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Association of anti-Mullerian hormone and small-dense low-density lipoprotein cholesterol with hepatosteatosis in young lean women with and without polycystic ovary syndrome

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ABSTRACT

Objective: To study the association of anti-Mullerian hormone (AMH) and small-dense low-density lipoprotein cholesterol (sd-LDL) with hepatosteatosis among young, lean, polycystic ovary patients.
Study design: A prospective, case control study was carried out including 79 young lean women. Fifty-eight women with polycystic ovary syndrome (PCOS) and 21 age-and BMI-matched healthy controls were recruited. Anthropometric variables, biochemical and hormonal parameters, insulin-resistance indices, lipid profiles including sd-LDL levels and serum AMH levels were determined. Hepatic lipid content was evaluated by abdominal ultrasonography (USG). Determining the best predictor(s) which discriminate normal USG and hepatosteatosis was analyzed by multiple logistic regression analyses. Adjusted odds ratios and 95% confidence intervals were also calculated.
Results: PCOS patients had an increased prevalence of hepatosteatosis by 41.4% ($P = 0.006$) and they had significantly higher levels of sd-LDL and AMH when compared with the control group ($P < 0.001$). AMH and sd-LDL levels were positively and significantly associated with hepatosteatosis in young lean women with and without PCOS (OR: 2.877, 95%CI: 1.453–5.699, *P*: 0.02 and OR: 1.336, 95%CI: 1.083–1.648, *P*: 0.007, respectively). AMH and sd-LDL levels were positively correlated in PCOS patients ($r = 0.626$, $P < 0.001$). Both sd-LDL and AMH levels were the most predictive parameters for the determination of hepatosteatosis within the PCOS group. (OR: 3.347, 95%CI: 1.348–8.313, $P = 0.009$ and OR: 1.375, 95%CI: 1.072–1.764, $P = 0.012$, respectively). Statistically significant higher levels of AMH were associated with hepatosteatosis both in insulin resistance (IR) positive and IR negative PCOS patients ($P < 0.001$).
Conclusion: Hepatosteatosis is common in young lean PCOS patients. Increased AMH and sd-LDL levels may independently predict hepatosteatosis in young lean women with and without PCOS.

Introduction

Anti-Mullerian hormone (AMH), a member of transforming growth factor β (TGF β) family, is produced by the granulosa cells of the early developing preantral and small antral follicles [1]. Circulating levels of AMH do not vary throughout a menstrual cycle [2] and even between cycles [3], indicating that it may be considered as a surrogate marker for antral follicle count in both polycystic ovary syndrome (PCOS) [4] and in normally menstruating women [5]. In addition it has been suggested that serum AMH levels may be used as a potential cardiovascular risk predictor in women as it is found to be associated with dyslipidemia and IR [6]. AMH has been reported as a regulator of the sex-linked biases in the nervous system [7] and lungs [8]. Recent studies implicate serum AMH in human nongonadal development, suggesting an inverse correlation with maturation in boys [9]. AMH also has been suggested to have functions in men as a regulator of the cardiovascular system [10]. Despite the growing number of studies about the clinical usefulness of AMH, the regulation and dynamics

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of its secretion as well as the hormonal functions and effects on other tissues remain poorly understood.

PCOS is the most common endocrinopathy among women of reproductive age presenting with hyperandrogenism, oligo-/anovulation and/or polycystic ovaries on ultrasonography and associated with multiple cardiovascular risk factors[11]. Although IR and abdominal obesity, the essential features of metabolic syndrome, are markedly increased in PCOS [12], less and conflicting data is available regarding the presence of IR in lean women with PCOS [13,14]. The paradoxal relation between IR and PCOS in lean women is recently explained by the "adipose tissue expandibility" hypothesis [15]. It states that when individuals reach their adipose tissue expansion limit which is determined by environmental and genetic factors, then lipids cannot be stored appropriately in adipose tissue and rather deposited in non-adipose organs such as liver and muscle and causes IR by a lipotoxic mechanism. Besides, it has been suspected that hyperinsulinemia as a consequence of IR, augments the premature differantiation of granulosa cells and may play a role in AMH secretion [16] and AMH is related to IR in women with and without PCOS [17].

