Original Article

Evaluation of HDL-C and Gene Expression of HER2 in Male and Female Breast Cancer and Risk Individual for Breast Cancer Development

Elaph Ali AL-Esawi, Buthainah Abbas Al-Azzawi[1]

Department of Clinical Biochemistry, AL-Imam AL-Sadiq Teaching Hospital, Babylon, [1]Department of Clinical Biochemistry, College of Medicine, University of AL-Qadisiyah, Diwaniyah, Iraq

Abstract

Background: High-density lipoprotein cholesterol (HDL-C) has been suggested to be associated with breast cancer. However, the roles of HDL-C on breast cancer still have been controversial. **Objective:** The objective of this study was to assess the difference in the level of (HDL-C) and gene expression of HER2 in the risk group compared with breast cancer patients and control. **Materials and Methods:** This study included 90 participants (female = 60 and male = 30) with an age range of 20–70 years. The participants are divided into three main groups: 40 risk group (individuals at increased risk for developing breast cancer), 25 breast cancer group, and 25 control (healthy group). A Zybio EXC 200 Biochemistry Analyzer measures the concentration of HDL-C for the risk group, breast cancer group, and control using standard enzymatic methods. Lipids were analyzed in one designated laboratory during December 2022 in the control and breast cancer group and January 2023 in the risk group (case study). HER2 gene expression was estimated by quantitative real-time polymerase chain reaction. This study was conducted during the period from the first of September 2022 to the end of February 2023 at Babylon Oncology Center and Imam Al-Sadiq (Peace Be Upon Him) Educational Hospital in Babylon governorate, and National Hospital for Oncology and Hematology in Najaf governorate, Iraq. **Results:** Estimation of serum HDL-C concentration (mg/dL) reveals that the risk group showed a strong significant increase in serum HDL concentration ($P < 0.0001$; mean = 83.78 ± 16.68 mg/dL) that is more than the control (mean = 59.66 ± 8.120 mg/dL) and breast cancer group (mean = 44.70 ± 14.01 mg/dL). Also, it showed a significant decrease ($P < 0.0001$) in serum HDL concentration of the breast cancer group less than the control group. Our results demonstrate overexpression of HER2 in the risk group, whereas downexpression in the breast cancer group. **Conclusion:** HDL-C elevation and HER2 overexpression may be indicators for breast cancer development in the individual at increased risk for developing breast cancer who do not have diagnosis yet, and could be predictive biomarkers. In contrast, downexpression of HER2 and low HDL concentration (HDL-C < 70 mg/dL) in breast cancer patients indicate cancer progression and metastasis.

Keywords: Chromosome 17, dyslipidemia, human epidermal growth factor receptor 2 (HER2), male breast cancer, scavenger receptor type 1 class B (SR-BI)

Introduction

Breast cancer (BC) is still most common tumor in woman, breast cancer can be affected not only female but also male. Male breast cancer more likely diagnosed at advanced-stage.[1] Low disease incidence in male and decreased awareness lead to delayed detection as a result of that, routine screening not performed in men which could be attributed to progression breast cancer in male.[2] First-degree family history of breast cancer remarkably increases the risk for men and women.

Lipids are a type of biological molecule with a wide range of actions. First, lipids are used to store energy in lipid droplets, primarily as triacylglycerol esters. In addition, lipids serve as metabolic signaling messengers in addition to being

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Address for correspondence: Mrs. Elaph Ali Al-Esawi, Department of Clinical Biochemistry, AL-Imam AL-Sadiq Teaching Hospital, Babylon, Iraq. E-mail: tamatored87@gmail.com

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structural elements of cellular membranes.[3,4] Physiological processes, such as the formation of milk or endometrial cell proliferation, require *de novo* synthesis of lipids to produce fatty acids.[5] However, increased lipid formation consider a hallmark of many cancers, one of them is breast cancer.[6,7] Known two main hallmarks of breast cancer progression are oxidative stress and lipid peroxidation, and high-density lipoprotein (HDL) exerts pleiotropic roles such as antioxidant and anti-inflammatory properties.[8] Decreased HDL cholesterol (HDL-C) levels had a significant association with worse overall survival in breast cancer patients.[9]

