The Role of BMI and TNF-α in Breast Cancer Development: A Case–Control Study

Keywords
obesity, risk factor, BMI, TNF-α, Keywords: breast cancer

Abstract

Introduction

A high body mass index (BMI) is closely linked to increased breast cancer risk. Despite the established links between BMI and TNF-α with breast cancer, few studies have explored their combined effects on breast cancer development. The aim of our study was to evaluate the separate and combined associations of BMI and tumor necrosis factor-alpha (TNF-α) with breast cancer risk.

Material and methods

This study conducted a case–control analysis involving 794 women diagnosed with breast cancer and 268 age–matched healthy controls from Sun Yat-sen University's affiliated hospitals between October 2008 and March 2018. Data on demographic characteristics, clinical features, and TNF-α levels were collected. Logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between BMI, TNF-α, and breast cancer risk.

Results

High levels of TNF-α (≥58.45 μg/ml) were significantly associated with an increased risk of breast cancer (OR 1.500; 95% CI 1.112–2.022). Elevated TNF-α levels are linked to early clinical stage, ER-positive, PR-positive, HER2-positive, high Ki67 expression, and the absence of lymphatic and distant metastases. No significant association was found between BMI and breast cancer risk (OR 0.947; 95% CI 0.685–1.310), nor was there a significant interaction effect between BMI and TNF-α.

Conclusions

TNF-α plays a significant role in breast cancer development, particularly in early clinical stages, and in specific pathological features. BMI alone is not a significant predictor of breast cancer risk. These findings underscore the importance of TNF-α as a potential target for breast cancer prevention and treatment strategies.

The Role of BMI and TNF-α in Breast Cancer Development: A Case–Control Study

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Abstracts

Objectives: A high body mass index (BMI) is closely linked to increased breast cancer risk. Despite the established links between BMI and TNF-α with breast cancer, few studies have explored their combined effects on breast cancer development. The aim of our study was to evaluate the separate and combined associations of BMI and tumor necrosis factor-alpha (TNF-α) with breast cancer risk.

Methods: This study conducted a case–control analysis involving 794 women diagnosed with breast cancer and 268 age–matched healthy controls from Sun Yat-sen University's affiliated hospitals between October 2008 and March 2018. Data on demographic characteristics, clinical features, and TNF-α levels were collected. Logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between BMI, TNF-α, and breast cancer risk.

Results: High levels of TNF-α (≥58.45 μg/ml) were significantly associated with an increased risk of breast cancer (OR 1.500; 95% CI 1.112–2.022). Elevated TNF-α levels are linked to early clinical stage, ER-positive, PR-positive, HER2-positive, high Ki67 expression, and the absence of lymphatic and distant metastases. No significant association was found between BMI and breast cancer risk (OR 0.947; 95% CI 0.685–1.310), nor was there a significant interaction effect between BMI and TNF-α.

Conclusions: TNF-α plays a significant role in breast cancer development, particularly in early clinical stages, and in specific pathological features. BMI alone is not a significant predictor of breast cancer risk. These findings underscore the importance of TNF-α as a potential target for breast cancer prevention and treatment strategies.

Keywords: breast cancer; TNF-α; BMI; obesity; risk factor

1 Introduction

Obesity affects more than 600 million adults globally (13% of the population) and is measured by BMI, which classifies individuals into different weight categories, with both low and high BMIs linked to an increased risk of breast cancer (1-3). Obesity is a significant risk factor for breast cancer due to its association with systemic inflammatory factors and metabolic alterations, such as insulin resistance, hyperglycemia, and dyslipidemia, which promote tumor growth(4). The relationship between obesity and breast cancer involves mechanisms that contribute to metabolic syndrome, cardiovascular and endocrine diseases, and increased risk due to adipocytokine dysregulation and elevated estrogen levels in obese women (5). Three primary cellular mechanisms connect obesity to cancer: the insulin-IGF-1 axis, sex hormones, and adipocytokines, all of which contribute to endocrine dysregulation in obese patients(6, 7). Metabolic changes in adipose tissue in obese individuals lead to systemic changes such as insulin resistance, hyperglycemia, dyslipidemia, and chronic inflammation, which increase cancer risk(8). Adipose tissue produces sex hormones and cytokines that promote tumor initiation and progression, and a high BMI increases the levels of inflammatory mediators and comorbidities that contribute to breast cancer (9, 10).

Tumor necrosis factor-alpha (TNF-α), a crucial proinflammatory cytokine, plays a multifaceted role in the tumor microenvironment. Imbalances in TNF-α and other cytokines can lead to immune system dysregulation, promoting infection and tumor growth, with cancer cells using cytokine communication to shape the tumor microenvironment and facilitate metastasis(11-13). TNF-α affects tumor cell proliferation, survival, metastasis, and recurrence. Its chronic overexpression is linked to lymph node metastasis in breast cancer (14). TNF-α is secreted by various cell types, including fibroblasts, macrophages, lymphocytes, and tumor cells, and influences primary tumor progression through its action on both tumor and stromal cells(3). The overexpression of TNF-α in breast tumor tissues and systemic circulation is linked to all stages of breast cancer progression, including cell proliferation, survival, motility, maintenance of the inflammatory state, acquisition of stemness, and resistance of cancer cells to chemotherapy(9). Additionally, obesity often leads to macrophage activation and the release of free fatty acids, hormones and cytokines (including TNF-α), which cause local and systemic chronic inflammation(15, 16). Chronic inflammation induced by obesity is significantly involved in cancer development, increasing cancer risk by interacting with oxidative DNA damage

pathways or altering the methylation status of oncogenes, creating a microenvironment conducive to cancer development(17). High levels of TNF-α in obese individuals increase the risk of breast cancer by inducing insulin resistance through the inhibition of insulin receptor tyrosine kinase activity, the downregulation of IGF-1, and the impairment of the GH/IGF-1 axis, leading to reduced GLUT4 translocation and impaired glucose uptake(18-20).