Another entity strongly associated with IR and metabolic syndrome is nonalcoholic fatty liver disease (NAFLD) [18]. Although obesity and type 2 diabetes are well-known risk factors for the developement of NAFLD, it is closely associated with metabolic disorders even in lean, nondiabetic patients. Furthermore, metabolic disorders such as IR and dyslipidemia were found to be higher in nonobese NAFLD patients, compared with the overweight group [19]. The pathogenetic pathway could be explained with the same hypothesis with PCOS, namely the "adipose tissue expandibility" hypothesis. Also it has been suggested that there is gender associated distribution among patients with nonalcoholic steatohepatitis (NASH), the more severe form of NAFLD, and high prevelance of it in females, indicating that female sex steroids may promote NASH [20]. Fertility is shown to be an important factor in fatty liver damage of NAFLD together with IR, suggesting that estrogen may exacerbate the clinical progress in NAFLD [21]. Due to the factor that estrogen is a well-known anti-oxidant and is shown to be a protective factor for NAFLD in women [22] and even in healthy men [23] the question "may another factor about fertility be related with this condition?" comes into mind. At this point of view we suggested that serum AMH levels can be associated with hepatosteatosis in young lean PCOS patients.

The aim of the present study is to investigate whether there is an association between AMH, dyslipidemia and hepatosteatosis in young lean women with PCOS.

Materials and methods

Fifty eight lean PCOS patients, aged between 20 and 32 years and with a mean body mass index (BMI) 21.94 ± 2.05 kg/m^2 were recruited consecutively from the outpatient clinic of Obstetrics and Gynecology unit of Ufuk University, between February 2009 and January 2011. The diagnosis of PCOS was made due to the presence of clinical and/or biochemical hyperandrogenism together with one of the following criteria as proposed by Androgen Excess Society: (i) oligo- or amenorrhea (ii) polycystic ovaries on ultrasound. Oligo- or amenorrhea was defined as a cycle length in excess of 35 days or less than 8 spontaneous menstrual cycles per year or the absence of menstruation for more than 3 months. Clinical hyperandrogenemia is defined as a Ferriman–Gallway score higher than 8 and biochemical hyperandrogenemia is defined as a total testosterone level above 0.8 ng/ml, and/or a free testosterone level above 3.6 ng/ml, and/or a dehydroepiandrosterone sulphate level more than 360 μm/dl. Ovaries were considered polycystic on ultrasound if there were 12 or more follicles measuring 2–9 mm in diameter in each ovary and/or enlarged ovarian volume (>10 mm^3).

Patients were excluded if any disorders causing androgen excess or irregular menses (hyperprolactinemia, uncontrolled thyroid disease, non-classical congenital adrenal hyperplasia, premature ovarian failure, Cushing's syndrome, androgen secreting tumors or pregnancy) and any other systemic diseases, were detected. Twenty one age and BMI matched healthy, lean women were also recruited as control group. Control women evaluated to ensure normal ovulatory cycles and the menstrual pattern, previous medical and obstetric history were also recorded. Besides all women in the control group were evaluated both by gynecological and physical examination and also by ultrasonography of the genital system. Exclusion criteria for both the study and the control groups were: (i) history of alcohol consumption, (ii) history of known liver disease, (iii) history of other diseases or medications causing an elevation of the liver enzymes.

All participants provided a written informed consent and the study protocol was approved by the institutional review board of the university.

All participants included in the study were evaluated in the early follicular phase, on the day 3 of a spontaneous menstrual cycle or after a withdrawal bleeding. Clinical examination was performed and anthropometric measurements were recorded. Blood samples were obtained after an overnight fasting at least 12 h by venipuncture for biochemical evaluation, and processed within 1 h after withdrawal for AMH and sd-LDL. Serum was stored in −80 °C. Biochemical evaluation consisted of complete blood counting, fasting glucose and insulin, total cholesterol, low-density cholesterol (LDL), high-density cholesterol (HDL), triglycerides (TG), C-reactive protein (CRP), FSH, LH estradiol, total and free testosterone (total-T and free T), 17-hydroxyprogesterone, dehydroepiandrosterone sulphate (DHEA-S), serum aspartate (AST) and alanine aminotransferases (ALT) and γ-glutamyltransferase (GGT).