Human epidermal growth factor receptor 2 (HER2) is a proto-oncogene, which produces HER2 proteins, also known as HER2/neu proteins, which act as receptors on breast tissue cells. Normally, breast cell growth, division, and self-repair are tightly regulated by HER2 receptors.[10] The HER2 gene malfunctions and overproduces copies of itself in 10%–20% of breast tumors particularly invasive ductal carcinoma (HER2 gene amplification).[11,12]

Breast cells are instructed to produce an excessive number of HER2 receptors by the additional HER2 genes (HER2 protein overexpression). Overexpression is associated with aggressive biological behavior and resistance to certain chemotherapeutic agents.[13] The HER2/neu status is of great clinical value in breast tumor patients.[14]

Materials and Methods

The subjects

The Medical Human Research Ethics Committee at the Faculty of Medicine, University of Al-Qadisiyah, Iraq, authorized the study. A case–control study was undertaken on 90 participants who include individuals with risk for breast cancer development, breast cancer patients, and healthy individuals (control).

The age of the women and men in the study population was between 20 and 71 years. Blood specimens of about 5 mL were collected from study participants. Blood samples of 3 mL were collected in clot activator tubes, left at room temperature for 15 min for clot formation then centrifuged at 3000 rpm for 10 min, and stored in an Eppendorf tube at −20°C for further HDL-C analysis. Within an ethylenediaminetetraacetic acid anticoagulant tube, 2 mL of fresh blood was homogenized and then stored at –80°C for a short period for use in genetic analysis. Samples for genetic analysis were put in a cooling box containing dry ice and also when transported to the laboratory after a short period of sample collection.

Determination of HDL

A Zybio EXC 200 Biochemistry Analyzer using commercial kits from Zybio Diagnostic Products was used to estimate circulating HDL concentration.

HER2 analysis by quantitative real-time polymerase chain reaction

Quantification of HER2 gene expression was estimated by quantitative real-time polymerase chain reaction (qRT-PCR) amplification by using GoTaq 1-Step RT-qPCR kit (Promega, Madison, WI, USA) that was accomplished in a single step. TRIzol RNA Purification (TRI) reagent BD was used for the isolation of total RNA. PCR reaction tubes contained a master mix that was prepared of 6.25 µl SYBR green, 1.25 µl of each relevant primer (10µM). 10 µl of the master mix was added to each sample after thoroughly mixed with 0.4 µl/sample of the transcriptase enzyme, immediately before the transfer the tube reaction to the PCR machine. PCR conditions were 37°C for 15 min, 95°C for 10 min, 95°C for 20 s, 60°C for 1 min, and, finally, primer extension 70°C for 37 s, the cycles were 44 cycles. HER2 was detected using the following primers.

HER/neu F: GCTCCCCATATGTCTCCCG;

HER2/neu R: CCGGACATGGTCTAAGAG GC;

Housekeeping gene β-actin F: ATGCAGAAGGAGATTACTGC;

β-actin: R TAAAACGCAGCTCAGTAACA.

All samples of the reference gene and interest gene were measured in duplicate. To compare the difference in gene expression between samples, the Livak method was calculated.[15]

Inclusion criteria

- Individuals with a positive family history of breast cancer.
- ny breast cancer patients with confirmed diagnosis.
- Patient with first-degree relationship for any patients with confirmed diagnosis of breast cancer and under the age of 80 years.

Exclusion criteria

The study excluded all patients with the following conditions:

- Unconfirmed diagnosis of breast cancer.
- Benign breast cancer.
- Diabetes mellitus.
- Inflammatory diseases.
- Other cancers.

