological (gender, age, disease duration, smoking habits) and clinical (presence of lumbar and buttock pain, coxalgia, peripheral arthritis, dactylitis, enthesitis, ocular and skin involvement or psoriasis, presence of HLA B27) and treatment data was obtained through chart review. Clinical data was considered in cumulative way. ASDAS (Ankylosing Spondylitis Disease Activity Score)-CRP (C Reactive Protein) (5) and ASDAS-ESR (Erythrocyte Sedimentation Rate) (5) were measured at inclusion, simultaneously with a doctor's global judgment of disease activity through VAS (Visual Analogic Scale - from zero to 10, where 0 means no disease activity and 10 the worst scenario).

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The parameters of disease activity (ASDAS-ESR, ASDAS-CRP, doctors VAS) were compared according to the presence or not of each clinical finding. For this, we selected the sample of patients with the presence of the studied clinical finding and tailored the comparison sample (patients without the clinical finding studied) in order to be paired for gender, age and disease duration.

Obtained data was analyzed in frequency and contingency tables. Fisher and chi-squared tests were used to compare nominal data and unpaired t test and Mann-Whitney test to compare numerical data. The software Graph Pad Prism 6.0 was used for calculations. The adopted significance was of 5%.

RESULTS

One hundred and forty one patients were included: 71 with pure axial form and 70 with mixed (axial and peripheral) involvement. The patients mean age was of 49.5±12.6 years and the median disease duration was of 17 years (range 12-58). About 26.9 (38/141) were exposed to tobacco (smokers and ex-smokers). HLA B27 was present in 64/111 (57.6%).

In this sample. 132/141(93.6%) were using nonsteroidal anti-inflammatory drugs (AINHs); 83/141 (58.8%) were using glucocorticoids; 100/141(70.2%) were using methotrexate; 13/141 (9.2%) were using leflunomide and 76/141 (53.9%) were using anti TNF-α drugs.

1-Comparison of disease activity and treatment requirement in SpA patients according to musculoskeletal complaints:

The comparison of patients with lumbar pain (n=108;76.5%) with a sample of 33 patients without it paired for gender (p=0.14) , age (p=0.19) and disease duration (p=0.35) showed no differences in the comparison of ASDAS-PCR (p=0.24), ASDAS-ESR (p=0.09); doctor's VAS (p=-0.78) , use of conventional DMARDs (p=0.09) and anti TNF-α drugs (p=0.47).

The comparison of 39/141 (27.6%) patients with buttock pain with 86 patients without it paired for gender (p=0.43); age (p=0.62) and disease duration (p=0.17) showed no differences in the comparison of ASDAS-CRP (p=0.13), ASDAS-ESR (p=0.22) and doctor's VAS (p=0.37). Also no differences in the requirement of conventional DMARDs (p=-60) and anti TNF-α drugs (p=0.68) were noted.

The comparison of patients with cervicalgia (n=62/141= 43.9%) with 79 patients without it paired for gender (p=0.99); age (p=0.12) and disease duration (p=0.35) demonstrated no differences in the ASDAS-CRP (p=0.61); ASDAS –ESR (p=0.66) and doctor's VAS (p=0.77), neither in the DMARDs (p=0.25) or anti TNF-α (p=0.88) use.

The comparison of patients with peripheral arthritis, dactylitis and enthesitis is on **TABLE 1**. In this table, it is possible to see that patients with upper limb arthritis have higher ASDAS-ESR and ASDAS-CRP and used more conventional DMARDs. Those with lower limb arthritis have equivalent ASDAS-CRP and ESR but the doctor's global evaluation classified them with worse disease. Patients with dactylitis required more DMARDs and anti-TNF-α drugs and patients with enthesitis showed no difference with those without it.

TABLE 1- COMPARISON OF INFLAMMATORY ACTIVITY PARAMETERS, DOCTORS GLOBAL EVALUATION (VAS) AND TREATMENT REQUIREMENT ACCORDING TO THE PRESENCE OF ARTHRTIIS, DACTYLITIS AND ENTHESITIS.

