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Survey the bacterial types in breast tissue and CA13-5 for women with breast disease in Babylon province

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***Abstract*---**Breast tumor may be malignant and benign so these tumor and breast diseases are harboring bacteria ,but types of bacteria may differ among breast tumors so the current study was aimed to detect type of bacteria in breast tissue . Specimens(Breast tissue and blood) was collected from 50 female (aged ranged between 14 to 60) undergoing breast surgery at AL-Hilla Teaching Hospital and Al-Sadiq Hospital in Babylon Province during period from November 2021 - March 2022 and blood sample also collected from healthy women as control . tissue obtained was combined outside the marginal zone, approximately 5 cm away from the neoplasm. After enucleation, the new tissue was immediately placed in a sterile plane tube or urine cup it contain a normal saline solution and it was cut , homogenized by using a sterile surgical scalpel and wooden sticks within 30 min of collection.2 it was cultivated on blood agar &MacConkey media ,and then on selective media .and Identification for bacteria by microscopic ,biochemical test and confirmed by Vitec 2Compact System . The results appeared the percentage of malignant tumor (17.14%) and benign (82.85%) , The growth percentage (88.57%) while no growth was (11.42%).The most common bacteria present in tissue was *Pseudomonas florescence* was 32.25% then *Staphylococcus aureus* was 29% while less percentage 3.22% was *Enterococcus feacales* , Cancer antigen 15-3 were measured in the serum of women with breast tumors and healthy by using Enzyme Linked Immunosorbent assay (ELISA) ,the results appeared concentrations of CA15-3were significantly increased in patients(146.79pg/ml) than control (94.17pg/ml).

***Keywords*---**breast cancer, benign tumor, cancer antigen, bacterial infection.

Introduction

Breast tumor includes malignant tumor involved Invasive ductal carcinoma(IDC) and invasive lobular carcinoma (ILC) (Makki, 2015) ,and the benign breast tumor and diseases inculde Fibrocystic change ,Fibroadenoma , Granulomatous mastitis ,and other disease (Lipoma and Fat necrosis) groups of benign breast tumors divided by (Stachs *et al* .,2019) Benign non-proliferative breast lesions include (1) inflammatory fat necrosis, which follows surgical or blunt trauma, and generally resolves automatically; (2) lymphytic mastitis, which can be seen in diabetic patients; and (3) granulomatous mastitis, associated with foreign body reactions, sarcoidosis , or certain infections. Other benign non -proliferative breast lesions present as tumor-like processes, including (4) highly diffuse fibroadenoma, (about 25% of women), usually an isolated, well-defined smooth lesion in younger age groups , (5) phyllodes tumor (usually known as cystsarcoma sheets); (6) intraductal papilloma, solitary lesions that may be accompanied with bloody discharge from the nipple (7) fibrocystic breast disease, which was now more appropriately calledfibrocystic changes because it is see clinically in up to 50% and histologically in 90% of women, formed of varying amounts of fibrosis and cysts sometimes associated with calcifications and inflammation, and (8) simple or complex cysts, which which must be suctioned with ultrasound guidance and when the liquid is not clear (Goldman ,2020).

The human body is home to a large and diverse population of bacteria with properties that are both harmful and beneficial to health (Hooper ,2004 ;Zhu et al ,2013), which is why there has been a strong push in recent years to make this fully happen Distinguish between bacteria associated with different parts of the body under different health condition . Previously, the breast tissue was suggested to be a sterile site, which is difficult to be colonized by the microbiota residing mainly the gut and the skin, This is not surprising given that the skin and oral bacteria can access the mammary ducts through the nipple (Ramsay et al., 2004), study indicates that it comes from the mother's digestive system (Donnet-Hughe *et al* ,2010). Looked at the nutrient-rich fatty composition of the women breast, Vascular and lymphatic proliferation, the diffuse location of the lobules and ducts coming from the nipple, the spread of bacteria within the mammary glands, regardless of lactation. Urbaniak *et al.*, 2014 confirmed that the breast tissue has its own exclusive microorganisms.

