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Relationship between Bacterial Profile and Some Immunological Parameters in Women with Breast Tumors

A thesis

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By

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Dedication

To the prophet of peace "Mohammed" Peace and prayer be on him and his purified family.

To the eyes that stayed wakeful to help me go on in my dreams, to the heart and lips that kept praying so that my dreams come true, to my source of power and first teacher

my dearest father

To the heart that beats to give me hope, to the cuddle that gave me assurance, to the hands that suffered for my comfort, to the candle that lights my way, to the spring of

my life and my paradise … my precious mother

To my love, who encourage and support me my dear wife

To my dears, childern

To whose laughs make me happy and whose love gives

me power and support …my sisters and my brothers

To My Superviser ….Dr Frial Gemeel .

Ask Allah to protect them.

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Thamer . 2022

Summary

Breast diseases was one of the most common diseases in women and the malignant breast diseases is the leading cause of cancer-related death in development Countries with the most diagnosed cancer among women all over the world. Breast cancer in Iraq is the most common type of cancer Compared to other types of cancer. 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. And estimation the concentrations of CA 15-3 as diagnostic marker for breast cancer . As well as estimation the concentrations of TLR-4 .TLR-6 and IL12 in women with breast disease by Enzyme Linked Immunosorbent assay (ELISA) . Specimens(Breast tissue and blood) was collected (50 biopies and 100 serum samples) it was taken from women with breast diseases and 50 control involved (50 blood sample only) from healthy women (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 .

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 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. it was cultivated on blood agar &MacConkey media ,and then on selective media .and Identification for bacteria by microscopic ,biochemical test and confirmed by Vitek2 Compact System .The results appeared the percentage of malignant tumor (18%) and benign (82.%). The benign breast disease divided to the four group from total ratio in this study according to the histological examination involved

Fibrocystic change was 36 (43.90%) while less percentage Fibroadenoma was 28 (34.14%),and Granulomatous mastitis was 11 (13.41%) and other (Lipoma ,fat necrosis) was 7 (8.53%) .

The growth of bacteria 44 (88%) while no growth was 6 (12%).The most common bacteria present in tissue was *Pseudomonas florescence* was 14 (31.81%) then *Staphylococcus aureus* was 12 (27.27%) while less percentage 2 (4.54%) was *Enterococcus feacales*, Cancer antigen 15-3 were measured in the serum of women with breast tumors and healthy, the results appeared concentrations of CA15-3were significantly increased in patients(146.79U/ml) than control(94.17U/ml). CA 15-3 In patients serum it differed according to the type of breast diseases where it was mean higher in ductal and lobular carcinoma and Granulomatous mastitis compared with other benign breast diseases .

In this study also been measured TLR4 and TLR6 concentration serum and tissue . The results appeared mean of TLR4 and 6 concentrations in serum of patient was (4.58 , 2.11) ng/ml than control(4.18 , 1.58) ng/ml respectively , with significant differences in concentration of TLR6. The results of TLR4 concentrations also appeared serum higher than tissue in different tumor .while TLR6 concentrations were higher increased in tissue than serum with different tumor types .

The mean of IL12 concentration in serum of patient was 16.57 ng/L while higher in control was 22.02 ng/L with significantly(0.0002). The mean of interleukin 12 was higher in tissue compare with serum according to the types of tumors.

In this study the results found positive relationship between IL-12 and TLR6 in serum, while negative correlation between IL-12 and TLR4 significant at $p>0.05$. Correlation between IL-12 in serum and IL-12 tissue was negative.

Correlation between tissue IL12 and TLR6 was positive ,also positive correlation between IL12 tissue and TLR4 tissue . Correlation between TLR6

systemic and TLR4 serum was positive correlation .while correlation between TLR6 tissue and TLR4 tissue was negative correlation. correlation betweenTLR6 serum and TLR6 tissue was negative. There was positive correlation between TLR4 serum and TLR4 tissue.

The conclusion of this study found bacterial infection increased the concentration of toll like receptors 6(TLR6) in breast tumors

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.