	ARTHRITIS UPPER LIMB n= 59/82 (*)			ARTHRITIS LOWER LIMB n=81/55(*)			DACTYLITIS n= 24/117(*)			ENTHESITIS n= 54/87(*)		
Pairing	Gender	Age	DD.	Gender	Age	DD.	Gender	Age	DD.	Gender	Age	DD.
P value	0.85	0.93	0.35	0.46	0.28	0.87	0.43	0.87	0.17	0.52	0.27	0.55
	WITH	**WITHOUT**	**P**	**WITH**	**WITHOUT**	**P**	**WITH**	**WITHOUT**	**P**	**WITH**	**WITHOUT**	**P**
ASDAS CRP Median (IQR)	2.59 (1.79-3.57)	2.07 (0.94-2.98)	**0.01**	2.10 (1.15-3.32)	2.36 (1.50-3.20)	0.64	2.20 (1.07-3.07)	2.20 (1.20-3.32)	0.70	2.23 (1.58-3.34)	2.15 (1.07-3.30)	0.51
ASDAS ESR Mean ± SD	2.95±1.19	2.51±1.00	**0.01**	2.71±1.15	2.68±1.02	0.87	2.37±1.04	2.76±1.10	0.11	2.77±1.13	2.65±1.08	0.52
VAS Doctor	4 (2-6)	3.0 (2.-5)	0.15	4 (2.5-6)	3.0 (1.0-5)	**0.01**	4 (2.2-6)	3 (2-5)	0.15	3 (1-5)	3 (2-6)	0.30
DMARds (n)	48 (81.3%)	52 (63.4%)	**0.02** (**)	61 (75.3%)	35 (63.3%)	0.14	22 (91.6%)	78 (66.6%)	**0.02** (§)	40 (74.0%)	60 (68.9%)	0.73
Anti TNF-α (n)	32 (54.2%)	44 (53.6%)	0.94	43 (53.0%)	31 (56.3%)	0.70	18 (75%)	58 (49.55)	**0.02** (§§)	33 (61.1%)	43 (49.4%)	0.25

(*) refers to number of patients with the finding of studied sample/controls; n= number; IQR= interquartile rate; SD= standard deviation; DD.= disease duration.

ASDAS= Ankylosing Spondylitis Disease Activity Score; ESR= erythrocyte sedimentation rate; CRP= C reactive protein; VAS= Visual analogic scale; DMARds- Disease modifying anti rheumatic drugs.

(**) OR=2.5; 95% CI=1.1-5.5; (§)- OR=5.2; 95%CI= 1.16-23.3; (§§)´OR=3.0; 95CI= 1.1-8.2.

2- Comparison of disease activity and treatment requirement in SpA patients according to extra articular manifestations.

The comparison of patients with uveitis and skin disease is on **TABLE 2**. Only five (3.5% of the sample) had inflammatory bowel disease not allowing comparisons.

TABLE 2 – COMPARISON OF INFLAMMATORY ACTIVITY DATA, DOCTOR'S VAS AND TREATMENT REQUIREMENT IN SpA PATIENTS WITH AND WITHOUT UVEITIS AND SKIN DISEASE.

	UVEITIS N=31/87 (*)			SKIN DISEASE N=37/41 (*)		
Pairing	**Gender**	**Age**	**DD**	**Gender**	**Age**	**DD.**
P value	0.57	0.18	0.10	0.22	0.08	0.67
	With	**Without**	**P**	**With**	**Without**	**P**
ASDAS-CRP Median (IQR)	2.17 (1.33-2.82)	2.20 (0.94-3.30)	0.75	13.0 (7.5-19.0)	15.0 (8.5-18.0)	**0.04**
ASDAS-ESR Mean ± SD	2.50±1.12	2.74±1.06	0.28	3.01±1.23	2.46±0.85	**0.03**
DOCTOR'S VAS Median (IQR)	3 (0-5)	4 (2-5)	0.35	3.0 (2.0-5.0)	4.0 (2.0-5.5)	0.44
DMARDs users (n)	20 (64.5%)	70 (80.4%)	0.07	35 (94.5%)	25 (60.9%)	**0.0004 (§)**
Anti-TNF-α users (n)	24 (77.4%)	43 (49.4%)	**0.006** (**)	20 (54.0%)	26 (63.4%)	0.40

(*) refers to number of patients with the finding/ controls; n= number; IQR= interquartile rate; SD= standard deviation; DD.= disease duration; AS-DAS= Ankylosing Spondylitis Disease Activity Score; ESR= erythrocyte sedimentation rate; CRP= C reactive protein; VAS= Visual analogic scale; DMARDs- Disease modifying anti rheumatic drugs.
(**) OR= 3.5; 95%CI=1.3-8.9; (§)- OR=11.2.7;(5%CI=2.3-53.1).

