Nonalcoholic fatty liver disease in women with polycystic ovary syndrome ☆,☆☆

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***Background/Aims*: Insulin resistance is a common feature of both nonalcoholic fatty liver disease (NAFLD) and polycystic ovary syndrome (PCOS), therefore, we hypothesize that PCOS and NAFLD may coexist. The aim of the present study was to determine the frequency and characteristics of NAFLD in women with PCOS.**

***Methods*: A prospective study of patients with PCOS and no current pharmacological treatment was conducted. NAFLD was diagnosed by abdominal ultrasound following exclusion of alcohol consumption, viral, or autoimmune liver disease. Anthropometric variables, serum levels of glucose, insulin, lipids and aminotransferases, and HOMA index were determined.**

***Results*: Forty-one PCOS patients (mean age: 24.6 ± 7.2 yr, mean body mass index [BMI]: 30.3 ± 7.0 kg/m^2) were included; 26 of 41 PCOS patients (63.4%) had insulin resistance and 17 (41.5%) had NAFLD. Nine of the NAFLD patients (64%) also had abnormal aminotransferases. Women with NAFLD and PCOS had a higher HOMA index and a higher waist-hip ratio than those with normal ultrasound. Patients with PCOS showed a higher frequency of NAFLD (41% vs. 19%) and insulin resistance (63% vs. 35.5%) than a control group.**

***Conclusions*: NAFLD is frequent in patients with PCOS confirming a relevant clinical association between these two conditions. Women with PCOS should be screened for liver disease.**

***Keywords*: Fatty liver; Polycystic ovary syndrome; Insulin resistance**

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is now recognized as one of the most common causes of chronic liver disease in western countries [1,2]. Pathologically, NAFLD comprises various degrees of progressive steatosis, lobular inflammation, and fibrosis of the liver [2,3]. Nonalcoholic steatohepatitis (NASH) is considered a

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Abbreviations: NAFLD, Nonalcoholic fatty liver disease; NASH, Nonalcoholic steatohepatitis; PCOS, polycystic ovary syndrome; BMI, body mass index; ALT, alanine aminotransferases; hsCRP, high-sensitive C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance.

more aggressive form of the disease, and is defined by the presence of necroinflammatory changes and pericellular fibrosis [4]. Patients with NASH are considered to be at a higher risk of developing advanced fibrosis, cirrhosis, and hepatocellular carcinoma [5]. NAFLD is strongly associated with insulin resistance, which is thought to have a key role in its pathogenesis and progression [6–8]. Moreover, considering that a high proportion of patients exhibit the cluster of risk factors that defines metabolic syndrome, NAFLD is considered the hepatic manifestation of metabolic syndrome [9].

Another entity strongly associated with insulin resistance and metabolic syndrome is polycystic ovary syndrome (PCOS), which is among the most common endocrine diseases in women, affecting up to 10% of women of reproductive age [10,11]. The main features of PCOS include chronic anovulatory cycles, oligo- or amenorrhea, and hirsutism [12]. Existing data indicate that about 50% of patients with PCOS have insulin resistance and fulfil the criteria for metabolic syndrome [13]. The latter is believed to be the major risk factor for the occurrence of cardiovascular disease in PCOS [14].

Considering that insulin resistance is a common feature of both NAFLD and PCOS, it is very likely that both entities coexist in a given patient. This is an important issue which may have relevance for clinical management in terms of when and how to screen for liver disease in patients with PCOS. However, data on this issue are scarce [15,16]. The current prospective study was conducted with the aim of determining the frequency and characteristics of NAFLD in Chilean women with PCOS. Our findings indicate that NAFLD is frequent in patients with PCOS, and confirms a relevant clinical association.

2. Methods

2.1. Patients

Forty-one consecutive non-pregnant patients with diagnosis of PCOS and no current pharmacological treatment, attending an endocrinology clinic in two university hospitals in Santiago, were recruited and prospectively studied between May 2005 and December 2006. In addition, a group of 31 non-pregnant women of similar age and body mass index (BMI) was recruited to serve as controls. PCOS was defined according to the Rotterdam criteria, which is based upon the presence of irregular menses and hyperandrogenism [17]. The study was approved by the Institutional Review Board Ethics Committee for Human Studies of the Pontificia Universidad Catolica de Chile, and informed consent was obtained from all participants. Subjects were excluded if they had a history of alcohol intake higher than 20 g per day. Women with a history of chronic viral hepatitis, hemochromatosis, autoimmune liver disease, other chronic liver disease, or those taking hepatotoxic drugs were excluded.