List of Ab.reviations

Symbol	Description
APC	Antigen- presenting cells
BC	Breast cancer
Bcl-2	B-cell lymphoma 2
BFT	*Bacteroides fragilis* toxin
BHIB	Brain heart infusion broth
BRCA1	Breast related cancer antigen
CA15-3	Cancer antigen 15-3
CBE	Clinical breast examination

CD8	Cluster of differentiation
CD14	Cluster of differention 14
CEA	Carcinoembryonic antigen
C3H8C3	Glycerol
CagA	Cytotoxin-associated gene
CNF	Cytotoxic necrotiziong factor
DAMPS	Damage- association molecular pattern
DCs	Dendritic cells
DCIS	Ductal carcinoma in situ
DNA	Deoxyribose Nucleic Acid
D.W	Distilled water
ELISA	Enzyme-linked Immunosorbent Assay
ER	Estrogen receptor
EMT	Epithelial Mesenchymal Transition
EDV	Epstein-Barr Virus
G+ve	Gram positive
G_ve	Gram negative
Gm	Gram
H.pyloria	*Helicobacter pyloria*
HBV	Heptitis B virus
HCV	Heptitis C virus
HTLV-1	Human T-cell lymphotropic virus type 1
HHV-8/KSHV	Human herpes virus -8/Kaposis sarcoma Herpes virus
HPr	Human papilloma virus
Her2	Human Epidermal Growth Factor
HRP	horseradish peroxidase
HMGB1	High mobility group box 1
HR+	Hormon receptor +

IARC	International Agency for Research on Cancer
IFN-γ	Interferon - -Gamma
IL12	Interleukin 12
IDC	Invasive ductal carcinoma
ILC	Invasive lobular carcinoma
KIA	Kligler iron agar
LBS	Lipopolysaccharides –binding protein
LPS	Lipopolysaccharides
LCIS	Lobular carcinoma in situ
LRR	Leucine –rich repeat
MR-VP	Methyl red –Vogas Proskauer
MAPK	Mitogen-activated protein kinase
MRI	Magnetic resonance imaging
MYD88	Myeloid –Associated molecular patterns
MD-2	Myeloid differentiation 2
MUC-1	Mucine rich repeat
NK	Natural killer cell
ng/ml	Nanograms per milliliter
Nm	Nanometer
NF-kB	Nuclear factor- kapa chain activated B-cells
O .D	Optical density
PAMPS	Pathogen –associated molecular patterns
PRb	Retinoblastoma protein
PR	Preventive fraction
Pg/ml	Picograms per milliliter
Psi	Per square
Rs	Reference SNP(single nucleotide polymorphism)
ROS	Reactive oxygen species

RNOS	Reactive oxygen and nitrogen species
SD	Standard Division
SPSS	Statistical package for the social Science
STAT4	Signal Transducer And Activator Of Transcription 4
TGF- β	Transforming growth factor beta
TLR	Toll Like Receptor
TNF- -α	Tumor Necrosis Factor alpha
Th 1	T helper cell 1
Th 2	T helper cell 2
TDLU	Terminal duct lobular unit
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Breast cancer which is considered the second most common cancer in women all over the world, with an estimated 2.4 million cases reported in 2015, with the vast majority occurs in women compared to cases in men. Through 2005 to 2015, breast cancer has been the fifth leading cause of global cancer death. It was the most popular cancer for women in approximately 183 countries and the most frequent cause of cancer deaths in women in 115 countries or territories (Fitzmaurice *et al*., 2017).

Breast tumors includes malignant and benign tumors,and also in turn it was divided into many types according to the location of the tumor and the type of disease (Makki, 2015, Stachs *et al* .,2019) **.**

There has been a strong push in recent years to fully characterize the bacteria associated with different parts of the body under different health conditions because the human body is home to a large and diverse population of bacteria with traits that are both harmful and beneficial to health (Zhu *et al*., 2013). It was once believed that breast tissue was sterile and that it was difficult for the microbiota, which primarily resided in the gut and skin, to colonize it. This was understandable given that the nipple provides access for bacteria from the skin and mouth to the mammary ducts (Ramsay *et al*.,2004),. The existence of unique bacteria in the breast tissue was established by Urbaniak *et al*. in 2014.

