Correlations between serum lipid and Ki-67 levels in different breast cancer molecular subcategories

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Abstract. Breast cancer has the highest incidence rate among all cancer types worldwide, seriously threatening women's health. The present retrospective study explored differences in serum lipid contents in different breast cancer (BC) subcategories and their correlation with Ki-67 expression levels in patients with invasive BC with the aim of identifying novel diagnostic and prognostic indicators for personalized BC treatment. The study included 170 patients diagnosed with BC who were diagnosed with invasive BC by postoperative pathological examination. Data on patient age, body mass index and menopausal status were collected, in addition to estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 (HER2) and antigen Ki-67 expression levels and pathological tumor type. Preoperative circulating lipid levels, specifically the levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and apolipoproteins A1 (ApoA1) and B (ApoB) were also obtained. Molecular subcategories of BC were grouped based on their immunohistochemistry. Differences in serum lipid levels between the groups were assessed, and correlations between serum lipid and Ki-67 expression levels were explored. While TC, LDL-C, HDL-C and ApoA1 levels differed significantly among molecular subcategories. TG and ApoB levels did not. Circulating TC and LDL-C levels were considerably higher in patients with triple-negative BC (TNBC) and HER2-positive [hormone receptor (HR)-negative] BC than in those with luminal A and B (HER2-negative) BC. Serum HDL-C levels were significantly diminished in the TNBC and HER2-positive (HR-negative) groups compared with the luminal A and B (HER2-negative) groups. ApoA1 levels were significantly reduced in cases of TNBC and HER2-positive (HR-negative) BC compared with luminal A and B BC. Ki-67 expression levels were positively correlated with circulating TC and LDL-C levels and inversely correlated with circulating HDL-C and ApoA1 levels but exhibited no correlation with serum ApoB and TG levels. The results indicate that elevated TC and LDL-C levels and diminished HDL-C and ApoA1 levels were high-risk factors in patients with TNBC and HER2-positive (HR-negative) BC, but not patients with luminal subcategories of BC. Abnormal serum lipid levels were correlated with Ki-67 expression levels, with elevated circulating TC and LDL-C levels and reduced circulating HDL-C and ApoA1 levels indicating a poor prognosis in patients with BC.

Introduction

The GLOBOCAN 2020 estimates of cancer burden suggest that there were ~4.57 million new cases of cancer in China in 2020. These include >410,000 patients with breast cancer (BC), accounting for 9.1% of all newly diagnosed cancers in China and 19.9% of all newly diagnosed cancers in Chinese women, making it the most prevalent cancer among women. In addition, the estimated number of deaths from BC was >110,000, accounting for ~3.9% of all cancer-associated deaths in China and 9.9% of all Chinese female deaths, ranking fourth in female cancer mortality (1). The incidence of BC and its associated deaths are increasing annually, seriously endangering the lives and health of Chinese women. Developments in medical technology have facilitated advances in BC treatment, from a single surgical treatment to a systemic, comprehensive mode of treatment based on surgery and including chemotherapy and endocrine, targeted, radiation and immune therapies. Therefore, BC is among the malignant tumors with the most clinical treatments and the greatest likelihood of a curative effect (2).

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Serum lipids are essential blood-borne lipid components in the human body. Along with proteins and nucleic acids, lipids are important biological membrane constituents and cellular building materials. Furthermore, lipids store energy, are crucial for cell metabolism and are critical signaling agents for numerous cellular functions (3). The modulation of lipid metabolism, such as lipid absorption, production and hydrolysis, is essential for maintaining cellular homeostasis (4).

The tumor microenvironment (TME) is crucial for the metabolic adaptation of tumor cells. Various substrates in addition to glucose are necessary to meet the nutritional requirements of highly proliferative tumor cells. Lipids have been suggested to be the most important alternative fuel supporting tumor cell proliferation. Tumor cell-based lipid metabolism and its significance in tumor progression and metastasis have gained extensive attention (5). Tumor cells use lipid metabolism to produce biofilm components, energy and signaling molecules required for proliferation, survival, invasion and metastasis, as well as to alter the TME and respond to cancer treatment (6). Changes in lipid metabolism, particularly fatty acid (FA) synthesis and oxidation, have been identified as essential metabolic reorganization phenomena within tumor cells (7). Previous studies have shown that serum lipid metabolism is associated with colon, ovarian, prostate and BC tumor malignancy (8,9).

The incidence of BC is affected by numerous factors, including estrogen levels, heredity and lifestyle. Lifestyle is considered an increasingly important factor in BC, with obesity, metabolic syndrome (MS) and hyperlipidemia increasing BC risk (10). However, the mechanism by which abnormal serum lipid metabolism leads to BC remains unclear. It has been suggested that the relationship between blood lipid metabolism and BC occurrence and development is reflected in blood lipid metabolism components such as 27-hydroxycholesterol, phosphatidylinositol, adiponectin and leptin (11-13). Indeed, some studies have indicated that serum lipid metabolism has a key role in the prognosis and treatment of BC metastasis (14,15).

BC exhibits clinical, morphological and molecular heterogeneity. Since the advent of microarray technology, gene expression studies have increasingly been used to elucidate the behavior of breast tumors. The identified gene expression profiles have provided an improved understanding of the complex heterogeneity and biological behavior of BC. Molecular typing can be used to further confirm these characteristics. In 2000, Perou *et al* (16) studied BC typing and classified BC into luminal, human epidermal growth factor receptor 2 (HER2)-positive, basal cell-like and normal breast-like types. However, obtaining intrinsic subcategories by gene expression profiling is complex and expensive and its clinical application is limited.

Methods that determine subcategories via clinicopathological examination have become the basis for the diagnosis and treatment of BC. Estrogen receptor (ER), progesterone receptor (PR), HER2 and proliferation marker protein Ki-67 statuses determined by pathological examination are associated with BC prognosis (17). In 2011, a new BC molecular subtyping standard was proposed at the St. Gallen International BC Conference. In this standard, according to the ER, PR, HER2 and Ki-67 expression of the tumor, BC is divided into the following types: Luminal A type, which is ER and/or PR positive and HER2 negative with low Ki-67 expression; luminal B (HER2-negative) type, which is ER and/or PR positive and HER2 negative with high Ki-67 expression; luminal B (HER2-positive) type, which is ER and/or PR positive and HER2 positive; HER2 overexpression type, which is ER and PR negative with HER2 upregulation or amplification; and triple-negative type, which is ER, PR and HER2 negative (18). This diagnosis and treatment consensus was the first to perform molecular BC typing through routine pathological diagnosis results, confirming that molecular typing can serve an important role in BC treatment decision-making.

The clinicopathological BC subcategories vary in invasiveness, treatment options and prognosis. Luminal subcategories often exhibit enhanced prognosis compared with the non-luminal subcategories because they are hormone receptor-positive and more sensitive to hormonal intervention. Since cases of HER2 overexpressing and triple-negative BC (TNBC) often experience early and frequent recurrence and metastasis, their prognosis is poor. The HER2 overexpressing type has an improved prognosis compared with TNBC because it can benefit from HER2-targeted therapy. TNBC is considered the most severe BC subtype because it does not express ER, PR or HER2. Also, it is more aggressive, has elevated recurrence and distant metastasis risks and a worse outcome, relative to other BC subcategories. Systemic treatment methods for TNBC are limited; since endocrine and targeted therapies are ineffective, chemotherapy is currently its primary treatment modality (19,20).

Ki-67 is a macromolecular nuclear protein that serves as an antigen in Hodgkin's lymphoma and is closely associated with cell proliferation. Ki-67 is involved in the cell cycle and is present in all cell cycle phases with the exception of G_0 (17). Ki-67 levels are lower in the G_1 and S phases, peak during prophase mitosis and decrease sharply during anaphase mitosis (21). Ki-67 has been widely employed as a proliferation capacity indicator for human tumor cells for numerous years, and its expression in normal cells is low. The faster the tumor cell growth rate and the lower the tissue differentiation, the higher the Ki-67 expression (22). Ki-67 is used as a proliferation marker in BC tissue (23), with its expression level reflecting tumor cell proliferation. Studies have shown that the proliferation of BC cells is closely associated with invasiveness, which is an indicator of patient prognosis. Therefore, Ki-67 has been shown to be a key prognostic and predictive marker for BC (24,25).

Researchers have performed several studies on the association between circulating lipid concentrations and BC. However, few studies have explored the correlations between circulating lipid concentrations in BC subcategories and Ki-67 expression. To the best of our knowledge, there is only one study on this topic, which reported that serum lipid levels differ among BC molecular subcategories (26). Therefore, the present study explored differences in preoperative circulating lipid concentrations in patients with different BC molecular subcategories and their correlations with Ki-67 expression. The aim was to identify BC diagnostic and prognostic indicators and provide a more accurate reference and guidance for the diagnosis, prognosis and individual treatment planning of patients with BC.

Materials and methods

Research objects. The study included the clinical data of 170 patients with an initial BC diagnosis at the Second Affiliated Hospital of Shandong First Medical University (Ti'an, China) from January 2019 to January 2021. The patients all underwent surgical treatment and were subsequently diagnosed with invasive BC by postoperative pathological examination.

Patient inclusion criteria were: i) First radical surgery for BC; ii) invasive non-specific BC, including invasive ductal carcinoma, invasive lobular carcinoma, sclerosing carcinoma and medullary carcinoma, confirmed by postoperative pathology; iii) no neoadjuvant therapy before surgery; iv) measurement of blood lipid levels within 1 week before surgery; v) no use of drugs affecting blood lipid levels within half a year before surgery; vi) no primary hyperlipidemia, diabetes, coronary heart disease, thyroid disease, MS or other diseases affecting blood lipid levels; v) complete clinical data.