2.2. Clinical, anthropometric, and laboratory data

The protocol included a pre-coded questionnaire with socioeconomic data, ethnicity, medical history including diagnosis of hypertension and diabetes, a detailed history of alcohol consumption with an estimation of daily intake in grams per day, physical examination, and blood tests. Excessive alcohol consumption was defined as more than 20 g per day. Anthropometric measurements included height, weight, body mass index (BMI), and waist-to-hip ratio. Obesity was defined in women as a BMI >30 kg/m^2 [18]. Fatty liver was diagnosed by abdominal ultrasound (US) using standardized criteria. US was performed in all subjects with the same equipment (Tosbee ultrasound scanner Toshiba, Japan), and all ultrasound examinations were performed by one of the authors (RMP), who was unaware of the clinical and laboratory results. The presence of fatty liver was determined in a qualitative manner using accepted criteria including a bright hepatic echo pattern (compared with the right kidney), a homogeneous or coarse echo pattern, increased attenuation of the US beam, and loss of intrahepatic architectural details [19]. Fasting glucose serum levels and insulin, oral glucose tolerance test (OGTT), 2-h glucose and insulin, cholesterol (total, LDL and HDL), triglycerides, serum alanine aminotransferases (ALT), and high-sensitive C-reactive protein (hsCRP) levels were measured. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to: insulin (μU/mL) $\times$ fasting plasma glucose (mmol/L)/22.5 [20]. HOMA-IR is a method to assess insulin sensitivity, and has a strong correlation with clamp measured total glucose disposal [20]. In this study, insulin resistance was diagnosed as HOMA-IR >2.6 [21]. Determination of hs-CRP was performed with a latex particle enhanced nephelometric immune assay with BN ProSpec equipment by Dade Behring® (Deerfield, IL). Serum hs-CRP values >10 mg/L were excluded under the assumption that they represented acute inflammatory illness [22]. Presence of antinuclear antibodies was investigated using a commercially available ELISA, while antibodies to anti-hepatitis C virus were detected by a third-generation immunoassay test using the MEIA (Microparticle Enzyme Immunoassay) technique (Abbott AxSYM™, Abbott Park, IL). Hepatitis B was detected by testing for the presence of hepatitis B surface antigen (HBsAg) using a commercially available assay (Abbott AxSYM™, Abbott Park, IL).

Metabolic syndrome was defined as the presence of 3 or more of the following abnormalities: waist circumference greater than 102 cm in men and 88 cm in women; serum triglyceride levels $\geqslant 150$ mg/dL; HDL cholesterol concentration <40 mg/dL in men and 50 mg/dL in women; blood pressure $\geqslant 130/85$ mm Hg; or serum glucose level $\geqslant 110$ mg/dL, following the criteria of ATP III [23]. Abnormal aminotransferase levels in women were defined as ALT >25 U/L according to normal values for healthy Chilean women. Diabetes mellitus was defined using the American Diabetes Association criteria [16]. Previously known hypertension was defined as a blood pressure equal to or higher than 140/90 mm Hg on two different occasions.

2.3. Statistical analysis

The clinical characteristics and laboratory measurements of patients with PCOS and subjects of the control group were analyzed, continuous variables were presented as means $\pm$ standard deviation (SD), and dichotomic variables were presented as percentages. Student's t-test was used to test continuous variables from independent samples and proportion differences were tested using Pr test. A p-value <0.05 was considered statistically significant. All statistical analyses were performed with SPSS version 10.0 (standard version, SPSS Inc.).

