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Comparative Study of Gut Microbiota in Breast Cancer Patients versus Controls in Abidjan, Côte d'Ivoire

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Abstract

Context: The gut microbiota represents a complex ecosystem encompassing all unicellular microorganisms residing in the digestive tract, primarily bacteria, fungi, archaea, and even viruses. The relationship between the host and the microbiota is symbiotic: bacteria benefit from a stable environment, while the host gains numerous capabilities in terms of digestion, metabolism, nutrition, and immunity. However, numerous studies suggest that the gut microbiota plays a crucial role in various non-communicable diseases, including obesity, chronic inflammatory bowel diseases, allergic and immune disorders, behavioral disorders, and even certain cancers. The objective of our study was to characterize the gut microbiota of a group of breast cancer patients by comparing it to that of control subjects in Côte d'Ivoire, using a metagenomic approach. **Method:** A case-control study was conducted from May 2020 to September 2023. A total of 85 women (39 cases and 46 controls) were recruited, and stool samples were collected from both breast cancer patients and healthy women. Among these, ten (10) samples from patients and ten (10) samples from healthy women were randomly selected for the study of the gut microbiota. The gut microbiota was characterized by sequencing the V4 region of 16S rRNA using metagenomic NGS technology, and bioinformatic analysis was performed using the mothur pipeline. **Results:** In women with breast cancer,

we observed a reduction in the relative abundance of the phyla *Firmicutes* and *Bacteroidetes*, as well as an increase in the phyla *Actinobacteria* and *Verrucomicrobia*. Additionally, their microbiota exhibited lower Chao1 and Sobs diversities compared to the control women ($p < 0.05$). Molecular variance analysis (AMOVA) revealed a significant difference between the case and control groups ($p < 0.001$). This study has highlighted a significant difference in the relative abundance of major phyla within the gut microbiota of cases compared to healthy controls. It will contribute to enriching African and global data, thus promoting a better understanding of the role of gut microbiota in breast cancer.

Keywords

Gut Microbiota, 16S Metagenomic Sequencing, Breast Cancer, Dysbiosis

1. Introduction

The gut microbiota (GM) represents a complex ecosystem encompassing all unicellular microorganisms residing in the digestive tract, primarily bacteria, fungi, archaea, and even viruses [1]. Bacterial concentration is the highest at the distal end of the digestive tract [2]. The relationship between the host and the microbiota is symbiotic, with bacteria benefiting from a stable environment (temperature, CO_2, pH, nutrients), while the host gains numerous capabilities in terms of digestion, metabolism, nutrition, and immunity [1] [3]. However, numerous studies suggest that the gut microbiota plays a crucial role in various non-communicable diseases, including obesity [4], chronic inflammatory bowel diseases, allergic and immune disorders [5] [6], behavioural disorders [2] [7], and even certain cancers [8] [9].

Breast cancer is the leading cause of cancer mortality among women in almost all countries, with 2.2 million cases and approximately 685,000 deaths reported in 2020 [10]. According to the latest report from the World Health Organization (WHO) in February 2018, cancer is the second leading cause of death worldwide after cardiovascular diseases, accounting for 10 million deaths in 2021. In Africa, 1,055,000 people were affected by cancer in 2018, with a mortality rate of approximately 70%. Breast cancer is on the rise in developing countries, including Côte d'Ivoire, where it ranks first among cancers in women, followed by cervical cancer [11]. According to data from the Abidjan Cancer Registry, the age-standardized incidence rate was 44.7 per 100,000 women, with approximately 74% at late stages (III and IV), and 1,785 deaths with a mortality rate of 25.3 per 100,000 women in 2020 [12] [13].

In the field of breast cancer, research on the gut microbiota focuses on the variations in microbiota composition between different populations, highlighting imbalances in the microbiota of women with this cancer. Understanding the complex role of the gut microbiota in the context of breast cancer is an expanding

field, requiring the analysis of a large volume of sequencing data. However, the majority of this data comes from studies carried out in America, Asia and Europe. There is little research on the gut microbiota in Africa, particularly in breast cancer research. The objective of our study was to characterize the gut microbiota of a group of breast cancer patients by comparing it to that of control subjects in Côte d'Ivoire, using a metagenomic approach.