The relationship between breast cancer and microbiome has been an important area of research (Blekhman *et al.*, 2015), and more attention has been paid to studying the microbiota differences between healthy and diseased breast. It has been suggested that breast tissue harbors unique microorganisms, which upon alteration (dysbiosis) can be distinctive of breast cancer or at least the subtype of cancer (Bannerjee *et al.*, 2015).So the current study was aimed to survey bacteria in different types of breast tumor .

Materials and Methods

Samples of Breast tissue was collected from fifty female (aged ranged between 14 to 60) undergoing breast surgery at AL-Hilla Teaching Hospital and Al-Sadiq Hospital in Babylon Province during period from November 2021 through March 2022. The women had lumpectomies or mastectomies for benign or cancerous tumors, and some female had history of breast cancer other had no history cancer . For female with tumors, the tissue obtained for analysis was combined outside the marginal region, approximately 5 cm away from the tumor. post circumcision , the fresh tissue was immediately placed in a sterilized plane tube or urine cup it contain a normal saline solution and it was cut , homogenized by using a sterile surgical scalpel and wooden sticks within 30 min of collection.2 it was cultivated on blood agar &MacConkey media ,and then on selective media .and Identification for bacteria by microscopic ,biochemical test according method (MacFadden,2000) and confirmed by Vitec 2Compact System .Blood samples were collected from patients(50) and 20 healthy women as control , serum was separated by centrifuge and then used to detect CA15-3 by The ELISA (Enzyme-Linked-ImmunoSorbent Assays) method was used according the manufacturer's instructions supplied by BioSource Company

Ethical approval

All the procedures performed in this study involving human participants Scientific Research Ethics Committee with ethical approval from the Ministry of Health and MOHSER in Iraq.

Result and Discussion

Breast tissue disease diagnosis by histological examination the participating women suffered from breast tumor. divided into malignant tumor (17.14%) and benign (82.85%). Other study (Shakir ,2019) in Baghdad where found high rate of malignant tumors compared with benign tumors because the size of samples taken the patients group with special oncology surgery centers such as Al –Amal oncology center and medical Education city in Baghdad had sites where samples were collected and nature of nutrition ,housing density and increased pollution in addition to other factors that vary from place to other so the results in Baghdadi society and its openness to smoking tobacco and drinking alcohol are all major factors in increasing the rate of breast cancer(Liu *et al.*, 2015; Gram *et al.*, 2019) .

In addition ,most studies focus on cancerous tumors rather than benign tumors therefore the rate of malignant tumors was higher in most studies. but it agree with another study in the southern Thailand found bengin breast disease ratio 72.9% of the women and (27.1%) women with breast cancer (malignant tumors)(Kotepui *et al*,2014).

The malignant tumor of the type disease of breast tissue distribution on percentage according age highest percentage was (28.57) in age (41-60) and less in age (21-40) was (15.38) and the ratio was zero in age (≤20).while the benign

tumor was percentage in age (≤ 20)highest ratio was (100%) and less in age (21-40) was the ratio (84.61) while in age (41-60) was (71.42) this results as table1

The malignant and benign breast tumors percentage differences according marital status and type of breastfeeding .the malignant tumor percentage of married women was (25%) while ratio the benign tumor was(75%). the malignant tumor ratio was zero of unmarried women while the benign tumor ratio was (100%) .in women who were breastfeeding the incidence of malignancy was (33.33) while the percentage of benign tumor was (66.66) and the percentage was in women with non-breastfeeding (zero) while ratio the malignant tumor was (100%) , either percentage of mixed breastfeeding was (11.11%) while ratio the benign tumor was (88.88%) . the percentage show in table (1).

Table 1: Socio-demographic characteristics and other risk factor from women the benign and malignant breast specimens were taken

A variable	Benign %	Malignant %
Age group		
≤20	100	Zero
21-40	84.61	15.38
41-60	71.42	28.57
Married	75	25
Unmarried	100	Zero
Breastfeeding	66.66	33.33
Non-breastfeeding	Zero	100
Mixed feeding	88.88	11.11

These results of the present study were agreement with (Shakir , 2019,Carroll and co-workers ,2008) who recorded that the age group of ≤20 years the highest benign tumor rate compared with other age .and highest ratio of malignant tumor at age (41-60) compared with other age . while no agree with study in Baghdad (Shakir ,2019) in ratio of breast tumors according to marital status. Late marriage or unmarried women was more likely to developed to breast cancer because increased estrogen in their body but in this study ,samples were taken from unmarried women are under 22 years old compared with married women were lower risk of breast cancer , these results do not agree with study in Baghdad (Salman ,2021). while breastfeeding maintain breast health and menstruation rate decreases during breastfeeding thus the estrogen level decreases (American cancer society, 2019).

