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Lipid profile and apolipoprotein A1 and B levels in women with pre- and postmenopausal breast cancer undergoing neoadjuvant therapy

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Abstract

Breast cancer is associated with modifiable risk factors such as diet and obesity. In this sense, the circulating levels of apolipoproteins and lipoproteins such as LDL cholesterol or HDL cholesterol could be involved in tumor development and be modified by them. Also, the hormonal profile of the patients seems to be a decisive factor in the progression of the disease, as well as determining, to a certain extent, the presence of obesity in postmenopausal women.

In this context, the purpose of this work has been to determine the levels of lipoproteins, specifically, LDL cholesterol and HDL cholesterol, using standard enzymatic colorimetric methods, as well as apolipoproteins ApoA1 and ApoB, using specific ELISA kits, in pre- and postmenopausal women with breast cancer treated or not with neoadjuvant chemotherapy in comparison with healthy pre- and postmenopausal women.

The significant differences obtained between groups of patients studied at the levels of lipoproteins and apolipoproteins, lead us to conclude that the hormonal profile is decisive to regulate lipid metabolism and that it is possibly involved in the progression of the disease according to the administration or not of neoadjuvant chemotherapy, mainly in postmenopausal women.

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Introduction

Diet and obesity are considered risk factors for breast cancer (BC), being both of them modifiable[1][2]. In this sense, the metabolism of tissue lipids as well as their circulating levels would be involved in the relationship between obesity and the risk of pathology [3]. On the other hand, the metabolism of sex hormones is altered by obesity, as are the pathways of inflammatory factors and other endocrine metabolism schemes [4].

Many studies suggest a relevant role of nutrition in the progression and development of breast cancer[5]. A high cholesterol intake is positively associated with the risk of breast cancer, especially among postmenopausal women [6][7]. Cholesterol is transported mainly by low-density and high-density lipoproteins LDL and HDL). and in studies evaluating the association between the lipid profile and the tumor process, it is observed that LDL cholesterol levels affect the prognosis of the progression of carcinoma, unlike HDL cholesterol, which plays an inverse role in carcinogenesis, by regulating cell cycle entry and cell apoptosis through the mitogen-activated protein kinase (MAPK) pathway [8]. In premenopausal women, recent studies propose a protective role for HDL against breast cancer [9][10], suggesting the modulation of cholesterol metabolism in breast cancer cells and its implication in consequence with the tumor process [11].

Apolipoproteins are the protein component of HDL, LDL, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL) and lipoproteins [12], which precisely explains how lipids and lipoproteins are transported. These apolipoproteins are not only involved in coronary-artery disease, atherosclerosis, metabolic syndrome (MS) and Type 2 diabetes (T2D) but also participate in various types of carcinoma [4]. Apolipoprotein A-I (ApoA-1), a subtype of ApoA, is involved in the reverse transport of cholesterol from peripheral tissues to the liver [13][14], being the most abundant in HDL particles [13]. In addition to releasing cholesterol from cells, ApoA-1 participates in physiological or pathological anti-inflammatory, anti-oxidation and anti-apoptosis processes [15].

Apolipoprotein B (ApoB) is responsible for transporting lipids into the cells of the human body[16], and it has been reported that some ApoB polymorphisms significantly increase the risk of breast cancer, especially in women who are in menopause [17]. Once breast carcinoma develops intraocular metastasis, ApoB acts as a risk factor[18] and could

establish indirect effects on the development of BC through 27-hydroxycholesterol (27-HC) and LDL.

Some authors point out the comparison of serum profiles, acquired before and after preoperative therapy, as a putative determination to evaluate tumor progression. In this sense, studies such as that of Mazouni et al [19] reveal that proteins such as α2-macroglobulin, complement 3, hemopexin, and C and A chains of apolipoprotein A-1 are modified. All proteins were decreased after therapy, except chain C apolipoprotein A that increased in women with breast cancer.

Given the implication of both HDL and LDL and the apolipoproteins that transport them, specifically Apo A-I and Apo B. in breast carcinogenesis. the purpose of this work is to determine the effect of neoadjuvant chemotherapy (NACD in pre- and postmenopausal women with breast cancer on these molecules and discuss the information they could provide on breast cancer evolution.

