LETTER TO THE EDITOR

Contribution of apolipoprotein estimation to cardiovascular risk assessment in women with endometriosis

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To the Editor,

Endometriosis is a widespread anomalous gynecologic disorder whose etiology is unknown. It is characterized by the implantation of endometrial tissue outside the uterus, particularly within the pelvis (1). Randomized clinical trials have improved our knowledge in better understanding the factors involved in the manifestation of cardiovascular disorders. The great achievement in this regard was to find out the association of elevated cholesterol levels with cardiac manifestations (2). Scarce information is available regarding the protein components associated with lipids, the apolipoproteins, which are also documented risk factors of cardiovascular disorders (CVD) (3).

Apolipoprotein B plays a vital role in the body because total cholesterol is the sum of all cholesterol in the HDL-C and Apo-B lipoproteins. Several clinical investigations have shown maximum cardiovascular risks when Apo-B was high and non–HDL-C was normal. Hence, Apo-B is a better biomarker of CVD risk as compared to non–HDL-C (4). Lipoprotein a [Lp(a)] is a lipoprotein full of cholesterol and is different from low-density lipoprotein by having a glycosylated protein apolipoprotein (A) [Apo(A)], that is bonded covalently to it. The direct association between elevated levels of serum Lp(a) and LDL with increased CVD risk has also been reported (5).

The present study aims to demonstrate the concentrations of apolipoproteins Apo-A1, Apo-B, and Apo-B:Apo-A1 ratio in females with endometriosis and healthy subjects and its prognostic efficiency in the establishment of cardiovascular disorders. Thus, the concept that Apo-B, Apo-A1, or Apo-B:Apo-A1 ratio may be exploited as the prognosticator of cardiovascular disorder, can be supported with this study.

MATERIALS AND METHODS

In this study, 81 premenopausal females aged 20 to 40 years, laparoscopically confirmed with endometriosis (revised American Fertility Society 1 [rAFS]) were recruited. As hormonal therapy can derange the lipid metabolism, none of the women had taken any medication or drug during the six months prior to sample collection.

A control group consisting of premenopausal healthy females was matched for body mass index, age, and systolic/diastolic blood pressure. None of the control subjects had taken any medication or oral contraceptives known to modify or upset the metabolic rate of lipids for 6 months. The investigation was permitted by the Ethics

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Committee of the tertiary care Hospital and informed written consent was obtained from each of the subjects included in the study. Women with chronic disorders such as diabetes mellitus, cardiovascular and autoimmune diseases were excluded from the study. Likewise, females with a history of pre-eclampsia or having ovarian cysts other than endometrium were also excluded. 5 ml blood samples were taken from each subject by venipuncture, using a sterilized disposable syringe (BD, Becton and Dickinson, Singapore), and were placed in a serum vacutainer. The blood samples were given special IDs marked on each of the blood tubes.

Within 2-3 h of phlebotomy, serum was separated. The blood sample was centrifuged at 3500 rpm for 10 min. RNase-free, 1.5 mL microfuge (Ambion, USA) tubes, were utilized to preserve separated serum which was stored at -80°C until further biochemical assessment. Apolipoprotein (apo) A1 and B levels in serum were analyzed by enzyme-linked immunosorbent assay (ELISA) kits using Glory Biosciences, USA, (cat # PRS-01940hu and cat# PRS-01939hu, respectively). Data obtained by biochemical analysis were subjected to statistical interpretation by applying the unpaired Student's *t*-test using Graph Pad Prism version 6 software.

RESULTS

The levels of various parameters of the study group are shown in Table I. The Apo-A1 level was estimated to be 1245±49.44 **µ**g/mL in the control group, which showed a significant reduction of 22% (P<0.0001) in patients. The level of Apo-A1 in the endometriosis group was 973.8±40.74 **µ**g/mL (Fig. 1). The average level of Apo-B was found to be 885±25.52 and 1484±46.04 **µ**g/mL in the control and patient groups, respectively. A significant difference (P< 0.0001) was observed with a 68% increase of Apo-B in the patient group (Fig. 2).

The average level of Apo-B:Apo-A1 ratio in the endometriosis and control group was found to be

Table I. *Demographic variables and lipid profile of newly diagnosed female patients with endometriosis and healthy controls.*

Parameters	Control (n=80)	Patient (n=81)	*P*-value	Percentage difference
Age (Yrs)	31.01±0.62	32.1±0.58	0.2	3.5
SBP (mmHg)	122.6±0.98	123.1±0.98	0.7	0.4
DBP (mmHg)	80.63±0.98	82.04±0.90	0.2	1.74
Waist (cm)	84.8±0.55	85.17±0.76	0.6	0.4
BMI (kg/m^2)	22.5±0.28	23.26±0.30	0.09	3.3
Apo-A1(µg/mL)	1245±49.44	973.8±40.74	< 0.0001	22↓***
Apo-B (µg/mL)	885.0±25.52	1484±46.04	< 0.0001	68↑***
Apo-B:Apo-A1	0.83±0.05	1.805±0.11	< 0.0001	125↑***

****indicate significance at P< 0.0001, SBP: Systolic BloodPressure,DBP: Diastolic Blood pressure, BMI: Body Mass Index, Apo-A1: Apolipoprotein A1, Apo-B: Apolipoprotein B, Apo-B:Apo-A1 (ratio) Values are mean±SEM.*

1.80±0.11and 0.83±0.05, respectively. A significant difference (P<0.0001) was observed with a 125% increase of Apo-B:Apo-A1 in endometriosis patients (Fig. 3).