The following patients were excluded from the study: i) Those with other cancers; ii) those given BC-associated adjuvant therapy before surgery; iii) those without original blood biochemical test samples collected before surgery; iv) those with hyperlipidemia, diabetes, coronary heart disease, MS or other diseases affecting serum lipid levels; v) those who had taken lipid-lowering drugs within half a year before surgery; vi) those without complete clinical data.

Patient clinical information included age, body mass index (BMI), menopausal condition (menopausal or non-menopausal), preoperative serum lipid levels and pathological data. Serum lipid levels and pathological examination results were extracted from patients' laboratory test results. Patient pathological data included ER, PR, HER2 and Ki-67 expression levels and pathological tumor type.

This retrospective clinical investigation only gathered existing clinical information; it did not intervene in the therapeutic regimens of the patients and caused no risks to their physiology. The researchers did their best to protect the information provided by the patients and not to breach their personal privacy. The requirement for ethics approval was waived due to the retrospective nature of the study, and informed patient consent was also waived by the Science Research Ethics Committee of The Second Affiliated Hospital of Shandong First Medical University (ref. no. 2022-088).

Serum lipid level determination. Fasting peripheral venous blood was collected from all participants at hospital admission, and serum lipid levels were determined using an automatic biochemical analyzer. Serum lipid contents included total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and apolipoproteins A1 (ApoA1) and B (ApoB). The normal ranges of these lipids were defined on the basis of the 2016 revised edition of the Chinese Adult Dyslipidemia Prevention and Control Guidelines for stratifying circulating lipid concentrations (27) and the reference ranges of the serum lipid indicator tests used in the hospital's laboratory: TC, 3.10-5.20 mmol/l; TG, 0.56-1.70 mmol/l; HDL-C, 1.29-1.55 mmol/l; LDL-C, 2.70-3.13 mmol/l; ApoA1, 1.0-1.60 g/l; and ApoB, 0.60-1.10 g/l. Dyslipidemia typically

Table I. Subcategories of breast cancer.

| | Indicator expression level | | | |
Subtype	HER2	ER	PR	Ki-67
HER2-positive (HR-negative)	+	-	-	Any
HER2-positive (HR-positive)	+	+	Any	Any
TNBC	-	-	-	Any
Luminal A	-	+	+	Low
Luminal B (HER2-negative)	-	+	-	High

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; Ki-67, proliferating cell nuclear antigen; PR, progesterone receptor; TNBC, triple-negative breast cancer.

refers to elevated circulating TC, TG and LDL-C concentrations and diminished circulating HDL-C concentrations.

Immunohistochemical result determination. Immunohistochemistry (IHC) was used to measure the ER, PR, HER2 and Ki-67 levels in tumor tissues according to the 2021 Chinese Society of Clinical Oncology (CSCO) criteria for BC diagnosis and treatment (27). The positive ER and PR levels standards are ≥1% of tumor cells showing nuclear staining; 20% PR positive cells is the standard threshold used to define high and low PR expression. HER2 status is described as follows: An IHC score of 3+ is considered HER2 positive; an IHC score of 0 or 1+ is considered HER2 negative; an IHC score of 2+ is considered uncertain and requires confirmation using fluorescence *in situ* hybridization (FISH); a positive FISH outcome is considered HER2 positive. Ki-67 positivity was defined as any degree of brown staining infiltrating the cancer cell nuclei. The expression of Ki-67 was calculated as the percentage of positively stained cells, with >30% indicating high expression and <15% indicating low expression. BCs were then stratified into five subcategories according to their ER, PR, HER2 and Ki-67 expression (Table I).

Other indicators. The height (m), weight (kg) and BMI of the patients were routinely measured at admission. BMI (kg/m^2) was computed as the weight in kg divided by height squared in m^2. Menopause is generally described as the permanent cessation of menstruation, indicating a persistent reduction in estrogen synthesis by the ovaries. For the diagnosis of menopause, it was necessary for a patient to meet at least one of the following conditions: i) Bilateral oophorectomy; ii) aged ≥60 years; iii) aged <60 years with natural menopause for ≥12 months without chemotherapy, tamoxifen, toremifen or ovarian castration within the last year and serum follicle-stimulating hormone and estradiol contents within the postmenopausal range; iv) aged <60 years, consuming tamoxifen or toremifene, and serum follicle-stimulating hormone

Table II. Clinical profiles of patients in the five breast cancer groups.

Subtype	Cases (n)	Age (mean ± SD)	BMI (kg/m²; mean ± SD)
HER2-positive (HR-negative)	18	53.44±8.77	24.63±3.12
HER2-positive (HR-positive)	28	50.43±9.71	25.47±3.26
TNBC	22	53.00±11.31	25.77±3.66
Luminal A	37	49.62±9.46	25.91±3.43
Luminal B (HER2-negative)	65	49.91±10.21	25.23±3.15
F-value		0.848	0.582
P-value		0.497	0.676

BMI, body mass index; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; TNBC, triple-negative breast cancer.

Figure 1. Comparison of TC concentrations in patients with five subtypes of breast cancer. $^*P<0.05$, $^{**}P<0.01$. TC, total cholesterol; TNBC, triple-negative breast cancer; HER2, human epidermal growth factor receptor 2; HR, hormone receptor.

and estradiol levels within the postmenopausal range for two consecutive measurements.

Statistical analysis. IBM SPSS Statistics Version 26 (IBM Corp.) was employed for all data analyses. Data with a normal distribution are presented as the mean ± standard deviation. Multi-group assessments were conducted via one-way analysis of variance with Tukey's test employed for post hoc analyses. Enumeration data are expressed as the case number (n) and percentage (%) and were analyzed using the Chi-square test. Linear correlation analyses were performed using Pearson's correlation test. In all results, P<0.05 was considered to indicate a statistically significant result.

Results

Clinical profile of each subgroup. None of the 170 patients with invasive BC had a family history of genetic disease or a history of other cancers. The age range of the patients was 30-83 years, with a median of 50 years and a mean of 50.71±9.96 years. The average BMI of the patients was 25.43±3.28 kg/m². A total of 92 patients were premenopausal and 78 were postmenopausal. Age, BMI and menopausal status did not show any significant differences among the various subtypes (Tables II and III). Based on the American Joint Committee on Cancer TNM stages, 91 patients had stage I cancer and 79 had stage II cancer. A Chi-square test indicated no significant differences in BC subtype in the two stages (Table III).

Serum lipid levels in each subgroup. TG and ApoB levels were comparable among patients in the different BC subtype cohorts (P>0.05; Table IV). However, TC, LDL-C, HDL-C and ApoA1 levels differed significantly among patients in the different BC subtype groups (P<0.05).

Serum TC and LDL-C levels were significantly elevated in the TNBC and HER2-positive [hormone receptor (HR)-negative] groups compared with the luminal A and B
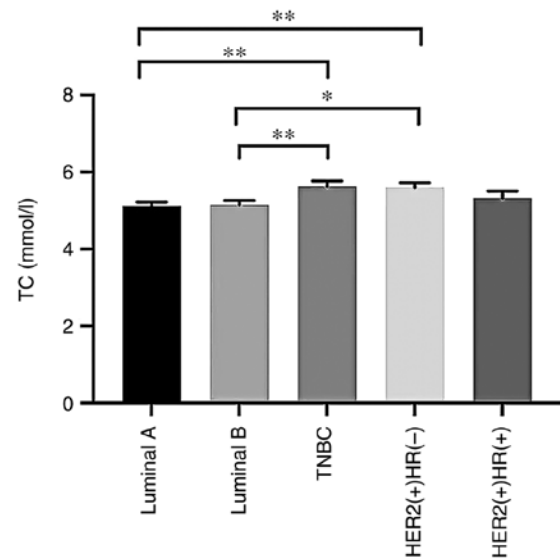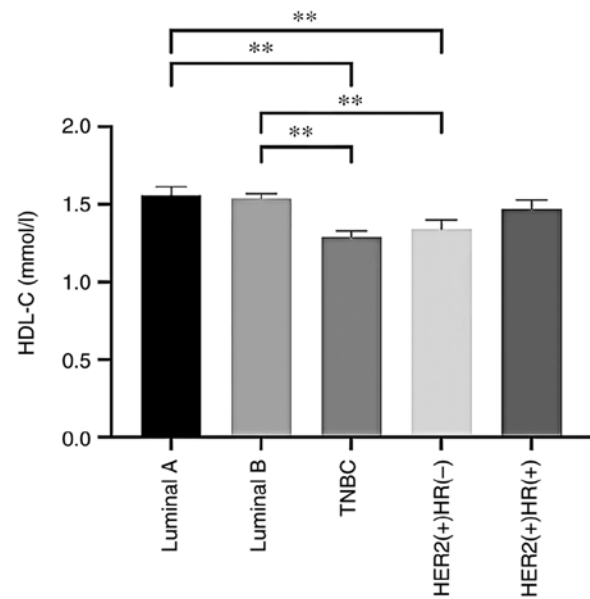

Figure 2. Comparison of HDL-C concentrations in patients with five subtypes of breast cancer. $^{**}P<0.01$. HDL-C, high-density lipoprotein cholesterol; TNBC, triple-negative breast cancer; HER2, human epidermal growth factor receptor 2; HR, hormone receptor.

(HER2-negative) groups. Serum HDL-C levels were significantly reduced in TNBC and HER2-positive (HR-negative) cases compared with luminal A and B (HER2-negative) cases. ApoA1 levels were significantly diminished in patients in the TNBC and HER2-positive (HR-negative) groups relative to those in the luminal A group. Furthermore, ApoA1 levels in patients with TNBC were markedly reduced compared with those in patients with luminal B (HER2-negative) BC. Differences in TC, HDL-C, LDL-C and ApoA1 levels among patients with different subcategories of BC are evident in Figs. 1-4.