Despite the strong theoretical links, the current study provides inconclusive evidence regarding the association between BMI and the development of breast cancer. These inconsistencies may be attributed to differences in the study populations, which are influenced by factors such as menopausal status, age, and population differences(21-23). While numerous studies have investigated TNF-α in breast cancer, they have focused on progression and prognosis rather than demographic and clinicopathological characteristics and the impact of TNF-α on the onset of breast cancer(24-26). Moreover, few studies have explored the combined role of BMI and TNF-α in breast cancer development. This study aimed to document clinical data from breast cancer patients to identify associations between relevant risk factors and breast cancer prognosis, investigate the relationships among breast cancer risk factors, different cancer types and stages, and assess the simultaneous effects of TNF-α levels, BMI, and other risk factors on breast cancer.

2 Materials and methods

2.1 Research design

A case–control study was conducted on patients with BC referred to First and Second Hospitals and Cancer Hospital affiliated with Sun Yat-sen University from October 2008 to March 2018 versus healthy individuals. The study was approved by the Ethics Committee of the School of Public Health, Sun Yat-sen University.

Female patients with newly diagnosed breast cancer (BC), confirmed by an expert physician based on histopathological assessment, were eligible for inclusion in the study. Exclusion criteria comprised a prior history of malignant neoplasms or psychiatric disorders, severe physical conditions such as shock or coma, as well as cognitive or behavioral impairments, including dementia or communication difficulties. Ultimately, 794 patients who met all inclusion and exclusion criteria were enrolled, and written informed consent was obtained from all participants.

The control group consisted of age-matched, healthy female individuals without evidence of breast cancer, as verified by imaging performed during routine medical examinations. Individuals with a previous diagnosis of breast cancer or any other malignancy were excluded. A total of 268 healthy controls were recruited, and all provided written informed consent prior to participation. Both the case and control groups were selected according to matching methods according to age group (5 years), regional origin in administrative districts of residence, and time to pathological diagnosis and physical examination (3 months).

2.2 Data collection

Baseline information from participants was collected via a structured questionnaire designed to capture demographic, clinical, and lifestyle factors relevant to breast cancer risk. The questionnaire was developed by Vandenberg University in the United States on the basis of the current epidemiological research hotspots of breast cancer, supplemented with the characteristics of the Guangdong region, and revised after the presurvey. A professionally trained and qualified investigator visited the wards of the above hospitals to conduct one-by-one interviews with the study subjects according to the questionnaire, and the survey time for each patient was 30–60 minutes. The survey included general demographic information (age at diagnosis, education level, marital status, height, weight, etc.), history of previous diseases, and menstrual and reproductive history (age at menarche, menstrual cycle, menopausal status, age at menopause, history of pregnancy, number of live births, history of breastfeeding, contraceptive methods, hormone use, etc.), and family history of malignant tumors and lifestyle habits (smoking, drinking tea, and sleeping).

Simultaneously, clinical test information, such as pathological information (ER, PR, HER2, P53, and clinical stage), was obtained from the electronic medical record systems of the three hospitals. The status of ER, PR, and HER2 was determined on the basis of the results of the immunohistochemical test, with the following criteria: ER/PR positive (≥1%), ER/PR negative (<1%), HER2 negative (HER2- or +), HER2 positive (++), and HER2 positive (++++). Clinical staging was performed according to the breast TNM staging guidelines (8th edition) (27).

2.3 Blood sample collection and TNF-alpha testing

Blood samples were collected from patients undergoing venipuncture for other tests to determine TNF-α levels. See Annex 1.

2.4 Data processing and statistical analysis

Statistical analyses were performed via the Statistical Package for the Social Sciences version 26 (IBM, SPSS, Inc.). The normality of continuous variables was assessed via the Kolmogorov–Smirnov test. Descriptive statistics for continuous variables, including age, TNF-α level, and BMI, are presented as the means± SDs and medians (interquartile ranges, IQRs). The differences in BMI and TNF-α levels between the study groups were analyzed via the Mann–Whitney test, and age was calculated via Student's t test. The study groups were categorized on the basis of a structural survey. Categorical variables are expressed as frequencies and component ratios. The associations between demographic and categorical variables and BC incidence were evaluated via the chi-square test (χ^2) followed by Fisher's exact test. To identify whether serum levels of TNF-α are associated with the development of BC, a multinomial logistic regression analysis was performed, and related parameters, such as the odds ratio (OR) and 95% confidence interval (CI), were calculated and adjusted for age, education, BMI, age at menarche, menopausal status, breastfeeding status, family history of BC, and marital status. Further analysis was performed considering the cutoff values for continuous variables, including age, BMI, and serum levels of TNF-α. In these fields, three distinct age groups (mean age ≤40, 41–60, ≥60 years), two distinct BMI groups (median BMI <24, ≥24 kg/m^2), and two categorized TNF-α groups (median TNF-α <58.45, ≥58.45 μg/ml) were considered, and the associations between BC and the abovementioned groups were investigated via chi-square tests (χ^2) followed by Fisher's exact test. For all the statistical tests, P values less than 0.05 were considered significant.

2.5 Quality control

The survey protocol and questionnaire used in this study were evaluated for validity and reliability according to standard guidelines (28) and were provided by well-trained investigators who had a medical education background. Blood sampling and storage were performed with

strict criteria. All the information was double-checked by two independent investigators, who were qualified and subjected to EpiData software (version 3.0; EpiData Association), a suitable software for real-time logic checking and consistency testing. The negative control was used to ensure that the control comparison was comparable to that of the case group.

