

## Carotid intima-media thickness in patients with psoriasis with and without metabolic syndrome

### *Grosor de intima-media carotídea en pacientes con psoriasis con y sin síndrome metabólico*

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### Abstract

**Introduction:** Patients with psoriasis have an increased prevalence of cardiovascular risk factors as well as cardiovascular disease. **Objective:** To determine if patients with psoriasis and metabolic syndrome (MS) have a higher frequency of sub-clinical atherosclerosis compared with those with psoriasis without MS. **Materials and Methods:** A cross-sectional study was conducted in patients with psoriasis; MS was defined according to ATP III criteria. Demographic, clinical, and anthropometric data were obtained. Blood chemistry, high sensitive C-reactive protein (hs-CRP), and insulin were measured. Subclinical atherosclerosis was defined as high carotid intima-media thickness (CIMT) by Mode B ultrasound. **Results:** 92 patients with psoriasis were included, 67 (72.8%) with MS and 25 (27.2%) without MS. Subjects with psoriasis and MS had significantly higher weight, body mass index, waist circumference, systolic blood pressure, glucose, insulin, triglycerides, insulin resistance, hs-CRP, and lower level of high-density lipoprotein cholesterol, compared with subjects without MS. High CIMT was greater in patients with psoriasis and MS than in those without MS. Age and MS were independent predictors of increased CIMT after multiple linear regression analysis. **Conclusions:** MS is associated with greater inflammation and subclinical atherosclerosis in patients with psoriasis.

**Keywords:** Psoriasis. Carotid intima-media thickness. Metabolic syndrome. Subclinical atherosclerosis.

### Resumen

**Introducción:** Los pacientes con psoriasis tienen prevalencia incrementada de factores de riesgo y enfermedad cardiovascular. **Objetivo:** Determinar si los pacientes con psoriasis y síndrome metabólico (SM) tienen mayor frecuencia de atherosclerosis subclínica comparados con pacientes con psoriasis y sin SM. **Material y Métodos:** Estudio transversal, en pacientes con psoriasis; SM fue definido con base en criterios ATP III. Se obtuvieron datos demográficos, clínicos y antropométricos. Se realizó química sanguínea, proteína C reactiva de alta sensibilidad (PCR-hs) e insulina. Atherosclerosis subclínica fue definida como grosor de íntima-media carotídeo (GIMC) elevado, medido por ultrasonido tipo B. **Resultados:** Se incluyeron 92 pacientes con psoriasis, 67 (72.8 %) con SM y 25 (27.2 %) sin SM. Los sujetos con psoriasis y SM tuvieron valores

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Date of reception: 02-04-2021

Date of acceptance: 17-08-2021

DOI: 10.24875/ACM.21000106

Available online: 09-09-2021

Arch Cardiol Mex (Eng). 2022;92(3):305-311

[www.archivoscardiologia.com](http://www.archivoscardiologia.com)

significativamente más elevados de peso, índice de masa corporal, circunferencia de cintura, tensión arterial sistólica, glucosa, insulina, triglicéridos, resistencia a insulina, PCR-hs y menores niveles de colesterol de alta densidad, comparados con sujetos sin SM. El GIMC fue mayor en pacientes con psoriasis y SM. La edad y el SM fueron predictores independientes de mayor GIMC después de realizar múltiples análisis de regresión lineal. **Conclusiones:** Síndrome metabólico está asociado con mayor inflamación y atherosclerosis subclínica en pacientes con psoriasis.

**Palabras clave:** Psoriasis. Grosor de íntima-media carotídea. Síndrome metabólico. Atherosclerosis subclínica.

## Introduction

Psoriasis is a chronic inflammatory disease, immune-mediated disorder; the most common clinical presentation is in plaques. The estimated prevalence is 2% in Mexico<sup>1</sup>. The etiology is unknown but scientific evidence involves immune, genetic, psychosomatic, environmental, and bacterial factors<sup>2,3</sup>. Patients with psoriasis have a higher incidence of obesity, diabetes mellitus, cardiovascular disease and stroke, and those with severe grades have increased mortality risk, particularly younger patients<sup>4,5</sup>. The presence of pro-inflammatory cytokines (TNF $\alpha$  and IL-6) and systemic inflammation associated with psoriasis increased immunological and metabolic changes as insulin resistance, causing endothelial dysfunction, atherosclerosis, and coronary artery disease<sup>6</sup>. In a previous study<sup>7</sup>, patients with psoriasis had a frequency of MS of 41.7% define by National Cholesterol Education Program, Adult Treatment Panel III criteria (NCEP ATP III), significantly increased than the control group without psoriasis (25.2%). Gisondi<sup>8</sup> reported that patients with psoriasis had more cardiovascular risk factors and the Framingham 10-year risk was higher. Increased carotid intima-media thickness (CIMT) is a well-established marker of subclinical atherosclerosis and is predictive of subsequent cardiovascular events in asymptomatic subjects<sup>9</sup>. However, the presence of subclinical atherosclerosis in patients with psoriasis has not been studied in Mexican population. The objective of the study was to determine whether the presence of MS is associated with subclinical atherosclerosis evaluated with a noninvasive method as the CIMT, in patients with psoriasis.