DISCUSSION

The results of this study show that SpA patients with upper limb arthritis have more disease activity measured by ASDAS-ESR and ASDAS-CRP and require more use of conventional DMARDS than those without it, but that this was not recognized by the global judgment made by the attending clinician. On the other hand, the physician considered patients with lower limb arthritis worse than those without it, but their inflammatory activity is considered the same by ASDAS-ESR and CRP. This is an interesting finding as it suggests that the attending physician probably values the lower limb complaints more than the upper ones. Contrary to our findings, Shali et al. (6) studying foot involvement in SpA patients found that this type of manifestation was associated with higher inflammation in blood tests (ESR and CRP). However, these authors studied the foot involvement as a whole, including in their observation entheseal disorders and not just arthritis. Ozaras et al., (7) studying feet function in SpA, could not correlate its impairment with inflammatory tests showing that foot involvement may not be directly affected from the disease activity but suffers influence of mechanical and ligament derangements. Probably such aspects influence the global health judgment by the physician. This also accentuates the need to evaluate foot involvement separately from global disease activity in order to improve patients' care.

The occurrence of dactylitis did not alter the measurements of disease activity but implicates in higher treatment requirement of both traditional DMARDs and biologic drugs. This manifestation results from inflammation in the finger flexor tendon sheaths (8), and according to some authors may be associated with synovitis (9). It is considered a severity marker in SpA mainly in the psoriatic form where it is more common (8). So, it is not surprising that such patients will need more treatment as demonstrated in the present study. Traditionally, dactilyitis are treated with nonsteroidal anti-inflammatory drugs and local corticosteroid injections; DMARDs and anti TNF-α drugs have also been used (8,10).

According to the present data, SpA patients with uveitis are more frequently treated with anti TNF-α drugs despite equal disease activity than those without it. This is easily explained, as uveitis inflammatory activity does not reflect in the parameters evaluated by ASDAS. On the other hand, anti TNF-α drugs are frequently indicated in more severe cases of uveitis (11,12). Our data also shows that patients who developed uveitis do not have a more active articular disease and do not agree with the findings of Chen et al. (13) that observed association between uveitis with SpA activity and functional indexes. However, this latter author studied a different activity index - the BASDAI (Bath Ankylosing Spondylitis Disease activity index) – and a sample of patients with diverse ethnic background (from Taiwan). This may explain the differences found; it is well known that uveitis occurrence suffers genetic influence (14).

Skin involvement was associated with more inflammatory activity measured by both ASDAS-ESR and ASDAS-CRP and also with higher requirement of DMARDs. It is possible that the inflammatory process in the skin itself (15) increased the results of the inflammatory blood test resulting in higher values of activity indexes.

Limitations of this study are due to its cross-sectional design and to the small studied sample. However, it highlights the idea that the knowledge of SpA patients' clinical profile may help the clinician to judge disease activity and/or treatment requirement.

CONCLUSION

Concluding, in this sample it was detected that the occurrence of upper limb arthritis and skin psoriasis was linked to higher ASDAS-CRP and ASDAS-ESR than in those without it. Those with lower limb arthritis were judged to be worse by doctors' global evaluation. Patients with dactylitis required more biologic and non-biologic treatment and those with uveitis received more anti TNF-α drugs.

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TOPICS IN MEDICAL CLINIC

ORIGINAL ARTICLES
METABOLIC SYNDROME PREVALENCE IN PSORIASIS PATIENTS WITH AND WITHOUT ARTHRITIS: A COMPARATIVE ANALYSIS

ESTUDO COMPARATIVO DA PREVALÊNCIA DE SÍNDROME METABÓLICA EM PACIENTES DE PSORIASE COM E SEM ARTRITE.