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. (Banerjee *et al*., 2015). Breast cancer occurs as a result of acombination of factors and causes one of these causes are bacteria related to the occurrence of breast cancer(Enby, 2002). Concerning breast tumor, either benign or malignant, different bacterial strain were identified,such as *Bacillus spp.,*

Micrococcus luteus, Propionibacterium acnes,P. granulosum, Staphylococcus spp., S. saprophyticus,Streptococcus oralis, and S. agalactiae. In addition, *Enterobacteriaceae,Listeria welshimeri,* and *Pseudomonas spp.* were also found in breast tumor tissues taken from other women (Urbaniak *et al.,* 2014).

Many methods are used to diagnosis breat cancer one of these methods is cancer antigen detected, Cancer antigen 15-3 (CA15-3) is a protein made by a variety of cells, particularly breast cancer cells. And its levels are higher than normal in most women with breast cancer that has spread to other parts of the body (called metastatic breast cancer) (American Association for Clinical Chemistry.2013) . Cancer antigen 15-3 is an antigen expressed in benign and malignant breast ductal epithelium. It is a mucin belonging to a large family of glycoproteins encoded by the MUC 1gene, that are heterogeneously expressed on the apical surface of normal epithelial cell types, including those of the breast (Prabasheela and Arivazhagan,2011). CA 15-3 should be used in conjunction with diagnostic imaging, clinical history and physical examination. CA 15-3 is particularly valuable for treatment monitoring in patients that cannot be evaluated using existing radiological procedures(Duffy, *et al.*,2010) .

Toll-like receptors are expressed by immune cells (macrophages, dendritic cells, mast cells and eosinophils)as well as some epithelial cells (Ayala-Cuellar *et al*. ,2019). TLRs play a central role in the recognition of harmful molecules from invading microorganisms or internal tissue damage, activating specific transcriptional responses including the NF-κB, Mitogen-activated protein kinase and interferon regulatory factor pathways, which induced inflammation (McGettrick and O'Neill ,2010).

SemlaliI *et al*.2018 suggested a strong association between rs5743810 and protection against breast cancer risk in Saudi Arabian women, Importantly, the rs5743810 Pro allele could be a potential breast cancer diagnostic biomarker in this ethnic population.

Interleukins12 (IL-12) is secreted as an early pro-inflammatory cytokine in response to infections (Medzhitov ,2001). IL-12 plays a key role in the transition between innate immunity and adaptive immunity. IL12 and IL18 stimulates NK cells and T cells to produce IFN-γ, which activates macrophages to kill phagocytosed foreign substances including microbes. It also increases cytolytic activity by stimulating CD8 cells. the principal role of IL-12 in breast cancer is that, breast cancer induces a local IL12-dependent type I immune response likely directed towards tumor associated antigens (Carpi *etal* .,2009).

1.2 Aim of study

The study was aimed to survey the bacteria that present in breast tumor patients and its effect on serum and tissue level of TLR 4 , 6 and interleukin 12 .

To achieved the above aim, the following objectives were concluded .

1- Collect specimens (blood and tissue) from women with breast benign and malignant tumors . and blood only from healthy women as control group .

2- Isolations and diagnosis of bacteria by routine methods and confirmed by Vitec 2 compact system.

3- Estimation the serum level of CA 15-3 as diagnostic marker for breast cancer .

4- Estimation the levels of TLR-4 .TLR-6 . and IL12 by ELISA .