DISCUSSION

In the present study, the level of Apo-A1 was significantly reduced, while marked elevation in the Apo-B concentration was found in the patients group, suggesting more cardiovascular risk and associated disorders in these patients. Apo-B is synthesized in the gut and liver and shows the overall atherogenic activity capturing the lipoproteins in the arterial wall (6). It has been documented from clinical trials and epidemiological studies that Apo-B is superior to other cholesterol biomarkers in establishing the risk of cardiovascular disorders and affecting the suitability of therapeutics involved in minimizing the lipids (7).

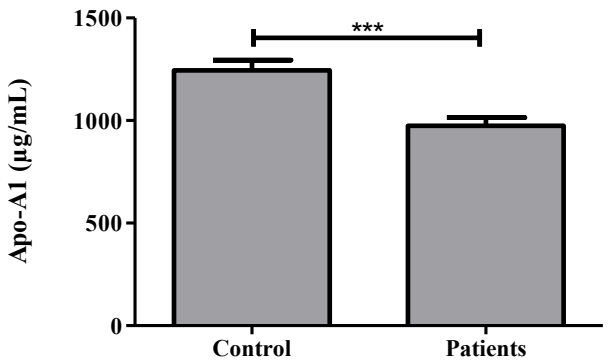

Fig. 1. *Apo-A1 (µg/mL) in control subjects and patients. ***indicates significance at P < 0.0001*

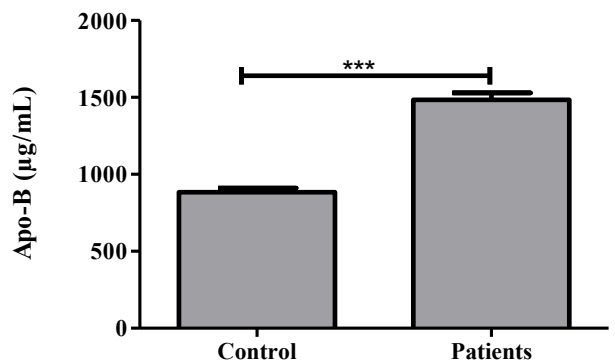

Fig. 2. *Apo-B (µg/mL) in control subjects and patients. ***indicates significance at P < 0.0001*

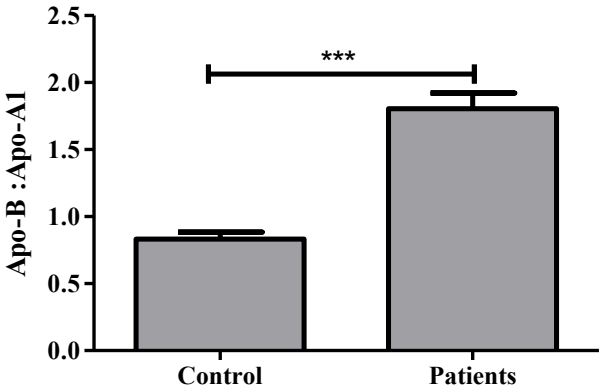

Fig. 3. *Apo B:Apo A1 ratio in control subjects and patients. ***indicates significance at P < 0.0001*

Allan et al. documented that the rate at which Apo-B particles move through the arterial wall and are captured is the number of the Apo-B particles in the lumen of arteries (8). Hence, the higher the Apo-B particles in the arterial lumen, the chances of capturing are higher. Apo-B particles smaller in size will contain a small amount of cholesterol, easily go through the arterial wall, and then keenly attach to the glycosaminoglycans as compared to bigger Apo-B particles (9). Thus, there is a higher number of trapped smaller cholesterol-depleted particles as compared to larger cholesterol-enriched particles in the arterial wall. Conversely, the higher the cholesterol trapped within Apo-B particles in the arterial wall, the higher cholesterol will be delivered at that position to damage the wall. As a result, there is correlation between greater damage per particle from the trapping of cholesterol-depleted as well as cholesterol-richer particles. Eventually, all LDL particles present, approximately, the same risk (2).

In the present study, the endometriosis group demonstrated higher levels of Apo-B as compared to control subjects. By considering the above-mentioned facts, these women may have high cardiovascular risk and may develop cardiovascular disorders in later life.

It has been suggested that Apo-B:Apo-A1 is the most precise diagnostic marker of cardiovascular risk (10). It considers both sides of the risk equation, the Apo-A1 side, which is antiatherogenic and, the Apo-B side which is atherogenic. The higher

Apo-B:Apo-A1 ratio shows the more circulating cholesterol that tends to be accumulated in the arterial wall, inducing the risk of atherosclerosis and CVD (11).

The results specify that the Apo-B:Apo-A1 is a lucid, precise, and novel marker for CVD events, the higher the Apo-B:Apo-A1, the higher the risk of CVD events. In the present investigation, the Apo-B:Apo-A1 ratio is elevated significantly in endometriosis subjects versus healthy controls, demonstrating their risk of CVD. Our investigation reinforces the idea that Apolipoprotein-B, Apolipoprotein-A1, and Apo-B:Apo-A1 can be utilized as strong prognosticators of CVD assessment in subjects with endometriosis.

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