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Abstract

Introduction: The high prevalence of metabolic syndrome (MS) is a serious problem in psoriasis and is responsible for the decrease in survival of these patients. **Objective:** To verify whether the association of psoriasis (Pso) with arthritis (Psoriatic arthritis or APso) influences the prevalence of MS. **Material and Methods:** This is an observational cross-sectional study that establishes a comparative analysis of the prevalence of MS in Pso patients with (n=95) and without APso (n=97) and control population (n=105). Epidemiological and clinical data on cutaneous involvement (psoriasis subtype, and extent of skin lesion), arthritis (type of joint involvement and extra-articular manifestations), presence of comorbidities such as diabetes mellitus (DM) and hypertension (SAH) and use of medications were obtained by analysis of medical records and direct interview with patients. Weight, height, BMI (body mass index), abdominal circumference and blood pressure were collected through physical examination. Laboratory tests (total cholesterol, HDL and LDL cholesterol, triglycerides and fasting glycemia) were obtained simultaneously with data collection. The prevalence of MS was estimated using the Adult Treatment Panel of the National Cholesterol Education Program III (NCEP-ACT III). **Results:** The prevalence of MS in patients with psoriasis and skin involvement only was 48/97 (49.4%), in APso was 50/95 (52.6%) and in controls of 36/105 (34.2%) with p=0.01. No difference was found in the prevalence of MS between the patients with only skin psoriasis and APso groups (p=0.6). Comparing controls with patients with psoriasis with and without APso, it was found that the controls presented lower body mass index (BMI) (p<0.0001), systolic blood pressure (p<0.01) and abdominal circumference (p<0.0001). Comparing patients with psoriasis without arthritis and with APso, significance was found for BMI (p<0.01), systemic blood pressure (p<0.04), dyslipidemia (p<0.0043), fasting glucose (p <0.003)) all with higher values in patients with APso. **Conclusion:** There is a high prevalence of MS in patients with Pso and the association with arthritis does not alter this prevalence. However, patients with Apso have more hypertension, dyslipidemia, higher BMI, and higher serum glucose levels than those without arthritis **Endocrinol diabetes clin exp 2021 / 2222 - 2225.**

Resumo

Introdução: A alta prevalência de síndrome metabólica (SM) é um problema grave em psoríase sendo responsável pela diminuição da sobrevida destes pacientes. **Objetivo:** Verificar se a associação da psoríase (Pso) com artrite (Artrite psoriásica ou APso) influi na prevalência de SM. **Material e Métodos:** Este é um estudo transversal osbervacional que faz uma análise comparativa da prevalência de SM em pacientes com Pso (n=95) e sem APso (n=97) e população controle (n=105). Dados epidemiológicos e clínicos do envolvimento cutâneo (forma, e extensão da lesão de pele), da artrite (tipo de envolvimento articular e manifestações extra-articulares), presença de comorbidades como diabetes mellitus (DM) e hipertensão (HAS) e de uso de medicamentos foram obtidos por análise dos prontuários e entrevista direta com os pacientes. Através de exame físico coletaram-se valores de peso, altura, IMC (índice de massa corporal), circunferência abdominal e pressão arterial. Exames laboratoriais (colesterol total, HDL e LDL colesterol, triglicérides e glicemia de jejum) foram obtidos simultaneamente com a coleta dos dados. A prevalência da SM foi estimada usando o *Adult Treatment Panel do National Cholesterol Education Program III* (NCEP-ACT III). **Resultados:** A prevalência de SM na Pso só de pele foi de 48/97 (49,4%), na APso de 50/95 (52,6%) e 36/105 (34,2%) nos controles com p=0,01. Nenhuma diferença foi encontrada na prevalência da SM entre os grupos Pso pura e APso (p=0.6). Comparando-se controles com pacientes com Pso com e sem APso, verificou-se que os controles apresentaram menor índice de massa corporal (IMC) (p<0,0001), pressão arterial sistólica (p<0,01) e circunferência abdominal (p<0,0001). Comparando-se pacientes com PSo sem e com APso encontrou-se significância para o IMC (p<0,01), pressão arterial sistêmica (p<0,04), dislipidemia (p<0,0043), glicemia de jejum (p <0,003)) todos com valores mais elevados em pacientes com APso. **Conclusão:** Existe uma alta prevalência da SM em pacientes com Pso e a associação com artrite não altera esta prevalência. Entretanto, pacientes com Apso apresentam mais HAS, dislipidemia, maior IMC e níveis séricos de glicose mais elevados que aqueles sem artrite. **Endocrinol diabetes clin exp 2021 / 2222 - 2225.**