Table III. TNM stages and menopausal status of patients in the five breast cancer groups.

Clinical features	Cases (n)	TNM stage [n (%)]		Menopausal status [n (%)]	
		I	II	Menopausal	Premenopausal
HER2-positive (HR-negative)	18	13 (72.2)	5 (27.8)	12 (66.7)	6 (33.3)
HER2-positive (HR-positive)	28	22 (78.6)	6 (21.4)	14 (50.0)	14 (50.0)
TNBC	22	20 (90.9)	2 (9.1)	13 (59.1)	9 (40.9)
Luminal A	37	19 (51.4)	18 (48.6)	14 (37.8)	23 (62.2)
Luminal B (HER2-negative)	65	17 (26.2)	48 (73.8)	25 (38.5)	40 (61.5)
χ^2-value		2.688		7.274	
P-value		0.112		0.122	

HER2, human epidermal growth factor receptor 2; HR, hormone receptor; TNBC, triple-negative breast cancer.
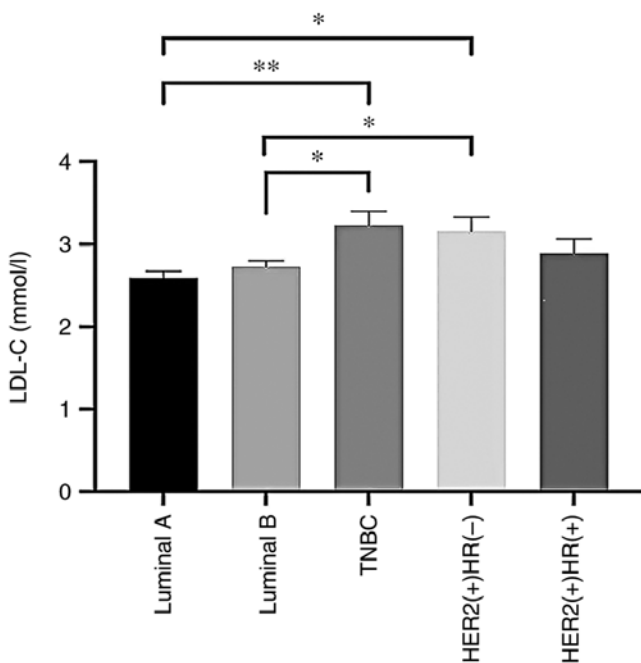

Figure 3. Comparison of LDL-C concentrations in patients with five subtypes of breast cancer. [*]P<0.05, [**]P<0.01. LDL-C, low-density lipoprotein cholesterol; TNBC, triple-negative breast cancer; HER2, human epidermal growth factor receptor 2; HR, hormone receptor.
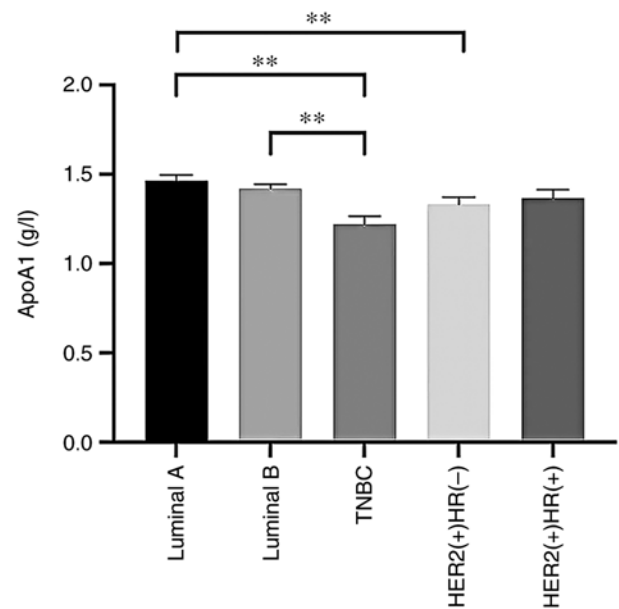

Figure 4. Comparison of ApoA1 concentrations in patients with five subtypes of breast cancer. [**]P<0.01. ApoA1, apolipoprotein A1; TNBC, triple-negative breast cancer; HER2, human epidermal growth factor receptor 2; HR, hormone receptor.

Correlations between serum lipid and Ki-67 levels in BC patients. Among the 170 patients, 65 had high Ki-67 levels and 35 had low Ki-67 levels. Correlation analyses between serum lipid and Ki-67 expression levels showed that circulating TC (r=0.186, P=0.015) and LDL-C (r=0.157, P=0.041) were significantly positively correlated with Ki-67 expression levels. By contrast, HDL-C (r=-0.262, P=0.001) and ApoA1 (r=-0.181, P=0.019) were significantly negatively correlated with Ki-67 expression levels. However, ApoB (r=-0.038, P=0.625) and TG (r=-0.146, P=0.057) levels were not significantly associated with Ki-67 expression levels (Table V). Plots showing the correlations of circulating TC, LDL-C, HDL-C and ApoA1 levels with Ki-67 expression levels in patients with BC are presented in Figs. 5-8.

Discussion

Cancer is one of the foremost global public health problems. Notable progress has been made in cancer diagnosis and treatment in recent decades. Early cancer detection via the use of predictive and diagnostic biomarkers is one of the most robust methods for early-stage cancer identification and personalized treatment. However, specific biomarkers with 100% diagnostic accuracy are lacking due to tumor heterogeneity and genetic instability induced by various carcinogenic factors (28,29). Lipid metabolomics may provide an improved molecular definition of tumors. The correlation between abnormal lipid metabolism and the occurrence and development of malignant tumors is a current topic of interest.

Lipids play numerous important roles in cell survival, proliferation and apoptosis and contribute to chemical energy storage, cell signal transduction, cell membrane and

Table IV. Comparison of serum lipid levels of patients in the five breast cancer groups.

Indicator	HER2-positive (HR-negative)	HER2-positive (HR-positive)	TNBC	Luminal A	Luminal B (HER2-negative)	F-value	P-value
Cases (n)	18	28	22	37	65	-	-
TC (mmol/l)	5.60±0.52	5.33±0.99	5.63±0.67	5.12±0.61[a,b]	5.15±0.93[a,b]	2.682	0.041
TG (mmol/l)	1.19±0.40	1.14±0.45	1.26±0.52	1.05±0.55	1.10±0.50	1.055	0.382
HDL-C (mmol/l)	1.34±0.26	1.47±0.29	1.29±0.20	1.56±0.32[a,b]	1.54±0.26[a,b]	5.443	<0.001
LDL-C (mmol/l)	3.15±0.73	2.89±0.92	3.23±0.80	2.59±0.49[a,b]	2.73±0.61[a,b]	4.376	<0.001
ApoA1 (g/l)	1.33±0.17	1.37±0.25	1.22±0.21	1.46±0.21[a,b]	1.42±0.18[a]	5.982	<0.001
ApoB (g/l)	0.96±0.24	0.93±0.29	0.97±0.26	0.94±0.20	0.97±0.22	0.265	0.902

[a]$P<0.05$ vs. TNBC; [b]$P<0.05$ vs. HER2 positive (HR-negative) breast cancer. ApoA1/B, apolipoprotein A1/B; HDL-C, high-density lipoprotein cholesterol; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; TNBC, triple-negative breast cancer.
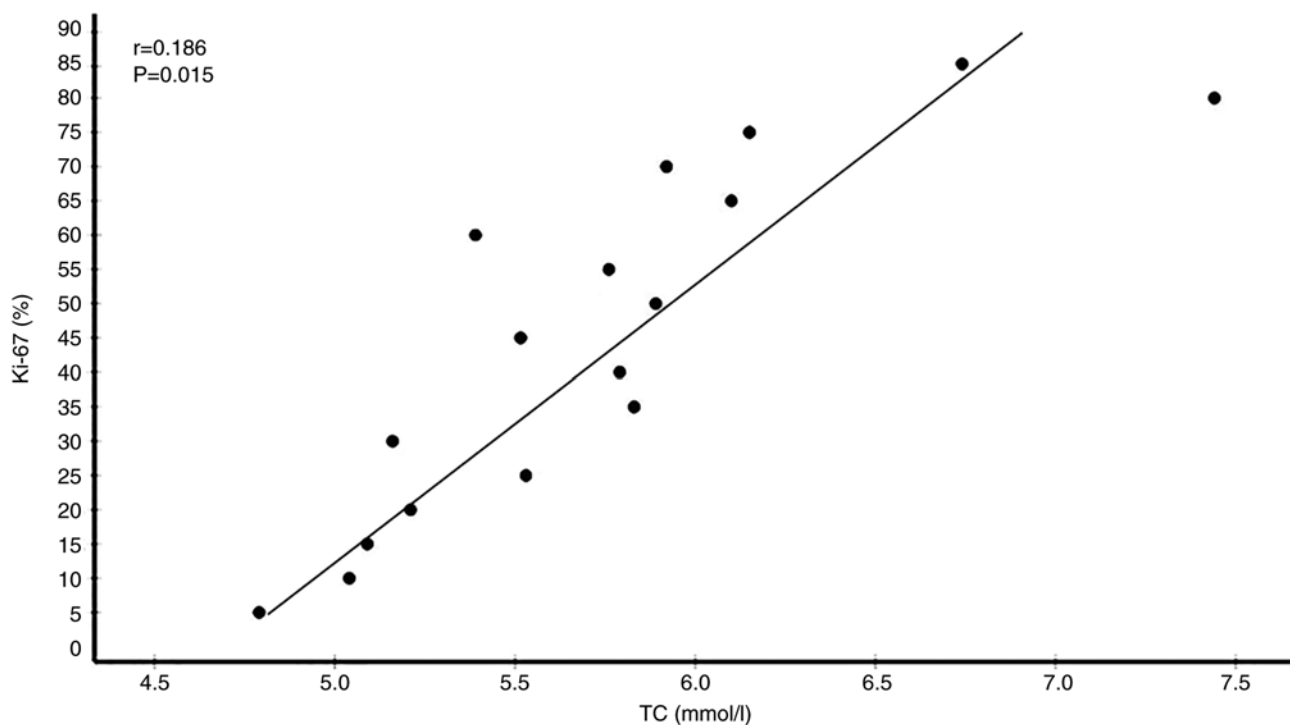