3. Results

Forty-one patients with PCOS met our inclusion criteria. Baseline clinical and biochemical characteristics of the PCOS patients are shown in Table 1. The clinical, anthropometric, and biochemical features of these patients were compared with a group of 31 clinically healthy women of similar age and BMI. In addition, the waist-to-hip ratio and blood pressure were similar in both groups. Aminotransferase levels (ALT) were

Table 1
Baseline clinical and biochemical characteristics of patients with polycystic ovary syndrome (PCOS) and controls

Variables	Patients with PCOS ($N = 41$)	Controls ($N = 31$)	p value
Age (yr)	24.68 ± 7.22	27.96 ± 6.99	0.057
Body mass index (kg/m^2)	30.39 ± 7.07	29.32 ± 5.26	0.46
Waist-to-hip ratio	0.87 ± 0.08	0.86 ± 0.08	0.49
Systolic blood pressure (mm Hg)	116.66 ± 14.79	116 ± 16.53	0.86
Diastolic blood pressure (mm Hg)	73 ± 11.85	73.1 ± 13.08	0.97
ALT (IU/L)	27.83 ± 20.66	15.5 ± 20.02	0.018
Log High sensitive CRP (mg/L)	0.2 ± 0.51	0.13 ± 0.51	0.31
Total cholesterol (mg/dL)	179.09 ± 44.5	185.96 ± 43.31	0.57
LDL cholesterol (mg/dL)	106.7 ± 34,63	119.85 ± 33.03	0.18
HDL cholesterol (mg/dL)	47.97 ± 10.82	47.29 ± 11.88	0.83
Triglycerides (mg/dL)	125.57 ± 96.61	127.62 ± 62.23	0.93
Fasting glucose (mg/dL)	86.7 ± 12.83	82.08 ± 8.75	0.16
Fasting insulin (mIU/L)	19.55 ± 17.15	12.68 ± 8.09	0.038
Log HOMA-IR	0.48 ± 0.32	0.34 ± 0.31	0.02

All values are expressed as means ± SD. p values reflect Student's t-test for independent samples or Pr test for discrete variables. ALT, alanine aminotransferase; hsCRP, high-sensitive C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance.

found to be higher among patients with PCOS compared to control group (27.83 ± 20.66 vs. 15.5 ± 20.02, $p = 0.018$). Fasting insulin and log HOMA-IR in the PCOS group were also significantly higher than the control group ($p = 0.038$ and 0.02, respectively), indicating that women with PCOS have more severe insulin resistance as indicated by HOMA-IR score than women of similar age and body weight without PCOS. The proportion of patients and controls demonstrating obesity, chronic arterial hypertension, diabetes, insulin resistance, metabolic syndrome, and fatty liver is shown in Table 2. Insulin resistance, fatty liver, and abnormal aminotransferase levels were significantly more frequent in PCOS patients. Insulin resistance was present in 26 of 41 (63.4%) patients with PCOS compared to 11 (35.5%) in controls ($p = 0.019$). The overall prevalence of hepatic steatosis as assessed by abdominal ultrasonography in patients with PCOS reached 41.5% (17/41) which doubled the frequency observed in control women of similar age and BMI (6/31, 19.4%, $p < 0.05$). The proportion of patients with PCOS having abnormal levels of ALT reached 39% (16/41) and was also significantly higher in patients with PCOS than controls where only one patient (3.2%) exhibited abnormal levels of ALT.

A subgroup analysis of patients with PCOS according to the presence or absence of liver steatosis is shown in Table 3. Analysis of clinical, anthropometric, and biochemical variables showed that women with PCOS and NAFLD had significantly higher BMI ($p = 0.002$), waist-hip ratio ($p = 0.013$), ALT levels (0.013), hsCRP levels (0.035), fasting insulin ($p = 0.038$), OGTT 2-h insulin ($p = 0.44$), and log HOMA-IR ($p = 0.024$) than those patients with PCOS without hepatic steatosis. In addition, 9 of 17 (52.9%) patients with fatty liver and PCOS, and 7 of 24 (29%) patients with PCOS and normal ultrasound had abnormal ALT levels. The latter group of patients included predominantly obese women (mean BMI: 33.6 ± 4.2) with insulin resistance (HOMA-IR > 2.6 in all subjects).