CHAPTER TWO

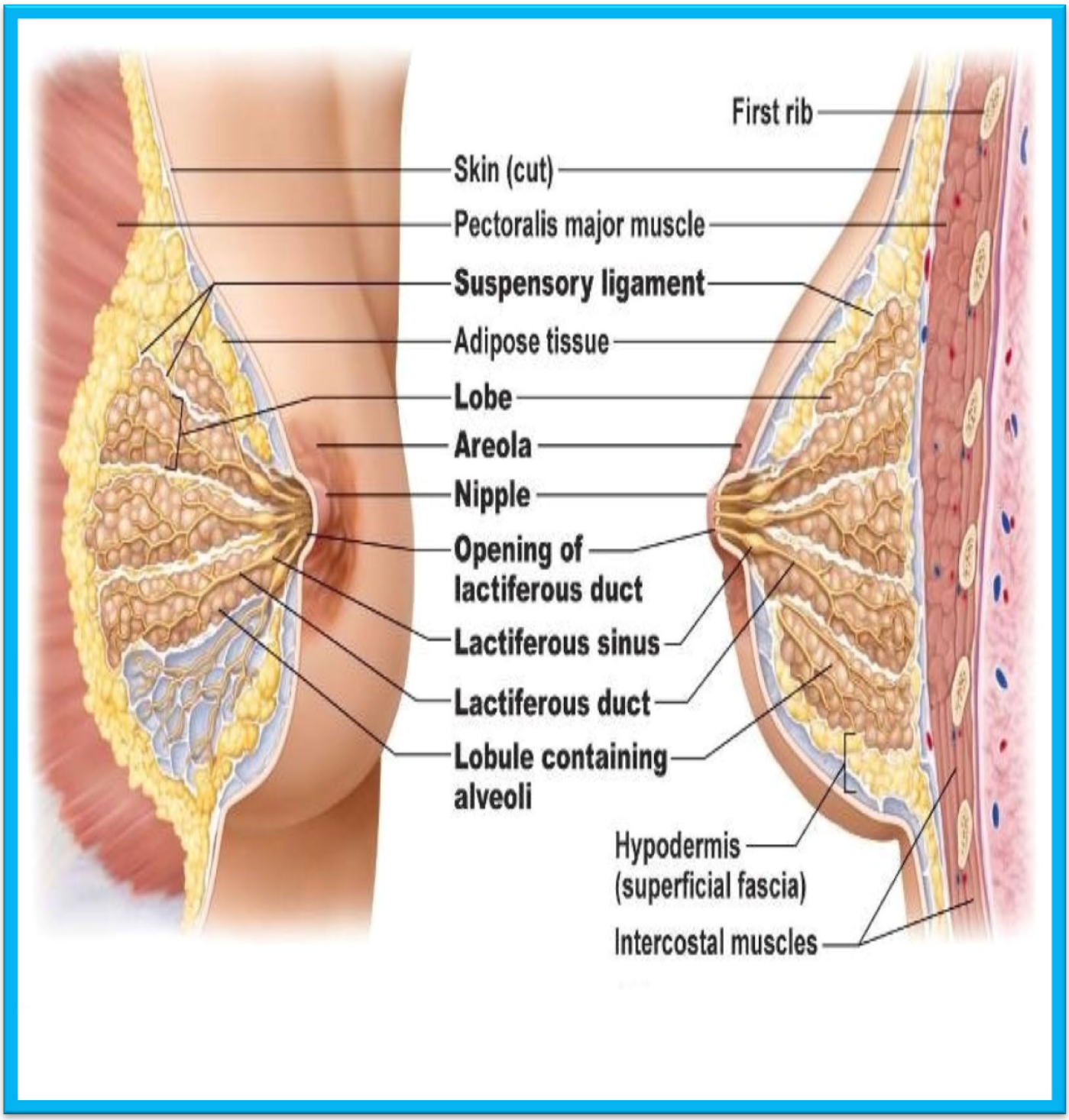
LITERATURES REVIEW

2-Literatures Reviews

2.1 **Breast Anatomy**

The mammary glands are modified sweat glands that are part of the skin. Each mammary gland is contained within a rounded skin-coveredbreast anterior to the pectoral muscles. Slightly inferior to the center of each breast is a pigmented area, the areola, which surrounds a central protruding nipple (Figure 2-1). Internally, each mammary gland consists of15 to 25 lobes that radiate around the nipple. The lobes are padded andseparated from one another by connective tissue and fat. Within each lobeare smaller chambers called lobules, which contain clusters of alveolar glands that produce milk when a woman is lactating. Milk produced by the alveolar glands exits each lobule by passing into lactiferous ducts, which open to the outside at the nipple. Just deep to the areola, each duct has a dilated region called a lactiferous sinus, where milk accumulates during nursing (Marieb and Keller, 2018) .