3 Results

3.1 Basic characteristics of the study subjects

A total of 1062 study participants were included in this study between October 2008 and March 2018. The control group included 268 healthy controls, and the case group included 794 female patients with BC. Table 1 presents the distributions of participants based on their general demographic characteristics, categorized into healthy and breast cancer groups. Most of the participants were premenopausal females aged 41--60 years. **No significant difference in age was observed between the case and control groups (P value = 0.886).** More than half of the control and case group patients had a BMI ranging from 18.5--23.9. The median BMI in the control and case groups (22.6 and 22.66, respectively) did not differ significantly. **The mean ± SD age at menarche was earlier in the control group (15.0 ± 9.10) than in the case group (16.9 ± 14.6, P = 0.009).** Additionally, **the proportion of participants who breastfed was greater in the control group than in the case group (P = 0.026).**

3.2 Associations of BMI and TNF-α with breast cancer risk

Logistic regression analysis was performed with the occurrence of breast cancer as the dependent variable and BMI and the TNF-alpha level as factors. The relationships between BMI and TNF-α levels and the risk of breast cancer are shown in Table 2. The results of one-way logistic regression analysis suggested that high levels of TNF-alpha were associated with an increased risk of BC (P value <0.05; OR 1.006, 95% CI 1.002–1.010). When participants were categorized on the basis of a TNF-α level≥58.45, the frequency of BC patients with elevated TNF-α was greater in the case group than in the control group (one-way logistic regression: OR 1.442, 95% CI 1.091–1.906; logistic regression model: OR 1.500, 95% CI 1.112–2.022). These findings suggest a significant role for elevated TNF-α levels in the development of BC. There was no statistically significant difference in BMI between the case and control groups (P

value=0.68). Moreover, logistic regression analysis for estimation of the relative risk of BC according to BMI did not reveal any discrepancies (one-way logistic regression: OR 1.000, 95% CI 0.991–1.010; logistic regression model: OR 0.988, 95% CI 0.976–1.000).

3.3 Associations between stratified levels of TNF-α and clinicopathological characteristics of patients with breast cancer

As shown in Table 2, patients with elevated levels of TNF-α were more susceptible to the development of BC (OR 1.500, 95% CI 1.112–2.022). The TNF-α-based stratified frequencies of BC patients were subsequently assessed according to their clinicopathological characteristics, including clinical stage, tumor diameter, ER status, PR status, HER2 status, Ki67 status, lymph node metastasis status, and distant metastasis status. In addition, after adjustment for demographic features (age, menopausal status, family history, breastfeeding, age at menarche, and number of live births), a comparison of the risk of BC was performed, the results of which are shown in Table 3. TNF-α levels were statistically significant in the stratification of clinical stages I/II (one-way logistic regression: OR 1.433, 95% CI 1.074--1.911), stage III/IV (one-way logistic regression: OR 1.476, 95% CI 1.002--2.175), ER-positive (one-way logistic regression: OR 1.508, 95% CI 1.128--2.015), PR-positive (one-way logistic regression: OR 1.475, 95% CI 1.097--1.983), HER2-negative (one-way logistic regression: OR 1.386, 95% CI 1.024--1.877).

3.4 Association between BMI and the risk of breast cancer stratified by clinicopathological characteristics

In this study, the risk of breast cancer incidence by BMI subgroup was estimated by logistic regression modeling, and a comparison of the risk of incidence between control study subjects and case group patients was performed by stratifying by clinicopathological characteristics and adjusting for other demographic factors. As shown in Table 4, there was a significant association between BMI and any clinicopathological characteristic and the risk of developing breast cancer (P value <0.05).

3.5 Associations of TNF-α with basic and clinicopathological features of patients with breast cancer among nonoverweight participants

To further analyze the associations between BMI and TNF-α and the risk of breast cancer development and possible interactions in each stratum, age, menopausal status, clinical stage, ER status, PR status, and HER2 status were used as secondary strata to stratify the risk of breast cancer development after adjusting for possible confounders. As shown in Tables 5 and 6, the associations between different levels of TNF-α and the risk of breast cancer incidence were stratified by basic and clinicopathological features and evaluated in nonoverweight (BMI<24) and overweight and obese (BMI>24) participants. High TNF-α levels (≥58.45) were able to increase the risk of breast cancer incidence in people with lower BMIs (OR 1.538, 95% CI 1.059–2.233) after adjusting for possible confounders. In addition, individuals with high TNF-α levels (≥58.45) may also have an increased risk of breast cancer in the higher BMI population compared with those with low TNF-α levels (<58.45), with an adjusted OR and 95% CI of 1.540 (0.909–2.607).

Patients aged ⩾50 years, having a higher BMI and having high levels of TNF-α had an increased risk of developing breast cancer, with an OR and 95% CI of 2.009 (1.039--3.884, P value = 0.042). However, in other age groups, BMI and TNF-α levels were not significantly associated with breast cancer development. An evaluation of the relationship between BMI and the risk of TNF-α in breast cancer development according to postmenopausal status revealed that a low BMI and high TNF-α are associated with an increased risk of breast cancer occurrence (OR 1.641, 95% CI 1.029–2.617, P value = 0.029), whereas a high BMI and high TNF-α are able to increase the risk of breast cancer occurrence in the postmenopausal population (OR 2.045, 95% CI 1.009–4.144, P value = 0.045). Other BMI and TNF-α levels were not significantly associated with breast cancer occurrence in the menopausal status stratum. The relationship between BMI and the risk of TNF-α-related breast cancer incidence after stratification by clinical stage indicated that in those with an early clinical stage, a low BMI with high TNF-α increased the risk of breast cancer (OR 1.535, 95% CI 1.046–2.253, P value = 0.036); similarly, in those with an early stage, a high BMI with high TNF-α increased the risk of breast cancer, which approached statistical significance (OR 1.619, 95% CI 0.935–2.803, P value = 0.067). A low BMI with high TNF-α increased the risk of breast cancer in the ER-positive population (OR 1.589, 95% CI 1.079–2.341, P value = 0.027); similarly, in the positive population, a high BMI