## Materials and methods

A cross-sectional study was conducted. Men and women, aged 18–74 years with clinical and/or histopathological diagnosis of psoriasis, regardless of the clinical variety were sequentially included from the Dermatology outpatient clinic of the General Hospital Dr. Manuel Gea Gonzalez, in Mexico City, from October 2015 to September 2016. Women with oral

contraceptive treatment were excluded as well as subjects with incomplete analysis results. The protocol was approved by the Ethics and Research Committees of General Hospital Dr. Manuel Gea Gonzalez. All participants signed voluntary informed consent before participation.

## Collection data

Participants completed questionnaires to obtain information about demographics and clinical information (type of psoriasis, time of evolution, and treatment; the severity of the disease was calculated with the psoriasis area severity index [PASI])<sup>10</sup>. A physical examination was conducted to obtain anthropometric parameters: weight, height, and waist circumference, performed by trained personnel with standardized methods. Body mass index (BMI) calculated as weight (kg)/height(m<sup>2</sup>). Blood pressure was measured three times with a digital sphygmomanometer Welch Allyn®, after at least 5 min of rest, in sitting position. The average of the last two measurements was used for the analysis. Metabolic syndrome (MS) was defined by NCEP ATP III<sup>11</sup>, by the presence of at least three or more components: increased waist circumference (>90 cm in men or >80 cm in women, modified with cutoff points for Asian population, which are the same cutting points used in the National Health Surveys of our country), hypertriglyceridemia (triglycerides  $\geq$  150 mg/dL), high-density lipoprotein cholesterol (HDL-C) ( $<40$  mg/dL in men or  $<50$  mg/dL in women), hypertension (blood pressure  $\geq$  130/85 mmHg), and hyperglycemia (fasting levels of serum glucose  $\geq$  100 mg/dL). After a 12 h fast blood samples were obtained for measuring serum glucose, glycosylated hemoglobin, urea, creatinine, uric acid, total cholesterol, HDL-C, triglycerides; total, direct and indirect bilirubin, albumin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, insulin, and high sensitive C-reactive protein. Low lipoprotein density cholesterol (LDL-C) was calculated. All samples were performed in a centralized laboratory with standardized methods and automated equipment

DXC 800 Beckman Coulter®. All patients underwent liver ultrasound to determine the presence of nonalcoholic fatty liver disease (NAFLD). Hepatic steatosis was defined as an increase in the echogenicity of the hepatic parenchyma compared with neighboring structures such as the right kidney<sup>12</sup>.

### **Subclinical atherosclerosis**

CIMT is a marker of subclinical atherosclerosis. The study was performed with the patient in supine position with the extended neck using high-resolution ultrasound equipment in B mode (Sonosite MicroMaxx®), with a 13-6 MHz transducer. Measures of the intima-media common carotid artery (CCA) were obtained on the longitudinal plane in the distal wall of the carotid artery to 2 cm from the carotid bulb bifurcation. Between the arterial-intima lumen interface and the media-adventitia interface of the distal wall, five measurements were done in the right and left CCA (5 on each side). The CIMT was the average of all measurements, CIMT values greater than 75<sup>th</sup> percentile specific for age group and gender were considered high (hCIMT) and indicative of atherosclerosis subclinical<sup>13</sup>. Carotid plaque was defined by Mannheim consensus as a focal structure that encroaches into the lumen by at least 0.5 mm or 50% of the surrounding intima-media thickness value or that has a thickness >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface. Measurements were obtained by a single trained physician without knowledge regarding the presence of MS. The intraobserver correlation coefficient was 0.96.