INTRODUCTION

Psoriasis (Pso) is a chronic inflammatory disease of the

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skin, with a genetic background that is characterized by reddish and desquamative lesions generated by the proliferation and altered differentiation of keratinocytes (1). The extent of cutaneous involvement is diverse ranging from some localized plaques to the general, erythrodermic, skin involvement (1). It can occur at any age, although most cases develop in individuals under forty years of age, being uncommon in children (1).

Psoriatic arthritis (Pso) happens in 20-30% of patients with cutaneous psoriasis (2) being more common in those with nail involvement, scalp and intergluteal skin lesions. (3). Several subtypes of arthritis can be seen such as asymmetric oligoarthritis (most common), arthritis of distal interphalangeal joints; symmetrical polyarthritis; spondyloarthritis and mutilans form (4). It also involves extra-articular structures promoting the appearance of uveitis, entesitis and dactylitis (5).

Patients with Pso may have metabolic disorders, which compromise their morbidity and mortality. Obesity, diabetes mellitus (DM), hypertension (SAH) and hepatic steatosis are well-recognized complications of this disease (6). The presence of chronic inflammatory process is linked to increased insulin resistance, and endothelial dysfunction (7).

Although the association between cardiovascular risk factors and psoriasis is well recognized, there are few studies to verify whether or not arthritis concomitance modifies this profile. If patients with APso are believed to have a more pronounced inflammatory process than those with pure cutaneous Pso, one might hypothesize that the presence of arthritis would have an aggravating role in this context. Therefore, this study aims to verify whether the presence of arthritis modifies the prevalence of metabolic syndrome (MS) of the population with Pso.

MATERIAL AND METHODS

The present study was approved by the local Ethics and Research Committee (protocol number 2.346.248) and all participants signed informed consent form. It was performed in the Rheumatology and Dermatology outpatient clinics of a single tertiary care hospital and had a convenience sample composed of all Pso patients with and without APso who attended to the outpatient clinic for a period of one semester and consented to participate in the research. They were invited to participate in the study according to the order of arrival for routine consultations. Individuals of both sexes over 18 years of age were included; pregnant women and individuals with untreated hypothyroidism were excluded. As a control group, self-declared healthy individuals who served as a companion for patients during the consultation were studied. Epidemiological data (gender, race, smoking, age and time of disease), clinical data of psoriasis and history of comorbidities such as arterial hypertension (HAS), dia-

betes mellitus (DM) and dyslipidemias were obtained from charts.

Patients were examined and weight and height values were obtained to calculate body mass index (BMI) and measurement of abdominal circumference. Blood pressure measurement was done with the individual seated and after 10 minutes of rest. BMI was obtained by dividing weight into kilograms by squared height. Waist circumference was measured at the midpoint of the last rib and iliac crest with the patient standing. Simultaneously, data were obtained regarding fasting glycemia, lipid profile (cholesterol, HDL and LDL cholesterol and triglycerides), These measurements were performed after 12 hours of fasting.

The presence of metabolic syndrome was judged according to the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ACT III) (8). Current ATP III criteria (8) defines the metabolic syndrome as the presence of any three of the following five items:

1-Abdominal obesity, defined as a waist circumference in men ≥102 cm and in women ≥88 cm;

2-Serum triglycerides ≥150 mg/dL or drug treatment for elevated triglycerides;

3-HDL cholesterol <40 mg/dL in men and <50 mg/dL (in women or drug treatment for low HDL cholesterol;

4-Blood pressure ≥130/85 mmHg or drug treatment for elevated blood pressure;

5-Fasting plasma glucose ≥100 mg/dL or drug treatment for elevated blood glucose.