4. Discussion

Considering that insulin resistance has been recognized as a frequent and key feature in both NAFLD [6,7] and PCOS [24–26], we sought to establish the frequency of NAFLD in PCOS patients. We found that 41.5% of patients have ultrasonographic evidence of

Table 2
Clinical and biochemical features among patients with PCOS and controls

Variables	PCOS (%)	Control (%)	p value
Obesity (BMI >30)	24 (58.5)	17 (54.8)	0.754
Chronic arterial hypertension	7 (17)	3 (9.7)	0.369
Diabetes	3 (7.3)	0 (0)	0.124
Insulin resistance (HOMA-IR > 2,6)	26 (63.4)	11 (35.5)	0.019
Metabolic Syndrome	6 (14.6)	5 (16.1)	0.647
Hepatic steatosis	17 (41.5)	6 (19.4)	0.046
Abnormal aminotransferases (ALT > 25 IU/L)	16 (39)	1 (3.2)	0.002

p values reflect Pr test for discrete variables. BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; ALT, alanine aminotransferase. PCOS group [$n = 41$], control group [$n = 31$].

Table 3
Comparison between patients with polycystic ovary syndrome (PCOS) with or without hepatic steatosis

Variables	Steatosis absent ($N = 24$)	Steatosis present ($N = 17$)	p value
Age (yr)	23.04 ± 7.4	27 ± 6.47	0.077
Body mass index (BMI) (kg/m^2)	27.48 ± 5.59	34.51 ± 7.02	0.002
Waist-to-hip ratio	0.845 ± 0.08	0.9 ± 0.06	0.013
Systolic blood pressure (mm Hg)	112.88 ± 13.25	122 ± 15.57	0.058
Diastolic blood pressure (mm Hg)	71.58 ± 12.42	75 ± 11.05	0.36
ALT (IU/L)	19.96 ± 11.76	38.94 ± 25.39	0.013
Log hsCRP (mg/L)	0.12 ± 0.53	0.31 ± 0.45	0.035
Total cholesterol (mg/dL)	169.05 ± 34.02	191.43 ± 54.03	0.28
LDL cholesterol (mg/dL)	99.35 ± 28.78	116.31 ± 40.21	0.367
HDL cholesterol (mg/dL)	49.59 ± 11.1	45.88 ± 10.47	0.49
Triglycerides (mg/dL)	98.58 ± 47.31	162.21 ± 131.78	0.097
Fasting glucose (mg/dL)	83.97 ± 13.79	88.19 ± 8.2	0.429
Fasting insulin (mIU/L)	13.8 ± 7.02	27.46 ± 23.26	0.038
OGTT, 2-h glucose (mg/dL)	110.2 ± 32.07	132.53 ± 33.74	0.065
OGTT, 2-h insulin (mIU/L)	99.97 ± 82.45	162.67 ± 80.55	0.044
Log HOMA-IR	0.44 ± 0.29	0.72 ± 0.38	0.024

All values are expressed as means ± SD. p values reflect Student's t-test for independent samples or Pr test for discrete variables. ALT, alanine aminotransferase; hsCRP, high-sensitive C-reactive protein; OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment of insulin resistance.

NAFLD. Moreover, half of these patients exhibited abnormal ALT levels, thereby suggesting that they might have NASH, the more severe form of NAFLD, although this remains to be proven. These findings are interesting since the women included in this study were young (mean age 24.6 yr), and therefore eligible for early detection and treatment of a potentially progressive liver disease [1,19]. Thus, the first implication of this study is that physicians that provide care for patients with PCOS must be aware of the need to evaluate NAFLD in this population.