with high TNF-α increased the risk of breast cancer (OR 1.726, 95% CI 0.999–2.982, P value = 0.055). In the PR-positive population, a low BMI with high TNF-α increased the risk of breast cancer (OR 1.508, 95% CI 1.014-2.242, P value = 0.034). Similarly, in the early-stage population, a high BMI with high TNF-α levels significantly increased the risk of breast cancer (OR 1.619 and 95% CI 0.935–2.803, P value = 0.067). In the PR-negative population, low BMI and high TNF-α levels also increased the risk of breast cancer (OR 1.618, 95% CI 1.017–2.574, P value = 0.038). Patients who were negative for HER2, had a low BMI and had high TNF-α levels had an increased risk of developing breast cancer (OR 1.561 and 95% CI 1.035–2.354, P value = 0.045). Although low BMI and high TNF-α may increase the risk of breast cancer in the HER2-positive population, the difference was not statistically significant (OR 1.496, 95% CI 0.972–2.301, P value = 0.067).

4 Discussion

This study investigated the associations between breast cancer (BC) and various demographic and clinical factors, focusing on BMI and TNF-α levels. The results indicated that high TNF-α levels were associated with an increased risk of breast cancer, whereas BMI was not significantly related to breast cancer risk. The results indicate that elevated TNF-α levels correlate with early clinical stages; ER-positive, PR-positive, HER2-negative, and HER2-positive status; high Ki67 expression; and the absence of lymphatic and distant metastases. However, the analysis revealed no significant association between BMI and the risk of developing breast cancer, as indicated by the confidence intervals crossing 1. The interaction between BMI and TNF-α was further analyzed by stratifying confounders that might affect the relationships among BMI, TNF-α, and breast cancer. No significant interaction was observed between BMI and TNF-α when the data were stratified by age, menopausal status, clinical stage, ER status, PR status, or HER2 status.

There is strong evidence of a mutual link between obesity, TNF-α, and inflammation, particularly concerning breast cancer risk. The interplay among obesity, inflammation, and hormonal factors forms a microenvironment that promotes breast cancer development and progression (29). These factors seem to significantly contribute to increased breast cancer risk and poor prognosis in obese women. In healthy breast tissue, TNF-α contributes to cell proliferation and morphogenic branching (30). However, in the context of obesity-associated inflammation, TNF-α promotes tumor growth and progression. TNF-α, a crucial

proinflammatory cytokine, is expressed in subcutaneous and visceral adipose tissues, particularly in monocytes and macrophages(31). Obesity results in a 2.5-fold increase in TNF-α levels in adipose tissue, strongly correlating with hyperinsulinemia (29-31).

TNF-α has become an important cellular link between inflammation and various cancers, including prostate cancer (32) and colorectal cancer (33). However, in endometrial (34) and ovarian cancer (35), TNF-α contributes to apoptosis and cell-mediated immune responses. A meta-analysis revealed that while increased TNF-α levels are associated with the risk of colorectal, pancreatic, and prostate cancers, there is no significant association with breast cancer risk (36). In contrast, another meta-analysis indicated that elevated TNF-α levels might be related to the clinicopathological features of tumorigenesis and the risk of cancer development in specific situations (37). Although it is unclear whether the breast is particularly susceptible to high TNF-α levels, some findings indicate that high TNF-α levels are associated with an increased risk of premenopausal breast cancer (38) and can interact with enzymes that synthesize estrogens(39). Cohort studies have also indicated an association between high TNF-α levels and a reduced risk of breast cancer (39). Recent studies have shown that chronic inflammation and endogenous TNF-α produced by tumor tissue are more likely to promote tumor action, providing the interstitial tissue and diffusion conditions required for tumor growth(39). Endogenous TNF-α can stimulate nuclear factor κ in activated B cells (NF-κB), which is involved in tumor cell proliferation, apoptosis, metastasis, and angiogenesis-related gene expression (40). The present study revealed that high TNF-α levels were associated with breast cancer development, which is consistent with the findings of several previous studies.

Obesity is a hallmark risk factor for many diseases, and a higher BMI is strongly associated with an increased risk of various cancers. A meta-analysis of 13 studies revealed that a high BMI was associated with an increased risk of contralateral breast cancer (OR=1.37; 95% CI: 1.20–1.57) in women previously diagnosed with breast cancer (41). Li et al. reported in a case–control study of East Asian women that overweight individuals had an increased risk of malignant tumors; overweight women were 2.96 times more likely to develop triple-negative breast cancer than were those with a normal weight (42). Compared with nonobese patients, obese patients with breast cancer have a greater risk of mortality and distant metastasis, with a 10% increase in cancer mortality per 5 kg/m2 increase in BMI (43). However, unlike some

current studies, the present study's logistic regression analysis of BMI indicated no statistically significant relationship between obesity and the development of BC. In postmenopausal women, adipose tissue becomes the primary source of estrogen due to the expression of aromatase, an enzyme that converts androgens to estrogens. Higher estrogen levels resulting from increased body weight can promote hormone receptor-positive breast cancers(44). Additionally, obesity induces a chronic inflammatory state, leading to the release of reactive oxygen species (ROS) and resulting in DNA damage within breast epithelial cells. This inflammation also increases aromatase expression, further increasing estrogen production and reinforcing the carcinogenesis cycle(45). Together, these hormonal and inflammatory mechanisms significantly increase breast cancer risk in obese postmenopausal women. A meta-analysis involving 3,318,796 subjects revealed no significant association between BMI and breast cancer risk in premenopausal women (summary RR 0.94, 95% CI 0.81–1.11)(46). Various factors, such as hormone replacement therapy (HRT), physical activity, and dietary habits, can also influence the relationship between BMI and breast cancer risk. Studies often adjust for these confounders to better understand the direct effects of BMI(47).