Plasma glucose levels were determined with the glucose hexokinase method (Cobas Integra 400 Plus, Roche Diagnostics, Mannheim, Germany). Serum levels of FSH, LH, E2, PRL, DHEAS, total-T, insulin and TSH were measured with electrochemiluminescence assays (ELECYS 2010 HITACHI, Roche Diagnostic, Germany). Serum levels of 17OH-P and free-T were measured by radioimmunoassay. The inter- and intraassay coefficients of variation (CV) were; for FSH 4.8% and 4.6%, for LH 2.9% and 2.4%, for E2 3.7% and 3.1%, for total-T 6.3% and 6.1%, for free-T 2.9% and 2.6%, for 17OH-P 4.3% and 3.7% and for DHEAS 7.1% and 4.2%, respectively. Homeostasis model assessment (HOMA-IR) (insulin × glycemia in (μmol/l)/22.5), and quantitative insulin sensitivity check index (QUICKI) (1/log insulin + log glycemia in mg/dl) were estimated. HOMA-IR > 2.5 was considered to indicate the presence of IR [24]. The serum levels of total cholesterol, HDL, LDL, and TG were determined with enzymatic colorimetric assays (Roche Diagnostic, Mannheim, Germany). ALT and AST levels were determined by the colorimetric IFCC approved method (Cobas Integra Plus, Roche Diagnostic, Mannheim, Germany). The GGT assay involved the transfer of the gamma-glutamyl group from the donor substrate to the glycylglycine acceptor to yield 3-carboxy-4-nitroaniline. The intra- and interassay coefficients of variation (CV) were for ALT 2.1% and 2.9%, for AST 2.4% and 3.1% and for GGT 2.5% and 2.7%, respectively. The other biochemical parameters were determined by routine laboratory methods.

A simple and inexpensive method for the quantification of sd-LDL using heparin–magnesium precipitation was performed in this study. This method is consisted of two steps. The first one is the settlement of the lipoproteins that have a density lower than 1.044 g/ml by using heparin-magnesium as a precipitation reagent and the second step is the determination of LDL cholesterol with

automatic colorimetric method (COBAS Integra 400 Plus) directly from the supernatant [25]. This method was found to be the best one correlated with the ultracentrifugation method and identical to the sd-LDL fraction (density 1.044 to 1.063 g/ml) isolated by ultracentrifugation [25,26].

Serum AMH concentrations were determined by using ultra-sensitive ELISA (AMH ELISA kit; Diagnostic System Laboratories, Texas USA). The coefficients of variability (CV) for AMH were; functional sensitivity 0.2 ng/ml, intra-assay and inter-assay CV were 4% and 8%, respectively.

Participants with elevated levels of liver enzymes or/and hepatosteatosis were further evaluated by a gastroenterologist and additional laboratory tests were performed when necessary, in order to exclude liver diseases other than nonalcoholic fatty liver disease.

Imaging was conducted using a high resolution ultrasound machine (Logic Q7, General Electric, USA) with a 7.5 MHz mechanical sector transducer in all cases. Fatty infiltration of the liver was considered as positive if the echogenity of the liver was higher than the echogenity of the right kidney. Ultrasonographic measurements were performed by the same experienced radiologist.

IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp.) was used for statistical analysis. Whether the distributions of metric discrete and continuous variables were normally or not was determined by Kolmogorov Smirnov test. Data were shown as mean ± SD or median (min-max), where applicable. While, the mean differences between groups were compared by Student's *t* test, otherwise, Mann Whitney *U* test was applied for comparisons of the median values. Categorical data were analyzed by Pearson's chi-square test. Determining the best predictor(s) which discriminate normal USG and hepatosteatosis was analyzed by multiple logistic regression analyses. Adjusted odds ratios and 95% confidence intervals were also calculated. A P value <0.05 was considered as significant.

Results

A total of seventy nine participants (58 PCOS patients and 21 age- and BMI-matched healthy controls) were enrolled in the study. The baseline anthropometric, biochemical and hormonal characteristics of PCOS patients and controls are given on Table 1. AMH levels were measurable in all participants with values ranging from 1.0 to 26.14 ng/ml as well as sd-LDL levels ranging between 2 and 50 mg/dl. The women with PCOS had significantly higher levels of sd-LDL, AMH, HOMA-IR, high sensitive C-reactive protein (hsCRP), ALT and AST levels when compared with the control group ($P = 0.025$, 0.013, 0.007, 0.012, 0.003, 0.014 and 0.03, respectively). Hepatosteatosis according to ultrasonography was detected in 24 PCOS patients (41.4%) and in only 2 (9.5%) controls ($P = 0.006$) (Table 1). In PCOS group, median AMH levels were 3.5 ng/ml (1.0–8.6) in patients with a normal liver ultrasonography and 7.6 ng/ml (5.2–26.1) in patients with hepatosteatosis ($P < 0.001$).

