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The Level of 27-hydroxycholesterol and Oxysterol 7α-hydroxylase (CYP7B1) in Tissues of Women with Breast Tumors

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Abstract

Background In Iraq, breast cancer is the commonest type of malignancy in females. The disease is a genetically and clinically heterogeneous. The cholesterol metabolite 27-Hydroxycholesterol (27HC), a primary metabolite of cholesterol and an estrogen receptor (ER) and Liver X receptor (LXR) ligand, increases ER-positive breast cancer. 27-hydroxycholesterol (27HC) is metabolized by oxysterol 7α-hydroxylase (CYP7B1), CYP7B1 expression has been decreased in ER-positive tumors compared with normal breast tissues.

Objective To study and investigate the possible association of clinic-pathological parameters 27HC and CYP7B1 level in sera of women with benign and malignant breast tumors and in control group by ELISA technique and Investigate the possible relationships of 27HC with CYP7B1.

Methods This case control study was conducted on sixty patients with breast diseases were divided into three group, group I contained twenty patients with benign breast diseases, group II consisted of twenty premenopausal patients with breast cancer. Group III comprised twenty postmenopausal patients with breast cancer with the mean age and standard deviation (25.25±7.87, 38.65±6.28, 58.5±7.02 years). 27HC and CYP7B1 were measured in tissues by instrument ELISA technique.

Results The tissue homogenates of women with premenopausal and postmenopausal breast cancer groups showed a significant elevation of 27HC in comparison with benign ($p > 0.001$), whereas there is no significant difference observed between both breast cancer groups ($p = 0.542$). The tissue homogenates of women with premenopausal and postmenopausal breast cancer groups showed a significant decrease of CYP7B1 concentration in comparison with benign ($p = 0.003$) and ($p = 0.001$), whereas there is no significant difference observed between both breast cancer groups ($p = 0.868$).

Conclusion A higher incidence of 27HC and a lower incidence CYP7B1 were obtained in malignant than benign breast tumor tissues with positive estrogen receptors. These indicate that the levels of 27HC and CYP7B1 in breast tumor tissues may be used as new biochemical markers for breast tumor prognosis.

Keywords 27-Hydoxycholesterol, CYP7B1, breast cancer, estrogen receptor

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List of abbreviations: 27HC = 27-Hydoxycholesterol, CYP7B1 = oxysterol 7α-hydroxylase, CYP27 = Cytochrome P450 sterol 27-hydroxylase.

Introduction

The cholesterol metabolite 27-hydroxycholesterol (27HC) formed by the mitochondrial cytochrome P450 sterol 27-hydroxylase (CYP27), an enzyme particularly expressed in the vascular endothelium, macrophages and the liver [1,2]. Introduction of a hydroxyl group allows the otherwise hydrophobic cholesterol molecule to pass amphiphilic membranes more easily [2,3]. Because of these physicochemical properties,

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27HC has been postulated to be secreted from cells independently of transporters and extracellular lipoprotein acceptors and thereby to facilitate an alternative route for apolipoprotein (apo) A-I/high density lipoproteins (HDL)-mediated transport of cholesterol from macrophages to the liver [2]. In the liver, 27HC is an important intermediary product of the so-called alternative bile acid synthesis pathway, which contributes ~10% to de novo bile acid biosynthesis [2]. In addition, 27HC is an important ligand of at least two types of nuclear hormone receptors. It activates liver-X-receptors (LXR) alpha and beta, which regulate the transcription of several genes involved in lipid and lipoprotein metabolism [4,5]. Most recently 27HC was identified as the first endogenous selective estrogen receptor modulator (SERM). Both in vitro and in vivo 27HC was found to modulate the transcriptional activity of estrogen receptors tissue-specifically either as an agonist or antagonist [6].