Previous studies have reported few conclusions on the interaction between BMI and TNF-α in BC. However, many studies indicate that adipokines produced by adipose tissue likely play a role in the association between obesity and cancer development, although most studies have not focused on TNF-α as a cytokine (48). Current studies on BMI and TNF-α mechanisms indicate that TNF-α can self-regulate transcriptional processes and secretion levels in adipose tissue, leading to increased TNF-α levels in obese individuals(49). Adipose tissue is an important site for estrogen conversion in women (50), and increased TNF-α expression and aromatase activity in the mammary gland were found to be associated with obesity in an animal study (51), where increased TNF-α expression led to elevated levels of estrogen, increasing the volume of adipocytes (52), which ultimately led to an increased risk of breast cancer in obese women (53). This study revealed no interaction effect between BMI and TNF-α and revealed that high TNF-α levels play a greater role in increasing the risk of BC. Other studies indicate that obesity, particularly increased central obesity, is strongly associated with increased inflammatory cytokine production in adipose and breast tissues, which may contribute to an elevated risk of BC (54-56). The study results revealed that high TNF-α levels significantly increased the risk of breast cancer in individuals with a low BMI. However, while the risk of breast cancer in

subjects with higher BMIs and high TNF-α did not significantly increase, this finding still suggests that the risk is greater in the higher BMI group than in the lower BMI group.

The clinicopathological characteristics of the study population were subsequently stratified. High levels of TNF-α at different clinical stages increase the risk of breast cancer development, which is consistent with the results of other studies (57). Studies of the associations between ER-positive and PR-positive women and breast carcinogenesis have indicated that high levels of TNF-α in ER-positive and PR-positive women are more likely to lead to the development of uterine fibroids than those in ER-negative and PR-negative women are (58), which may be due to the increased effects of estrogen and progesterone resulting from the release of inflammatory factors such as TNF-α (59). However, more studies are needed on high levels of TNF-α in ER-positive and PR-positive subjects.

HER2 is overexpressed in 15–20% of breast cancers (60) and is not only an important gene expression receptor associated with the prognosis of breast cancer but also highly specific for the diagnosis of breast cancer (61). However, previous studies of HER2 and TNF-α in breast carcinogenesis have reported less on their role in the risk of occurrence, and more research has focused on their effects on survival and cancer progression (62). After stratification by pathological characteristics, the results of the present study revealed that HER2 status plays a role in increasing the risk of breast cancer development in both populations with higher levels of TNF-α. In our study, high levels of TNF-α were associated with a greater OR for developing breast cancer in the earlier HER2-positive study subjects (OR=1.507) than in the HER2-negative population (OR=1.457), whereas high levels of TNF-α were associated with a greater OR for developing breast cancer in the high-Ki67-status study subjects (OR=1.683) than in the low-Ki67-status population (OR=1.572). These two aspects have the same conclusions as those in the present study on the basis of the results of other studies (63).

Earlier age at menarche is also associated with various physiological changes, such as higher BMI, which has been documented and noted as a potential risk factor for breast cancer development (64). The onset age at menarche is less than 13 years and has been reported as a strong risk factor for BC (60% greater than) (65). A possible biological justification is that women with an earlier age at menarche have a longer reproductive duration, increasing the exposure of breast tissue to steroid hormones such as estrogen, which is secreted by the ovaries, increasing the risk of BC development (65). Chakor et al. provided evidence on the effect of

menarche in adolescents, supporting the importance of good hygiene on overall well-being (66). In a cohort study of women with BC, the effect of endogenous ovarian hormones was shown to be a more important risk factor than the age at menarche (67). The results of this study revealed a significant difference in the distribution of age at menarche between the control and case groups, with the control group being at menarche earlier than the case group was. One possible reason for these different results may be recall bias in the reporting of the age of menarche by the female subjects, including inaccurate memory at the time of menarche and the relatively small number of subjects in the control group, which is less representative than the 794 in the case group. However, evaluating the associations between sex hormones and BC may be useful for risk prediction.

Many previous studies have shown a highly significant relationship between TNF-α and cancer metastasis and invasion (72), which is consistent with the results of the present study. The presence of TNF-α and the risk of distal metastasis may be due to its induction of signaling pathways that can alter the lipid raft composition of cell membranes by upregulating the expression of proteases, thereby increasing the risk of metastasis in breast cancer cells (73). Evidence suggests that obesity is an inflammatory disease and that increased circulating inflammatory cytokines such as lipocalin and TNF-α increase the expression of aromatase, which leads to increased levels of estrogen in the breast and, in turn, increases the risk of breast tumorigenesis, insulin resistance and interleukin synthesis (54, 74). In our study, higher TNF-α levels were observed to be a significant risk factor for breast cancer development in the early stages but not in cases of lymph node and distant metastases.

