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REVIEW ARTICLE

Evaluation Low Density Lipoprotein Cholesterol and Triglyceride Concentrations and Gene Expression of p53 may Predict the Risk for Breast Cancer

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Abstract

Background: Breast cancer is a condition in which cancerous cells in the breast tissue proliferate and divide uncontrollably. Through blood arteries or lymph, these cells infiltrate harmless surrounding tissues, where they cause the development of secondary cancer metastases. Abnormality in lipid metabolism (typically by poor diet or obesity) and cholesterol changes can be risky factor for cancer in breast have been subject to the substantial research. Circulating lipids not only associated with etiology but also with prognosis in cancer. Lipids are crucial in the emergence and progression of cancer. LDL-C has been linked to a higher risk of breast carcinoma. Overexpression of p53 gene could be predictive tumor marker for breast cancer diagnosis in risk group.

Aims of the study: Evaluation of LDL-C and TG concentrations (mg/dl) and gene expression of p53 in higher risk group for breast cancer.

Materials and Methods: A Zybio EXC 200 Biochemistry Analyzer measures the concentration of LDL-C and TG for the risk group, breast cancer group and control by using standard enzymatic methods, RT-qPCR for measuring gene expression of p53 in the three main group. This study was conducted during the period from first of September 2022 until 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: The outcome of this research show decreased LDL-C levels in risk group as compared to the healthy control and cancer group and no significant difference in TG concentration in risk group. Over-expression of p53 in risk group while downexpression of p53 in cancer group as compared to control.

Conclusions: Overexpression of p53 and LDL-C could be one of the biological parameters that can be used in the clinical laboratory diagnosis of cancer in breast.

Keywords: Lipoprotein; Risk Individuals; p53.

Introduction

Mostly common malignancy in women still is tumor in breast. Breast carcinoma is the second most common carcinoma in females (1) mostly increases with age and reaches the peak after the age of 50 (2) but it has never meant no occurrence in earlier age groups. And more surprisingly that is male in comparison to female are greater likelihood of receiving an advanced-stage breast cancer diagnosis (3), decreased awareness and delayed detection as a result of low disease incidence in male made routine screening not performed in men which could be attributed to this progression in male. First-degree family history of breast carcinoma remarkably elevated the risk for men and women (4).

Breast carcinoma classifies to early and advanced disease. Early or “primary” indicates disease that is identified to cause localized breast lesion and those detected clinically or through mammography in areas away from breast tissue known as “advanced” or “metastatic” (5) .

Lipids, a type of biological molecules with a wide range of actions. Firstly, lipids are used to store energy in lipid droplets, primarily as triacylglycerol esters. Additionally, lipids serve as metabolic signaling messengers in addition to being structural elements of cellular membranes (6). Physiological processes,

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like the formation of milk or endometrial cell proliferation, require de novo synthesis of lipids to produce fatty acids (7). However, increased lipid formation is considered a hallmark of many cancers one of them is breast cancer (8).

Lipids once absorbed in the intestine, are carried in plasma by lipoproteins. Identification of serum lipid panel capable of distinguishing benign lesions from breast cancer in its early stages and serving as useful indicators for early detection of breast cancer (9).

Identifying circulating lipid profiles that may distinguish between benign lesions and breast cancer in its early stages and serve as helpful biomarkers for early diagnosis of the disease (9). Fast, high-efficiency, high-throughput detection using lipid profiles was possible with only a minimally intrusive method. A Mendelian randomization study discovered a link between genetically elevated LDL-C and a higher chance of breast cancer development (10). Triglycerides (TG) increase the risk for breast cancer recurrence and are associated with a poor prognosis of breast cancer (11).

The p53 gene encodes the P53 protein that regulates the cell cycle and functions as a tumor suppressor; hence, it is also known as tumor protein p53, so it is referred to as " Cellular Gatekeeper" and " The Guardian of the Genome" (12) according to its role in conserving stability by preventing genome mutation (13).