Figure 5. Correlation between TC concentrations and Ki-67 expression levels. TC, total cholesterol. Data from 170 patients were divided into 10 segments, and each point represents the mean of 10 adjacent values.

cell-to-cell associations. These cellular activities are closely associated with oncogenic pathways, particularly transformation, progression and metastasis (28). Studies have found aberrant levels of enzymes associated with lipid production, storage, activation and destruction in BC, indicating that the overexpression of these enzymes is closely associated with BC tumor progression (30,31). Heterogeneity among different molecular subcategories of BC is partly reflected by differences in serum lipid metabolism. An analysis of raw metabolomic data from 92 patients with primary BC who received surgery at the National Institute of Oncology in Milan, Italy, identified metabolic differences between BC subcategories. The results revealed that the magnitude of these metabolic changes was more pronounced in TNBC and HER2-positive BCs than in

luminal BCs. In particular, luminal B subtype tumors showed a greater dependency on FA metabolism for energy. By contrast, the HER2 overexpressing and TNBC subcategories exhibited general alterations in glucose and glutamine metabolism (30). In another study, Eiriksson *et al* (32) showed that while TNBC depends more on exogenous FA absorption and storage, the luminal subtype upregulates FA production and oxidation while the HER2-positive subtype depends on additional FA production and enhanced FA storage and oxidation.

Heterogeneity among BC subcategories is also associated with changes in the transcript and protein expression levels of lipid metabolic enzymes (33,34) and subtype-specific lipid profiles (35). The most widely studied during carcinogenesis is FA synthetase (FASN), an essential enzyme for
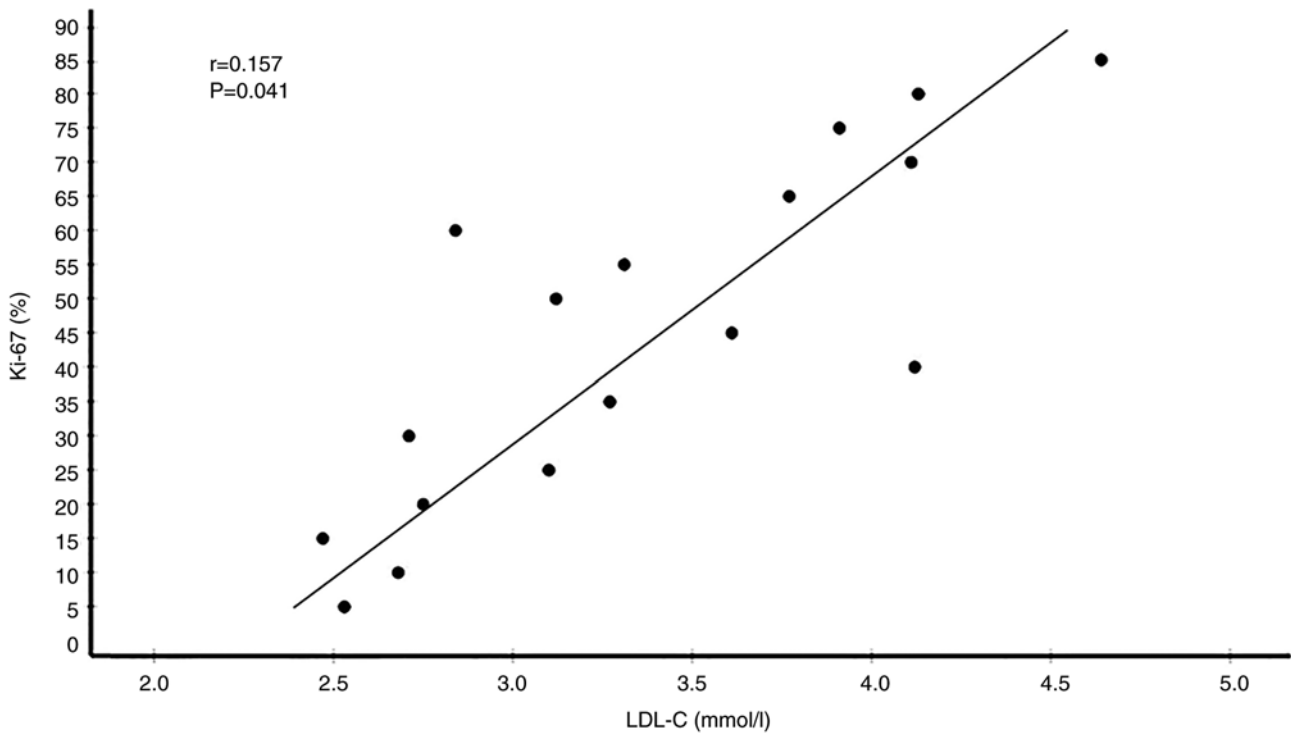
Figure 6. Correlation between LDL-C concentrations and Ki-67 expression levels. LDL-C, low-density lipoprotein cholesterol. Data from 170 patients were divided into 10 segments, and each point represents the mean of 10 adjacent values.
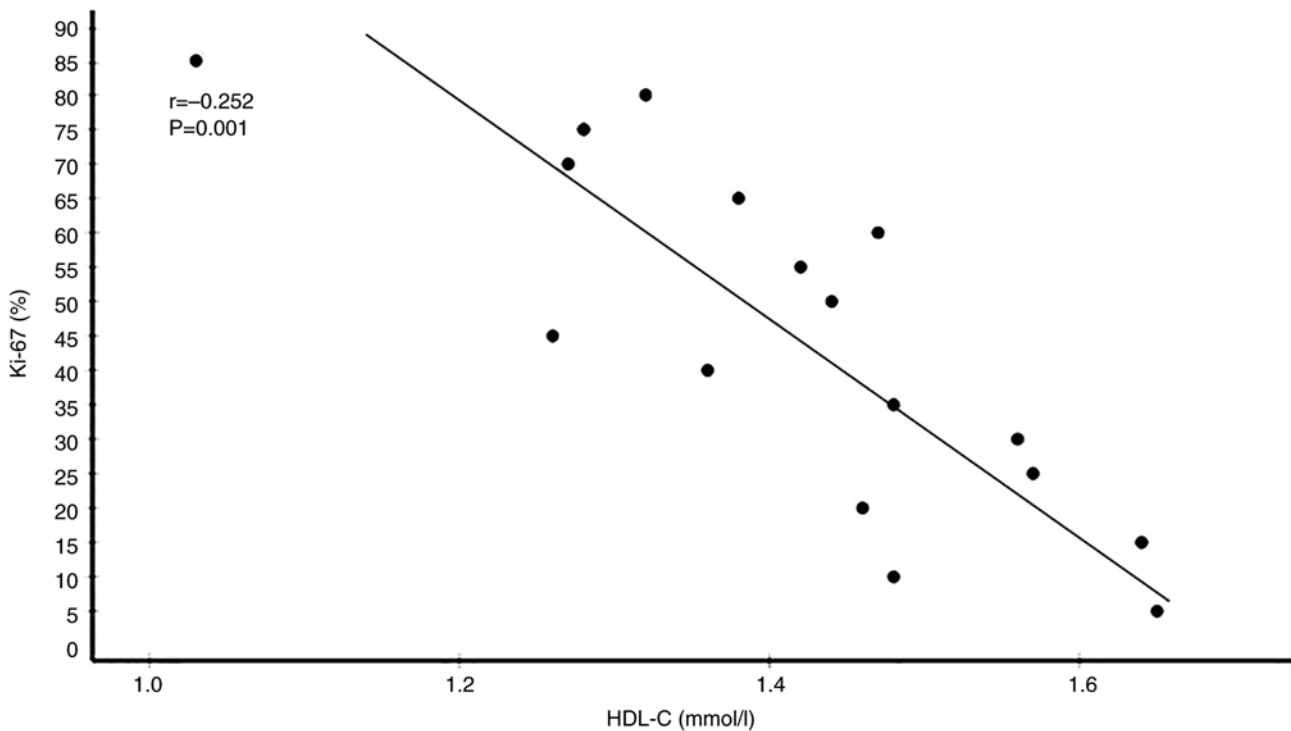

Figure 7. Correlation between HDL-C concentrations and Ki-67 expression levels. HDL-C, high-density lipoprotein cholesterol. Data from 170 patients were divided into 10 segments, and each point represents the mean of 10 adjacent values.