Despite yielding significant findings, this study has several limitations that warrant consideration. First, the control group sample size (268 participants) was relatively small compared with the case group (794 participants), which may affect the reliability of the statistical analyses and the representativeness of the results. Additionally, discrepancies in demographic characteristics between the control and case groups, such as recall bias in reporting age at menarche, may introduce bias into the findings. Second, the study employed a case–control design, which inherently has limitations such as selection bias and recall bias. Specifically, the reliance on participants' memories for reporting critical events such as age at menarche may lead to inaccuracies, compromising the reliability of the results. Furthermore, case–control studies cannot establish causality, only correlations. Third, although the study

accounted for several confounding factors (e.g., age, menopausal status, family history, breastfeeding, age at menarche, and number of live births), other potential confounders that could significantly impact breast cancer risk were not adequately controlled. These include dietary habits, physical activity levels, and environmental exposures, which may have a substantial influence on outcomes. One possible explanation for the lack of statistical association between BMI and clinicopathological characteristics in our study may be the influence of underlying biological heterogeneity among patients, such as differences in fat distribution, metabolic health, and hormonal milieu, which BMI alone does not adequately capture. Additionally, BMI does not distinguish between adipose and lean mass, and may thus obscure obesity-related inflammatory mechanisms that are more directly relevant to tumor characteristics. Finally, the study did not find a significant interaction effect between BMI and TNF-α levels on breast cancer risk. However, these results might be limited by the sample size and study design. Future studies should explore longitudinal data to establish causal relationships between TNF-α levels, BMI, and breast cancer development, as well as investigate the role of other inflammatory mediators in breast carcinogenesis

.5. Conclusion

High levels of TNF-α (≥58.45) are strongly associated with the development of breast cancer, and its role is especially distinct in clinically advanced cases. In addition, ER positivity, PR positivity, or high Ki67 levels suggest a greater risk for breast cancer, especially in patients without lymph node metastasis or distal metastasis. The statistical association between BMI and breast cancer development was not significant, both when stratified on the basis of clinicopathological characteristics and when stratified according to factors such as age, menopausal status, clinical stage, and ER, PR, and HER2 status. In addition, no interaction between BMI and TNF-α has been observed in breast cancer patients. These results highlight the importance of TNF-α levels and provide new clues for further studies on the pathogenesis of breast cancer.

Declaration

Ethics approval and consent to participate

All the patients were informed about the purposes of the study. All investigations conformed to the principles outlined in the Declaration of Helsinki and were approved by the Ethics

Committee of Sun Yat-sen University (No: 2019B030316002).

Consent for publication

Written informed consent was obtained from the patient for the publication of this report.

Data availability

The data that support the findings of this study are available upon request from the corresponding author.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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None.

Authors' contributions

Shen Wang, Yunqian Li, Hengming Ye, and Zefang Ren participated in the design of this study, and Shen Wang, Yunqian Li and Zefang Ren performed the statistical analysis. Shen Wang, Hengming Ye, and Zefang Ren carried out the study and collected background information. Shen Wang drafted the manuscript. All the authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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640 **Tables**

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Table 1 Distribution of demographic characteristics of the control and case groups

Demographic characteristics		Control N=268(%)	Case N=794(%)	P value*
Age				0.899
≤40		63 (23.5)	185 (23.3)	
41-60		164 (61.2)	496 (62.5)	
≥60		41 (15.3)	133 (14.2)	
Mean ± SD		49.1±10.96	49.2±11.04	0.886
Educational level				0.145
Junior high school and below		99 (38.1)	310 (42.5)	
Senior high school		96 (36.9)	221 (30.3)	
College and above		65 (25.0)	198 (27.2)	
Age at menarche (years)				0.387
≤12		40 (15.1)	225 (13.0)	
>12		225 (84.9)	670 (87.0)	
Mean ± SD		15.0±9.10	16.9±14.6	**0.009****
Menopausal state				0.708
Pre-menopausal		150 (57.3)	451 (58.6)	
Post-menopausal		112 (42.7)	319 (41.4)	
Number of live births				0.735
Nulliparous		13 (4.9)	42 (5.4)	
1 or more		235 (95.1)	732 (94.6)	
Breast feeding				**0.026***
Yes		33 (13.1)	58 (8.3)	
No		219 (86.9)	642 (91.7)	
Family history of breast cancer				0.890

Yes	14 (5.3)	39 (5.1)	
No	250 (94.7)	728(94.9)	
Marital status			0.799
Unmarried	8 (3.0)	20 (2.5)	
Married or cohabiting	239 (89.2)	715 (90.1)	
Widowed, divorced	17 (6.3)	42 (5.3)	
Unaccounted	4 (1.5)	17 (2.1)	

Control: healthy participants without breast cancer; Case: Patients with Breast cancer; *: P-value < 0.05; **: P-value <0.01

664 **Table 2 Association of BMI and TNF-α with breast cancer development**

Variant	Control group N=268 (%)	Case group N=794 (%)	OR value (95%CI)[a]	OR value (95%CI)[b]
TNF-alpha(μg/ml)				
＜58.45	152 (56.7)	378 (47.6)	1.00 (reference)	1.00 (reference)
≥58.45	116 (43.3)	416 (52.4)	**1.442 (1.091-1.906)**	**1.500 (1.112-2.022)**
Median (IQR)	52.03 (33.86-72.72)	59.32 (39.02-85.31)	**1.006 (1.002-1.010)**	**1.006 (1.002-1.011)**
BMI（kg/m^2）				
<24	174 (67.4)	519 (68.0)	1.00 (reference)	1.00 (reference)
≥24	84 (32.6)	244 (32.0)	0.974 (0.720-1.317)	0.947 (0.685-1.310)
Median (IQR)	22.60 (20.94-25.25)	22.66 (20.70-25.00)	1.000 (0.991-1.010)	0.988 (0.976-1.000)

Control: Healthy participants without breast cancer; Case: Patients with breast cancer; a One-way logistic regression analysis; b Logistic regression model: adjusted for age, menopausal status, family history, breastfeeding, age at menarche, and number of live births.