The normal cell cycle is hypothesized to be regulated by the tumor suppressor gene p53. Almost all cancers have dysregulated p53. About 50% of cancers still exhibit p53 wild-type activation (14). It was demonstrated that p53 dysfunction plays a significant role in the regulation of numerous other cellular processes and functions, including metabolism, autophagy, inhibiting angiogenesis, which is crucial for breast cancer, and inflammation. P53 dysfunction has severe consequences by enabling unchecked tumor cell growth. Importantly, the tumor microenvironment's makeup and performance can be impacted by the p53 status of the tumor cells (15).

p53 expression in breast cancer regulates tumorigenesis in a manner specific to oncogenes, so it influences the tumor immune landscape and ultimately affects patient survival (16). For individuals with breast cancer, particularly those with the triple-negative breast cancer (TNBC—tumors negative for ER, PR, and HER2) subtype, it is taken into consideration as a possible biomarker and therapeutic target (17). p53 abnormalities are very prevalent in breast cancer and colorectal cancers and are deducted to be the majority of them not encoded in the germline, and their frequencies may be driven by immunogenicity (18). Recent studies have shown that breast cancers which progress from primary to advanced metastatic lesions characterized by the increased expression of mutant p53 from 13% to 50% (19). It is crucial to note that p53 is a sort of housekeeping gene, which is expressed in all tissues of an organism (20). When there is DNA damage in the cell, gene, or chromosome, the p53 gene expresses itself and produces more amounts of p53 tumor suppressor protein (21).

Materials and Methods

Sample Preparation

A case-control study was undertaken on 90 participants who included three groups: 40 risk group included (Female= 30, Male=10), risk group who are individuals at risk for breast cancer development and were first-degree relatives for the breast cancer patients, was classified mainly according to having a positive family history for breast cancer, 25 breast cancer group included (Female= 17, Male=8) and 25 control group included (Female= 13, Male= 12).

The Medical Human Research Ethics Committee at the Faculty of Medicine, University of Al-Qadisiyah, Iraq, authorized the study.

The age of the study population ranged from 25-50 years for risk group, 40-67 years for breast cancer group and 20-39 years for control. Blood specimens about 5ml collected from study participants. Blood samples 3ml were collected in clot activator tubes and left to coagulate at ambient temperature for 15 minutes before being centrifuged at 3000 rpm for 10 minutes and stored in an Eppendorf tube at - 20°C for further LDL-C and TG analysis. Within an Ethylene Di-amine Tetra Acetic acid anti-coagulant (EDTA) tube 2 ml of fresh blood was homogenized 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 TG and LDL:

A Zybio EXC 200 Biochemistry Analyzer using commercial kits from Zybio Diagnostic Products was used to estimated circulating Triglyceride and Low Density Lipoprotein concentration .

p53 analysis by RT-qPCR:

Quantitative real-time PCR (qRT-PCR) amplification to estimate the expression level of the p53 gene by using GoTaq® -\Step RT-qPCR kit (Promega, USA) that accomplished in single step. TRIzol RNA Purification (TRI) reagent BD used for isolation of total RNA. PCR reactions contained master mix prepared of 6.25 µl SYBR green, 1.25 µl of each primer each, 0.4 µl/sample of the enzyme was added to the master mix before the transfer to the PCR machine, thoroughly mixed. PCR conditions were 37C°for 15min, 95C° for 10min, 95C° for 20sec, 60C°for one minute and finally primer extension 70 C° for 37sec, the cycles was 44 cycles. p53 were detected using the following primers.

p53 F: CTGGGCAGGTCTACTTTGGG

p53 R: CTGGAGGCCCCAGTTTGAAT

β-Actin F: ATGCAGAAGGAGATTACTGC.