2. Materials and Methods

2.1. Materials

2.1.1. Recruitment of Women

This case-control study was conducted from May 2020 to September 2023 at the Oncology Department of Treichville University Hospital for the recruitment of cases, at the blood donation service of the National Blood Transfusion Center of Treichville, and at the Reception, Welcome, and Sampling Unit of the Institut Pasteur de Côte d'Ivoire for the recruitment of controls.

Two groups of women were included in this study: the first group consisted of postmenopausal and premenopausal women with breast cancer (cases), while the second group comprised postmenopausal and premenopausal women without the disease (controls). For the cases, women of all ages diagnosed with breast cancer at any stage were included, and for the controls, women of any age with a normal mammogram/breast ultrasound less than one year old were included in the study.

Pregnant women, those who had used hormones in the six months preceding their inclusion, and those who had started chemotherapy were excluded from the study. Before proceeding with the interview and sampling, each participant provided informed consent. The study protocol was approved by the National Ethics Committee for Life and Health Sciences (Côte d'Ivoire) under the number IRB000111917.

2.1.2. Biological Material

The biological material consisted of fresh stool samples collected from the participants.

2.2. Methods

2.2.1. Interview and Data Collection

Sociodemographic information and the weight and height of the participants were recorded during the interview. Clinical status was obtained from medical records.

2.2.2. Collection, Transport, and Preservation of Samples

The stool sample collection was carried out using a specially designed collection kit. This kit included a collection procedure sheet, a sterile container for the samples with a spatula attached to the lid, plastic bags with a zip closure, and a cooler equipped with a cold pack. The samples were transported to the laboratory within less than 2 hours after emission and stored at a temperature of −80 degrees Celsius

at the Biological Resource Center of the Institut Pasteur de Côte d'Ivoire.

2.2.3. 16S rRNA Metagenomics Analysis

The extraction of total bacterial DNA was performed using the Quick DNA™ Fecal/Soil Microbe Microprep kit (ZYMO RESEARCH), and the quantification of the extracts was carried out using the Qubit fluorometer. The sequencing libraries were prepared using the Quick-16S™ Plus NGS Library Prep Kit (V4) (ZYMO RESEARCH) targeting the hypervariable V4 region of the 16s rRNA gene (515f: GTGYCAGCMGCCGCGGTAA; 806r: GGACTACNVGGGTWTCTAAT). The libraries were sequenced on the Illumina® MiSeq platform.

2.2.4. Bioinformatics Analysis

Bioinformatics analyses were conducted using the mothur pipeline version 1.48 [14] [15]. A truncated reference sequence from the silva seed version 132 [16] to the V4 region of the 16S rRNA gene was used for alignment, and the RDP version 18 reference files were used for taxonomy.

2.2.5. Statistical Analysis

Statistical analyses were performed using R software (4.3.1) and R Studio version 2023. Alpha diversity was calculated using the Sobs and Chao1 diversity indices to estimate community richness. Beta diversity was studied using Molecular Analysis of Variance (AMOVA) and Non-metric Multidimensional Scaling (NMDS). Statistical differences between cases and controls were examined using Student's t-test for normally distributed variables and the Wilcoxon-Mann-Whitney test for non-normally distributed variables, with a significance threshold of $\alpha = 0.05$.

3. Results

3.1. Characteristics of Included Women

In total, we collected 85 stool samples from patients with breast cancer (cases) and healthy women (controls), among which ten (10) from the cases and ten (10) from the controls were randomly selected through multi-stage sampling for this preliminary stage of gut microbiota characterization. The average age of the cases included in the study was 53.7 years ± 12 years. For the women selected for metagenomic analyses, there were no significant differences between the two groups in terms of age and body mass index (Table 1).

Table 1. Age and BMI of women included in the metagenomic study.