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677 **Table 3 Association between TNF-α and risk of breast cancer, stratified by**
678 **clinicopathological characteristics**

Clinicopathological features	TNF-α (μg/ml)		OR value（95%CI）[a]	OR value（95%CI）[b]
	<58.45 N (%)	≥58.45 N (%)		
Clinical Stages				
I/II Stages	304(48.6)	322(51.4)	**1.433(1.074-1.911)**	**1.481(1.090-2.014)**
III/IV Stages	85(50.6)	83(49.4)	**1.476(1.002-2.175)**	1.486(0.964 - 2.290)
Tumor diameter(cm)				
≤2.0	162(46.7)	185(53.3)	1.162(0.844-1.601)	1.239(0.879 - 1.746)
>2.0	225(50.8)	218(49.2)	1.310(0.182-9.441)	1.294(0.170 - 9.837)
ER				
Negative	92(47.4)	102(52.6)	1.257(0.868-1.822)	1.212(0.800-1.834)
Positive	297(49.5)	303(50.5)	**1.508(1.128-2.015)**	**1.567(1.151-2.135)**
PR				
Negative	139(51.7)	130(48.3)	1.380(0.983-1.939）	1.405(0.970-2.037)
Positive	250(47.6)	275(52.4)	**1475(1.097-1.983）**	**1.525(1.111-2.094)**
HER2				
Negative	241(52.3)	220(47.7)	**1.386(1.024-1.877)**	**1.457(1.050-2.021)**
Positive	148(44.4)	185(56.6)	**1.523(1.102-2.105)**	**1.507(1.065-2.131)**
Ki67 status				
≤14.0%	84(48.3)	90(51.7)	1.206(0.849-1.711)	1.310(0.895-1.918)
＞14.0%	277(50.0)	277(50.0)	**1.416(1.061-1.889)**	**1.435(1.054-1.954)**
Lymph node metastasis				

No	204(46.2)	238(53.8)	1.334(0.984-1.809)	**1.415(1.019-1.963)**
Yes	184(52.9)	164(47.1)	0.437(0.045-4.253)	0.685(0.057-8.283)
Distant metastases				
No	375(49.5)	383(50.5)	**1.472(1.112-1.949)**	**1.512(1.121-2.040)**
Yes	14(38.9)	22(61.1)	0.936(0.462-1.895)	0.926(0.420-2.044)

The TNF-α<58.45 was considered as reference (OR value= 1.000); a: One-way logistic regression analysis; b: Logistic regression model: adjusted for age, menopausal status, family history, breastfeeding, age at menarche, and number of live births.

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Table 4 Association between BMI and risk of breast cancer, stratified by clinicopathological characteristics

Clinicopathological features	BMI (kg/m^2)		OR value（95%CI）[a]	OR value（95%CI）[b]
	< 24 N=268（%）	≥ 24 N=794（%）		
Clinical Stages				
I/II Stages	418(69.3)	185(30.7)	0.917(0.671-1.253)	0.920(0.659 - 1.284)
III/IV Stages	101(63.1)	59(36.9)	1.210(0.800-1.830)	1.296(0.817 - 2.057)
Tumor diameter(cm)				
≤2.0	239(72.6)	90(27.4)	0.779(0.546-1.111)	0.814(0.556 - 1.189)
>2.0	277(64.4)	153(35.6)	1.139(0.822-1.579)	1.134(0.797 - 1.613)
ER				
Negative	131(71.2)	53 (28.8)	0.838(0.555-1.265)	0.876(0.554 - 1.384)
Positive	388(67.0)	191(33.0)	1.020(0.746-1.394)	1.008(0.722 - 1.407)
PR				
Negative	177(68.9)	80(31.1)	0.936(0.646-1.357)	0.850(0.567-1.275)
Positive	342(67.6)	164(32.4)	0.993(0.721-	1.054(0.747 -

			1.368)	1.487)
HER2				
Negative	295(66.9)	146(33.1)	1.025(0.739-1.422)	1.007(0.705-1.439)
Positive	224(69.6)	98(30.4)	0.906(0.637-1.289)	0.936(0.644 - 1.362)
Ki67 status				
≤14.0%	119(71.3)	48(28.7)	0.857(0.582-1.260)	0.821(0.539-1.249)
＞14.0%	357(66.9)	177(33.1)	1.015(0.743-1.386)	1.036(0.743-1.445)
Lymph node metastasis				
No	300(70.4)	126(29.6)	0.871(0.624-1.215)	0.865(0.606-1.236)
Yes	217(65.0)	117(35.0)	1.116(0.792-1.573)	1.160(0.799 - 1.683)
Distant metastases				
No	493(67.7)	235(32.3)	0.987(0.729-1.337)	1.000(0.724-1.381)
Yes	26(74.3)	9(25.7)	0.717(0.322-1.598)	0.760(0.309-1.871)

The BMI<24 was considered as reference (OR value= 1.000); a One-way logistic regression analysis; b Logistic regression model: adjusted for age, menopausal status, family history, breastfeeding, age at menarche, and number of live births.

