# Detection of Bacterial and Level of CA15-3 in Serum of Patients with Breast Tumors

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**Summary**
Breast cancer, which is the most common type of cancer in women and the leading cause of cancer deaths in women globally, is a global problem. A growing body of research links the micro biome to the development and spread of certain cancers. The current study set out to ascertain how the development of breast cancer may be affected by the microbiome. Ages of the 170 patients with breast cancer in the research ranged from 14 to 65.. The research was done in the Iraqi city of Babil between September 10, 2021, and August 20, 2022. 50 women who were similar to the women in the sick group and were in the 25–75 age range made up the control group. Patients with benign breast lesions and breast cancer had their blood and breast tissue samples obtained. Immunological techniques were used to analyses blood samples, and the CA15-3 enzyme-linked immunosorbent assay (ELISA) was used. Patients with benign breast lesions and breast cancer had their breast tissue samples collected in order to isolate and identify microorganisms.. Gram negative bacteria were more prevalent than Gram positive bacteria, according to the results, with Klebsiella pneumonia accounting for 24% of all bacteria, Staphylococcus aureus 22%, E. coli 14%, Serratia marcescens 14%, Staphylococcus warneri 7%, Pseudomonas auroginosa 12%, and Proteus mirabilis 7% being the most prevalent. There was no difference in the concentration of CA15-3 between patients before and after chemotherapy, although it was much higher for patients than for the control before and after chemotherapy. age groups do not differ in terms of CA15-3 concentration.
Key words: breast tumors , bacteria , CA 15-3

## 1. Introduction

There are numerous different types of breast diseases. Breast problems are typically benign. Because some of these lesions were clinically unimpressive, they only required minimal therapy. However, some symptoms may be clinically serious and require the patient's and the treating physician's attention, especially if they continue. (1). Breast ultrasound pictures' morphological and textural characteristics are frequently utilized to distinguish between benign and malignant cancers. The simple method for classifying tumors as benign or malignant is to physically examine the texture and morphological features in pictures and rely on highly qualified and experienced radiologists (2). A simple alternative to manually grading ultrasound images is to create classifiers based on morphological and textural characteristics using a computer. This will automatically divide tumors into benign and malignant groups (3).

Breast tissue, traditionally believed to be sterile, now appears to have a distinctive microbial community, according to some data (4). In addition, unlike normal mammary gland tissue, breast cancers have their own unique microbial community (5)(6). The emergence and progression of BC may be caused by a particular colony of microbes in breast tissue. According to studies, the gut microbiome may have an effect on breast cancer. in addition to the breast microbiota. Additionally, different breast cancer patients may have diverse microbiological signatures. For instance, fecal microbiota study reveals that the gut microbiota of postmenopausal women with breast cancer is different from that of healthy volunteers and exhibits enrichment of different bacterial species ( 7). Additionally, breast cancer is a diverse illness with a variety of subtypes, and intriguingly, the microbial signatures of the various subtypes may vary (8).

The microbiota of human breast tissue is diverse and distinct from that of other parts of the body, regardless of the sample site within the breast, age, location, or previous pregnancies. Other microbial compartments' diversity of flora is not comparable to that of the local breast (e.g., gut, skin, vagina). Proteobacteria, Firmicutes, Actinobacteria, and Bacteroide make up the distinctive breast microbiome pattern in decreasing order of abundance. The presence of particular microorganisms in human milk has been recognized for some time, even though research on the microbiota in breast tissue has just lately started. Additionally, the human milk microbiota's makeup resembles that of the breast microbiota in that proteobacteria are the most prevalent phylum, followed by the other phyla (9,10).