	Cases	*Controls*	*p-value*
Characteristics of women included (n)	10	10	
Age: mean (sd)	45.3 (13.72)	49.9 (13.28)	***0.45***
BMI: mean (sd)	25.8 (4.49)	26.47 (2.54)	***0.68***

3.2. Metagenomics Analysis

3.2.1. α Diversity

Calculating the alpha diversity indices revealed that there was a significant

difference between the microbiota of cases and controls for both the Sobs and Chao1 diversity indices (Sobs index $p = 0.005$; Chao1 index $p = 0.004$) (Figure 1).

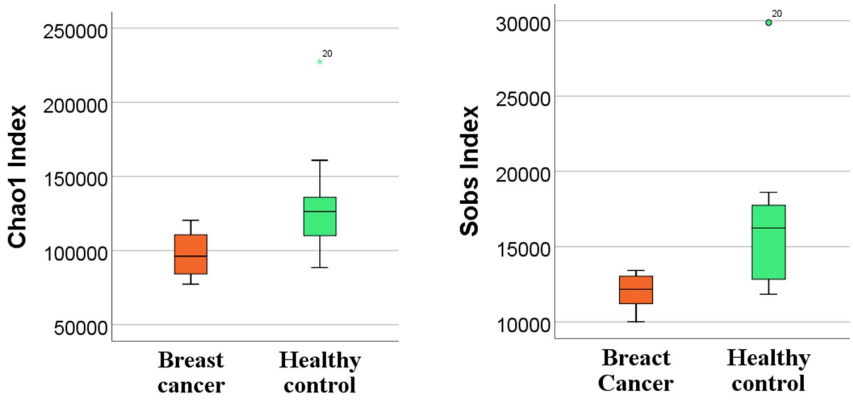

Figure 1. Comparison of alpha diversity between cases and controls.

3.2.2. β Diversity

Non-metric multidimensional scaling (NMDS) analysis using the Bray-Curtis dissimilarity matrix revealed a difference in the distribution distance of the microbiota of cases compared to controls. The gut microbiota samples from the women were distributed in space, representing two distinct groups. One group consisted predominantly of cases samples, while the other group consisted predominantly of control samples (Figure 2). Additionally, molecular variance analysis (AMOVA) showed a significant difference between the two groups ($p = 0.001$).

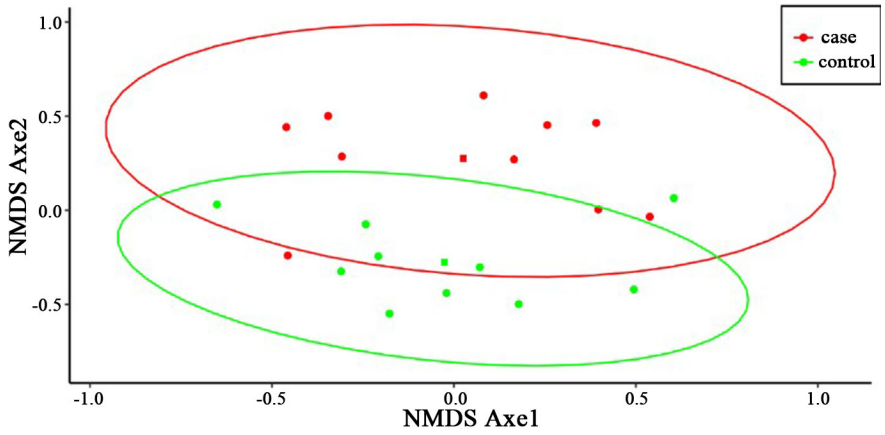

Figure 2. Non-metric Multidimensional Scaling (NMDS) of samples.

3.2.3. Taxonomic Composition Analysis

The analysis of taxonomic composition highlighted five major phyla (>3%) in the microbiota of both population groups: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*. The other phyla and unclassified genera in this study represent the remaining 3%.