Material and methods

Subjects and Study design

A total of 198 women were recruited at the Unit of Breast Pathology at the University Hospital of Jaen, and 78 volunteers women without breast cancer were also included as control groups. This study was approved by the Ethical Committee of the University Hospital of Jaen and all subjects signed a term of free, informed consent.

All women with breast cancer were diagnosed with ductal infiltrating carcinoma. A total of 83 of these women (39 premenopausal and 44 postmenopausal) did not receive NACT, whereas 115 of them (63 premenopausal and 52 postmenopausal) received NACT before surgery. The clinicopathological characteristics of studied patients have been previously reported [20] and are shown in Table 1. Patients treated with NACT received an anthracycline/taxane-based regimen including 4 courses of EC (epirubicin 90 mg/m2 and cyclophosphamide 600 mg/m2, every 21 days), followed by 8 courses of 100 mg/m2 paclitaxel once a week or 4 courses of 75 mg/m2 docetaxel every 21 days. Patients with a HER2/neu-overexpressing tumor also received trastuzumab (14 courses at 6 mg/kg every 21 days). Women with triple-negative breast cancer received 6 cycles of 75 mg/m2 docetaxel plus carboplatin (AUC 6).

Control groups consisted of 78 women, aged 28 to 69 years old (premenopausal women with regular menstrual periods n=38; postmenopausal women with spontaneous menopause for at least one year, n=40), with no previous history of any type of cancer, chemotherapy, hormonal or antioxidant therapy, or chronic diseases. Thus, women were excluded if they were current smokers, regular alcohol consumers, antioxidant supplement users, pregnant or lactating, presented hepatic, cardiac or renal dysfunction, hormonal therapy, use of drugs, hypertension, diabetes, and other eventual chronic conditions.

Sample acquisition

Samples from patients treated with NACT were obtained after completion of chemotherapy treatment and in parallel to

samples from patients not treated with NACT and control volunteers to be processed under the same conditions.

Blood samples were obtained after an overnight fast by venous arm puncture in tubes without anticoagulants. Blood specimens were allowed to clot and centrifuged at 3000 g, for 10 min, at 4 °C to obtain the serum. Serum samples were collected, rapidly frozen in liquid nitrogen and kept at -80°C until usage for assays.

Apolipoprotein A1 (ApoA-1) assay

Samples were measured by a human apolipoprotein A1 ELISA kit (Invitrogen), according to manufacturer instructions. The sensitivity of detection is 0.08 ng/mL; intra-assay coefficient of variation is <10%; inter-assay coefficient of variation is <12%.

Apolipoprotein B (ApoB) assay

Samples were measured by a human Apolipoprotein B ELISA Kit (Abeam), according to

manufacturer instructions. The sensitivity of detection is 5.28 ng/mL; intra-assay coefficient of variation is 5.9%; inter-assay coefficient of variation is 2.4%.

Cholesterol assay

Total cholesterol, high-density lipoprotein cholesterol and triglycerides were assayed with the use of standard enzymatic colorimetric methods using commercially available kits according to Roeschlau [21], Sugiuchi [22] and Siedel [23]. The low-density lipoprotein (LDL) cholesterol levels were calculated according to the Friedewald formula. Results are expressed in mg/dL.

Statistical analysis

To analyze the differences between groups, we have used multiple analyses of variance plus LSD post-hoc test, using IBM SPSS V.23 software. All comparisons with p-values below 0.05 were considered significant.

Results

Figure 1 shows circulating levels of apolipoprotein A I (A) and apolipoprotein B (B) measured in pre-and postmenopausal control women and women diagnosed with infiltrating ductal carcinoma treated or untreated with NACT.

Regarding ApoA-1 in premenopausal women with breast cancer a significant increase ($p<0.01$) is found when compared with premenopausal control women. However, we observe a significant decrease ($p<0.05$) in ApoA-1 in postmenopausal women with breast cancer untreated and treated with NACT respect to the same group in premenopausal women. On the contrary, no changes are observed in circulating levels of ApoB in premenopausal women groups. Figure 1B shows a

significant ($p<0.01$) lower concentration of circulating ApoB in premenopausal control women when compared to postmenopausal control women. On the contrary, we observe a significant increase in ApoB levels ($p<0.01$) in treated postmenopausal women with breast cancer when compared with postmenopausal control women.