In order to boost survival rates for breast cancer patients, early detection of primary and recurrent tumors is therapeutically extremely important. (11) Tumor markers are thought to provide a non-invasive, affordable way to forecast how a treatment will be received in order to plan treatment, track the progression of the disease, and assess the prognosis (12). (13). Tumor size, axillary lymph node status, lymphatic and vascular invasion, hormone receptor status, and HER2 expression are traditional breast cancer prognostic markers, although they don't

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always reliably predict the outcome. The two most often utilized tumor markers for breast cancer are plasma CEA and the cancer antigen CA 15-3. (34) Numerous types of epithelial cells express CA 15-3, a member of the mucin-1 (MUC-1) glycoprotein family. However, 90% of breast cancer cases had aberrantly high levels of it (15). This study aims to compile a list of the bacteria found in breast tissue.

## 2. Material and methods

A cross-sectional study was carried out in Babylon, Iraq, between September 10, 2021, and August 20, 2022. The study's 50 controls and 170 breast cancer patients, whose ages ranged from 14 to 65. During an interview with these patients, the researcher used a questionnaire form that she had designed. Twenty women with malignant breast tumors were among the patients, who had ages ranging from 14 to 65 and 50 with benign breast lesions. Patients with benign and malignant breast lesions as well as those with breast tumors had their blood and breast tissue samples obtained. Immunological techniques and the enzyme-linked immunosorbent test (ELISA) were utilized to analyze the blood samples to detect CA15-3. 5 ml of blood samples were placed in plain tubes and centrifuged for 15 minutes at 3000 rpm after being kept at 37 °C for 30 minutes. The obtained sera were pipetted into two clean test tubes using an automated micropipette in order to run serological assays on them. Before being frozen at -20°C for later serological testing to determine the level of CA15-3 using the ELISA method, each test tube had labels applied to it.

70 fresh breast tissue samples were donated by the same women undergoing breast surgery at Hilla Teaching Hospital, patients with breast tumors, and patients with benign and malignant breast diseases, respectively. After that, excised tissue samples were placed in tubes filled with physiological saline solution for bacterial isolation. Following sample collection, the isolated breast tissue samples were incubated both aerobically and anaerobically for around 2 hours at 37°C. Then, utilizing methods from the VITEK 2 system, culture, biochemistry, and microscopy, the emerging microbial cultures in the culture media were observed and confirmed. ELISA kits were utilized in the CA15-3 detection procedure.

## 3. Result

The results of the current study's bacterial culture showed that the most common bacteria created more malignant breast tumors than benign ones (table 1).

Figure 1 and Table 4 demonstrate that Gram negative bacteria are more prevalent in breast tissue tumors than gram positive bacteria. Gram positive bacteria in breast tissue included Staphylococcus aureus (22%), Staphylococcus warneri (7%), Proteus mirabilis (7%), E. coli, and Serratia marcescens (14%), and both Gve+ and Gve- were identified utilizing selected and enhanced media (Blood agar, MacConkey). Gram-positive cocci were discernible as Staphylococcus under a light microscope, and the species was catalase-positive and oxidase-negative. S. aureus and S. epiderms are coagulase positive, whereas S. feacalis, which is coagulase negative, catalase negative, and develops on mannitol salt agar. Gram negative bacteria could be found using the Vitec 2 compact system, and successful results from biochemical tests such the indole, methyl red, Voges-Proskauer, Simmons' citrate test and Kligler iron agar test were obtained. Simmon Citrate positive results in color medium after a 24-hour incubation change from blue to green, but Simmon Citrate negative results remain blue due to the indole development of a red ring with the addition of Kovacs reagent.. After adding the reagent, a red ring formed, signaling a positive Voges-Proskauer test result. The biochemical test for gram-negative bacteria was shown in Table. .