Published data on the coexistence of PCOS and NAFLD are limited to two retrospective studies [15,16] and one case-report [27]. The first published study was carried out by Setji et al. [15] and consisted of a retrospective chart review of PCOS patients attending an academic endocrinology clinic. In this study, 15% of PCOS patients had aminotransferase elevations and 6 women underwent liver biopsies. Histological examination of the liver found evidence of NASH with varying degrees of fibrosis in all. The authors focused the analysis on the latter group of biopsy-proven NASH, and found that these patients had lower HDL and higher triglycerides, fasting insulin, and aminotransferase levels. Besides some methodological limitations [i.e. retrospective nature, referral bias, availability of complete data on all patients] this study indicates that severe liver disease may occur in the setting of PCOS. In the case of the recent report by Gambarin-Gelwan et al. [16], where a similar methodology to that of the present study was used, the authors found NAFLD in 55% of subjects with PCOS, a similar figure to the 41.5% found in our patients. This may be considered as a relatively high frequency since the estimated prevalence of NAFLD in the general population ranges from 3% to 24%, with most estimates in the 6% to 14% range [28]. Although data regarding the prevalence of NAFLD in Chile are scarce, a recent epidemiological study designed to assess gallbladder disease in this country found ultrasonographic evidence of NAFLD in 22.5% of the population [29]. Interestingly, some studies indicate that Hispanic ethnicity, which is predominant in Chile, may increase susceptibility to NAFLD [30,31]. If this is the case, the high frequency of NAFLD seen in our patients with PCOS patients may be influenced by their Hispanic ancestry.

Also similar to our study, Gambarin-Gelwan et al. found that the presence of steatosis was associated with a greater BMI and HOMA-IR, indicating that obesity and insulin resistance are major determinants of NAFLD in PCOS patients. Interestingly, comparison of PCOS patients with a group of women without PCOS, but of similar age and BMI, showed that PCOS patients have higher HOMA-IR scores than their non-PCOS counterparts. Thus, it seems that for a given BMI, PCOS patients have a more severe insulin resistance which likely contributes to a greater prevalence of NAFLD. This is in agreement with evidence suggesting that androgens and insulin resistance seem to have synergistic effects in PCOS patients [32]. Thus, the higher prevalence of NAFLD in patients with PCOS may be related to the fact that insulin resistance has a higher frequency and is more severe in PCOS patients. This may also be related to the observed frequency of liver enzyme abnormalities (51%) in subjects with demonstrated hepatic steatosis by ultrasonography in this study. This subgroup of patients may potentially have NASH, the more severe form of NAFLD, raising the possibility of an increased frequency of NASH in patients with PCOS, since the reported frequency of NASH in NAFLD ranges from 20% to 30% of patients

[33]. However, this remains speculative and a liver biopsy would have been required to further characterize this issue. Although histological examination of the liver remains the most sensitive diagnostic tool in NAFLD, it was not considered in this study since the decision of when to perform a liver biopsy in NAFLD is controversial and has to be made on individual basis [34,35]. Interestingly, as mentioned earlier, in the report of Setji et al. [15], all 6 women with abnormal liver enzymes who underwent liver biopsy had evidence of NASH and fibrosis despite their young age. Thus, severe insulin resistance and obesity may contribute to the occurrence of NASH in PCOS patients. However, further studies are needed to better define the role of liver biopsy in diagnostic evaluation of these patients.

Our study has both strengths and limitations. First, patients and controls were prospectively recruited and all pertinent data were available at the time of analysis, including a serologic work-up to exclude other causes of liver disease. Second, both diagnoses of PCOS and NAFLD were made on a homogeneous basis by the same group of physicians which gives consistency to the clinical findings. Third, patients were not receiving any treatment like contraceptives, metformin, or other insulin sensitizers when recruited to the study. Among the limitations are the relatively small number of patients and their exclusive Hispanic ethnicity. Both factors may preclude the applicability of findings to the general population.

In conclusion, findings consistent with NAFLD are frequent in patients with PCOS confirming a relevant clinical association. Abnormal aminotransferase levels were found in more than half of patients with NAFLD and PCOS, thereby suggesting an increased frequency of NASH in patients with PCOS, which was likely related to more severe insulin resistance. Thus, women with PCOS should be screened for liver disease given the potentially progressive nature of the disease, and the possibility of early introduction of life style changes that may improve NALFD (i.e. moderate exercise and weight loss) [27]. In addition, some women may be candidates for specific drug treatment [36]. Further studies are needed to define the set of studies (including liver biopsy or non-invasive markers of fibrosis) to be carried out in patients with PCOS and NAFLD as well as the treatment strategy to be used in these patients.

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