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Lower Gut Microbiome Diversity and Breast Cancer Risk: A Critical Literature Review

Yingwen Shi

URIT Medical Electronic Co., Ltd., Guilin, China
shi_yingwen_epi@163.com

Abstract. Globally, breast cancer is the second-most common cause of cancer-related deaths among women. Recent research suggests that the gut microbiome may influence the development of breast cancer via hormonal, immunological, and metabolically mediated pathways. This review systematically summarizes the literature on the differences in gut microbiome diversity between women with breast cancer and without breast cancer. Women with breast cancer show consistently lower alpha diversity than women without breast cancer, particularly in postmenopausal women and those with metabolic dysfunction. These patterns suggest that gut microbiome diversity serves as an ecosystem-level indicator that interacts with the host context rather than acting as a standalone biomarker risk. This review also identifies the limitations in the current literature and the limitations of this review. For example, the evidence presented here is based on cross-sectional observational studies, and the methods for analyzing gut microbiota are highly heterogeneous. This review is non-systematic, focusing solely on gut microbiota diversity rather than functional/multi-site datasets. The insights presented in this review underscore the need for large, longitudinal studies of ethnically diverse cohorts and standardized methods to assess gut microbiota diversity, with the goal of determining how modifying gut microbiota diversity could provide a basis for developing personalized prevention/management strategies for breast cancer.

Keywords: Breast cancer, Epidemiology, Gut microbiome, Metabolic syndrome, Microbial diversity

1. Introduction

Globally, breast cancer is the second most diagnosed cancer among women, and currently accounts for the highest rate of cancer-related deaths in females; during 2022, approximately 2.3 million new cases and an estimated 665,684 breast cancer deaths are expected to occur [1]. Despite continuous advancements in breast cancer detection, diagnosis, treatment and survivorship care, a number of different factors remain responsible for the differences among countries regarding breast cancer incidence and breast cancer mortality rates. The Global Breast Cancer Initiative (GBCI) identifies three areas that need to be addressed as part of a complete approach to combat breast cancer: Health Promotion and Early Detection, Timely Diagnosis, and Comprehensive Management of Breast Cancer [1]. A number of studies support the theory that genetics, environment, and lifestyle factors

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contribute to the incidence of breast cancer in humans [2]. Recent studies indicate that there is an association between the microbiome of the human gut and the risk of developing breast cancer, and that dietary modifications may result in changes to the composition of the gut microbiome.

The gut microbiota affects several metabolic / immune / endocrine pathways related to the development of cancer [1, 2]. For example, in relation to breast cancer, the gut microbiome can alter the metabolism of estrogens, influence the regulation of the immune system, and modulate the signaling of inflammation, which all play a role in the initiation of breast tumors, as well as their subsequent growth and response to treatment [2, 3]. Both recent clinical and epidemiological studies have specifically noted that breast cancer patients generally present with altered gut microbial communities, when comparing women with breast cancer to breast cancer-free women; examples include variations in overall diversity and in community composition across both study groups [3-6]. From this information, the hypothesis has emerged that decreased gut microbiome diversity may increase susceptibility to breast cancer [7]. The relationship between gut microbiome diversity and cancer is undeniably complex. While there are many challenges currently affecting how these findings will ultimately be translated into routine clinical care, there is still considerable opportunity for future development of both this and related areas of research [1, 7, 8].

The available evidence indicates that there is likely a biological connection between the gut microbiome and increased risk of breast cancer. There are, however, still many unanswered questions regarding this topic. Most studies examining relationships between gut microbiome diversity and breast cancer have focused on premenopausal or postmenopausal women, where researchers noted that both alpha diversity (the number of different species present) as well as beta diversity (the overall structure of the community) were not as high in breast cancer patients compared with their healthy counterparts. As such, it could be suggested that both loss of microbial diversity and changes to the structure of the microbiome could occur due to tumor development [4-7]. There may, however, be variation between different study populations. For example, there are some populations in which a significant association between gut microbiome diversity and breast cancer remains after controlling for metabolic and lifestyle factors, whereas other populations show this association to be weak or completely absent [5, 6, 9, 10]. It has also been noted that differences in ethnic background, geographic location, obesity and metabolic syndrome status, and type of diet consumed by participants may be contributing factors to variability between studies [5, 10-12]. Thus, while there is consensus within the literature that the gut microbiome is important to the biology of breast cancer, it remains unclear as to whether diminishing diversity in the gut microbiome represents a causal mechanism or simply a reflection of existing risk or an unintended consequence resulting from treatment for the breast cancer itself.