The data obtained were collected in frequency and contingency tables and their distribution was analyzed by the Shapiro--Wilk test. The central trend was expressed in mean and standard deviation (SD) when the data were parametric and in median and interquartile range I (IIQR) when nonparametric. The Chi--square test was used to compare nominal data and those of /Mann Whitney and non-paired t for comparison of numerical data chosen according to the sample distribution. The level of significance adopted was 5%. The calculations were made with the help of graph pad prism software, version 5.0.

RESULTS

The sample was composed of 105 control individuals and 192 individuals with psoriasis, 97 with exclusively skin disease and 95 with APso. The data of the pairing of controls with total psoriasis sample are in **table 1**. This table also shows the comparative analysis between components of metabolic syndrome in these two populations and it is possible to verify that patients with psoriasis (with and without arthritis) have more metabolic syndrome than controls mainly the costs of obesity, HAS and DM.

Table 1 – Comparison of epidemiological data and items of metabolic syndrome between controls and psoriasis patients (with and without arthritis).

	Controls N=105	With psoriasis N=192	P
Gender: female/male	54/51	95/97	0.74
BMI – median (IQR) – kg/m^2	26.4 (22.8-29.2)	28.4(25.4-32.4)	<0.0001
SBP– median (IQR)-mm Hg	120 (115-130)	130 (120-140)	0.01
DBP- median (IQR)- mm Hg	80 (70-90)	80 (70-90)	0.45
Waist circumference - mean±SD - cm	92.6±16.1	100.2±12.4	<0.0001
Triglycerides– median (IQR) – mg/dL	116.0(83.0-16.0)	115.0 (80.0-165.0)	0.97
Total cholesterol – median (IQR) – mg/dL	179.0(155.1-211.4)	181.0 (161.0-202.5)	0.97
HDL cholesterol – median (IQR) – mg/dL	47.0 (39.0-59.7)	46.0 (38.0-55.0)	0.16
LDL cholesterol – median (IQR) – mg/dL	102.8 (84.9-125.0)	106.4 (87.0-130.5)	0.69
Fasting glucose – median (IQR) – mg/dL	91.0 (87.8-101.0)	93.0 (82.0-103.3)	0.86
History of Arterial Hypertension (n)	44/105 (41.9%)	85/192 (44.2%)	0.69
History of Diabetes Mellitus (n)	10/105 (9.52 %)	28/192 (14.5%)	0.27
Treatment for dyslipidemia (n)	24/105 (22.8%)	64/192 (33.3%)	0.05
Diagnosis of metabolic syndrome (n)	36/105 (34.2%)	98/191 (51.3%)	0.004 (*)

n=number; SD=standard deviation; IQR=interquartile rate; BMI= body mass index; SBP= systolic blood pressure; DBP=diastolic blood pressure; HDL=high density lipoprotein; LDL=low density lipoprotein.
(*)OR= 2.02; 95% IC=1.2- 3.3.

Comparative analysis of the psoriasis population with and without arthritis showed the results of **Table 2**. This table compares the data regarding MS and systemic treatment established in both populations.

TABLE 2 - Comparison of epidemiologic and metabolic profile of patients with psoriasis with and without arthritis.

	Psoriasis without arthritis (n=97)	Psoriasis with arthritis (n=95)	P
Gender female/male	45/52	50/45	0.38
BMI Median (IQR) Kg/m2	27.5 (24.9-30.9)	29.8 l (26.0-33.6)	0.01
SBP – median (IQR)- mm Hg	130 (120-140)	126 (110-140)	0.08
DBP- median (IQR) – mm Hg	80 (70-90)	5 80 (70-90)	0.57
Waist circunference- mean – SD (cm)	100.7±12.8	99.7±12.1	0.59
History HBP - (n)	36/97 (37.1%)	49/95 (51.5%)	0.04
Treatment for dyslipidemia (n)	23/97 (23.7%)	41/95 (43.1%)	0.004 (*)
History DM (n)	10/97 (10.3%)	18/95 (18.9%)	0.09
Fasting blood glucose – median (IQR) mg/dL	89.0(80.0-98.5)	96.0 (86.5-105.5)	0.003
Triglycerides – median (IQR)- mg/dL	118.0 (78.0-164.5)	115.0 (80.0-175.0)	0.55
Cholesterol - median (IQR) mg/dL	179.0 (155.1-211.5)	181.0 (160.0-203.5)	0.26
HDL cholesterol - median (IQR)- mg/dL	42.0 (37.0-50.5)	50.0 (39.2-60.0)	0.002
LDL cholesterol- median (IQR)- mg/dL	106 (79.6-130.5)	104.9 (92.8-138.8)	0.41
Metabolic syndrome (n)	48/97 (49.4%)	50 /94 (53.1%)	0.60