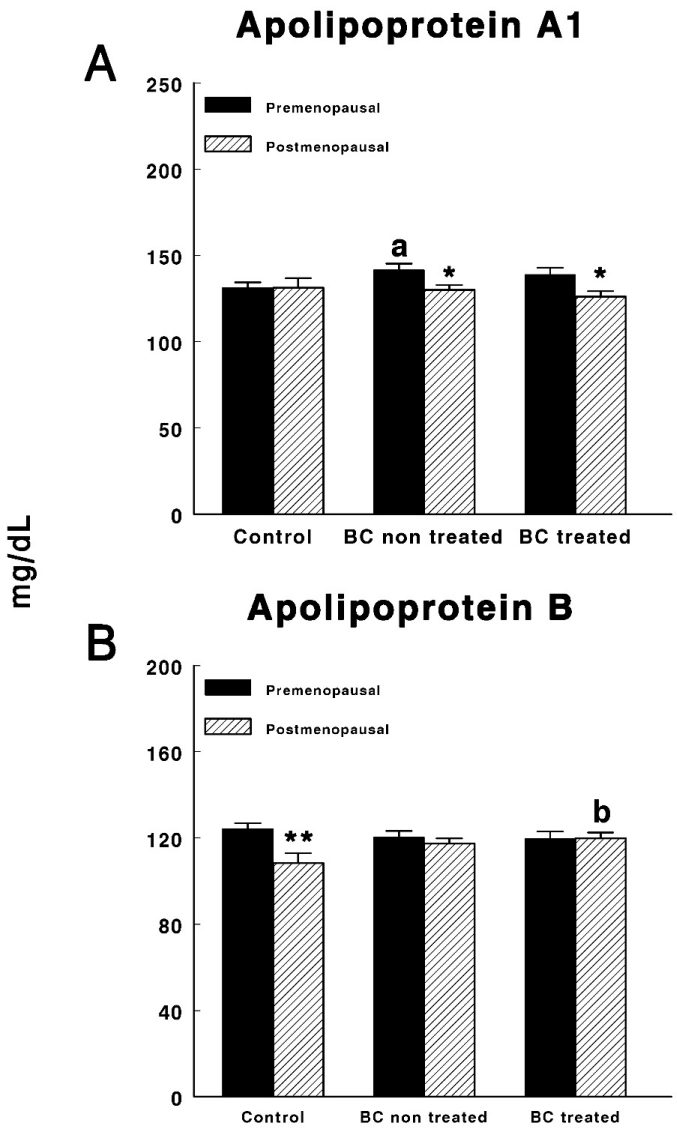

Figure 1. Circulating levels of apolipoprotein A-1 (A) and apolipoprotein B (B) were measured in healthy premenopausal and postmenopausal control women, premenopausal and postmenopausal women with breast cancer and premenopausal and postmenopausal women with breast cancer treated with neoadjuvant chemotherapy (Mean ± SEM; *$p<0.05$; **$p<0.01$; $^{a}p<0.01$ vs. control premenopausal women; $^{b}p<0.01$ vs. control postmenopausal women).

Figure 2 shows circulating levels of HDL cholesterol (A) and LDL cholesterol (B) measured in pre-and postmenopausal control women and women diagnosed with infiltrating ductal carcinoma treated or untreated with NACT.

A significant ($p<0.01$) lower level of HDL cholesterol was found in both treated and untreated premenopausal women with breast cancer when compared to their control group. Similar results are observed in postmenopausal women regarding HDL cholesterol with a significant decrease ($p<O.O\ 1$) in treated and untreated women with breast cancer when compared to the postmenopausal control group. Treated postmenopausal women with breast cancer found a significant decrease ($p<0.01$) when compared to treated premenopausal with breast cancer.

Figure 2B shows a significant increase ($p<0.01$) in postmenopausal control women with respect to premenopausal control women. However, a significant decrease ($p<0.01$) in untreated and treated women with breast cancer was observed when compared with postmenopausal control women.