In women with breast cancer, we observed a reduction in the relative abundance

of the phyla *Firmicutes* (47.97%) and *Bacteroidetes* (6.68%) compared to the controls, which had respective abundances of 56.83% and 19.42%. Conversely, we observed an increase in the phyla *Actinobacteria* (28.75%) and *Verrucomicrobia* (6.49%) compared to the controls, which had abundances of 12.56% and 2.87%, respectively. Regarding the phylum *Proteobacteria,* there were no large variations between cases (7.29%) and controls (6.07%) (**Figure 3**).

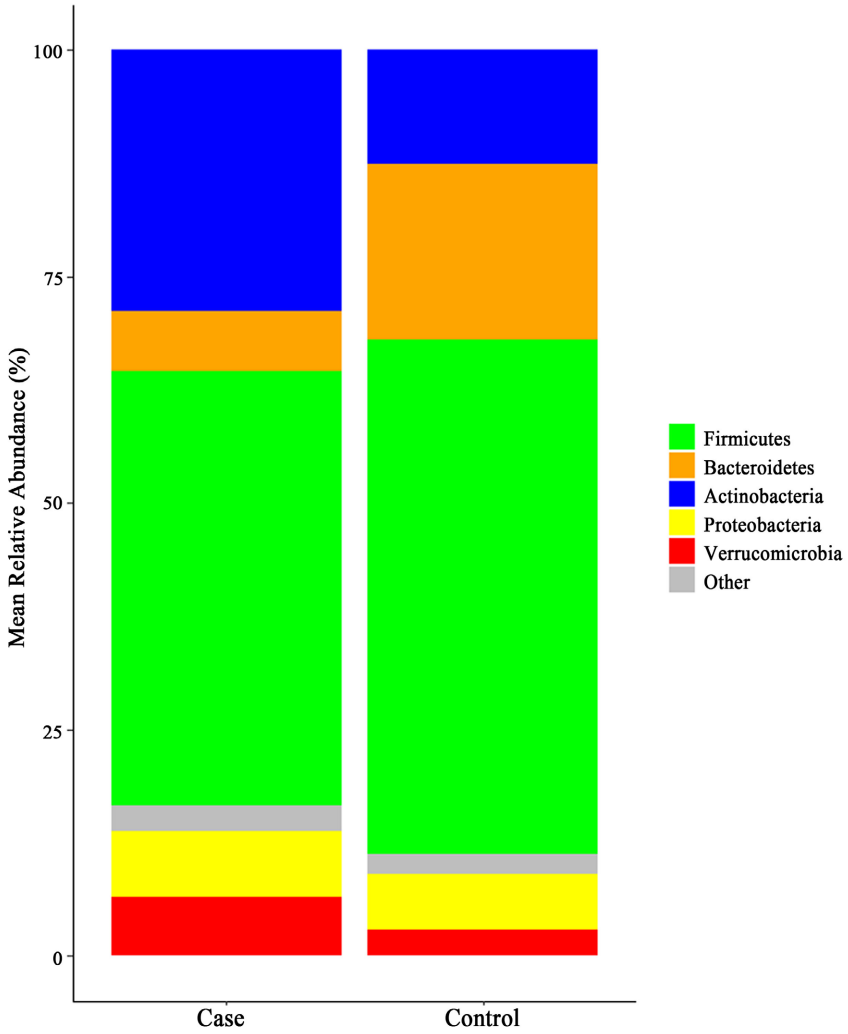

Figure 3. Comparison of the relative abundance of major phyla between cases and controls.

4. Discussion

The composition of the gut microbiota has already been highlighted in several digestive cancers, such as stomach cancer [17], liver cancer [18], and colon cancer [19] [20]. In breast cancer, some studies have suggested that the composition of the microbiota could modulate the reabsorption of estrogens at the level of enterohepatic circulation [21] [22]. In this regard, Goedert *et al.* (2015) demonstrated a difference in the composition of the microbiota in cases compared to healthy women [23], suggesting that the composition and stability of the gut microbiota

are crucial for maintaining good biological activities in the body.