### **Statistical analysis**

Continuous variables are reported as means  $\pm$  (standard deviation) or median (lower-upper quartiles), and categorical variables as frequencies and percentages, Student's t-test was used to compare the CIMT and other variables between patients with and without MS; Chi-square test for compare frequency of hCIMT as well as coronary risk factors frequencies. Pearson's correlation coefficient was used to correlate variables and multiple linear regression analysis to predict the independent predictors of CIMT. The outcome variable was CIMT and the significant predictors in bivariate correlation (age, MS, glucose, PASI, and duration of psoriasis) were included in the model  $\beta$  Standardized coefficient of determination was calculated. A  $p < 0.05$  was considered statistically significant. We used SPSS statistical program version 15.

## **Results**

92 patients with psoriasis were included, mean age was  $52.6 \pm 13.2$  years, and 52.3% of them were women. Patients were divided with MS ( $n = 67$ , 72.8%) or without MS ( $n = 25$ , 27.2%). The most frequent clinical variety was in plaques (89.6%) and the most used treatment was topical steroids, the duration and severity were similar in both groups; the clinical and anthropometric data are shown in **table 1** and metabolic characteristics in **Table 2**. The prevalence of MS components and cardiovascular risk factors in patients with psoriasis was: diabetes 28.3%, hypertension 25%, obesity 45.7%, abdominal obesity 90.2%, hypoalphalipoproteinemia 76.1%, hypertriglyceridemia 58.7%, non-alcoholic fat liver 81.5%, insulin resistance 57.6%, and hypercholesterolemia 15.2%. Underlying comorbidities in the study population were: acute myocardial infarction 14.1%, cerebrovascular event 3.3%, and hypothyroidism 14.1%. Medication reported by patients: metformin 24%, sulfonylurea 8.7%, insulin 5.4%, anti-hypertensive drugs 24%, statins 14.1%, and fibrates 10.9% (data not shown). The frequency of MS components is shown in **table 3**. **Figure 1** shows the CIMT values of patients with and without MS. Subjects with MS had significantly higher average values of CIMT compared to those without MS ( $0.67 \pm 0.12$  mm vs.  $0.60 \pm 0.10$  mm,  $p = 0.01$ ), as well the maximum values ( $0.74 \pm 0.14$  mm vs.  $0.65 \pm 0.10$  mm,  $p = 0.005$ ). The prevalence of carotid plaque was higher in patients with MS psoriasis (44.7% vs. 24.0%,  $p = 0.055$ ). Patients with psoriasis had a simple positive correlation with: age, MS, glucose, duration of the disease, and PASI with CIMT. However, only age and MS remained an independent predictor of CIMT after multiple linear regression analysis (**Table 4**).

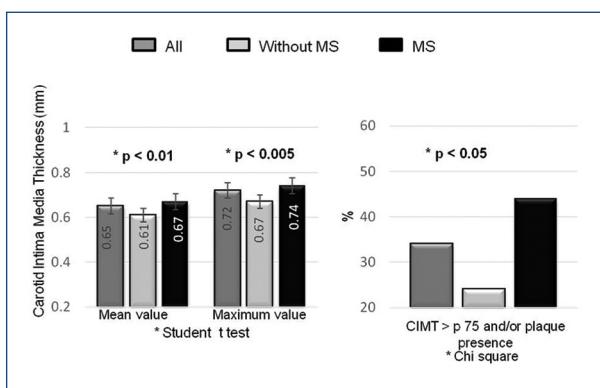
## **Discussion**

In this cohort of patients with psoriasis, we found a high frequency of MS (72.8%) and CIMT elevated (39.1%) which was higher in patients with MS (44.7%). Multiple linear regression analysis revealed that age was the most important independent predictor of CIMT followed by MS in our population. Using the ATP III criteria for MS, the prevalence in Mexican adults over 20 years were 26.6% in 1993<sup>14</sup>, 36.8% in 2006<sup>15</sup>, and 45% according to the latest Mexican nutritional survey (ENSANUT 2012)<sup>16</sup>. In a previous study Espinoza et al. reported a prevalence of MS of 41.7% in patients with psoriasis and 20% in those without psoriasis<sup>7</sup>. The

**Table 1.** Clinical and anthropometric characteristics of patients with psoriasis, according to metabolic syndrome (ATP III)