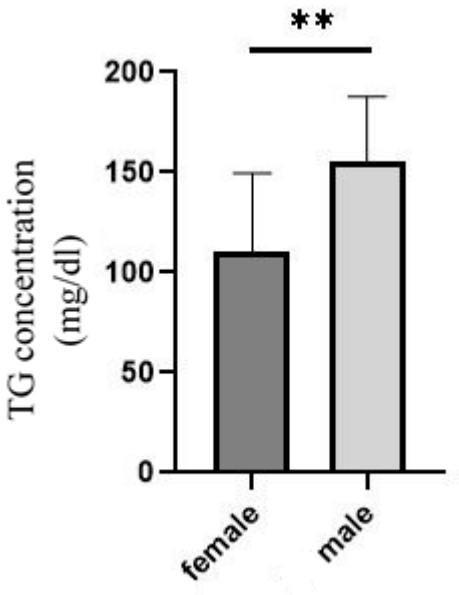
β-Actin R: TAAAACGCAGCTCAGTAACA.

Results:

Measurement of Serum Triglyceride Concentration Reveals a Significant Difference among the Different Groups

The measurement of serum TG concentration (mg/dl) reveals significant (P<0.022) decrease in the risk group (mean=138.09±78.61) as compared to the breast cancer group (mean=170±14.33 mg/dl) while no significant difference (P≥0.321) with control group (mean= 124.05±27.67 mg/dl) (Figure 1).

Comparison of TG according to gender in risk group (study group), show significantly difference (P<0.005) in the serum TG concentration (mg/dl) between male and female of risk group (Male: mean±SD=155.38±32.59; Female: mean±SD=132.32±88.57) (Figure 2)



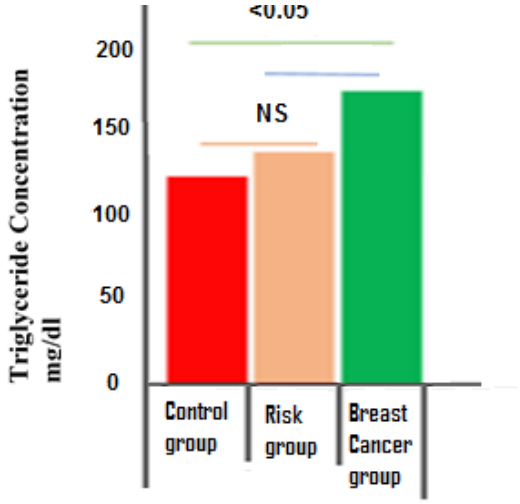
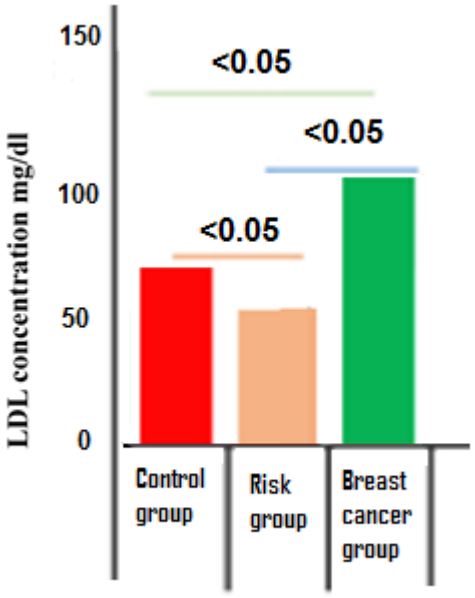


Figure (1): The measurement of serum Triglyceride concentration (mg/dL). Data are expressed as means ± SD. Indicates p value ≥ 0.05 not significant and p-value <0.05 is significant.

Figure (2): Comparison of Triglyceride Concentration with gender in the risk group. Results expressed as mean ± SD. Indicate **P < 0.005.

Estimation of Serum LDL Concentration Reveals a Significant Difference among the Different Groups

The measurement of serum Low Density Lipoprotein (LDL) concentration (mg/dl) reveals that risk groups showed highly significant (P<0.0001, mean=70.13±30.59mg/dl) decrease in LDL concentration as compared to the breast cancer group (mean=142.27±19.03 mg/dl) and control (mean=94.45±13.42 mg/dl) (Figure 3).



