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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