This review synthesizes the existing literature and outlines important findings about the relationship between lower levels of gut microbiome diversity and breast cancer risk. Various risk factors are examined in this review, including menopause, ethnicity, geographical location, obesity, and metabolic health, in their respective associations. Furthermore, this review provides a comprehensive overview of the methodologies utilized in this field, highlighting both commonalities and differences, and proposes potential directions for future research on this subject.

2. Risk factors in the association between gut microbiome diversity and breast cancer risk

2.1. Menopausal status and gut microbiome diversity

Gut microbiome diversity and breast cancer risk are shaped by a woman's menopausal status. For example, studies show that post-menopausal women who have breast cancer have lower gut

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microbiome diversity than healthy controls; this finding indicates that microbial changes may be associated with increased breast cancer risk, possibly due to altered estrogen metabolism [4]. Research also suggests that menopause alters the composition of the microbiome and that specific fungal families are associated with breast cancer risk [5]. Yet unknown is how changes in gut microbiota contribute to breast cancer. Most research has examined the effects of menopausal status on post-menopausal women; thus, there is little information on how hormonal changes during pre-menopause impact diversity and risk factors for breast cancer.

Although there has been considerable research conducted on the gut microbiome of post-menopausal women, there have been only a few studies conducted on the gut microbiome of pre-menopausal women. A study conducted by Hou et al. [6], found that pre-menopausal women with breast cancer had unique gut microbiome diversities when compared to healthy controls, even though no significant differences in terms of alpha-diversity were observed. The findings in this study support the idea that differences in the structure of the gut microbiome may be related to increased risk for breast cancer among these individuals. In addition, the transition to menopause has many hormonal changes, which, when interacted with ethnicity, diet, and lifestyle factors, have not been researched in detail. Future studies should include diverse cohorts of pre-menopausal and peri-menopausal women and investigate how changing levels of hormones affect gut microbiome diversity and ultimately breast cancer risk.

2.2. Ethnicity and population differences

The role of ethnicity in geographic locations was shown to be important for field research, as it affects the composition of the microbiome as well as the incidence of breast cancer. For instance, Mendelian randomization between European and East Asian populations indicates that the associations between some gut microbial taxa and breast cancer risk differ between these populations [11]. A second study of tumor microbiota has indicated that differences in breast cancer microbiome studies based on race continue to be under-researched [12]. Finally, in a case-control study with newly diagnosed patients diagnosed with breast cancer, it was found that both diet and lifestyle factors that differ greatly between regions and cultures have been found to associate with gut microbiome composition as well as breast cancer risk [9]. Overall, these studies demonstrate the need for more microbiome-breast cancer research that examines how regions, ethnicity, diet, and lifestyle affect the microbiome's ecosystem and influence breast cancer risk through greater inclusion of diverse groups.

There is a definite relationship between differences in the microbiome and breast cancer risk; however, different ethnic groups and regions exhibit significant variations. This leads us to believe that there is an interaction among the genetic make-up, environmental conditions, and lifestyle choices of individuals and the many ways in which these three influence the microorganisms of humans. Many cross-cultural studies have not yet been done, and this hampers our ability to understand how the various factors combine to determine breast cancer risk in a given population. In future research, researchers will need to focus on a wide variety of cohorts that are well-characterized and have diverse geographic locations, so that they can better understand how geographic and cultural variability affect the composition of the microbiome and how that affects the outcome of breast cancer.

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2.3. Obesity, metabolic syndrome, and gut microbiome diversity

Many studies have supported the notion that obesity is a significant risk factor for breast cancer. When looking specifically at the connection between postmenopausal women and their prevalence of breast cancer as compared to women who are not overweight, there appears to be a substantial correlation between the two. Recent research has further established that obesity can lead to alterations in gut microbiota composition and function [8, 13]. Furthermore, additional data indicated that excess adiposity increases chronic low-grade inflammation, insulin resistance, and peripheral (i.e., non-localized) production of estrogen, all of which have been implicated as risk factors for breast cancer development [13]. As reviewed by Gaber et al. [8], the biological pathways linking obesity, dysbiosis, and breast cancer include intestinal permeability, gastric metabolic activity (bile acid), SCFA production, and systemic inflammatory signaling pathways, which contribute to the creation of a pro-tumor environment. These pathways offer plausible explanations for how decreased gut microbial diversity or less favorable community composition can influence breast cancer risk through interactions with metabolic risk factors.