Variable	All (n = 92)	Without MS (n = 25)	MS (n = 67)	p
Age (years)	52.6 ± 13.2	49.1 ± 13.2	54.2 ± 13.2	0.107
Sex F/M n (%)	49(53)/43(47)	16(32)/19(21)	33(67)/33(78)	0.23
Weight (kg)	76.5 ± 16.2	68.1 ± 14.8	79.6 ± 15.5	0.002
Height (cm)	158.5 ± 9.4	158.8 ± 8.9	158.4 ± 9.6	0.876
BMI (kg/m <sup>2</sup> )	30.5 ± 5.4	27.0 ± 4.9	31.7 ± 5.0	<0.001
Waist (cm)	99.6 ± 13.6	89.9 ± 13.2	103.4 ± 11.8	<0.001
SBP (mmHg)	120.1 ± 14.3	115.5 ± 10.7	121.7 ± 15.1	0.033
DBP (mmHg)	72.0 ± 8.5	70.4 ± 6.2	72.4 ± 9.3	0.239
Psoet (months)	96 (36-240)	120(26-300)	90(36-195)	0.277
PASI	6.4 (3.5-9.7)	6(2.7-10.6)	6.7(3.6-9.8)	0.954
DM2 n (%)	29 (31.9)	2 (8)	27(41)	0.002
HTA n (%)	23 (22.2)	4 (16)	19 (28.4)	0.173
Obesity n (%)	42 (31)	6(24)	36 (39.1)	0.016
IR n (%)	53 (45.5)	5 (20)	48 (71)	<0.001
Non-alcoholic liver fat n (%)	75 (82.5)	21 (84)	54 (81)	0.483

Data are expressed as means ± SD. MS: metabolic syndrome, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, DM: diabetes mellitus 2, HTA: hypertension, Psoet: psoriasis evolution time, PASI: psoriasis area and severity index, IR: insulin resistance: HOMA-IR: homeostasis model assessment of insulin resistance > 2.7. p value was calculated by Student t-test for independent samples or with Chi<sup>2</sup>.

**Figure 1.** Mean and maximum values of CIMT and prevalence of plaque in subjects with psoriasis according to the metabolic syndrome.

difference in the prevalence of MS between that and our study is that we used as cutting points for abdominal obesity for the Asian population that is 80 cm for women and 90 cm for men, which are the same cutting points used in the National Health Surveys of our country. Meanwhile, Espinoza used 90 cm for women and

102 cm for man. The high prevalence of MS in our study population could be explained by higher age, the prevalence of obesity (98%), and psoriasis. This is the first study in Mexican population that reports a higher CIMT and a greater frequency of hCIMT in patients with psoriasis and MS compared with those without MS. Several cases and control studies<sup>17</sup> had documented a significantly higher CIMT in subjects with psoriasis compared with the control group. Mongy *et al.*<sup>18</sup> spotlight the association between subclinical atherosclerosis with psoriasis. They compared patients with psoriasis without cardiovascular risk factors, with healthy control. The first group had higher CIMT ( $0.90 \pm 0.20$  mm vs.  $0.70 \pm 0.10$  mm,  $p < 0.001$ , respectively) as well higher prevalence of carotid plaque (27.8% vs. 14%,  $p = 0.07$ ). This data supports the scientific evidence that suggests psoriasis is not only a skin inflammatory disease, but also a systemic inflammation illness that increases the risk of cardiovascular disease. Indeed, moderate or severe psoriasis is associated with a high prevalence of cardiovascular risk factors such as DM2, obesity, smoking, and MS<sup>19</sup>. On the other hand, Yiu *et al.*<sup>20</sup> also provide

**Table 2.** Metabolic characteristics of subjects with psoriasis, according to metabolic syndrome (ATP III)

Variable	All (n = 92)	Without MS (n = 25)	MS (n = 67)	p
Glucose (mg/dL)	116.2 ± 39.7	99.0 ± 25.9	122.4 ± 42.0	0.002
Insulin (mIU/mL)	10.05 (7.5–15.6)	7.5 (5.9–11.2)	12.2 (8.2–78)	<0.001
HOMA-IR	2.9 (1.9–4.3)	1.8 (1.3–2.7)	3.4 (2.2–5.3)	<0.001
Uric acid (mg/dL)	5.7 ± 1.3	5.5 ± 1.3	5.8 ± 1.3	0.356
ALT (IU/L)	31.2 ± 15.9	23.9 ± 7.7	33.7 ± 17.5	<0.001
AST (IU/L)	28.9 ± 13.2	24.1 ± 7.0	30.6 ± 14.5	0.006
GGT (IU/L)	25 (18–36)	17 (12–27.5)	27.5 (20–39.5)	0.267
hs-CRP (mg/dL)	0.23 (0.11–0.51)	0.13 (0.054–0.37)	0.26 (0.13–0.57)	0.031
TC (mg/dL)	186.4 ± 38.7	188.2 ± 38.1	185.6 ± 39.0	0.783
TG (mg/dL)	166 (123–210)	116 (87–150)	181 (145–228)	0.007
C-HDL (mg/dL)	41.2 ± 11.2	50.3 ± 12.3	37.7 ± 8.6	<0.001
C-LDL (mg/dL)	110.1 ± 34.4	113.2 ± 32.0	108.8 ± 35.2	0.584
C- No HDL (mg/dL)	142.8 ± 34.6	137.8 ± 34.6	147.9 ± 34.6	0.236
C-LDL/C-HDL	2.79 ± 0.9	2.3 ± 0.7	2.9 ± 0.9	0.005
CT/C-HDL	4.7 ± 1.2	3.8 ± 0.9	5.0 ± 1.1	<0.001
TG/C-HDL	4.7 ± 2.8	2.6 ± 1.4	5.5 ± 2.8	<0.001
HbA1c (%)	6.3 ± 1.5	5.9 ± 1.3	6.4 ± 1.5	0.122