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716 **Table 5 Association between TNF-alpha and risk of breast cancer in non-overweight**
717 **participants**

	TNF-α (μg/ml) <58.45			TNF-α (μg/ml) ≥ 58.45		
	Control N (%)	**Case N (%)**	**OR （95%CI）**	**Control N (%)**	**Case N (%)**	**OR （95%CI）**
BMI	100(57.5)	250(48.2)	1.00(reference)	74(42.5)	269(51.8)	**1.538(1.059-2.233)**
Age						
<50 years	58(54.7)	142(46.3)	1.00(reference)	48(45.3)	147(53.7)	1.430(0.882-2.319)
≥50 years	42(61.8)	108(50.9)	1.00(reference)	26(38.2)	104(49.1)	1.802(0.976-3.328)
menopausal status						
pre-menopausal	63(56.3)	146(46.2)	1.00(reference)	49(43.8)	172(53.8)	**1.641(1.029-2.617)**
post-menopausal	35(61.4)	98(52.7)	1.00(reference)	22(38.6)	88(47.3)	1.125(0.484-2.613)
Clinical stage						
Early stage	100(57.5)	203(48.6)	1.00(reference)	74(42.5)	215(51.4)	**1.533(1.046-2.253)**
End stage	100(57.5)	47(46.5)	1.00(reference)	74(42.5)	54(53.5)	1.509(0.862-2.642)
ER						
Negative	100(57.5)	66(50.4)	1.00(reference)	74(42.5)	65(49.6)	1.317(0.785-2.209)

Positive	100(57.5)	184(47.4)	1.00(reference)	74(42.5)	204(52.6)	**1.589(1.079-2.341)**
PR						
Negative	100(57.5)	85(48.0)	1.00(reference)	74(42.5)	92(52.0)	**1.618(1.017-2.574)**
Positive	100(57.5)	165(48.2)	1.00(reference)	74(42.5)	177(51.8)	**1.508(1.014-2.242)**
HER2						
Negative	100(57.5)	142(48.1)	1.00(reference)	74(42.5)	153(51.9)	**1.561(1.035-2.354)**
Positive	100(57.5)	108(48.2)	1.00(reference)	74(42.5)	116(51.8)	1.496(0.972-2.301)

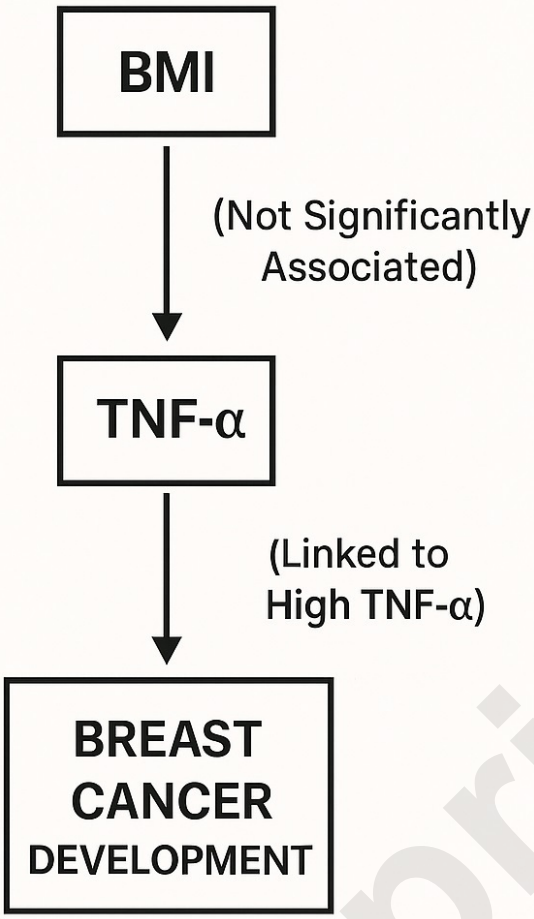
Control: Healthy participants without breast cancer; Case: Patients with breast cancer; Logistic regression model: adjusted for age, menopausal status, family history, breastfeeding, age at menarche, number of live births.

Table 6 Association between TNF-alpha and risk of breast cancer in overweight and obese participants

	TNF-α (μg/ml) <58.45			TNF-α (μg/ml) ≥58.45		
	Control N (%)	**Case N (%)**	**OR value （95%CI）**	**Control group N (%)**	**Case group N (%)**	**OR value （95%CI）**
BMI	49(58.3)	110(45.1)	1.00(reference)	35(41.7)	134(54.9)	1.540(0.909-2.607)
Age						
<50 years	10(40.0)	42(42.0)	1.00(reference)	15(60.0)	58(58.0)	0.969(0.376-3.771)
≥50 years	39(66.1)	68(47.2)	1.00(reference)	20(33.9)	76(52.8)	**2.009(1.039-3.884)**
menopausal status						
pre-menopausal	14(46.7)	49(41.5)	1.00(reference)	16(53.3)	69(58.5)	1.491(0.787-2.824)
post-menopausal	35(66.0)	59(49.2)	1.00(reference)	18(34.0)	61(50.8)	**2.045(1.009-4.144)**
Clinical stage						
Early stage	49(58.3)	82(44.3)	1.00(reference)	35(41.7)	103(55.7)	1.619(0.935-2.803)
End stage	49(58.3)	28(47.5)	1.00(reference)	35(41.7)	31(52.5)	1.363(0.654-2.837)
PR						
Negative	49(58.3)	39(48.8)	1.00(reference)	35(41.7)	41(51.2)	1.114(0.564-2.199)

Positive	49(58.3)	71(43.3)	1.00(reference)	35(41.7)	93(56.7)	**1.840(1.046-3.236)**
HER2						
Negative	49(58.3)	70(47.9)	1.00(reference)	35(41.7)	76(52.1)	1.544(0.864-2.758)
Positive	49(58.3)	40(40.8)	1.00(reference)	35(41.7)	58(59.2)	1.583(0.837-2.993)

Control: Healthy participants without breast cancer; Case: Patients with breast cancer; Logistic regression model: adjusted for age, menopausal status, family history, breastfeeding, age at menarche, number of live births.



Key Findings:

- High TNF-α (≥58.45 µg/ml) significantly associated with increased breast cancer risk.
- High TNF-α linked to early stage, ER+, PR+, HER2+, high Ki67, no metastases
- No significant association found between BMI and breast cancer risk.
- No significant interaction between BMI and TNF-α.