Research on the intersection between metabolic health, microbiome diversity, and breast cancer is backed by clinical studies. Abdelqader et al. [10] demonstrated that patients diagnosed with breast cancer who also have metabolic syndrome have signs of gut microbiota dysbiosis based on their diversity when compared to metabolically healthy patients. Their research indicates that metabolic syndrome, which is typically associated with central obesity and insulin resistance, may be a strong modifier of the association between microbiome diversity and the risk of developing breast cancer, rather than simply a confounding factor. Altinok Dindar et al. [9] conducted a complementary case-control study, in which they found that breast cancer patients had a reduced level of alpha diversity in their gut microbiota compared to controls. However, some of these diversity differences diminished after adjustments for dietary factors, body mass index, and other related lifestyle factors. Gamba et al. [7] further supported the findings of Altinok Dindar et al. [9] in their meta-analysis by noting that the most significant variability in reported diversity effects between studies occurred due to differences in the extent to which factors related to obesity were considered in making the results of each study valid. Overall, the results from these studies indicate that obesity and metabolic syndrome are both closely related to gut microbiome diversity and breast cancer risk, making it impossible to assign the observed association between microbiome diversity and breast cancer to the microbiome alone.

The research supports potential future studies to understand how obesity-related alterations in the gut microbiome contribute to the risk of developing breast cancer and possibly developing interventions to help prevent breast cancer by improving the gut microbiome through diet and/or weight management. Additionally, while the research supports future studies to delineate the role of microbiome diversity, metabolic function, and adiposity on breast cancer risk, a more comprehensive characterization of the health of the study cohort (metabolic function) through multiple measurements, including diet, exercise, and medication use, will be necessary to determine if improving dysbiosis related to obesity can reduce the risk of developing breast cancer or improve the outcome of a diagnosis.

3. Diversity metrics and their relevance

A key methodological consideration in how to quantify and assess the gut microbiome and breast cancer is the method used to identify the gut microbiome's diversity and diversity metrics. Many of the ways in which researchers have characterized the structure of the gut microbiome have focused

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on two indices: alpha and beta diversity. For example, in Gamba et al. [7] systematic review and meta-analysis of gut microbiota and breast cancer, the investigative group found that, when taking into account case–control studies on gut microbiota in women diagnosed with breast cancer, the breast cancer patients had significantly less alpha diversity than that of their respective control group, and that the beta diversity profiles of women with breast cancer were significantly different than controls. However, the directionality and the magnitude of the finding were not consistently produced across the many studies. In addition, the investigators also compared alpha and beta diversity and the composition of the gut microbiota in premenopausal women diagnosed with breast cancer to that of their respective age-matched controls, and found only slight or non-significant differences in alpha diversity; however, they did find significant differences in beta diversity and the composition of the gut microbiota relative to their control group. When taken together, these two studies indicate that both the richness and diversity structure of the gut microbiome may provide insight into breast cancer development and risk, rather than just a singular metric representing diversity.

In terms of Alpha diversity metrics and the use of the three alpha metrics (Gamma, Beta, and Omega), it is essential to note that these metrics are not directly comparable and must be interpreted accordingly. The use of an Alpha metric with different values will yield different results for each allocated Alpha metric and may therefore lead to misinterpretation or miscalculations. In fact, a comparison between cases vs controls is based only on the selection of the chosen diversity indices and on how deeply the sequencing is performed. The study of comparative abundance within a sample can be influenced by a number of factors, including rarefaction, proportional normalization, etc., and can result in artificial differences in the abundance of each taxon type that have been generated. Therefore, there is a need for additional caution when including alpha metric analysis in a case study or comparative analysis between cases and controls. With regard to the study of Beta Diversity, it is important to remember that beta diversity is influenced very much by the metric and statistical methodology used. In particular, Gamba et al. [7] have identified an additional level of complexity due to the differences between sequencing platforms, different variable regions, and the use of varying bioinformatics pipelines when synthesizing findings of diversity observed in studies of breast cancer.