Comparison of LDL-C according to the gender in risk group, show no significant difference of LDL concentration in both gender (Male: mean±SD=75.38±30.66; Female: mean±SD=68.37±30.89). This shown in Figure 4.

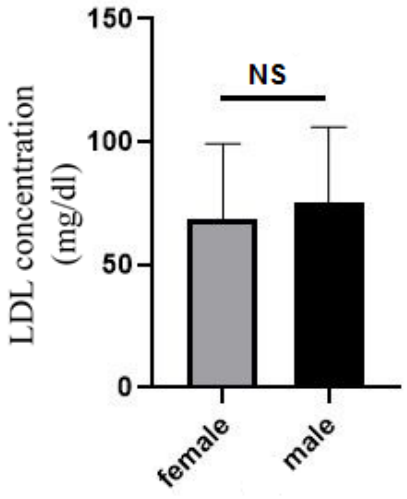
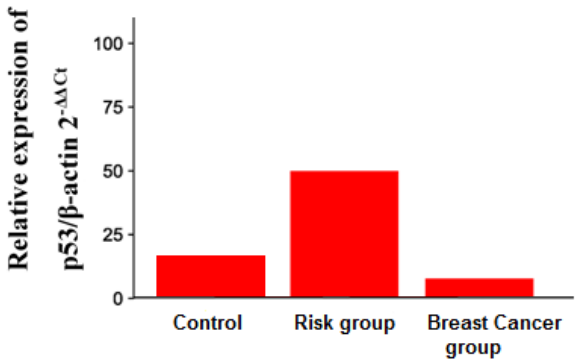


Figure (3): The measurement of serum very low-density lipoprotein concentration (mg/dl) in the three main groups (Control group, Risk group and Breast cancer group). Results expressed as mean ± SD.

Figure (4): Comparison of LDL Concentration with gender in the risk group. Results expressed as mean ± SD. NS Indicate P ≥ 0.005.

Gene expression quantitation



To assess the changes in p53 gene expression among the main groups, qPCR examination was done for healthy, breast carcinoma patients and their relatives groups for the p53 gene. The results indicate an increase in the gene expression for the p53 gene which was significantly higher (P value <0.05) in the risk group as compared to the breast cancer and control groups. However the risk group showed a significantly (P<0.05) overexpression for p53 gene. This shown in (Figure 5).

Figure 5: Estimation of gene expression. An increase in the expression of p53 was seen in risk group. Down expression was in breast cancer group

Roc curve

To test the diagnostic ability of the p53 gene in breast cancer, we used “receiver operating characteristic curve (ROC)” statistical test. The test was conducted to the risk and breast cancer groups

For the p53 gene, the result of the risk group showed that (AUC=0.7, P<0.0001) with 95% confidence interval= 0.69 to 0.90, indicating sensitivity (true positive), so would be predictive biomarker. Shown in Figure (6).

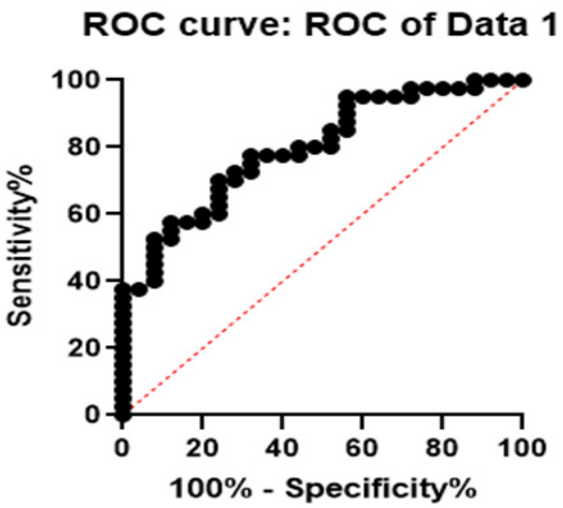


Figure 6: ROC curve for the results of p53 gene expression .plotting sensitivity and specificity for risk group

Discussion

It may be possible to get important knowledge about the systemic effects of breast cancer treatment by measuring the levels of circulating lipoproteins and metabolites. Circulating lipids are not only associated with etiology but also with prognosis in cancer (22).