Data are expressed as means ± SD. MS: metabolic syndrome. HOMA-IR: homeostasis model assessment of insulin resistance. ALT: alanine transaminase. AST: aspartate aminotransferase. GGT: gamma-glutamyl transferase, hs-CRP: high sensitivity C-reactive protein. TC: total cholesterol, TG: triglycerides C-HDL: high-density lipoproteins. C-LDL: low-density lipoproteins. TG: triglycerides, HbA1c: glycosylated hemoglobin. p value was calculated by Student t-test for or Mann–Whitney U-test.

**Table 3.** Frequency of metabolic syndrome components in subjects with psoriasis according ATP III

MS components n (%)	All (n = 92)	Without MS (n = 25)	MS (n = 67)	p
Abdominal obesity	80 (87)	16 (64)	64 (97)	<0.001
Hypoalphalipoproteinemia	70 (77)	8 (32)	62 (94)	<0.001
Fasting glucose >100 mg/dL or DM2	63 (69)	7 (28)	56 (85)	<0.001
Triglycerides > 150 mg/dL	57 (62)	7 (28)	50 (76)	<0.001
BP>130/90 mmHg or Hypertension	38 (42)	3 (12)	35 (53)	<0.001

Data are expressed as frequencies and (percentages). MS: metabolic syndrome, abdominal obesity: waist circumference men > 90 cm and women > 80 cm, hypoalphalipoproteinemia: C-HDL in women <50 mg/dL, men <40 mg/dL, DM 2: type 2 diabetes mellitus; BP: blood pressure, p value calculated by Chi<sup>2</sup>.

evidence of a greater CIMT in subjects with psoriasis compared with healthy controls (0.73 ± 0.11 mm vs. 0.67 ± 0.08 mm, respectively, p < 0.01).

**Table 4.** Simple and multiple linear regression analyses factors associated with CIMT and clinical variables in patients with psoriasis

Variable	Simple linear regression		Multiple linear regression	
	Standardized β coefficients	p	Standardized β coefficients	p
Age	0.62	<0.001	0.56	<0.001
Metabolic syndrome	0.29	0.005	0.2	0.01
Fasting glucose	0.24	0.01	-0.13	0.88
Psoet	0.23	0.02	0.07	0.42
PASI	0.19	0.06	0.13	

PASI: psoriasis area and severity index, adjusted by LDL-C: low lipoprotein density cholesterol. CIMT: carotid intima media thickness. Psoet: psoriasis evolution time.

Patients with psoriasis have been also reported an increased prevalence of NAFLD<sup>21</sup>. Our study population had a higher frequency of NAFLD (87%), compared

with those reported by Romero (43%)<sup>22</sup> and Roberts *et al.* 47%<sup>23</sup>. Both are systemic inflammatory illnesses and they have been also associated with MS, insulin resistance, and increased cardiovascular risk<sup>24</sup>. The NAFLD produces an imbalance between pro-inflammatory and anti-inflammatory cytokines; these molecules are implicated in the pathogenesis of psoriasis. Thus, their similar mechanisms could exacerbate alterations in the levels of inflammatory cytokines, leading to an increase in cardiovascular risk through the implication of obesity, MS and IR<sup>25</sup>. Insulin resistance may be a major underpinning link between the two conditions due to its central role in both MS and NAFLD pathogenesis<sup>26</sup> and in addition, it is frequent in psoriasis patients<sup>27</sup>. The increased prevalence of NAFLD probably could be explained by the higher frequency of obesity and IR in our population.