The cholesterol metabolite 27HC is the most abundant oxysterol in the circulation [4], the plasma levels of 27HC have been found to correlate with the cholesterol content in atherosclerotic lesions and the severity of coronary artery disease [7]. Patients with genetic CYP27 deficiency suffer from cerebrotendinous xanthomatosis and develop premature atherosclerosis despite having normal levels of plasma cholesterol [2]. In addition, there are greater 27HC levels in tumor samples compared with controls. Furthermore, survival of cancer patients is markedly poorer for patients with low versus high tumor CYP7b1 expression. In mouse models, 27HC promoted the tumor growth and metastasis by independent mechanism [8].

This study aimed to determine of 27HC levels and CYP7B1 in tissues of women with benign and malignant breast tumors. Also, to investigate the possible relationships of 27HC with CYP7B1.

Methods

The study was executed during the term from February 2017 to June 2017, it included 60 patients of woman with breast tumor that divided into 3 groups; Group I contained 20 patients with benign breast tumor, Group II consisted of 20 premenopausal patients with breast cancer, Group III comprised 20 postmenopausal patients with breast cancer with the mean age and standard deviation (25.25±7.87, 38.65±6.28, 58.5±7.02 years) respectively. All samples were collected from Al-Imamein Al-Kadimein Medical City, Medical City of Baghdad Teaching Hospital, Al-Kadimiyah Privet Hospital and Al-Numan Hospital. They were histologically proven, newly diagnosed and not underwent any type of therapy. Patients suffered from any disease that may interfere with this study were excluded.

Collection of specimens

The tumor tissues were surgically removed from breast tumor patients by either mastectomy or lumpectomy. The specimens were cut off and immediately immersed in ice-cold isotonic saline solution. They were collected individually in plastic receptacle and stored at -20 °C until homogenization. The level of 27HC and CYP7B1 was measured by monoclonal antibody Enzyme Linked Immuno Sorbent Assay (ELISA) technique using number of kit two for each 27HC and CYP7B1 from China.

Data were statistically analyzed using SPSS statistical software (version 23). Values were expressed as the mean ± standard deviation. A unpaired t test was used to compare mean levels of 27HC and CYP7B1 in women with breast tumors.

Results

In tissue homogenate samples, 27-HC was significantly elevated in postmenopausal breast cancer group compared with benign group ($p< 0.001$) and elevated in premenopausal breast cancer group compared with benign group ($p< 0.001$) as shown in table (1) and figure (1). In

tissue homogenate samples, CYP7B1 was significantly decreased in postmenopausal breast cancer group compared with benign group (p= 0.001) and CYP7B1 was significantly decreased in premenopausal breast cancer group compared with benign group (p= 0.003) as shown in table (2) and figure (2).

The possible relationships of 27-HC with CYP7B1 in tissue homogenates of women with breast tumors is shown in table (3).

Table 1. Comparison the concentration of 27-Hydroxy cholesterol (ng/l) in sera and tissues homogenate of women with breast tumors

Group	No.	Mean±SD in tissue	P value
Group I Benign	20	15.90±4.84	< 0.001
Group II Pre	20	26.95±5.68	
Group I Benign	20	15.90±4.84	< 0.001
Group III Post	20	28.25±7.55	