The benign breast disease divided to the four group from total ratio in this study according to the histological examination involved Fibrocystic change was (44.82%) while less percentage Fibroadenoma was (34.48%) , and Granulomatous mastitis was (13.79%) and other (Lipoma ,fat necrosis) was (6.89% ..show in Figure 1 .and the proportion of each group according to age was of age (≤20) fibrocysti change was (50%) and fibroadenoma was (50%) while was of age (21-40) fibrocystic change (33.33%) ,fibroadenoma (41.66%) , Granulomatous mastitis (25%) ,as for age (41-60) it was fibrocystic change percentage (40%) ,fibroadenoma was (10%) , Granulomatous mastitis was (20%) and other benign disease (lipoma and fat necrosis)was percentage (20%).show for table (2) no agree

with study (Kotepui *et al*,2014). found highest proportions fibroadenoma and fibrocystic change respectively.

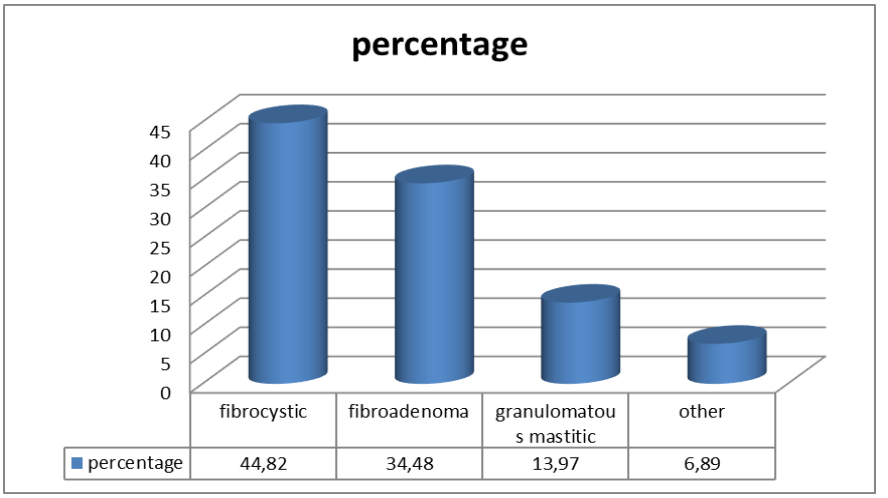


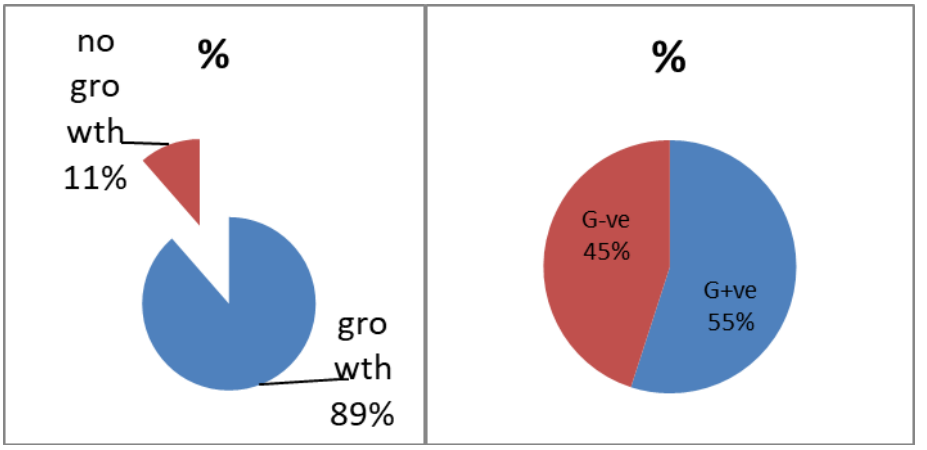
Figure (1) Distribution of type of benign breast tissue disease