Participants were further divided into two subgroups according to the presence of hepatosteatosis on ultrasonography. All anthropometric, biochemical and hormonal parameters were again compared among 2 groups [first with normal ultrasonography ($n = 53$) and the second group with hepatic steatosis ($n = 26$)], even the presence of PCOS was compared as well (Table 2). In univariate analysis, sd-LDL ($P < 0.001$), HOMA-IR ($P = 0.001$), and AMH ($P < 0.001$) levels were significantly higher while QUICKI ($P = 0.003$) was significantly lower in hepatosteatosis group.

The parameters exhibiting significant differences between two groups in univariate analysis were further evaluated with logistic regression analysis (Table 3). Only HOMA-IR and ALT levels were included because there was close association between HOMA-IR and QUICKI as well as ALT and AST levels. In logistic regression analysis, AMH (OR: 2.877, 95%CI: 1.453–5.699, *P*: 0.02) and sd-LDL levels (OR: 1.336, 95%CI: 1.083–1.648, *P*: 0.00) were positively and significantly associated with hepatosteatosis.

Table 1
Baseline characteristics, laboratory values and ultrasonographic evaluation in polycystic ovary syndrome cases and the control group.

Parameter	PCOS (*n*: 58)	Control (*n*: 21)	*P* value*
Age (mean)	24.3 ± 3.3 (20–32)	24.5 ± 2.8 (20–30)	0.84
Body mass index (kg/m^2)	21.9 ± 2.1	21.8 ± 1.0	0.90
Waist–hip ratio	0.74 ± 0.06	0.71 ± 0.07	0.17
sd-LDL (mg/dl)	17.5 (2–50)	11 (2–27)	0.02
AMH (ng/ml)	4.6 (1.0–26.1)	3.4 (1.4–7.0)	0.01
HOMA-IR	1.9 (0.5–14.0)	1.7 (0.5–2.1)	0.007
QUICKI	0.346 (0.27–0.43)	0.352 (0.34–0.43)	0.01
FSH (mIU/ml)	5.7 (2.1–10.9)	5.4 (3.1–8.1)	0.44
LH (mIU/ml)	6.1 (1.7–23.8)	6.5 (1.1–11.5)	0.88
Estradiol (E2) (pg/ml)	36.7 (5–562)	59.7 (14–230)	0.04
Total testosterone (ng/ml)	0.3 (0.03–19)	0.2 (0.1–0.6)	0.15
Free testosterone (pg/ml)	2.6 ± 1.0	2.3 ± 0.8	0.33
17-OHP (ng/ml)	1.6 (0.5–4.0)	1.8 (1.0–2.6)	0.53
DHEA-S (μg/dl)	248.5 (64–677)	268 (55–526)	0.68
T. Cholesterol (mg/dl)	168.6 ± 33.3	164.5 ± 23.2	0.55
HDL (mg/dl)	52.4 ± 10.5	59.8 ± 11.7	0.009
LDL (mg/dl)	99 ± 29.4	99.8 ± 25.9	0.91
Triglycerides (mg/dl)	78 (30–233)	66.5 (33.8–137)	0.07
hsCRP (mg/l)	1.4 (0.2–8.4)	0.6 (0.2–2.1)	0.003
ALT (U/l)	15.0 (5.8–89)	12 (7.3–19)	0.01
AST (U/l)	16.0 (8.6–53)	14 (8.5–23)	0.003
GGT (U/l)	11.9 (6–28)	11.2 (6.5–18)	0.08
Ultrasonography			
Normal	34 (%58.6)	19 (%90.5)	0.006
Hepatosteatosis	24 (%41.4)	2 (%9.5)	

sd-LDL: small dense low density lipoprotein, AMH: anti-Mullerian hormone, HOMA-IR: homeostasis model assessment, QUICKI: quantitative insulin sensitivity check index, FSH: follicle stimulating hormone, LH: luteinizing hormone, 17-OHP: 17 hydroxyprogesterone, DHEA-S: dehydroepiandrosterone-sulphate, HDL: high density lipoprotein, LDL: low density lipoprotein, hs-CRP: high sensitive C reactive protein, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma glutamyltransferase.
* $P < 0.05$ statistically significant.

Table 2
Univariate analyses of possible independent risk factors for hepatosteatosis.