FA biosynthesis. It is ubiquitous in various cancers, namely, prostate, liver, ovarian, colon, endometrial and breast cancer, and is associated with malignant transformation and poor outcomes (33). A strong relationship has been identified between FASN mRNA levels and HER2 overexpressing, luminal A and luminal B subcategories, with IHC analyses of tumor samples confirming that FASN protein levels were the highest in the HER2 overexpressing subtype and most diminished in TNBC (34). FASN is often upregulated and activated in HER2-positive BC and is critical for maintaining
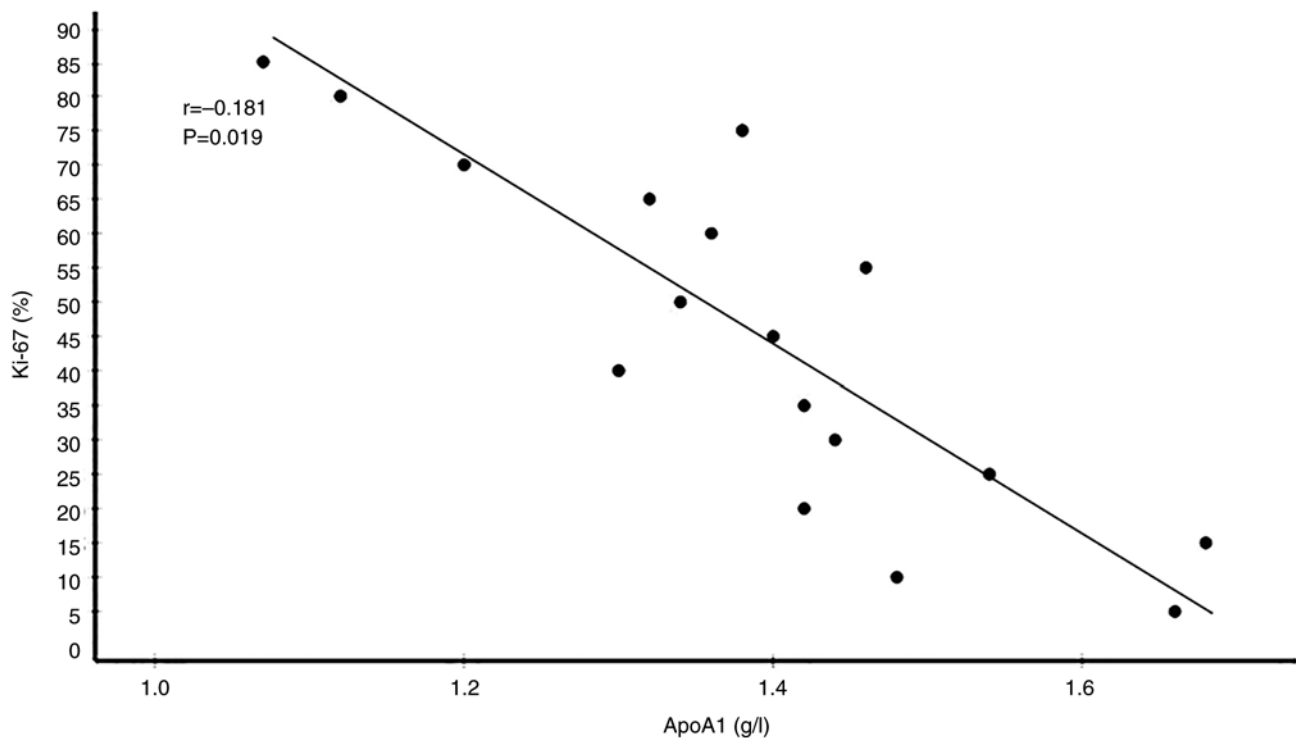
Figure 8. Correlation between ApoA1 concentrations and Ki-67 expression levels. ApoA1, apolipoprotein A1. Data from 170 patients were divided into 10 segments, and each point represents the mean of 10 adjacent values.

the growth, proliferation and viability of HER2-positive BC cells (36).

Acetyl-coenzyme A (CoA), critical for FA synthesis, is generated from citrate and CoA via ATP-citrate lyase (ACLY) catalysis. Upregulated ACLY levels and function have been observed in numerous cancers, including BC, suggesting that ACLY is important in cancer metabolism (37). *ACLY* mRNA has been shown to be the most upregulated in HER2-overexpressing subcategories of BC and downregulated in TNBC. Enzymes that release free FAs from TGs, including monoglyceride lipase and fatty triglyceride lipase, have also been found to be downregulated in TNBC. This finding indicates that free FA mobilization predominates in luminal-type and HER2-positive BCs (31). Kang *et al* (35) examined 34 pairs of breast surgical tissue samples comprising BC and adjacent normal tissue to distinguish cancerous and normal epithelial tissue and classify different BC subcategories. Lipidomic analyses revealed elevated phosphatidylcholine (PC) levels in BC, with significantly higher PC levels in TNBC than in luminal and HER2-positive subcategories. In addition, the study also found that matrix-assisted laser desorption/ionization mass spectrometry lipid profiles differed significantly between luminal, HER2-positive and TNBC subcategories, which may have important prognostic relevance.

A meta-analysis identified a strong link between HDL-C levels and BC risk (38). Other studies have reported that LDL-C levels are not associated BC risk, whereas circulating LDL levels may predict BC progression (39,40). However, few clinical investigations have explored the association between circulating lipid concentrations and different BC molecular subcategories. The present study performed a retrospective analysis to investigate differences in serum lipid levels between invasive BC molecular subcategories. The results demonstrated that serum lipid levels differed between BC subcategories. TC and LDL-C levels were elevated while HDL-C levels were diminished in TNBC and HER2-positive (HR-negative) BCs relative to the other types of BC. In addition, ApoA1 levels were particularly high in patients with luminal-type BC. However, the levels of TG and ApoB did not differ among the BC subcategories.

Cholesterol is important in promoting cell proliferation, migration and invasion, and so is essential in cancer occurrence and development. During carcinogenesis, cancer cells exhibit dysregulated cholesterol homeostasis and increased cholesterol synthesis and uptake, which enables them to synthesize cell membranes. These changes lead to the accumulation of cholesterol in cancer cells, which increases tumor cell survival, proliferation, metastasis and invasion, thereby promoting tumor survival and development (41). At present there is no clear evidence supporting an independent dyslipidemic effect of cholesterol in BC pathogenesis. However, clinical studies support a strong link between cholesterol and BC (42-46): A prospective trial in South Korean postmenopausal women assessed the relationship between total circulating cholesterol and BC risk and found a positive association in an age- and BMI-adjusted model (42). The first systematic review and meta-analysis of prospective trials investigating the relationship between cholesterol and BC by Touvier *et al* (38) showed a negative association between prediagnostic TC levels and BC risk. A number of studies have studied the association between cholesterol and BC types. For example, one study observed that cholesterol biosynthesis was significantly upregulated in TNBC tissues, and the high expression of genes involved in cholesterol synthesis was associated with short recurrence-free

Table V. Correlations between serum lipid and Ki-67 levels in patients with breast cancer.

Serum lipids	Levels (mean ± SD)	r	P-value
TC (mmol/l)	5.093±0.852	0.186	0.015[a]
TG (mmol/l)	1.161±0.496	-0.146	0.057
HDL-C (mmol/l)	1.482±0.265	-0.262	0.001[b]
LDL-C (mmol/l)	2.815±0.675	0.157	0.041[a]
ApoA1 (g/l)	1.405±0.210	-0.181	0.019[a]
ApoB (g/l)	0.955±0.236	-0.038	0.625

[a]$P<0.05$; [b]$P<0.01$. ApoA1/B, apolipoprotein A1/B; HDL-C, high-density lipoprotein cholesterol; Ki-67, proliferating cell nuclear antigen; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

survival in patients with TNBC (43). In another study, Fagherazzi et al (44) reported on 2,932 primary invasive BC cases after a 12-year follow-up in a prospective trial of the French E3N cohort. The study showed that women who used cholesterol-lowering drugs had a significantly lower risk of luminal BC than women who did not use them. Furthermore, a large French study collected 215 breast adipose tissue samples from patients with invasive BC and identified increased breast fat cholesterol levels in tissues obtained from patients with HER2 overexpressing and TNBC types. The authors concluded that increased cholesterol levels in breast adipose tissue might increase the aggressiveness of HER2-positive BC and TNBC (45). In the present study, patients with TNBC had the highest serum TC levels, and the TC levels of the patients with TNBC and HER2-positive (HR-negative) BC were significantly higher compared with those of patients with luminal A and B BC. Since HER2-positive BC and TNBC are more aggressive and with worse outcome then luminal BC, abnormal TC levels might be associated with enhanced aggressiveness and a worse outcome in patients with BC.

HDL-C is a small, dense lipoprotein comprising proteins and lipids that remove fat and cholesterol from cells and return them to the liver for excretion or reuse, which is known as reverse cholesterol transport. The levels of HDL-C are generally considered to be inversely associated with cardiovascular risk, and it has also been suggested that HDL-C function may be associated with BC (40,46-48). However, although most studies have found that circulating HDL-C levels are inversely associated with BC risk, some studies have reported opposing results, making this association unclear. Notably, a meta-analysis of observational studies revealed an inverse relationship between HDL-C levels and BC risk in postmenopausal women (46). In addition, Li et al (48) retrospectively assessed the blood lipid data of 1,044 patients who underwent BC surgery. They concluded that lower HDL-C levels were significantly associated with a worse overall survival (OS).

Katzke et al (47) found a strong association between HDL-C levels and BC risk. A large Mendelian randomization analysis also suggested that elevated circulating HDL-C levels may be linked to enhanced BC risk (40). However, a

Korean controlled study of 2,070 BC cases and controls found a marked inverse association between HDL-C levels and BC risk, with an increased risk of ER/PR negative BC compared with ER/PR positive BC (49). Maiti et al (50) investigated a possible association between highly aggressive TNBC and MS, collecting data on MS components and tumor profiles from 176 patients, including 86 with TNBC. A strong downregulation of HDL-C levels was evident in the patients with TNBC. Another study examined the association of MS and its components with TNBC and non-TNBC by reviewing 1,391 patients, including 394 with TNBC and 855 with non-TNBC. The results showed that among the patients with TNBC, those with lower HDL-C levels had worse 5-year recurrence-free survival and OS rates, suggesting that patients with TNBC and low serum HDL-C levels were at higher risk of death. One possible mechanism is the inverse association between HDL-C and angiotensin II, whose concentrations correlate positively with the vascular endothelial growth factor signaling pathway in TNBC cells (51). In the present study, serum HDL-C levels were the lowest in patients with TNBC and significantly lower in patients with TNBC and HER2-positive (HR-negative) BC than in those with luminal A and B BC. Based on the associations between HDL-C and molecular BC type reported in most prior studies, it can be concluded that a significant inverse relationship between HDL-C levels and TNBC exists. While elevated HDL-C levels are not a prerequisite for TNBC development, they have prognostic value in TNBC tumor recurrence and cancer-specific mortality. Therefore, the monitoring and normalization of HDL levels in TNBC subcategories may be particularly important.