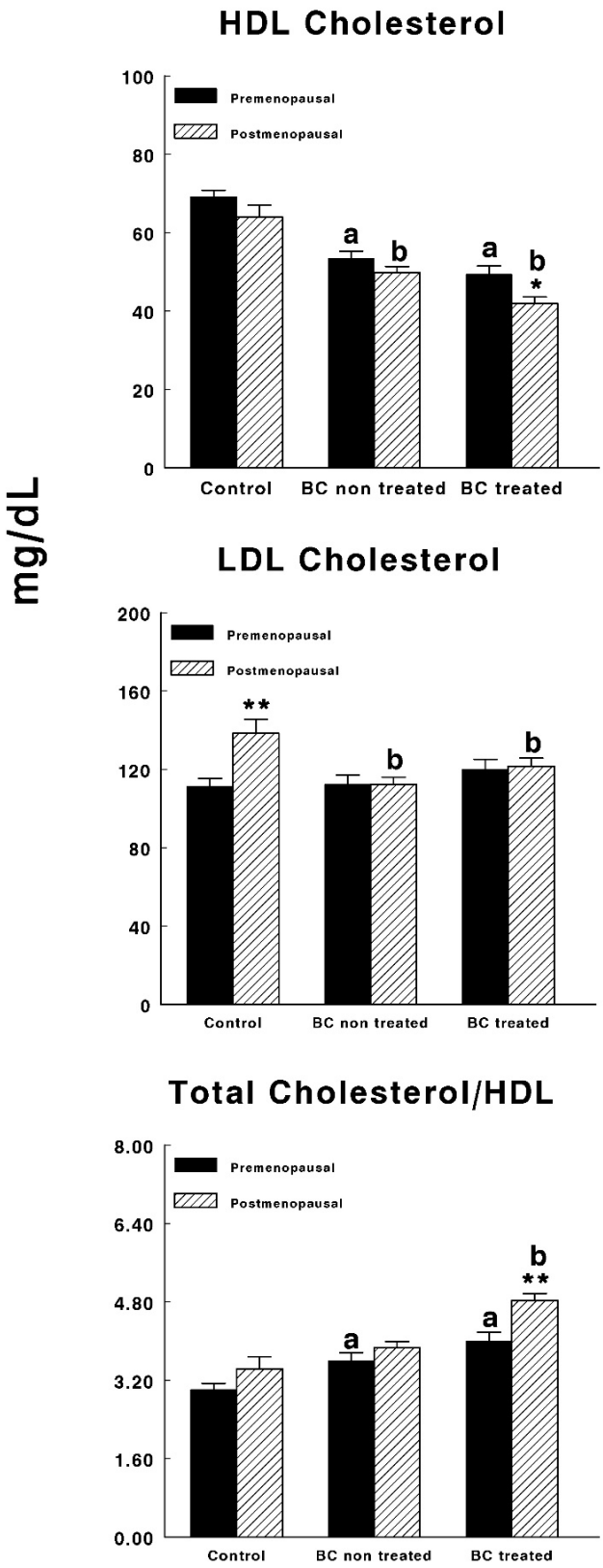

Figure 2. Circulating levels of HDL cholesterol (A), LDL cholesterol (B) and total cholesterol/HDL ratio, measured in healthy premenopausal and postmenopausal control women, premenopausal and postmenopausal women with breast cancer and premenopausal and postmenopausal women with breast cancer treated with neoadjuvant chemotherapy (Mean ± SEM; *p<0.05 **p<0.01; [a]p<0.01 vs. control premenopausal women; [b]p<0.01 vs. control postmenopausal women).

Discussion

Unhealthy lifestyles as well as hormonal and reproductive factors that trigger obesity are considered important risk factors for breast cancer [24]. In fact, during menopause, it is a factor that is closely associated with the prognosis of BC. Postmenopausal women are more likely to develop breast cancer, being more aggressive, possibly due to the hormonal imbalance typical of menopause. Furthermore, during menopause, the diagnosis of metabolic syndrome and type II diabetes also increases [25]. Consequently, the menopausal state, metabolic syndrome, type II diabetes and obesity are closely related to each other and affect the development of BC [26]. Since cholesterol is primarily transported by LDL and HDL, several clinical trials have associated them with breast cancer. In this sense, some studies do not find associations between lipoproteins and breast cancer, while others show a direct association between LDL cholesterol and the risk of breast cancer, as well as an inverse association with HDL cholesterol [27]. However, a complex relationship between HDL and menopause has also been described [28]. In this context, our results show a decrease in HDL cholesterol levels in pre and postmenopausal women with breast cancer treated and not treated with NACT, compared to their healthy controls. Our data are in agreement with those published by Tian et al. [29] since we also observed a decrease in HDL cholesterol levels in the groups treated with NACT, which points to the importance of the hormonal profile in NACT treatment. In addition, in postmenopausal women with breast cancer treated with NACT, we observed a significant decrease compared to premenopausal women with treated breast cancer, which redounds the importance of the hormonal profile in NACT treatment and its effect on the lipid profile. Most estrogen-dependent breast cancers occur after menopause, despite low levels of circulating estrogens. Studies such as those developed by Brown et al. [30], indicate that aromatase levels are higher in the breast tissue of postmenopausal women, regardless of BMI, with aromatase mRNA levels strongly associated with HDL cholesterol, among others. Therefore, in general and regardless of BMI, postmenopausal women have higher breast aromatase expression than their premenopausal counterparts [30]. The BMI data of our study has been previously described in Ramirez-Expósito et al {m/2020/} [31] presenting premenopausal women with breast cancer not treated and treated with NACT and postmenopausal women without breast cancer a BMI of 25-29.9 kg/m^2, while postmenopausal women with treated or untreated breast cancer had a BMI ≥30 kg/m^2 or more [32].