Reduced alpha diversity in biological systems is commonly viewed as a sign of "dysbiosis" when examining breast cancer, however, this interpretation is an overly simplistic view of how dysbiosis affects breast cancer. As outlined in a study by Hou et al. [6] were able to identify large differences in beta diversity and the presence or absence of specific taxa within breast cancer patients, even when studying alpha diversity, thus demonstrating that the importance of the gut microflora is likely to be more about which taxa are present than the total number of taxa present. Furthermore, Altinok Dindar et al. [9] demonstrated that the association of microbial diversity and breast cancer started to diminish when dietary and lifestyle variables as well as BMI were factored into the analysis. This adds to the difficulty of determining what role, if any, microbial diversity has on breast cancer independent of other host and environmental influences. Additionally, because breast cancer/microbiome studies are generally of a cross-sectional nature, it is not possible to determine whether changes in microbial diversity occur before the onset of breast cancer, as a result of physiological changes associated with breast cancer, or whether they have resulted from treatment modalities such as chemotherapy, hormonal therapies, or antibiotics.

While alpha- and beta-diversity measures can describe the overall state of the ecosystem globally, they do not have enough meaning independently to explain the mechanisms involved. In order for breast cancer to be studied in the future, investigators will need to perform integration of diversity

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with taxonomic and metabolic characteristics. Researchers must publish standardized analysis protocols and how they were made, such as the statistical analyses used or how all analyses were made. In addition, breast cancer studies must have longitudinal designs that reflect the changes in microbial diversity that are associated with risk/progression.

4. Discussion

Epidemiological, clinical, and mechanistic research on the gut microbiome's impact on breast cancer is pooled through this review. The previous studies have highlighted some key findings. Women diagnosed with breast cancer exhibit altered gut microbiome diversity relative to control populations. Studies performed on both pre-menopausal and post-menopausal women have shown that breast cancer patients have either less alpha diversity or distinct beta-diversity profiles [4-7, 9]. These results corroborate findings from clinical reviews that demonstrate how the gut microbiome alters estrogen metabolism, regulates immune response, and activates inflammatory pathways; therefore, providing an understanding of how the gut microbiome may influence breast cancer development [2, 3, 8].

Analysis of the literature by menopausal status, ethnicity and geographical area, metabolic health, and diversity metrics shows where the field has advanced beyond comparing only case–control studies. In women after menopause, there is evidence of lower alpha diversity, as well as evidence of dysbiosis, which has been shown to be associated with estrogen depletion and obesity-related conditions [4, 8-10, 13]. It has also been shown that even with similar richness, community composition and beta diversity can shift in response to changes related to hormonal activity during women's pre-menopausal lives [6]. Collectively, these studies indicate that host hormonal status is not merely an influence on how we interpret patterns of diversity but rather plays an essential role in determining the same.

Research involving the inclusion of non-Western and multi-ethnic populations has helped to elucidate subset population-specific patterns of disease. For example, in addition to the Ghana Breast Health Study [5], other studies have shown that the fecal microbiome profile associated with breast cancer in African women does not completely mirror that of breast cancer patients from Europe or North America [5]. Research using Mendelian randomization has supported this finding by revealing that while there is a strong correlation between specific taxa and breast cancer risk in many European populations, this correlation does not always exist between European and East Asian populations [11]. Results from these studies illustrate how ethnicity, geography, diet, and environmental exposures shape both the human microbiome and the association of the microbiome with disease development, thus demonstrating how the earlier studies that were limited in their exploration of population diversity (to date) may have been confounded due to their focus on homogeneous populations with higher incomes [1, 5, 7, 11, 12].

The information in the literature about obesity, metabolic syndrome, and gut microbiome diversity shows how far we have come with understanding these areas of health through scientific literature, with many reviews and primary research articles indicating associations between dysbiosis and metabolic syndrome, as well as breast cancer risk. Evidence has suggested that some of the metabolic risk factors associated with breast cancer could lead to an increased risk due to the presence of a dysbiotic gut microbiome. Furthermore, research examining metabolic syndrome and women who develop breast cancer has found that while metabolic health can potentially confound and influence the relationship between breast cancer and the gut microbiome, it appears that metabolic status can also alter the relationship between the two [9]. These findings have prompted ongoing debate regarding how best to understand the relationship between the microbiome and

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breast cancer and how to incorporate metabolic health into a more integrated approach rather than using the contrasting perspectives of the microbiome versus the host.