Variables	Normal ultrasonography (*n*: 53)	Hepatosteatosis (*n*: 26)	*P* value*
Age (median)	24.3 ± 2.9	24.3 ± 3.5	0.99
Body mass index (kg/m^2)	21.7 ± 1.5	22.4 ± 2.3	0.16
Waist–hip ratio	0.72 ± 0.06	0.75 ± 0.07	0.059
PCOS (*n*, %)	34 (64.2)	24 (92.3)	**0.008**
sd-LDL (mg/dl)	12 (2–27)	25 (10–50)	**<0.001**
AMH (ng/ml)	3.4 (1.0–8.6)	7.1 (1.7–26.1)	**<0.001**
HOMA-IR	1.7 (0.5–6.7)	2.8 (0.5–14.0)	**0.001**
QUICKI	0.352 (0.29–0.43)	0.328 (0.27–0.43)	**0.003**
FSH (mIU/ml)	5.7 ± 1.6	5.4. ± 1.2	0.48
LH (mIU/ml)	6.7 (1.1–23.8)	5.8 (1.7–23.2)	0.18
E2 (pg/ml)	37 (5–230)	39.5 (7.8–562)	0.72
Total testosterone (ng/ml)	0.2 (0.03–1.3)	0.3 (0.06–19)	0.72
Free testosterone (pg/ml)	2.5 (0.8–4.6)	2.4 (1–4.2)	0.65
17-OHP (ng/ml)	1.8 (0.5–2.8)	1.6 (0.5–4.0)	0.45
DHEA-S (μg/dl)	261 (55–677)	236 (82–562)	0.35
T. Cholesterol (mg/dl)	166.8 ± 29.3	169.0 ± 34.5	0.77
HDL (mg/dl)	55.2 ± 11.1	52.5 ± 11.6	0.33
LDL (mg/dl)	98.3 ± 29.2	101.0 ± 27.0	0.69
Triglycerides (mg/dl)	71 (33.8–196)	81.5 (30–233)	0.17
hsCRP (mg/dl)	1.3 (0.2–8.4)	1.1 (0.2–5.7)	0.93
ALT (U/l)	13 (6–59)	19 (8.2–89)	**0.02**
AST (U/l)	15 (9–37)	17 (8.5–53)	**0.03**
GGT (U/l)	11 (6–28)	12 (6.5–28)	0.55

PCOS: polycystic ovary syndrome, sd-LDL: small dense low density lipoprotein, AMH: anti-Mullerian hormone, HOMA-IR: homeostasis model assessment, QUICKI: quantitative insulin sensitivity check index, FSH: follicle stimulating hormone, LH: luteinizing hormone, E2: estradiol, 17-OHP: 17 hydroxyprogesterone, DHEA-S: dehydroepiandrosterone-sulphate, HDL: high density lipoprotein, LDL: low density lipoprotein, hs-CRP: high sensitive C reactive protein, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma glutamyltransferase.
* $P < 0.05$ statistically significant.

Table 3
Logistic regression analysis of possible independent risk factors for hepatosteatosis.

Variables	OR	CI (95%)	*P* value*
Presence of PCOS	0.290	0.017–5.013	0. 395
sd-LDL	1.336	1.083–1.648	**0.007**
AMH	2.877	1.453–5.699	**0.02**
HOMA-IR	2.741	0.715–10.513	0.141
ALT	1.023	0.946–1.106	0.571

PCOS: polycystic ovary syndrome, sd-LDL: small dense low density lipoprotein, AMH: anti-Mullerian hormone, HOMA-IR: homeostasis model assessment, ALT: alanine aminotransferase.
* $P < 0.05$ statistically significant.

Additional stastistical analysis was performed to determine the predictive factors of hepatosteatosis within the PCOS group. First, univariate analysis of possible risk factors for hepatosteatosis within PCOS group was made (Table 4). Serum AMH, sd-LDL, HOMA-IR and ALT levels were significantly higher in PCOS patients with hepatosteatosis group when compared with PCOS without hepatosteatosis (*P* levels were <0.001, <0.001, =0.002 and =0.039, respectively). All statistically significant parameters according to univariate analysis in the PCOS group with hepatosteatosis were further evaluated with multivariate backward logistic regression analysis (Table 5). In this stepwise regression analysis, the most unavailable parameters; at first ALT and secondly HOMA-IR levels excluded from the regression model. As a result both sd-LDL and AMH levels were found to be most predictive parameters for the determination of hepatosteatosis within the PCOS group. In return to every 1 unit increase in AMH level, the risk of hepatosteatosis was increased by 3.347 (95%CI: 1.348–8.313, $P = 0.009$) and also an increased risk in hepatosteatosis by 1.375 was obtained for each increased unit in sd-LDL levels (95%CI: 1.072–1.764, $P = 0.012$).