Table (1) type of bacteria in breast tumors tissue

| p. auroginosa | P. mirabilis | E. coli | S. marcescns | S.warneri | K. pneumonia | SAureus | Type of bacteria Type of tumors |
|---|---|---|---|---|---|---|---|
| 4 | 7 | 6 | 4 | 7 | 5 | 10 | Malignant breast tumor |
| 2 | 2 | 2 | 2 | 2 | 10 | 3 | Fibrocystic change |
| 2 | 1 | 2 | 1 | 1 | 5 | 2 | Fibro adenoma |
| 1 | 1 | 3 | 5 | 1 | 5 | 2 | Granulomastitis |
| 3 | 1 | 1 | 2 | 1 | 3 | 5 | Ductal invasive |

Table 2: Gram-negative bacteria isolated from breast tissue biopsy biochemical test

| p. auroginosa | P. mirabilis | E.coli | S. marcescns | K. pneumonia | S.Aureus | S.warneri | Test |
|---|---|---|---|---|---|---|---|
| - | - | - | - | - | + | + | Gram stain |
| - | - | - | - | - | - | - | Spore forming |
| + | + | + | + | + | + | + | Catalase |
| + | - | - | - | - | - | - | Oxidase |
| - | - | - | - | - | + | - | Coagulase |
| + | + | + | + | - | - | - | Motality |
| - | + | - | + | + | + | - | Urease |
| - | - | + | - | - | - | - | Indole |
| - | + | + | - | - | + | - | Methyle red |
| - | - | - | + | + | + | - | Vogas proscure |
| + | + | - | + | + | + | - | Cimon citrate |

The bacterium was identified using a variety of tests, including macroscopic and microscopic exams, biochemical investigations, and the Vitec 2 compact system

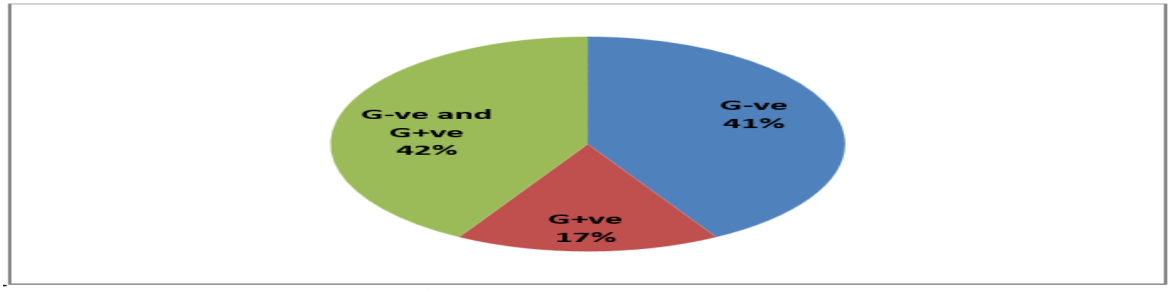


*Figure (1) percent of Gram positive and gram negative in breast tissue*

| Table (4) Bacterial content in breast tissue as a percentage | |
|---|---|
| Percent % | Types of bacteria |
| 22 | Staphylococcus aureus |
| 14 | E.coli |
| 24 | Klebsiella pneumonia |
| 14 | Serratia marcescens |
| 7 | Staphylococcus warneri |
| 7 | Proteus mirabilis |
| 12 | Pseudomonas auroginosa |

In the current investigation, table (5) demonstrates that the concentration of CA15-3 was significantly higher in patients than in controls before and after chemotherapy, whereas table (6) demonstrates that there was no difference in the concentration of CA15-3 between patients.

| Table (5) Comparison between patients and control with breast tumors before chemotherapy | | | |
|---|---|---|---|
| Before chemotherapy | | | |
| P value | M±SD | | Parameter |
| | Control | Patients | |
| 0.000*** | 62.802±17.629 | 106.307±28.881 | CA 15-3 |
| After chemotherapy | | | |
| P value | M±SD | | Parameter |
| | Control | Patients | |
| 0.05* | 62.802±28.881 | 101.107±28.881 | CA 15-3 |
| P value was significant at p ≥ 0.05 | | | |

| Table (6 ) Comparison in concentration of CA-15-3 between patients before and after chemotherapy | | | |
|---|---|---|---|
| P Value | M±SD | | Parameter |
| | After | Before | |
| 0.7 | 101.107±93.288 | 106.307±28.881 | CA15-3 |