Thus, following this sequence: menopause - increased aromatase activity in breast tissue

- modification of HDL/cholesterol levels, the modification of the lipid profile, specifically HDL levels, could provide us with indications on the progression of hormone-dependent breast cancer in postmenopausal women.

In this context. we must take into account that compound as paclitaxel inhibited basal and TNFα-stimulated aromatase activities in stromal fibroblasts derived from normal or malignant breast tissues [33].

Regarding LDL cholesterol levels, in our study, the group of healthy postmenopausal women shows a significant increase compared to healthy premenopausal women. In this sense, postmenopausal women often have significantly higher BMI than the premenopausal group [34]. Weight gain during the menopausal transition has been attributed to hormonal

changes, lower physical activity, and increased energy intake [35]. In the premenopausal group, we did not observe significant differences between healthy women, and women with breast cancer no-treated and treated with NACT. In contrast in breast cancer women postmenopausal group, the LDL levels are decreased in untreated and treated postmenopausal women, with respect to the healthy group. In this line, authors such as Pires et al. [36] showed that before chemotherapy, there is an overexpression of LDL receptor in the tumoral tissue compared to normal breast tissue in 8 of these patients and after chemotherapy, expression of LDL and LDL receptor-related protein I, decreased in the tumors of 6 patients, increased in 4 and was unchanged in 2, concluding that chemotherapy does not impair LDL receptors.

This fact could be related to the ability of 27-hydroxycholesterol (27 HC) to bind to estrogen receptors, accepting itself as an estrogenic ligand [37]. 27-HC is mainly transported by LDL and could stimulate ER-positive MCF-7 cells, being ApoB the major structural protein of LDL [27]. In our study, ApoB is significantly increased in postmenopausal women with breast cancer treated with NACT concerning the postmenopausal healthy group. No changes were observed in ApoB levels in premenopausal women groups.

About ApoA-1 levels, in premenopausal women with breast cancer not treated, we observed a significant increase compared to healthy women. ApoA-1 has been reported to significantly inhibit the growth and invasion of tumor cells, however, the overexpression of ApoA-1 in serum may not absolutely benefit BC patients, since redundant ApoA-1 increases 27- hydroxycholesterol (27-HC) expression, which favors tumor growth [27] becoming involved in tumor metastasis [4]. On the other hand, the reduction in oxidized LDL caused by the overexpression of ApoA-1 similar to the increase in the LDL level promotes multiplication and migration in ER-negative BC cells, and oxidized LDL may promote MCF-7 cell proliferation [38][39].

In contrast, postmenopausal women with breast cancer not treated and treated with NACT show a significant decrease in ApoA-1 levels, compared to their respective groups of premenopausal women. ApoA-1 is the main apoprotein constituent of high-density lipoprotein, and as we have described above, ApoA-I participates in several physiological or pathological processes of anti- inflammation, anti-oxidation and anti-apoptosis. Nevertheless, the effect of menopause and its relationship with aromatase activity and HDL levels could decide between pro or antitumor effects.

Conclusions

We can conclude that the hormonal profile is definitive in terms of the lipid profile of patients with breast cancer and NACT treatment. Specifically, on the levels of HDL and LDL, as well as the apolipoproteins that constitute them, which could condition tumor development. In this sense, we would highlight its importance in postmenopausal women, where the putative relationship between lipoprotein levels and aromatase activity, in our view, makes relevant a greater number of in-depth studies in this line of research.

Conflict of Interest

The authors declare no conflict of interest.

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