Results

Estimation of circulating high-density lipoprotein concentration

The measurement of serum HDL concentration (mg/dL) reveals a strong significant increase (HDL> 70 mg/dL) in the risk group more than control and breast cancer group ($P < 0.0001$; 83.78 ± 16.68 mg/dL). In contrast, results

revealed a significant decrease in serum HDL <70 mg/dL in the breast cancer group (44.70 ± 14.01 mg/dL) compared with the control group (mean = 59.66 ± 8.120 mg/dL) and risk group, as shown in Figure 1.

Comparison of HDL-C with gender show significant increase of HDL in both female ($P \leq 0.0005$; mean ± SD = 84.32 ± 18.34) and male of risk group ($P \geq 0.05$; mean ± SD = 82.14 ± 10.84) compared with control (female: mean = 56.53 ± 8.37; and male: mean = 63.03 ± 6.58) and breast cancer group (female: mean ± SD = 45.54 ± 10.32), as shown in Figures 2 and 3.

The breast cancer group shows a significant decrease of HDL-C in females only ($P < 0.05$; mean ± SD = 45.54 ± 10.32) compared with the control and the risk group. In contrast, results reveal a significant difference between males of breast cancer and risk groups ($P \leq 0.0005$) and no significant difference in the male breast cancer group ($P \geq 0.05$, mean ± SD = 48.99 ± 11.11) compared with the males of control.

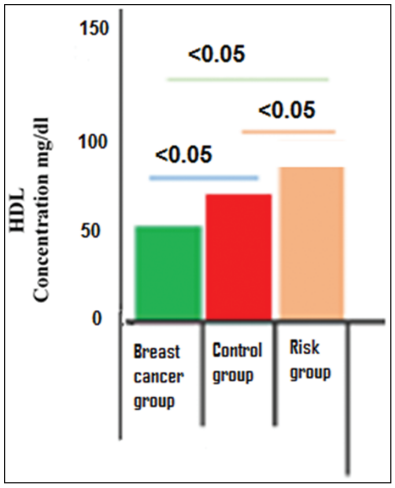

Figure 1: The measurement of serum high-density lipoprotein concentration (mg/dL). Data are expressed as means ± SD. $P \geq 0.05$ is not significant, and $P < 0.05$ is significant

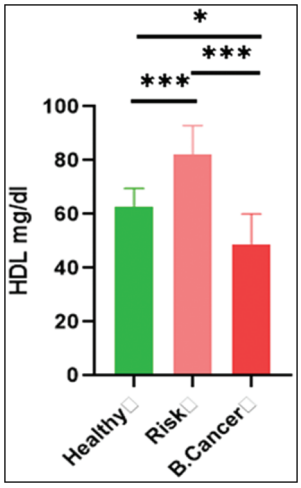

Figure 2: The measurement of serum high-density lipoprotein concentration (mg/dL) in females. The risk group shows a significant increase of HDL in females of the risk group ($P \leq 0.0005$). The breast cancer group shows a significant decrease of HDL-C in females only ($P < 0.05$). Data are expressed as means ± SD. *$P < 0.05$ and ***$P \leq 0.0005$

Gene expression quantitation

To assess the changes in HER2 gene expression among the main groups, qPCR examination was done for healthy, breast carcinoma patients and risk group (first-degree relatives) for the HER2 gene. The results indicate an increase in the gene expression for the HER2 gene, which were significantly higher ($P < 0.05$) in the risk group, whereas downexpression

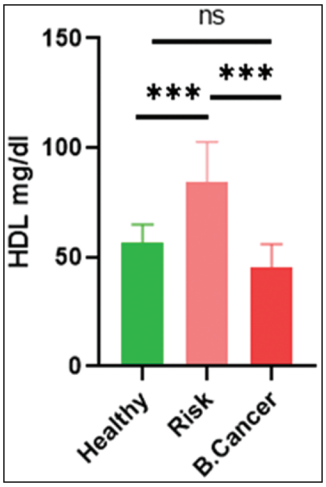

Figure 3: The measurement of serum high-density lipoprotein concentration (mg/dL) in males. The risk group shows a significant increase of HDL in both genders ($P \leq 0.0005$). The breast cancer group shows no signification in HDL-C concentration ($P \geq 0.05$). Data are expressed as means ± SD. $P < 0.05$ indicates no significant (ns) and ***$P \leq 0.0005$