The influence of age group on the concentration of CA 15-3 is shown in table (7), with the conclusion that there is no difference in the concentration of CA 15-3 between age groups.

| Table (7) Effect of age group on concentration of CA15-3 in patients with breast tumors | | |
|---|---|---|
| P value | M±SD | Age groups Year |
| 0.9 | 47.201±9.084 | 14-34 |
| | 55.439±9.800 | 35-55 |
| | 36.452±18.226 | 56-65 |

In table (8) show that no significant different in the concentration of CA15-3between malignant and benign breast tumors , in table (9) show that there are different significant between type of benign breast tumors .

| Table (8)Comparison in CA15-3 concentration between malignant and benign breast tumors | | | |
|---|---|---|---|
| P Value | M±SD | | Parameter |
| | Malignant breast tumors | benign breast tumors | |
| 0.7 | 118.799±26.153 | 101.765±28.752 | CA15-3 |

| Table (9)Comparison in CA15-3 concentration between type of benign breast tumors | | |
|---|---|---|
| P value | M±SD | benign breast tumors |
| 0.001** | 31.250±9.021 | Fibrocystic change |
| | 22.008±4.803 | Fibro adenoma |
| | 18.588±7.026 | Granulomastitis |
| | 18.202±9.101 | Ductal invasive |
| *P value was significant at p >0.05 | | |

## 4. Dissuasion

Breast cancer is the most common kind of cancer in women and the main reason why people die from cancer in women around the world. It causes 15% of all cancer deaths, on average (16). According to this study, breast tissue from both benign and malignant tumors had a wide variety of germs and wasn't

sterile. The microbiota of healthy mammary glands, breast milk, and breast tissue has been the subject of extensive research. have proven the presence of bacteria in the breast tissue (17,18.19, 20,21 ,22). Because it is primarily made up of fatty tissue, the breast itself provides a favorable environment for bacterial growth (23). The result of a bacterial culture In comparison to gram positive bacteria, Klebsiella pneumonia was the most prevalent gram negative bacterium. It was also the most common bacterium in both benign and aggressive breast cancer. This study contradicted the previous one (24) S. epidermidis was the most prevalent species in both benign and malignant tumors, as well as in swab and biopsy samples. S. epidermidis was found in 39 out of 77 culture-positive samples of benign and malignant breast tumors, indicating that it may be a key player in the development of breast cancer. Furthermore, study (25) shows that a distribution between 54.8% G+ve bacteria and 45.20% negative (G-ve) bacteria was found.

The study's results go counter to the idea that germs in tissue are identical (26) Hieken discovered that healthy individuals had much larger relative abundances of Chen Prevotella, Lactococcus, Streptococcus, Corynebacterium, and Micrococcus than did breast cancer patients. Women with malignant cancer had a very different breast microbiome from those with benign illness. Furthermore, it was found that the relative abundances of the Patients with breast cancer had considerably greater concentrations of the bacteria Bacillus, Staphylococcus, Enterobacteriaceae, Comamondaceae, and Bacteroidetes. very early

Many studies on primary breast cancer have shown a link between higher blood CA15-3 values at diagnosis and worse overall survival and disease-free survival, as well as higher breast cancer stage, tumor size, and positive axillary lymph nodes (27) (28). As opposed to a study (29) Our analysis revealed that CA15-3 levels were higher in patients before and after chemotherapy than in controls, consistent with a previous study that reported that the levels of CA15-3 in serum and saliva were slightly but not statistically higher in BC patients than in healthy controls. Furthermore, I disagree with (30) who asserted that there were no appreciable variations in serum and salivary CA15-3 levels between the BC patients and the control group. Our results concur with those of (31)(32), who found a notable variation in the levels of CA15-3 between patients and controls.

## 5. Conclution

The most prevalent bacterium was Klebsiella pneumonia, and the amount of this bacterium was higher in malignant breast tumors than benign breast tumors. Before and after chemotherapy, patient blood levels of CA 15-3 were also higher than control levels.

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