Further analysis was performed to see whether effects of AMH on hepatosteatosis are stronger in IR PCOS group or not. Statistically significant higher levels of AMH were determined in the presence of hepatosteatosis both in IR(+) and IR(−) PCOS patients ($P < 0.001$) (Table 6).

The relation between AMH and sd-LDL levels were also evaluated. Statistically significant positive correlations between AMH and sd-LDL levels were determined among all participants involved in the study ($r = 0.486$, $P < 0.001$) and among PCOS patients as well ($r = 0.626$, $P < 0.001$).

Comments

In the present prospective case-control study, increased prevalence of hepatosteatosis was detected in young lean women with PCOS compared with age- and BMI-matched controls. In addition AMH and sd-LDL levels were found to be directly associated with hepatosteatosis even in the absence of PCOS.

In consistence with the majority of the previous reports we found an increased prevalence of hepatosteatosis by 41.4% in lean PCOS patients [27,28]. As well as ultrasonographic evaluation, serum alanine aminotransferase (ALT) levels have been widely used in the diagnosis of NAFLD and increased levels of aminotransferases were reported in women with PCOS as 15–39%, varying due to the upper limits of ALT as defined to be abnormal [27–29]. In our study abnormal ALT levels were detected in 23/58 (39.7%) PCOS patients, a result consistent with the report of Cerda et al. [28]. But in contrast with the aforementioned studies, all of the PCOS patients recruited in our study were lean with a mean BMI of 21.94 ± 2.05 kg/m^2. The most probable explanation for this high percentage of elevated ALT levels, is the use of a lower cut-off value for ALT (19 U/l) in our study as defined by Prati et al. [30].

Although all patients in our study were young and lean, the prevalence of hepatosteatosis is nearly the same with the previous studies consisted of patients with higher BMI and age. It has been already shown that the majority of cases with PCOS are overweight/obese and therefore the relation of hepatosteatosis with PCOS was based on the markedly increased prevalence of IR and abdominal obesity by now. However recently, it has been published that the presence of NAFLD may occur irrespectively of obesity in PCOS patients and in lean, insulin resistant patients

Table 4
Univariate analysis of possible risk factors for hepatosteatosis within PCOS group.

Variables	Normal ultrasonography (*n*: 34)	Hepatosteatosis (*n*: 24)	*P* value*
Age (median)	24.3 ± 3.1	24.2 ± 3.5	0.869
Body mass index (kg/m^2)	21.6 ± 1.8	22.4 ± 2.4	0.171
Waist–hip ratio	0.72 ± 0.05	0.75 ± 0.08	0.103
sd-LDL (mg/dl)	12.0 (2.0–24.0)	26.0 (13.0–50.0)	**<0.001**
AMH (ng/ml)	3.5 (1.0–8.6)	7.5 (5.2–26.1)	**<0.001**
HOMA-IR	1.7 (0.5–6.7)	2.9 (0.8–14.0)	**0.002**
QUICKI	0.35 (0.29–0.43)	0.33 (0.27–0.40)	**0.009**
FSH (mIU/ml)	5.0 ± 1.7	5.4 ± 1.1	0.173
LH (mIU/ml)	7.0 (2.9–23.8)	5.8 (1.7–23.2)	0.172
E2 (pg/ml)	34.5 (5.0–146.0)	37.0 (7.8–562.0)	0.528
Total testosterone (ng/ml)	0.25 (0.03–1.3)	0.30 (0.06–19.0)	0.786
Free testosterone (pg/ml)	2.4 (0.8–4.6)	2.5 (1.0–4.2)	0.411
17-OHP (ng/ml)	1.7 (0.5–2.8)	1.6 (0.5–4.0)	0.751
DHEA-S (ng/ml)	254.0 (64.0–677.0)	246.2 (97.0–562.0)	0.962
T. Cholesterol (mg/dl)	169.0 ± 32.4	167.9 ± 35.3	0.895
HDL (mg/dl)	53.2 ± 10.3	51.2 ± 10.8	0.492
LDL (mg/dl)	98.5 ± 31.5	99.7 ± 26.7	0.887
Triglycerides (mg/dl)	75.1 (34.8–196.0)	81.5 (30.0–233.0)	0.507
hsCRP (mg/dl)	1.6 (0.2–8.4)	1.1 (0.2–5.7)	0.162
ALT (U/l)	13.0 (5.8–59.0)	22.7 (8.2–89.0)	**0.039**
AST (U/l)	15.3 (8.6–37.0)	16.8 (12.0–53.0)	0.132
GGT (U/l)	11.1 (6.0–28.0)	12.7 (6.5–28.0)	0.722