In our study, the average age of cases at diagnosis was 52.7 years. This result aligns with an African meta-analysis showing an average age at diagnosis ranging between 46 and 60 years [24]. However, this result differs from industrialized countries where the average age of breast cancer onset has been advancing in recent years [25]-[27], with an average age around 67 years in France, for example. This difference could be explained by the fact that in developing countries, populations are increasingly adopting a Western lifestyle with an increase in risk factors, while populations in developed countries are returning to a much healthier lifestyle as a result of awareness policies among at-risk populations.

In this study, alpha diversity analysis revealed a significant difference in the Sobs and Chao1 indices ($p < 0.05$). Women with breast cancer had lower alpha diversity than healthy women. This same finding has been reported by several authors in studies on similar populations in Ghana, the United States, and China [28]-[30]. Conversely, a study in China presented opposite results, where postmenopausal cases had a higher Sobs diversity index than postmenopausal controls, and the Shannon index was higher in premenopausal cases. However, in this study, microbiota characterization was performed using shotgun metagenomics, and the results presented were not adjusted for case-control groups [31]. The gut microbiota has often been mentioned in the regulation of estrogens, for example, in postmenopausal women, previous studies suggested a negative correlation between gut microbiota alpha diversity and estrogen concentrations in stool, while a positive correlation was observed in urine [32].

Non-metric multidimensional scaling (NMDS) analysis using Bray-Curtis dissimilarity indices revealed a difference in the distribution distance of the microbiota of the diseased women group compared to the microbiota of the healthy women group. Indeed, the gut microbiota samples from the women are visually distributed in space, representing two distinct groups. One group is predominantly formed by cases samples, and the other group is predominantly formed by control samples. The separation of samples into two groups could be explained by the observed and estimated richness differences, represented by the Sobs and Chao1 indices, respectively. In the study by He *et al.* (2021), a similar grouping of case and control samples in premenopausal women was shown [33]. However, in this study, the representation of dissimilarity data was done by redundancy analysis (RDA). Similarly, the study by Byrd *et al.* (2021) showed a significant difference in the distribution of samples from diseased and healthy women using principal coordinate analysis with the Bray-Curtis matrix [29].

Several hypotheses suggest that changes in the composition (dysbiosis) and functions of several bacterial genera in the gut can contribute to the development and progression of breast cancer through various pathways [34]. This study revealed a difference in composition between the microbiota of the two subject groups (case-control). This included a reduction in the relative abundance of the phyla *Firmicutes* and *Bacteroidetes*, as well as an increase in the phyla *Actinobacteria* and

Verrucomicrobia in cases. Two other studies had already observed a difference in composition within the gut microbiota in cases. However, comparing our Ivorian study to these, the relative abundance of major phyla differs. In the study by Ma *et al.* (2022) in China, the relative abundances of *Firmicutes* and *Proteobacteria* were reduced while that of *Bacteroidetes* increased [30]. Similarly, in the study by Bobin-Dubigeon *et al.* (2021) in France, the relative abundance of *Bacteroidetes* was reduced while that of *Firmicutes* increased [35]. Furthermore, molecular variance analysis (AMOVA) revealed a significant difference between the microbiota of cases and controls ($p = 0.001$). These differences between study results could be explained by factors such as diet and geographical distance between the studied populations [36] [37], implying that it is necessary to produce more data from multiple continents and various population types, which will promote a better understanding of the role of the gut microbiota in breast cancer.

5. Conclusion

This study highlighted a significant difference in the relative abundance of major phyla as well as in the diversity within the gut microbiota of breast cancer patients and control subjects. However, it is crucial to produce data from multiple continents and various population races. This study will contribute to enriching African and global data, thereby promoting a better understanding of the role of the gut microbiota in breast cancer.

Limitations of the Study

Although we used rigorous sampling methods to select the sample, we are aware that the small sample size may limit the statistical power of the results.

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Data Availability

Data are available at Institut Pasteur de Côte d'Ivoire and with authors. Authors are ready to share on demand at any moment.

Conflicts of Interest

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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