Many methodological problems have also been addressed in the literature that pertains to these theme areas, and these methodological problems now serve as the primary means of referencing across all these different types of studies. Systematic reviews published by Bose et al. [1] and Gamba et al. [7] highlight the variability in the sampling and sequencing, the choice of targeted regions, and the bioinformatics pipelines employed by the researchers affect diversity estimation and create difficulties when attempting to compare the findings of different studies. Studies examining diversity metrics have concluded that both alpha- and beta-diversity indices can provide useful summary descriptions of the sampled microorganisms, but that it is important to note that these metrics are very sensitive to the choice of index used, the method of normalizing sample size for statistical analysis, and the statistical model that is used to analyze the data [6, 7, 9] as well as the fact that the majority of studies in this area still tend to be cross-sectional, underpowered, and based on taxonomic rather than functional data [1, 7, 8].

This new information is an important step toward answering fundamental questions related to what has been discovered. In particular, the following research directions will benefit from larger prospective studies with multiple gut microbiome samples taken at different time points that correlate with the peri-menopausal period, periods of weight fluctuation, and periods of starting use of hormone or chemotherapy. Future studies should address the effect modification of menopause status, metabolic health, and ethnicity as potential covariates in addition to treating these variables as confounders. To further promote consistency and comparability of research data across studies, researchers should create harmonized laboratory and analytical processes and develop clear procedures for reporting and publishing their methods to provide a standardized way of assessing microbiome diversity in the same study, and for establishing meta-analytical conclusions across studies. Future research must integrate taxonomic, metabolic functions, and metabolomics data with microbiome findings to better understand if targeting obesity-related dysbiosis or low diversity will have an impact on reducing the risk of breast cancer and improving outcomes for those diagnosed with breast cancer.

5. Limitations

The limitations of this narrative review should be considered when interpreting its findings. The narrative approach (i.e., not systematic or meta-analysis) to reviewing the literature did not allow for a comprehensive evaluation of the literature, nor was the quality of studies included in this narrative review formally rated, and therefore it is unknown if there is publication or selection bias among the studies that were included. The emphasis on diversity of the gut microbiome has resulted in limited discussion of other important aspects of the gut microbiome-breast cancer relationship, such as specific taxa, functional and metabolomic measures and the microbiome in non-gut sites of the body. This narrative review is primarily based on the most recent human observational studies and does not systematically integrate preclinical, experimental and/or interventional evidence and thus limits the ability to fully assess mechanisms and causation. The organization of the discussion around pre-defined themes (menopausal status, geographical and ethnic diversity, diversity metrics, and metabolic health) inevitably means that other modifications of breast cancer and the gut microbiome-breast cancer relationship, such as previous exposure to antibiotics, treatment history, and tumor subtype, received less weight in this review than in previous reviews. Nonetheless, the synergism of epidemiological findings, mechanistic considerations, and methodological considerations clarifies what is currently known about the gut microbiome-breast cancer

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relationship, and frames important future research directions, including the development of supportive and diverse cohorts, the need for standardizing microbiome analysis techniques, and the need for an integrative analysis of diversity measures with biological and clinically relevant endpoints.

6. Conclusion

Analyzing recent evidence relating lower gut microbiome diversity to risk of developing breast cancer provided the basis for examination of the current literature by grouping studies based upon menopausal status, race, geographic location, metabolic health, and diversity metrics. Consistent with two primary areas of focus, the results of studies organized using these methods indicate that there are notable differences in gut microbial diversity and composition between women diagnosed with breast cancer and healthy control groups. These differences appear to be associated with hormonal, immune, and metabolic mechanisms linked to an increased risk of developing breast cancer. Although similar patterns are observed across various domains within the reviewed literature, the evidence indicates that these patterns are subject to influence by contextual factors such as host variables and methodology, and do not represent a singular universal signature associated with a diagnosis of breast cancer.

The results of our study, combined with previously published studies, indicate that instead of regarding gut microbiome diversity as an individual marker, it should be viewed as a component within a greater ecological system that integrates information about menopausal status; metabolic risk factors and community-based characteristics or context. Understanding these relationships will allow for developing an understanding of how to best utilize the gut microbiome as a potential preventative measure against breast cancer. We have identified areas of alignment; for example, we have noted the importance of research focused on connections between obesity-related dysbiosis and breast cancer (emerging convergence), while we also noted that we still need further research in areas of disparity; for instance, the lack of understanding regarding ethnic variations of diversity and the functional mechanisms driving these shifts.

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