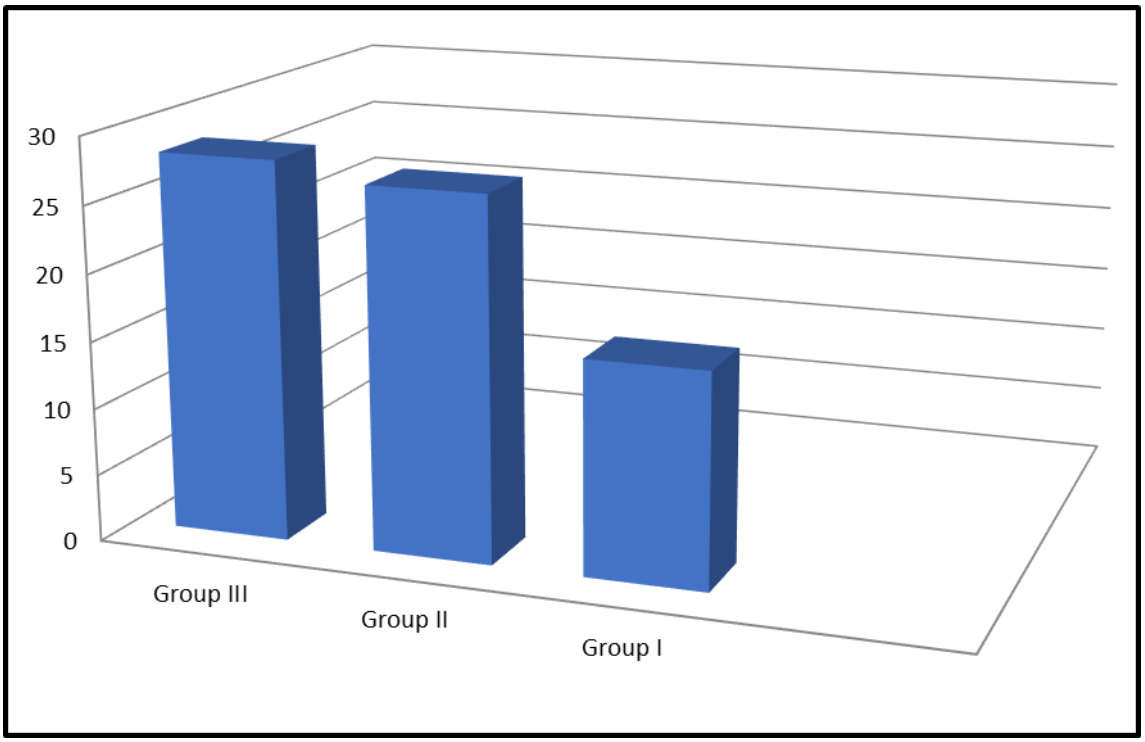

Figure 1. Comparison the concentration of 27-Hydroxy cholesterol (ng/l) in tissues of woman with breast tumor

Table 2. Comparison the concentration of CYP7B1 (U/ml) in tissues homogenate of women with breast tumor

Group	No.	Mean±SD in tissue	P value
Group I Benign	20	8.85±3.96	0.003
Group II Pre	20	5.30±2.36	
Group I Benign	20	8.85±3.96	0.001
Group III Post	20	5.19±1.75	