LDL is mainly responsible for distributing cholesterol to tissues and cells other than those in the liver. LDL binds to the LDL receptor (LDLR) in most tissues. LDLR upregulation often accompanies elevated serum LDL-C levels (52). In vitro analysis has shown that cancer cells show the dysregulated expression of genes associated with cholesterol regulation and metabolism, including LDLR and HMG-CoA reductase (53). Indeed, numerous types of cancer cells show elevated LDL-C uptake and LDLR levels (54). Antalis et al (55) revealed that ER-negative BC cells contained more lipid droplets and had higher oleic acid uptake, LDL-C uptake, cholesteryl ester to triacylglycerol ratios and acyl-CoA-cholesterol acyltransferase 1 expression than ER-positive BC cells. These data confirmed an association between lipid accumulation and invasive behavior in ER-negative BC cell lines. Therefore, LDL-C was suggested to be involved in the invasiveness of ER-negative BC cells. In another study, dos Santos et al (56) used well-established in vitro and in vivo cholesterol enrichment models to investigate the mechanisms by which LDL-C promotes BC growth and invasiveness. The results revealed that LDL-C induced the proliferation and migration of ER-negative cell lines from different BC subcategories and stages; an association between high LDL-C levels and HER2-positive BC was also identified.

The expression profile of the LDLR differs among BC subcategories. The abundance of LDLR mRNA is reportedly 3-5-fold higher in MDA-MB-231 TNBC cells than in ER-positive MCF-7 cells. Moreover, LDL has been shown to accelerate MDA-MB-231 cell proliferation but not MCF-7 cell proliferation. This difference may be due to the capacity of TNBC cells to absorb, store and use LDLR-mediated

exogenous cholesterol. Elevated LDLR levels in TNBC cells are associated with the invasive and metastatic properties of these cells (57). Furthermore, in a study using a mouse model of hyperlipidemia to establish the significance of upregulated serum LDL-C and LDLR levels in tumor cells in BC growth, LDLR expression was higher in TNBC and HER2-positive cell lines than in ER-positive cells. Moreover, high LDL-C levels increased the size of the tumors in the mice (52).

LDLR-related protein 1 (LRP1) is an LDLR family member. A study of ductal BC found that LRP1 expression was associated with aggressive TNBC and HER2-positive tumors but not with HR-positive carcinomas, and that LRP1 upregulation was associated with the proliferation and invasiveness of HER2 BC and TNBC (58). Since LDLR overexpression was often accompanied by increased serum LDL-C levels in the aforementioned studies, this suggests an association of abnormally elevated LDL-C levels with TNBC and HER2-positive BCs, consistent with the present study; LDL-C levels were markedly elevated in the patients in the TNBC and HER2-positive subcategories compared with the luminal subcategories. However, further studies are required to confirm whether there is an association between high serum LDL-C levels and TNBC and HER2-positive subcategories.

ApoA1 is the main protein component of HDL-C and has an essential function in reverse cholesterol transport; it retrieves cholesterol and phospholipids from peripheral tissues and transports them to the liver for excretion. The role of ApoA1 in cancer is a current topic of research interest. Reduced serum ApoA1 levels have been reported in patients with gall-bladder, lung and colon cancers (59-61). In addition, another study showed that ApoA1-deficient mice rapidly developed tumors and were at a significant survival disadvantage while ApoA1-expressing mice exhibited a significant reduction in tumor progression and improved survival in a dose-dependent manner (62).

Studies have reported mixed results concerning the association between ApoA1 and BC. A study conducted by Borgquist *et al* (60) showed that elevated ApoA1 expression levels were associated with higher BC incidence. A small case-control study also concluded that elevated plasma ApoA1 levels were associated with a higher BC risk in Chinese women (63). Conversely, a large, nested case-control study of multiple plasma lipid marker measurements prior to BC diagnosis showed that the BC risk was reduced in patients with higher ApoA1 levels (11). Another study similarly detected a negative association between ApoA1 levels and BC risk (64). Plasma ApoA1 binds to ATP-binding cassette proteins to regulate ER and PR levels in BC *in vitro* and *in vivo* by inducing the cell division control protein 42 protein, which is essential for cancer metastasis, and the PAK1 signaling pathway (65). This indicates that ApoA1 contributes more to ER/PR-positive BC than to ER/PR-negative BC.

A comparative study by Lin *et al* (66) found that plasma ApoA1 levels were negatively associated with the incidence of invasive ductal carcinoma and that most patients with ER/PR-positive invasive ductal carcinoma had elevated plasma ApoA1 levels. By contrast, the patients with TNBC more frequently had low ApoA1 levels. These results suggest that tumors with high plasma ApoA1 levels are more likely to be luminal-type BCs than other BC types. In the present study,

ApoA1 levels were markedly elevated in patients with luminal subtype BC compared with other subcategories, suggesting that ApoA1 levels could be used as a predictive marker for prognosis and hormone therapy sensitivity in HR-positive BC patients.

Ki-67 is a proliferation marker that is mainly used to predict cancer prognosis and treatment response. The association of Ki-67 with the prognosis of BC has been extensively studied (67,68). Studies have shown that elevated Ki-67 levels are linked with increased recurrence rates and poorer survival in BC and have confirmed that Ki-67 is of independent prognostic value (67,69). However, the current definition of the critical value of Ki-67 is inconsistent and lacks validity. A large meta-analysis that included 64,196 patients revealed that Ki-67 was a stand-alone prognostic indicator for OS in BC patients when a Ki-67 threshold of >25% was employed (70).

Various studies have investigated the function of Ki-67 expression in different molecular BC subcategories. They have shown a marked association between elevated Ki-67 levels and ER/PR negativity and a positive correlation with HER2 positivity (71,72). Soliman and Yussif (73) reported that upregulated Ki-67, defined as >15% Ki-67-positive nuclei, was negatively associated with ER and PR expression, and that 34% of HER2-positive BCs and 60% of TNBC cases had high Ki-67 expression. Hashmi *et al* (74) found that Ki-67 positivity in >90% of HER2-positive BCs and >14% of TNBCs. TNBC had the highest Ki-67 index (50.9±23.7%), followed by the HER2-positive type (42.6±21.6%). Another study confirmed that the Ki-67 expression levels in TNBC and HER2-positive subcategories of BC are higher compared with those in luminal subcategories (75). As an independent BC prognostic factor, Ki-67 indicates the increased invasiveness and recurrence rate of the TNBC and HER2-positive subcategories, as well as a reduced survival rate.

In the present study, circulating LDL-C and TC levels correlated positively with Ki-67 expression levels, while HDL-C and ApoA1 levels correlated negatively with Ki-67 expression levels. These results suggest that abnormal circulating lipid concentrations are associated with a poor outcome in patients with BC. However, additional clinical studies are necessary to determine whether they can be used as useful clinical indicators to predict BC prognosis.

In summary, the present study demonstrated that abnormal TC, LDL-C, HDL-C and ApoA1 levels are closely associated with different molecular subcategories of BC and correlated with Ki-67 expression levels. TC, LDL-C, HDL-C and ApoA1 abnormalities were more common in TNBC and HER2-positive BC subcategories than in luminal subcategories. The measurement of blood lipid levels in patients with primary invasive BC is useful for the discovery of diagnostic markers and therapeutic targets for BC, and provides new information that may be helpful for the prognosis and specific therapy of different molecular BC subcategories. It also enables patients' prognosis and survival rates to be improved via the adjustment of abnormal serum lipid levels. However, the present investigation used a retrospective clinical observation design. Consequently, the blood lipid levels of patients 2 and 6 months after treatment were not collected for further analysis. Furthermore, the

relationship between lipid levels and survival rate was not analyzed. Therefore, further studies are required to explore these important issues.

The present study explored the association between circulating lipid concentrations and BC patient prognosis. However, certain fundamental factors affect lipogenesis. For example, a study showed that the expression of oncogenic phosphatidylinositol 3-kinase (PI3K; H1047R) or KRAS (G12V) mutations in breast epithelial cells promotes lipid synthesis via the convergent activation of the mammalian target of rapamycin (mTOR) complex 1 downstream of these common oncogenes. These findings indicate that PI3K/protein kinase B/mTOR pathway activation is critical for the oncogenic network to produce additional lipids to accelerate abnormal cancer cell growth and proliferation (76). A limitation of the present retrospective study is that only the relationship between serum lipid level differences among molecular BC subcategories and Ki-67 expression was explored; its mechanistic basis was not investigated. In future clinical and experimental studies, explorations of the correlations between *PI3K* or *KRAS* genetic mutations and lipogenesis are planned.

In conclusion, the current study indicated that elevated TC and LDL-C and diminished HDL-C and ApoA1 levels are risk factors for TNBC and HER2-positive (HR-negative) BCs but not luminal subtype BCs. It also revealed that circulating TC, LDL-C, HDL-C and ApoA1 levels are correlated with Ki-67 expression, with abnormal serum TC, LDL-C, HDL-C and ApoA1 levels being intricately associated with worse BC patient outcomes. Based on these findings, it is suggested that changes in serum lipid concentrations should be closely monitored in BC patients, and TC, LDL-C, HDL-C and ApoA1 levels should be kept within the normal range.

Acknowledgements

Not applicable.