The well-known tumor suppressor gene that plays a crucial role in maintaining genomic stability and preventing the formation of cancer cells is the p53 gene (23). The present study indicates that the overexpression of p53 has diagnostic significance in addition to serum LDL-C level in assessing the risk group of breast cancer.

p53 acts as a transcription factor that responds to cellular stress and DNA damage by controlling the cell cycle, DNA repair, and apoptosis (24). Mutations or dysregulation of the p53 gene can be a cause for the impaired response to DNA damage and an increased risk of tumorigenesis (25).

Overexpression of p53 in the risk group may indicate an enhanced cellular response to DNA damage, highlighting the activation of protective mechanisms against breast cancer initiation (26).

A unique previous study included 244 patients with breast cancer in order to find out if plasma LDL-cholesterol levels may be a predictor of breast cancer. The findings of this previous study showed that the LDL-cholesterol quantity was strongly linked to the advancement of breast cancer, which may be important for identifying and monitoring high-risk breast cancer cases (27). As a result, LDL cholesterol levels at the time of diagnosis may be taken into account as a prognostic factor in cases of breast cancer.

LDL cholesterol (LDL-C) levels upon diagnosis were found to be a predictive factor of breast tumor development in another clinical trial in which the lipid profile of women with breast cancer was evaluated (28). These previous studies can be in line with our results that demonstrate low serum LDL-C concentrations in the risk group as compared to the control and breast cancer groups.

Contrary to LDL-C levels, the study indicates no significant difference in serum triglyceride concentrations in the risk group in comparison with the control and the breast cancer group (29). The absence of a significantly different circulating triglyceride level in the risk group suggests that triglyceride metabolism may not be directly associated with breast cancer risk or development (30).

Previous studies have suggested a possible link between elevated triglyceride levels and breast cancer risk (31). Triglycerides serve as a source of energy and are involved in various physiological processes. Elevated triglyceride levels have been associated with obesity, insulin resistance, and metabolic syndrome, which are known risk factors for breast cancer (32). However, the lack of a significant difference in serum triglyceride levels in the risk group suggests that triglycerides alone may not be a reliable biomarker for breast cancer risk assessment. It is important to note that the complexity of lipid metabolism and its interactions with breast cancer development necessitate further investigation. Future studies should explore the potential interplay between triglyceride metabolism, other lipid profiles, and breast cancer risk to gain a comprehensive understanding of the underlying mechanisms.

The finding of higher serum triglyceride levels in males of the risk group compared to females underscores the importance of considering gender-specific risk factors in breast cancer research. Hormonal differences, including variations in estrogen and androgen levels, between males and females may contribute to this gender disparity (33).

In males, testosterone plays a role in regulating lipid metabolism (34), and alterations in androgen levels may affect triglyceride levels. Testosterone has been shown to decrease triglyceride clearance rates, leading to higher serum triglyceride concentrations (35). Thus, the higher triglyceride levels observed in males within the risk group could be attributed to hormonal differences and their impact on lipid metabolism.

Conclusions

Overexpression of p53 and LDL-C could be one of the biological parameters that can be used in the clinical laboratory diagnosis of risk group of breast cancer.

Recommendations

This study could recommend the following:

1- Further studies are necessary for better patient treatment and overall management. An Extensive study of the lipid components and more related genes on a larger sample size is needed, and the outcome and prognosis should be recorded in order to create a practical guide for the evolution of the prognosis and treatment of breast carcinoma in our country.

2- We recommend additional studies of the p53 gene and plasma lipid levels as a possible risk factors for this disease.

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Adherence to Ethical Standards: The ethical committee approved the study at the University of Al-Qadisiyah.

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