PCOS: polycystic ovary syndrome, sd-LDL: small dense low density lipoprotein, AMH: anti-mullerian hormone, HOMA-IR: homeostasis model assessment, QUICKI: quantitative insulin sensitivity check index, FSH: follicle stimulating hormone, LH: luteinizing hormone, E2: estradiol, 17-OHP: 17 hydroxyprogesterone, DHEA-S: dehydroepiandrosterone–sulphate, HDL: high density lipoprotein, LDL: Low density lipoprotein, hs-CRP: high sensitive C reactive protein, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: Gamma glutamyltransferase.
* $P < 0.05$ statistically significant.

Table 5
Backward logistic regression analysis of possible independent risk factors for hepatosteatosis within PCOS group.

Variables	OR	95%CI	*P* value*
Step 1			
sd-LDL	2.030	0.771–5.344	0.152
AMH	9.768	0.606–157.386	0.108
HOMA-IR	15.400	0.239–993.158	0.198
ALT	0.994	0.863–1.145	0.929
Step 2			
sd-LDL	1.996	0.845–4.711	0.115
AMH	9.485	0.690–130.345	0.092
HOMA-IR	14.128	0.407–489.817	0.143
Step 3			
sd-LDL	1.375	1.072–1.764	0.012
AMH	3.347	1.348–8.313	0.009

PCOS: polycystic ovary syndrome, sd-LDL: small dense low density lipoprotein, AMH: anti-mullerian hormone, HOMA-IR: homeostasis model assessment, ALT: alanine aminotransferase.
* $P < 0.05$ statistically significant.

Table 6
The association of hepatosteatosis with AMH levels in PCOS patients with or without insulin resistance.

	Hepatosteatosis	AMH level•	*P**
IR(+) (*n*: 22)	Present (*n*: 15)	7.07 (5.19–26.14)	**<0.001**
	Absent (*n*: 7)	2.30 (1.01–4.28)	
IR(−) (*n*: 36)	Present (*n*: 9)	8.04 (5.77–17.20)	**<0.001**
	Absent (*n*: 27)	3.74 (1.02–8.64)	

PCOS: polycystic ovary syndrome, AMH: anti-Mullerian hormone, IR: insulin resistance.
• As median.
* $P < 0.05$ statistically significant.

[27,31]. Also lean NAFLD was shown to be independently associated with younger age, female sex and dyslipidemia in general population [32,33]. In addition, some clinical paradoxes contradict the association between obesity and IR such as: (i) lipodystrophic individuals develop severe IR and suffer from diabetes and dyslipidemia although they are extremely lean, (ii) some obese individuals do not present with metabolic syndrome [34,35]. This paradoxal relation was explained by the "adipose tissue expandibility" hypothesis suggesting that the ectopic deposition of lipids in organs except adipose tissue may cause IR by lipotoxicity [15]. As statistically significant higher levels of AMH were determined in the presence of hepatosteatosis both in IR(+) and IR(−) PCOS patients in our study, it is in consistence with the aforementioned previous studies suggesting that another mechanism and/or biomarker rather than obesity and IR may be responsible for the increased prevalence of NAFLD in PCOS.

Recent studies demonstrated that more than one-third of PCOS patients with normal lipids have hidden pro-atherogenic lipid disturbances including the increased levels of sd-LDL such as in our study [36,37]. However, Kim et al. recently reported that atherogenic changes in low-density lipoprotein particle profiles were not observed in non-obese women with polycystic ovary syndrome [38]. This difference may be due to the different ethnic and geographical origin of the patients. Besides Kim et al. also have been found increased levels of lipoprotein(a), a well-known independent risk factor for coronary heart disease in PCOS patients in consistence with the report of Rizzo et al. [37]. Eventually, atherogenic dyslipidemia is generally present in PCOS patients but probably the reflection of it to the laboratory test differs between different ethnic groups.