Table (2) distribution of types benign breast tissue disease according to the age of the patients

Age/ year	Fibroadenoma %	Fibrocystic change % %	Invasive carcinoma % %	Granulomatous mastitis%
≤ 20	50	50	0	0
21-40	41.66	33.33	25	60
41-60	10	40	20	20

Identification and isolation of bacteria in breast tissue

The isolation and diagnostic of bacteria from w omen with breast tumors (50 biopsies) A diagnosed to have either benign or malignant tumor, bacteria were found growth (88.57%) detected as Gram-positive (G^{+ve}) and Gram-negative (G^{-ve}) bacteria, while (11.42%) of the samples were show no growth. show in figure (2). On the other hand, found single isolates was (76%) and mixed isolate was(24%) .distribution between (54.8%) as G^{+ve} bacteria,while (45.20%) were detected as negative (G^{-ve}) bacteria, show in figure (2).



Figure(2) - Distribution of the bacteria growth in breast tissue ,percentage of G+ and G-bacteria

The breast has been suggested to have a variety collection of bacterial spp. (Kim *et al.*, 2009).).agree with study in Najaf city (Khdear,2021) found (82.8%) were detected as Gram-positive (G^{+ve}) and Gram-negative (G^{-ve}) bacteria, while (17.2%) of the samples were show no growth. and On the other hand, found (75.5%) as G^{+ve} bacteria, and the ratio (20.3%) belonging to G^{-ve} bacteria .

Gram positive bacteria(G+ve) involved. Growth , *Staphylococcus aureus, Staphylococcus epiderms, Stapylococcus warneri, Enterococcus faecalis, andGram negative bacteria involved Pseudomonas fluorescens and Acinetobacter baumannii* were identified using enriched and selective media (Blood agar ,, MacConkey agar ,,Eosin methylene blue and mannitol salt agar), Gram stain technique, as well as biochemical tests (catalase test, oxidase test, coagulase) and other biochemical tests , *staphylococcus* by Gram stain under light microscope seen as Gram positive cocci appaering in clusters and staphylococcus species was catalase positive and negative oxidase . *S.aureus* is coagulase positive but *S.epiderms* and *S.werneri* was coagulase negative ,*Enterococcus.feacalis*,is catalase negative and grow on mannitol salt agar .Gram negative bacteria were diagnostic by Vitec 2 compact system and biochemical test like indole, methyl red, Voges-Proskauer, Simmons' citrate test and Kligler iron agar test result was Positive. Result of indole formation of red ring after addition Kovacs reagent, Simmon Citrate positive result convert of color media after 24 hour incubation change from blue to green negative result remains blue. Voges-Proskauer test positive result formation of red ring after added the reagent Table. (2) showed biochemical test for gram negative bacteria.

Table (2) Biochemical test for Gram negative bacteria that isolated from breast tissue biopsy

Bacteria	Indole	methyl red	Voges-Proskauer	Simmon citrate	Catalase	Oxidase
Acientobacter baumannii	-	+	-	+	+	-
Pseudomonaus	-	-	-	+	+	-

flourescense						

The microorganism was diagnostic by different test such as macroscopic and microscopic ,biochemical test , and Vitec 2 compact system results were mentioned in Table(3) , probability of bacteria diagnostic by Vitec 2 compact2 system between (90-99%) as in table (4-5).in addition to the percentage of each isolated bacteria from breast tumor biopsy was *Staphylococcus aureus*29%, *S.epiderms*16.12% ,*S.warneri* 6.45%, *Enterococcus feacales* 3.22%, *Acientobacter bumanni* 12.90% ,and the higest percentage of bacteria was for *Pseudomonas florescences* 32.25% .the percentage of bacteria isolated from breast tumor biopsy show in table (3).