In the Nurse's Health Study<sup>28</sup> psoriasis was associated with an elevated risk of DM2, relative risks were 1.76 (95% CI: 1.48-2.09) and age-adjusted were 1.25 (95% CI: 1.05-1.49), respectively. Besides Armstrong *et al.*<sup>29</sup> reported in a meta-analysis an association between psoriasis and its severity with the prevalence of DM2: for mild psoriasis OR 1.53 (95% CI: 1.16-2.04) and for severe psoriasis OR of 1.97 (95% CI: 1.48-2.62). Furthermore, they could assess among the longitudinal studies for DM2 incident, OR of 1.27 (95% CI: 1.16-1.40). Furthermore, for every 10% increase in body surface area affected by psoriasis, there was an approximate 20% further increase in diabetes risk<sup>30</sup>. Estimated from this data, there are an additional 125 650 new diagnoses of type 2 diabetes worldwide per year attributable to psoriasis. Espinoza *et al.*<sup>7</sup> reported 30.1% of diabetes and 81% of abdominal obesity in its population; our study showed a similar frequency of DM (31.9%). Setty *et al.*<sup>31</sup> reported an association between general obesity ( $BMI > 30 \text{ kg/m}^2$ ) and abdominal obesity with the risk of psoriasis, suggesting that obesity precedes this pathology; however, another study concluded that obesity is a consequence<sup>32</sup>, therefore is not clear whether obesity is a risk factor for or comorbidity of psoriasis. BMI is a risk factor mediating diabetes and psoriasis independently; hence, immune-mediated inflammatory process, metabolic biomarkers, and environmental factors could be the potential links between psoriasis and diabetes.

Dyslipidemia is a further risk factor, which is shared by NAFLD, psoriasis, and cardiovascular disease. Patients with psoriasis often have significantly higher cholesterol concentrations in the very-low density lipoprotein and high-density-lipoprotein fractions at the

onset of skin disease<sup>33</sup>. Hypercholesterolemia has been associated with the increase in the incidence of psoriasis in women, primarily if the evolution of the disease is greater than 7 years<sup>34</sup>. In our population, the frequency of C-LDL  $> 100 \text{ mg/dl}$  was 57.6%. Although hypercholesterolemia is not a component of MS, it is a risk factor for the development of atherosclerosis. The subjects with MS compared with those without MS had a higher prevalence of hypoalphalipoproteinemia (94% vs. 32%) and hypertriglyceridemia (76% vs. 28%). Indeed atherogenic index was significantly higher in the MS group (Table 2). Espinoza *et al.*<sup>7</sup> found a hypertension prevalence of 26.0%, in our study was similar (22.2%).

The strengths of this study include a relatively large sample size, of a prospective study with a well-characterized population who were recruited from the outpatient clinic of a tertiary hospital. The evaluation of CIMT was done direct measurement by a trained person with experience in subclinical atherosclerosis. Our study has limitations and the results should be interpreted with caution. The main is lack a control group match by sex, age, without MS, without psoriasis, and without immunosuppressant treatment. We do not have historical control of the patients evaluated in this study. The cross-sectional design of our study does not provide longitudinal follow-up of atherosclerosis. There are other risk factors associated with atherosclerosis as dietary habits and physical activity; these factors were not assessed. We cannot dismiss that the cause of fatty liver is due to genetic or metabolic diseases, drug consumption, environmental toxins, food, or infections, so this information should be taken with caution.

## Conclusions

This is the first study in the Mexican population that documents a greater CIMT in patients with psoriasis who are carriers of MS, compared with those without MS. Elevated CIMT is a marker of subclinical atherosclerosis. Furthermore, it is alarming the frequency of metabolic disturbances and the components of MS in the population studied. Identification of MS components in these patients could be useful for offering cardiovascular primary prevention with an intensive treatment aimed to modify cardiovascular risk factors. All subjects studied are candidates for therapeutic, to improve the nutritional status, and to receive the appropriate treatment for psoriasis severity, obesity, diabetes, hypertension, and dyslipidemia.

## Acknowledgments

The authors would like to thank Rodríguez-Medina Judith, Jasso-Arenas Juana, Barrera-Reyes Néstor Omar, Escobar-Hernández Nidia.

## Funding

The authors declare have no commercial or financial relationship with any sponsor, or direct professional relationship with it.

## Conflicts of Interest

The authors declare that have no conflicts of interest.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

**Confidentiality of data.** The authors declare that they have followed the protocols of their work center on the publication of patient data.

**Right to privacy and informed consent.** The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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