Funding

Availability of data and materials

This datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

BQR designed the study. WWL and XBS conceived the article, performed the literature search and data analysis, drafted and critically revised the work, and confirm the authenticity of the raw data. BQR reviewed and edited the manuscript. BW, ZPY, HZT, SL, YYW and JXQ analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval was waived by The Science Research Ethics Committee of The Second Affiliated Hospital of Shandong First Medical University since researchers have open access to relevant data for research purposes, the study used retrospective clinical data and no ethical issues or other conflicts of interest were encountered. A waiver of informed patient consent was also obtained from the committee (ref. no. 2022-088).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Cao W, Chen HD, Yu YW, Li N and Chen WQ: Changing profiles of cancer burden worldwide and in China: A secondary analysis of the global cancer statistics 2020. Chin Med J (Engl) 134: 783-791, 2021.
2. Gang H, Jingyi G, Shujiang R, Xiaobin L and Fenli L: Advances in breast cancer treatment. J Front Med 10: 11-12, 2020 (In Chinese).
3. Long Y, Pan Z, Wenhua Z and Jingzhi G: Progress in the key enzymes in tumor lipid metabolism. Cancer Res Clin 28: 789-792, 2016 (In Chinese).
4. Röhrig F and Schulze A: The multifaceted roles of fatty acid synthesis in cancer. Nat Rev Cancer 16: 732-749, 2016.
5. Maan M, Peters JM, Dutta M and Patterson AD: Lipid metabolism and lipophagy in cancer. Biochem Biophys Res Commun 504: 582-589, 2018.
6. Bian X, Liu R, Meng Y, Xing D, Xu D and Lu Z: Lipid metabolism and cancer. J Exp Med 218: e20201606, 2021.
7. Beloribi-Djefaflia S, Vasseur S and Guillaumond F: Lipid metabolic reprogramming in cancer cells. Oncogenesis 5: e189, 2016.
8. Halama A, Guerrouahen BS, Pasquier J, Satheesh NJ, Suhre K and Rafii A: Nesting of colon and ovarian cancer cells in the endothelial niche is associated with alterations in glycan and lipid metabolism. Sci Rep 7: 39999, 2017.
9. Kouba S, Ouldamer L, Garcia C, Fontaine D, Chantome A, Vandier C, Goupille C and Potier-Cartereau M: Lipid metabolism and calcium signaling in epithelial ovarian cancer. Cell Calcium 81: 38-50, 2019.
10. Sung H, Siegel RL, Torre LA, Pearson-Stuttard J, Islami F, Fedewa SA, Goding Sauer A, Shuval K, Gapstur SM, Jacobs EJ, et al: Global patterns in excess body weight and the associated cancer burden. CA Cancer J Clin 69: 88-112, 2019.
11. Lingquan K, Xin L, Hongyuan L, Guosheng R and Kainan W: Progress on the study of breast cancer related dyslipidemia. Chin J Endocrine Surg 11: 89-91, 96, 2017.
12. Nelson ER: The significance of cholesterol and its metabolite, 27-hydroxycholesterol in breast cancer. Mol Cell Endocrinol 466: 73-80, 2018.
13. Quiroga-Morales LA, Sat-Muñoz D, Martínez-Herrera BE, Alcántara-Cadillo RR, Macías-López GG, García-Cobián TA, Bañuelos-Rizo M and Balderas-Peña LMA: Obesity and adipocytokines in breast cancer and benign breast disease. Rev Med Inst Mex Seguro Soc 56: 246-254, 2018 (In Spanish).
14. Qi L, Yanhui Z, Junzhe Y, Xian W, Xingmeng W, Chaoran Y, Ruyu C and Xiaoan L: Research progress on the relationship between serum lipid level and breast cancer treatment. Chin J Surg Oncol 11: 168-171, 2019 (In Chinese).
15. Chen M, Zhao Y, Yang X, Zhao Y, Liu Q, Liu Y, Hou Y, Sun H and Jin W: NSDHL promotes triple-negative breast cancer metastasis through the TGFβ signaling pathway and cholesterol biosynthesis. Breast Cancer Res Treat 187: 349-362, 2021.
16. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, et al: Molecular portraits of human breast tumours. Nature 406: 747-752, 2000.

17. Bustreo S, Osella-Abate S, Cassoni P, Donadio M, Airoldi M, Pedani F, Papotti M, Sapino A and Castellano I: Optimal Ki67 cut-off for luminal breast cancer prognostic evaluation: A large case series study with a long-term follow-up. Breast Cancer Res Treat 157: 363-371, 2016.

18. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B and Senn HJ; Panel members: Strategies for subtypes-dealing with the diversity of breast cancer: Highlights of the St. Gallen international expert consensus on the primary therapy of early breast cancer 2011. Ann Oncol 22: 1736-1747, 2011.

19. Lin NU, Claus E, Sohl J, Razzak AR, Arnaout A and Winer EP: Sites of distant recurrence and clinical outcomes in patients with metastatic triple-negative breast cancer: High incidence of central nervous system metastases. Cancer 113: 2638-2645, 2008.

20. Cao MD, Lamichhane S, Lundgren S, Bofin A, Fjøsne H, Giskeødegård GF and Bathen TF: Metabolic characterization of triple negative breast cancer. BMC Cancer 14: 941, 2014.

21. Li LT, Jiang G, Chen Q and Zheng JN: Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep 11: 1566-1572, 2015.

22. Yuan P, Xu B, Wang C, Zhang C, Sun M and Yuan L: Ki-67 expression in luminal type breast cancer and its association with the clinicopathology of the cancer. Oncol Lett 11: 2101-2105, 2016.

23. Juríková M, Danihel Ľ, Polák Š and Varga I: Ki67, PCNA, and MCM proteins: Markers of proliferation in the diagnosis of breast cancer. Acta Histochem 118: 544-552, 2016.

24. Inwald EC, Klinkhammer-Schalke M, Hofstädter F, Zeman F, Koller M, Gerstenhauer M and Ortmann O: Ki-67 is a prognostic parameter in breast cancer patients: Results of a large population-based cohort of a cancer registry. Breast Cancer Res Treat 139: 539-552, 2013.

25. Denkert C, Budczies J, von Minckwitz G, Wienert S, Loibl S and Klauschen F: Strategies for developing Ki67 as a useful biomarker in breast cancer. Breast 24 (Suppl 2): S67-S72, 2015.

26. Baek AE and Nelson ER: The contribution of cholesterol and its metabolites to the pathophysiology of breast cancer. Horm Cancer 7: 219-228, 2016.

27. Li JB and Jiang ZF: Chinese society of clinical oncology breast cancer guideline version 2021: Updates and interpretations. Zhonghua Yi Xue Za Zhi 101: 1835-1838, 2021 (In Chinese).

28. Perrotti F, Rosa C, Cicalini I, Sacchetta P, Del Boccio P, Genovesi D and Pieragostino D: Advances in lipidomics for cancer biomarkers discovery. Int J Mol Sci 17: 1992, 2016.

29. Szász AM, Győrffy B and Marko-Varga G: Cancer heterogeneity determined by functional proteomics. Semin Cell Dev Biol 64: 132-142, 2017.

30. Cappelletti V, Iorio E, Miodini P, Silvestri M, Dugo M and Daidone MG: Metabolic footprints and molecular subtypes in breast cancer. Dis Markers 2017: 7687851, 2017.

31. Monaco ME: Fatty acid metabolism in breast cancer subtypes. Oncotarget 8: 29487-29500, 2017.

32. Eiriksson FF, Nøhr MK, Costa M, Bödvarsdottir SK, Ögmundsdottir HM and Thorsteinsdottir M: Lipidomic study of cell lines reveals differences between breast cancer subtypes. PLoS One 15: e231289, 2020.

33. Furuta E, Okuda H, Kobayashi A and Watabe K: Metabolic genes in cancer: Their roles in tumor progression and clinical implications. Biochim Biophys Acta 1805: 141-152, 2010.

34. Kim S, Lee Y and Koo JS: Differential expression of lipid metabolism-related proteins in different breast cancer subtypes. PLoS One 10: e0119473, 2015.

35. Kang HS, Lee SC, Park YS, Jeon YE, Lee JH, Jung SY, Park IH, Jang SH, Park HM, Yoo CW, *et al*: Protein and lipid MALDI profiles classify breast cancers according to the intrinsic subtype. BMC Cancer 11: 465, 2011.

36. Ligorio F, Pellegrini I, Castagnoli L, Vingiani A, Lobefaro R, Zattarin E, Santamaria M, Pupa SM, Pruneri G, de Braud F and Vernieri C: Targeting lipid metabolism is an emerging strategy to enhance the efficacy of anti-HER2 therapies in HER2-positive breast cancer. Cancer Lett 511: 77-87, 2021.

37. Zaidi N, Swinnen JV and Smans K: ATP-citrate lyase: A key player in cancer metabolism. Cancer Res 72: 3709-3714, 2012.

38. Touvier M, Fassier P, His M, Norat T, Chan DS, Blacher J, Hercberg S, Galan P, Druesne-Pecollo N and Latino-Martel P: Cholesterol and breast cancer risk: A systematic review and meta-analysis of prospective studies. Br J Nutr 114: 347-357, 2015.

39. Orho-Melander M, Hindy G, Borgquist S, Schulz CA, Manjer J, Melander O and Stocks T: Blood lipid genetic scores, the HMGCR gene and cancer risk: A Mendelian randomization study. Int J Epidemiol 47: 495-505, 2018.

40. Beeghly-Fadiel A, Khankari NK, Delahanty RJ, Shu XO, Lu Y, Schmidt MK, Bolla MK, Michailidou K, Wang Q, Dennis J, *et al*: A Mendelian randomization analysis of circulating lipid traits and breast cancer risk. Int J Epidemiol 49: 1117-1131, 2020.

41. Meng Y, Wang Q and Lyu Z: Cholesterol metabolism and tumor. Zhejiang Da Xue Xue Bao Yi Xue Ban 50: 23-31, 2021.

42. Ha M, Sung J and Song YM: Serum total cholesterol and the risk of breast cancer in postmenopausal Korean women. Cancer Causes Control 20: 1055-1060, 2009.