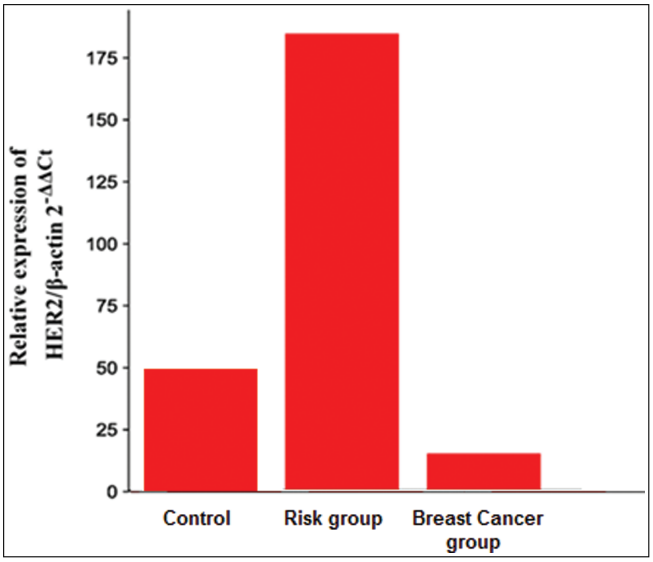

Figure 4: Estimation of gene expression. An increase in the expression of HER2 was seen in the risk group. Downexpression was in the breast cancer group

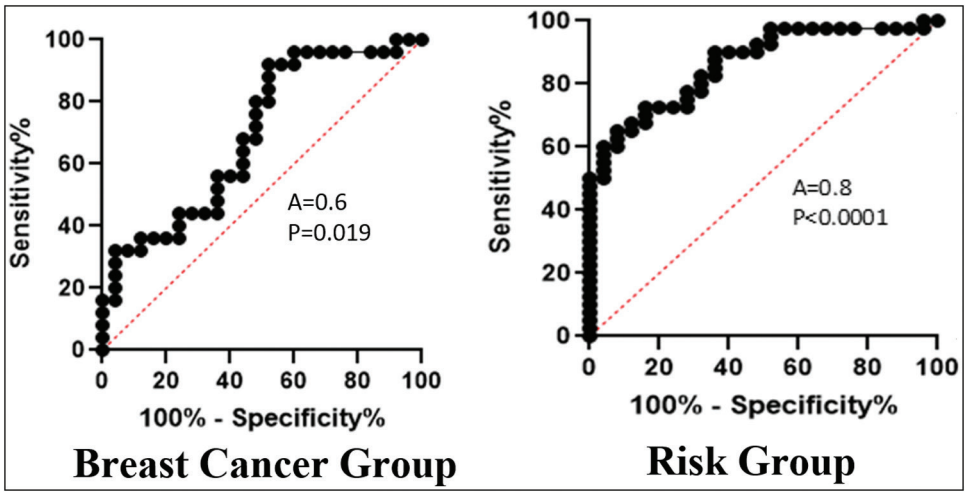

Figure 5: ROC curve for the results of HER2 plotting sensitivity and specificity for the risk and breast cancer groups

in the breast cancer group compared with control. However, the risk group showed a significant ($P < 0.05$) overexpression for HER2 gene as shown in Figure 4.

Receiver operating characteristic curve

To test the diagnostic ability of the HER2 gene in breast cancer, we used the receiver operating characteristic (ROC) curve statistical test. The test was conducted to the risk and breast cancer groups.

The area under ROC curve for HER2 in the risk group was A = 0.8, $P < 0.0001$, and showed sensitivity (true positive) with a confidence interval of 0.77–0.94, so would be a predictive biomarker, whereas HER2 in the breast cancer group revealed area under curve = 0.6, $P = 0.019$, with a confidence interval of 0.54–0.84 [Figure 5].