In our study, subgroup analysis demonstrated that among PCOS patients with hepatosteatosis HOMA-IR was significantly higher (median 2.95) when compared with both PCOS patients and controls without hepatic steatosis (median 1.72 and 1.68, respectively), a result in agreement with the previous studies [39,40]. The relatively lower value of HOMA-IR in our study when compared to others reporting HOMA-IR values above 4, may be due to the lower BMI levels of our study group.

In the present study, participants were further divided into two subgroups according to the presence of hepatosteatosis on ultrasonography. Sd-LDL, HOMA-IR, ALT and AST levels were again significantly higher in hepatosteatosis group, a result similar with the previous studies [39,40]. Also, the patients in hepatosteatosis

group had significantly higher levels of AMH and the presence of PCOS was also higher. Surprisingly, in multiple logistic regression analysis, only increased AMH rates and sd-LDL levels were found to be positively and significantly associated with hepatosteatosis in our study, but no significant correlations found between hepatosteatosis with ALT, AST, IR and even with the presence of PCOS. Further evaluation to determine the predictive factors of hepatosteatosis within the PCOS group in our study also showed that, both sd-LDL and AMH levels are predictive for the presence of hepatosteatosis. It has been already shown that dyslipidemia and the proatherogenic lipids are associated with NAFLD [36,37], but the role of AMH is not evaluated yet. As fertility is shown to be an important factor in fatty liver damage of NAFLD together with IR [21], but estrogen has a protective role [22,23], we concluded that "the another factor about fertility responsible for hepatic steatosis" may be AMH.

Statistically significant positive correlations between AMH and sd-LDL levels were determined among PCOS patients in the present study. This significant association of AMH and sd-LDL levels indicate the possible effect of AMH on hepatosteatosis may be due to the altered lipid composition.

Our study has both strengths and weaknesses. First, all participants were recruited prospectively, evaluated by the same clinicians and all data were available at the time of analysis that gave permission to further evaluation with additional laboratory tests when necessary, in order to exclude liver diseases other than NAFLD. Second, patients were not receiving any medications while participating. Third, both groups were homogenous and age- and BMI-matched with each other as well. Limitations were: (i) the relatively small number of patients that may defeat the applicability of the findings to general population, (ii) lack of information on other potential confounding factors, such as differences in diet, family history, and/or exercise patterns, (iii) only non-obese and young subjects are examined. Only young PCOS patients were enrolled in our study as metabolic impairment leading to increased risk of NAFLD reported to begin at earlier stages of the disease, so it is important to screen PCOS patients at an earlier age than is currently recommended for the general population [29]. Another limitation of the present study is that the serum AMH concentrations were determined by using ultrasensitive ELISA (AMH ELISA kit; Diagnostic System Laboratories, Texas USA) but this enzyme linked immunosorbent assay is not available anymore and AMH Gen II assay is in current use. The values obtained with the AMH Gen II assay were reported to be approximately 40% higher than those obtained simultaneously using the DSL assay [41]. Besides, several other authors suggested that Gen II assay exhibited a systematic positive bias across the concentration range despite using same standard [42]. The cause for this discordance between anticipated and actual population values for the AMH Gen II assay is unclear. For today, neither of these assays are the "gold test". So, it seems that there is urgent need for the introduction of new assays into this field.

To the best of our knowledge this is the first study examining the relation between hepatosteatosis and serum AMH levels in lean PCOS patients. The gold standard in the diagnosis of hepatosteatosis is known to be fine-needle aspiration biopsy but in our study fatty infiltration of the liver was evaluated by ultrasonography. However, considering the high prevalence of PCOS among women and that the liver biopsy is associated with serious risks including mortality, it was not feasible and ethical to perform biopsy in all PCOS and control patients. In addition, hepatic ultrasonography was shown to have a high sensitivity, specifity and positive predictive value for steatosis [43,44].

In conclusion, the current study shows that hepatosteatosis is common in PCOS patients and increased AMH and sd-LDL levels are associated with the presence of it. Since NAFLD is a potentially progressive disease and a factor that increases the probability of cardiovascular risks, assesment of liver enzymes and the evaluation of fatty liver infiltration by ultrasonography is suggested especially in patients with high levels of AMH for early diagnosis of hepatosteatosis. This study need to be validated with larger cohorts and serum samples for the measurement of AMH levels can be obtained from patients particularly with biopsy-proven hepatosteatosis, NAFLD or NASH, independent from the presence of PCOS.

Condensation

Increased anti-Mullerian hormone and small-dense low-density lipoprotein cholesterol levels are associated with hepatosteatosis in young lean women with and without polycystic ovary patients.

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