43. Ehmsen S, Pedersen MH, Wang G, Terp MG, Arslanagic A, Hood BL, Conrads TP, Leth-Larsen R and Ditzel HJ: Increased cholesterol biosynthesis is a key characteristic of breast cancer stem cells influencing patient outcome. Cell Rep 27: 3927-3938. e6, 2019.

44. Fagherazzi G, Fabre A, Boutron-Ruault MC and Clavel-Chapelon F: Serum cholesterol level, use of a cholesterol-lowering drug, and breast cancer: Results from the prospective E3N cohort. Eur J Cancer Prev 19: 120-125, 2010.

45. Goupille C, Ouldamer L, Pinault M, Guimares C, Arbion F, Jourdan ML and Frank PG: Identification of a positive association between mammary adipose cholesterol content and indicators of breast cancer aggressiveness in a French population. J Nutr 151: 1119-1127, 2021.

46. Ni H, Liu H and Rong G: Serum lipids and breast cancer risk: A meta-analysis of prospective cohort studies. PLoS One 10: e0142669, 2015.

47. Katzke VA, Sookthai D, Johnson T, Kühn T and Kaaks R: Blood lipids and lipoproteins in relation to incidence and mortality risks for CVD and cancer in the prospective EPIC-Heidelberg cohort. BMC Med 15: 218, 2017.

48. Li X, Tang H, Wang J, Xie X, Liu P, Kong Y, Ye F, Shuang Z, Xie Z and Xie X: The effect of preoperative serum triglycerides and high-density lipoprotein-cholesterol levels on the prognosis of breast cancer. Breast 32: 1-6, 2017.

49. Kim Y, Park SK, Han W, Kim DH, Hong YC, Ha EH, Ahn SH, Noh DY, Kang D and Yoo KY: Serum high-density lipoprotein cholesterol and breast cancer risk by menopausal status, body mass index, and hormonal receptor in Korea. Cancer Epidemiol Biomarkers Prev 18: 508-515, 2009.

50. Maiti B, Kundranda MN, Spiro TP and Daw HA: The association of metabolic syndrome with triple-negative breast cancer. Breast Cancer Res Treat 121: 479-483, 2010.

51. Fan Y, Ding X, Wang J, Ma F, Yuan P, Li Q, Zhang P and Xu B: Decreased serum HDL at initial diagnosis correlates with worse outcomes for triple-negative breast cancer but not non-TNBCs. Int J Biol Markers 30: e200-e207, 2015.

52. Gallagher EJ, Zelenko Z, Neel BA, Antoniou IM, Rajan L, Kase N and LeRoith D: Elevated tumor LDLR expression accelerates LDL cholesterol-mediated breast cancer growth in mouse models of hyperlipidemia. Oncogene 36: 6462-6471, 2017.

53. Scheinman EJ, Rostoker R and Leroith D: Cholesterol affects gene expression of the Jun family in colon carcinoma cells using different signaling pathways. Mol Cell Endocrinol 374: 101-107, 2013.

54. Hoque M, Rentero C, Conway JR, Murray RZ, Timpson P, Enrich C and Grewal T: The cross-talk of LDL-cholesterol with cell motility: Insights from the Niemann pick type C1 mutation and altered integrin trafficking. Cell Adh Migr 9: 384-391, 2015.

55. Antalis CJ, Uchida A, Buhman KK and Siddiqui RA: Migration of MDA-MB-231 breast cancer cells depends on the availability of exogenous lipids and cholesterol esterification. Clin Exp Metastasis 28: 733-741, 2011.

56. dos Santos CR, Domingues G, Matias I, Matos J, Fonseca I, de Almeida JM and Dias S: LDL-cholesterol signaling induces breast cancer proliferation and invasion. Lipids Health Dis 13: 16, 2014.

57. Ye J, Xia X, Dong W, Hao H, Meng L, Yang Y, Wang R, Lyu Y and Liu Y: Cellular uptake mechanism and comparative evaluation of antineoplastic effects of paclitaxel-cholesterol lipid emulsion on triple-negative and non-triple-negative breast cancer cell lines. Int J Nanomedicine 11: 4125-4140, 2016.

58. Catasus L, Gallardo A, Llorente-Cortes V, Escuin D, Muñoz J, Tibau A, Peiro G, Barnadas A and Lerma E: Low-density lipoprotein receptor-related protein 1 is associated with proliferation and invasiveness in Her-2/neu and triple-negative breast carcinomas. Hum Pathol 42: 1581-1588, 2011.

59. van Duijnhoven FJ, Bueno-De-Mesquita HB, Calligaro M, Jenab M, Pischon T, Jansen EH, Frohlich J, Ayyobi A, Overvad K, Toft-Petersen AP, et al: Blood lipid and lipoprotein concentrations and colorectal cancer risk in the European prospective investigation into cancer and nutrition. Gut 60: 1094-1102, 2011.
60. Borgquist S, Butt T, Almgren P, Shiffman D, Stocks T, Orho-Melander M, Manjer J and Melander O: Apolipoproteins, lipids and risk of cancer. Int J Cancer 138: 2648-2656, 2016.
61. Zuo M, Rashid A, Wang Y, Jain A, Li D, Behari A, Kapoor VK, Koay EJ, Chang P, Vauthey JN, et al: RNA sequencing-based analysis of gallbladder cancer reveals the importance of the liver X receptor and lipid metabolism in gallbladder cancer. Oncotarget 7: 35302-35312, 2016.
62. Zamanian-Daryoush M, Lindner D, Tallant TC, Wang Z, Buffa J, Klipfell E, Parker Y, Hatala D, Parsons-Wingerter P, Rayman P, et al: The cardioprotective protein apolipoprotein A1 promotes potent anti-tumorigenic effects. J Biol Chem 288: 21237-21252, 2013.
63. Han C, Zhang HT, Du L, Liu X, Jing J, Zhao X, Yang X and Tian B: Serum levels of leptin, insulin, and lipids in relation to breast cancer in China. Endocrine 26: 19-24, 2005.
64. Chang SJ, Hou MF, Tsai SM, Wu SH, Hou LA, Ma H, Shann TY, Wu SH and Tsai LY: The association between lipid profiles and breast cancer among Taiwanese women. Clin Chem Lab Med 45: 1219-1223, 2007.
65. Holm C, Rayala S, Jirström K, Stål O, Kumar R and Landberg G: Association between Pak1 expression and subcellular localization and tamoxifen resistance in breast cancer patients. J Natl Cancer Inst 98: 671-680, 2006.
66. Lin X, Hong S, Huang J, Chen Y, Chen Y and Wu Z: Plasma apolipoprotein A1 levels at diagnosis are independent prognostic factors in invasive ductal breast cancer. Discov Med 23: 247-258, 2017.
67. Luporsi E, André F, Spyratos F, Martin PM, Jacquemier J, Penault-Llorca F, Tubiana-Mathieu N, Sigal-Zafrani B, Arnould L, Gompel A, et al: Ki-67: Level of evidence and methodological considerations for its role in the clinical management of breast cancer: Analytical and critical review. Breast Cancer Res Treat 132: 895-915, 2012.
68. Haroon S, Hashmi AA, Khurshid A, Kanpurwala MA, Mujtuba S, Malik B and Faridi N: Ki67 index in breast cancer: Correlation with other prognostic markers and potential in pakistani patients. Asian Pac J Cancer Prev 14: 4353-4358, 2013.
69. de Azambuja E, Cardoso F, de Castro G Jr, Colozza M, Mano MS, Durbecq V, Sotiriou C, Larsimont D, Piccart-Gebhart MJ and Paesmans M: Ki-67 as prognostic marker in early breast cancer: A meta-analysis of published studies involving 12,155 patients. Br J Cancer 96: 1504-1513, 2007.
70. Petrelli F, Viale G, Cabiddu M and Barni S: Prognostic value of different cut-off levels of Ki-67 in breast cancer: A systematic review and meta-analysis of 64,196 patients. Breast Cancer Res Treat 153: 477-491, 2015.
71. Alco G, Bozdogan A, Selamoglu D, Pilanci KN, Tuzlali S, Ordu C, Igdem S, Okkan S, Dincer M, Demir G and Ozmen V: Clinical and histopathological factors associated with Ki-67 expression in breast cancer patients. Oncol Lett 9: 1046-1054, 2015.
72. Kanyilmaz G, Yavuz BB, Aktan M, Karaağaç M, Uyar M and Fındık S: Prognostic importance of Ki-67 in breast cancer and its relationship with other prognostic factors. Eur J Breast Health 15: 256-261, 2019.
73. Soliman NA and Yussif SM: Ki-67 as a prognostic marker according to breast cancer molecular subtype. Cancer Biol Med 13: 496-504, 2016.
74. Hashmi AA, Hashmi KA, Irfan M, Khan SM, Edhi MM, Ali JP, Hashmi SK, Asif H, Faridi N and Khan A: Ki67 index in intrinsic breast cancer subtypes and its association with prognostic parameters. BMC Res Notes 12: 605, 2019.
75. Mitrović O, Čokić V, Đikić D, Budeč M, Vignjević S, Subotički T, Gulan M, Radović S and Furtula S: Correlation between ER, PR, HER-2, Bcl-2, p53, proliferative and apoptotic indexes with HER-2 gene amplification and TOP2A gene amplification and deletion in four molecular subtypes of breast cancer. Target Oncol 9: 367-379, 2014.
76. Ricoult SJH, Yecies JL, Ben-Sahra I and Manning BD: Oncogenic PI3K and K-Ras stimulate de novo lipid synthesis through mTORC1 and SREBP. Oncogene 35: 1250-1260, 2016.