n=number; IQR= interquartile rate; SD= standard deviation; BMI- body mass index; SBP=systolic blood pressure; DBP=diatolic blood pressure; HDL=high density lipoprotein; LDL= low density lipoprotein; HBP=high blood pressure; DM=diabetes mellitus.
(*) OR=2,4; 95%CI= 1,31 to 4,53

DISCUSSION

Our data confirm the high prevalence of MS in the population with psoriasis when compared to the normal population, although it was not possible to establish a difference in the psoriasis population with and without arthritis. These findings are in agreement with those of Salunke et al (9) who studied 95 patients with psoriasis and 95 controls finding an OR of 2.39 for MS in psoriatics in general, a number quite similar to ours of 2.02. These authors also noted that the prevalence of MS increased along with the extent of the cutaneous process (9).

Analyzing the factors that lead the PSo population to have a higher prevalence of MS, it is observed that, in relation to the normal population, psoriatic individuals have higher BMI, higher abdominal circumference and a greater number of individuals with dyslipidemia, and these points treatment of this group of patients. *Madanagobalane S*, *Anandan S*. (10) observed an increase in obesity in individuals with psoriasis when compared to controls. *Balci et al*. (11) evaluated the increase in visceral fat

level in patients with psoriasis and its contribution to MS. In this study, the visceral adipose area was shown to be increased in patients with psoriasis and associated with the presence of MS. However, *Gönül et al*. (12) obtained different results, finding no significant difference in abdominal fat index between patients with psoriasis and controls and concluding that the presence of psoriasis was not an independent risk factor for increase in abdominal fat index in patients with MS. In the present study, an increase in the waist diameter was found, pointing to increased abdominal fat in psoriatic patients. It is known that increased abdominal fat is associated with insulin resistance and increased cardiovascular risk (13). Adipose tissue is responsible for a low-grade inflammatory process generated by the infiltration of immune cells and production of adipokines (14).

Lin et al. (15) found that patients with APso had a higher prevalence of MS and more thickening of the carotid media than those with skin-only psoriasis attributing this increase to the inflammatory process generated by joint inflammation. It was not possible to prove in our sample that the occurrence of arthritis aggravates the prevalence of MS in individuals with psoriasis. However, some differences between the two groups were found as increased dyslipidemia, history of high blood pressure, BMI and fasting glucose in individuals with arthritis. It is possible that the use of medications such as non-hormonal anti-inflammatory drugs and glucocorticoids contributed to these findings. In addition, individuals with arthritis tend to have a greater sedentary lifestyle since joint inflammation generates incapacity and pain to movement. On the other hand, these arthritis patients were being treated in a tertiary hospital where the "treat to target" strategy is adopted, i.e. to seek the lowest level of inflammatory activity possible. The restricted control of the inflammatory process may have contributed to minimize its metabolic consequences.

This study has several limitations; the first is generated by the characteristics of its cross-sectional design. Another is the small number of participants. However, he points to the fact that individuals with psoriasis are disadvantaged from a metabolic point of view and the physician who treats them should take a holistic approach, aiming not only at the treatment of psoriasis or arthritis, but also on the aspects that cover metabolic syndrome, in order to improve morbidity and mortality in this context.

CONCLUSION

In conclusion, it is possible to highlight that, in our study, there was an increase in the prevalence of MS in patients with psoriasis, but that the occurrence of psoriatic arthritis did not alter this prevalence. However, patients with psoriatic arthritis have more hypertension, dyslipidemia and higher BMI than patients with arthritis-free psoriasis.

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