Table (3) The probability of diagnostic bacterial isolates from breast tissue biopsy by vitec2compact system

Bacteria	Probability%
Staphylococcus epidermidis	95
Staphylococcus warneri	94
Staphylococcus aureus	99
Acinetobacter baumanni	93
Enterococcus feacales	91
Pseudomonas florescence	99

Table (4) The percentage of bacteria isolated from breast tissue biopsies

NO.	Bacteria	Percentage %
1	*Enterococcus feacales*	3.22
2	*Staphylococcus aureus*	29
3	*Staphylococcus warneri*	6.45
4	*Staphylococcus epidermidis*	16.12
5	*Acientobacter baumanni*	12.90
6	*Pseudomonas florescence*	32.25

This study found that the breast tissue from malignant and benign tumors containing many bacterial spp, and was not sterile, ,where was the growth rate 89% while no growth rate was 11%. The existence of bacteria within the breast tissue has been confirmed by many researchers who documented the microbiota of healthy mammary glands, breast milk and breast tissue, (Fernandez *et al.*, 2013; Xuan *et al.*, 2014; Hieken *et al.*, 2016; Urbaniak *et al.*, 2016; Wang *et al.*, 2017; Banerjee *et al.*, 2018). The breast has been suggested to have a variety of bacterial spp, (Kim *et al.*, 2009). The breast itself is an encouraging member for bacterial growth, because it is mainly consist of fatty tissue (Urbaniak *et al.*, 2014).The most common bacterial isolates was *Pseudomonas florescence* in breast tissue and *Staphylococcus aureus* was second this results were being different from Seng et al.,2015 who found opposite results .

Cancer antigen 15-3 (CA15-3) were measured in the serum of women with breast tumors by using Enzyme Linked Immunosorbent assay (ELISA) were used for quantification of human CA 15-3 the result of this test were calculated by using standard curve fit equation Figure (4-4) . Mean of CA 15-3 concentration in

serum of patient was 146.79Pg/ml compared to control was 94.17pg/ml with high significantly was (0.0066) .show in table (5) .

Table (5) Concentration of systemic CA 15-3 in patient and control

Cytokine	Patient M±SD	Control M±SD	P_value
CA 15-3 systemic	146.79±62.01	94.17±11.24	0.00665

CA 15-3 In patitents serum it differed according to the type of breast tumor where it was mean higher in ductal and lobular carcinoma and Granulomatous mastitis respectively (230.99 pg/ml and 227.88 pg/ml) while the mean less than in fibroadenoma was 104.58pg/ml and fibrocystic change as 103.10 pg/ml with no significant where was the p value (2.557) .show in table (6).

Table (6) Concentration of systemic of CA15-3 in womem with breast tumors according type of disease and control

Types of breast diseases	CA 15-3Systyemic pg./ml M±SD
Fibrocystic change	103.10±8.50 (a)
Fibroadenoma	104.58±10.98 (a)
Invasive carcinoma(IDC,ILC)	230.99±45.44 (b)
Granulomatous mastitis	227.88±32.71 (b)
Control	94.17±11.24 (a)

*Similar letters in the same column indicate that there is no significant difference (P >0.05) .(ANOVA-Duncan).

These results of the present study were agreement with(Atoum et al.,2012) where there was mean of patient was higher than control with higher significant (0.004)but in this study there was also significant between malignant tumors and benign tumors was (0.001) while in my studies nothing significant between groups and the mean cystic neutrophilic granulomatous it is one of groups of benign tumors asymptotic to the mean of groups of cancerous tumors.

As well as in other study found lower significant between groups of cancerous and benign tumors The mean CA 15-3 value was significantly lower in patients with benign breast tumors as compared with the breast cancer group: 16.8 +/- 8.2 vs. 23.9 +/- 20.9 U/ml ($p < 0.01$), also found CA15-3 levels are associated with tumor burden indicators including tumor size and lymph node status(Park et al ,2008and Lian et al,2019) and patients with locally advanced breast cancer exhibit significantly higher levels of CEA and CA 15–3(Hashim,2014) . in another study it was found The higher levels of CEA and CA 15–3 are more common in patients larger tumor size(Shao et al, 2015).

Conflict of interest

The authors declare that they have no conflict of interest.

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