Discussion

The measurement of circulating lipoproteins and metabolites may provide valuable insight into the systemic effects of treatment for breast cancer. Circulating lipids are not only associated with etiology but also with prognosis in cancer. HER2/neu activation is often brought on by HER2/neu gene amplification, which elevates the expression of the HER2 protein and eventually leads to uncontrolled division of breast cells.[16]

Paraoxonase-1 (PON1), apolipoprotein AI (Apo-AI), lecithin cholesterol acyltransferase (LCAT), and platelet-activating factor (PAF)-acetylhydrolase are components of HDL structure on the surface. Recently, serum amyloid A (SAA) protein that built in the liver during the acute phase in response to inflammatory stimulus. Cancer development is associated with inflammation, SAA is considered a marker of low-grade chronic inflammation and may be used as a potential predictor of survival in breast cancer patients.[17] Under the oxidative status brought on by cancer etiology, PON1 and Apo-AI are reduced because of HDL oxidation.[17,18]

Previous research showed that elevated SAA levels were the cause of changes in HDL functions and antioxidant capacity. HDL and SAA interactions result in Apo-AI displacement, and these compositional alterations are accompanied by a decline in PON1 and LCAT activities. As a result, membrane scavenger receptor type 1 class B (SR-BI) is activated, which functions as an HDL receptor and mediates HDL binding.[19] SR-BI, in turn, activates multiple inflammatory signaling pathways that provide inflammatory environment for cancer, leading to cell proliferation, invasion, and migration, as well as inhibiting apoptosis.[20] All these studies can demonstrate our result of significant decrease in HDL-C in breast cancer patients ($P < 0.05$).

As mentioned above, HDL-C employs antioxidant and anti-inflammatory roles under normal conditions. PAF acylhydrolase (PAF-AH) degraded PAF, that is, well-known potent inflammatory mediator. PAF-AH degraded PAF by its phospholipase activity (calcium-independentphospholipase A2). The beneficial effect was closely related to HDL-C antioxidative and anti-inflammatory properties. One previous study suggested that HDL-C could prevent lipid peroxidation by inhibiting LDL-C oxidative damage.[8,21] Furthermore, several researches observed that high level HDL-C was associated with greater production of anti-inflammatory cytokines such as interleukin-10 that are considered to play a protective role against breast cancer.[22,23] This finding could come together with our result of elevated HDL levels in the risk group for breast cancer development and the elevation may be considered an indicator for cancer initiation and progression in risk individuals.

HER2 proto-oncogene was detected in terminal duct lobular units as well as in atypical ductal hyperplasia.[24] In contrast, HER2 is amplified and overexpressed in ductal carcinoma *in situ* (DCIS) and invasive breast cancer.[25] Typically, overexpression of HER2 occurs at the transition from hyperplasia to DCIS.[26]

Overexpression of HER2 may accelerate the development and progression of premalignant breast disease and increase the proliferation and migration of cells. Our results demonstrated the overexpression of HER2 in the risk group. Approximately all patients participating in the study undergo chemotherapy with a minimum of two doses, so we hypnotized downexpression of HER2 in the breast cancer group, which may be due to chemotherapy or certain HER2-target drugs.

Activation of HER2 seems in human tumors to be a dominant transformation mechanism, and the mechanism for overexpression of HER2 is still complex and unclear. Retrospective study to investigate the relationship between HER2/neu breast cancer overexpression and amplification. This study carried on human breast cancer patients conducted expression levels of HER2/neu mRNA by RT-PCR higher than the levels estimated by immunohistochemistry and fluorescence in situ hybridization-negative tumors.[14] These findings and our results of overexpression of interested gene (HER2) detected by real-time PCR can question the relevance of HER2 assessment in routine diagnosis of individuals at high risk for breast cancer development in the clinical laboratory setting.

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Conflicts of interest
There are no conflicts of interest.

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