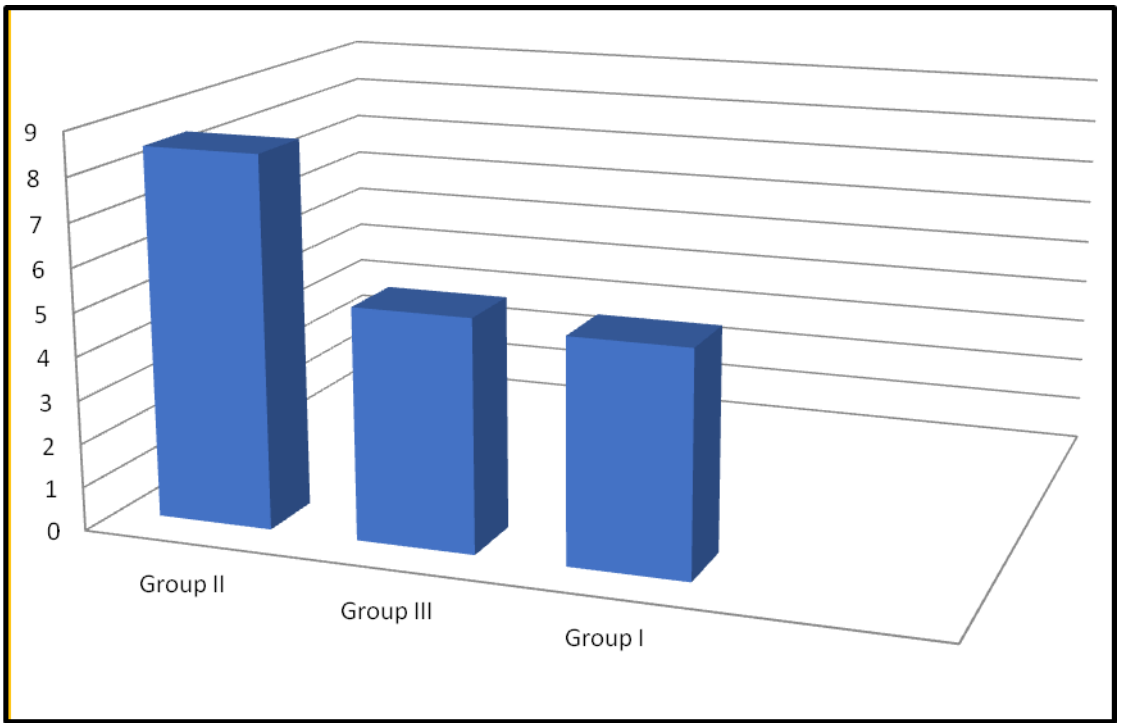

Figure 2. Comparison the concentration of CYP7B1 (U/ml) in tissues of women with breast tumor

Table 3. Correlation of 27-hydroxycholesterol with CYP7B1 in tissue homogenates of women with breast cancer

Value	Group I Benign N=20	Group II Pre N=20	Group III Post N=20
r	-0.135	-0.492	-0.314
P	0.570	0.028	0.178

Discussion

In tissue homogenate samples, in present study the mean and standard deviation of 27-HC in benign, pre and postmenpausal women with breast cancer in ng/l (15.90±4.84, 26.95±5.68, 28.25±7.55 ng/l) respectively. The result of the present study found that 27-HC was significantly elevated in pre and postmenopausal breast cancer group compared with benign and control group at ($p< 0.001$), and this agreed with studies by (Wu et al. [8] Schor [9] and Kimbung et al. [10].

27HC abundance is also predictably elevated in the setting of hypercholesterolemia and with obesity, which is frequently a comorbidity with hypercholesterolemia [11,12].

In women, both dyslipidemia and obesity raise breast cancer risk and severity, with obesity particularly having an adverse impact in postmenopausal women [13-16].

Study by Lee et al showed when applied on animal model enabled us to compare the impact of 27HC with or without hypercholesterolemia. Now found that elevations in 27HC via the deletion of CYP7b1 caused exaggerated atherosclerosis with-out altering lipid status in the setting of normo- and hypercholesterolemia; in addition,

estrogen-related atheroprotection is markedly attenuated [17].

The results of the present study revealed that the mean and standard deviation of CYP7B1 in tissues of benign, pre, postmenopausal women with breast cancer were (8.85±3.96, 5.30±2.36 and 5.19±1.75 U/ml) respectively. These results indicate that CYP7B1 was significantly decreased in postmenopausal breast cancer group compared with benign group (p= 0.001) and was significantly decreased in premenopausal breast cancer group compared with benign group (p= 0.003), and this agreed with studies accomplished by the following researchers Lee et al. [17] and Stiles et al. [18].

CYP7B1 is diminished in breast tumor compared with normal breast tissue. There is more than 7-fold poorer overall survival in women whose tumors display low CYP7B1, compared with women with high tumor CYP7B1 [19]. This may be related to estrogen deprivation because in mice E2 up regulates hepatic CYP7B1 expression in an ERα-dependent manner without impacting CYP27A1 [20].

The conclusion of this study revealed a higher incidence of serum 27-HC and a lower incidence of CYP7B1 found in malignant than benign breast tumors with positive estrogen receptors. These indicate that the presence of 27-HC and deficient of CYP7B1 in sera of patients with breast tumors may be used as a favorable prognostic indicators and diagnostic tools in breast tumors.

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Authors contribution

Dr. Al-Saeed suggests the study; Dr. Nile select the suitable patients and both of them co-writes the manuscript for study. Mohammed collected the blood samples, conducted the necessary analysis of the study, writes the paper and analyzed the results statistically.

Conflict of interest

There was no conflict of interest.

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