As well as the Breast contains blood and lymphatic vessels. Most lymphatic vessels within the breast lead to axillary lymph nodes, some also connect to supra- or infraclavicular nodes, and internal mammary nodes. They may enter lymphatic vessels and spread to lymph nodes. Beneath the tissues of the breast lie the muscles of the chest wall and between the two is the fascia (a layer of connective tissue). Two layers of suspensor ligaments (Cooper's ligaments) link the breast to the fascia, providing support. As these ligaments stretch with age or weight gain, the breast loses some of its firmness(Rehnke *et al*., 2018).



(a) (b)

Figure 2-1: Female mammary glands(breast) structure (a) Anterior view (b) Sagittal section (Marieb and Keller, 2018).

2.2 **Breast cancer**

Breast cancer is a group of diseases in which cells in the breast tissue divide and change uncontrollably, resulting in a lump or mass (American cancer society, 2019). Breast cancer can begin in a variety of locations within the breast (American cancer society, 2014).and most breast cancers begin in the lactiferous ducts (ductal carcinoma) that carry milk to the nipple. Some start in the cell of the lobules (lobular carcinoma), which are the milk-producing glands (American cancer society, 2015; Martini *et al*., 2018). There are also less common types of breast cancer, and a small number of cancers start in other tissues (fatty and fibrous connective tissues) in the breast, these cancers are called sarcomas and lymphomas and are not really thought of as breast cancers (American cancer society, 2014). Breast cancer is the most common cancer in women of all races and ethicities and leading cause of premature mortality among women, early detection is associated with reduced mortality (Tkaczuk *et al*., 2017). Even though breasts come in different sizes, they have approximately the same number of ducts and lobules, with adipose tissue causing the differences in size. Breast cancers are staged according to the size of the tumor, evidence of tumor spread to local lymph nodes, and evidence of distant metastases. Breast cancer spreads through the lymphatic system, so lymph nodes that drain the breast tissue are sampled to look for tumor cells (Martini *et al*.,2018)

2.2.1 Breast Cancer Incidence

The breast cancer was the most commonly diagnosed cancer throughout the world (Youlden *et al*., 2014). It is the second most common malignancy in women and the second leading cause of cancer death all over the world (Siegel *et al*., 2013). Importantly, one in eight women are predicted to be diagnosed with breast cancer during their lifetime, with 40,000 deaths annually (Jemal *et al*., 2010). In 2012 breast cancer among women worldwide was estimated

about 1.67 million (25% of all cancers) among new cancers diagnosed and it wasranked the fifth reason of woman's death from cancer. While in 2020 there were an estimated 2.3 million new breast cancer cases diagnosed constituting (11.7%) of all cancer cases in women and 685.000 (6.9%) breast cancer death worldwide, and surpassed lung cancer as the most commonly diagnosed cancer worldwide (I.A.R.C., 2020).

Incidence rates differ inthe world regions, with rates ranging from 27 in East Asia and Middle Africato 96 in Western Europe per 100000 (Ferlay *et al*., 2015). Although breast cancer incidence rates in the Eastern Mediterranean Region are substantially lower than rates in high-income countries in Europe and North America, an increasing trend is evident where data are available. The incident breast cancer case and deaths estimated number in the region in 2012 were 99300 and 42200, respectively. The number of breast cancer cases increased from 61000 in 2008 to 99300 in 2012; the increase was particularly notable in Bahrain, Egypt, Islamic Republic of Iran, Jordan, Kuwait and Qatar (WHO , 2017). In Iraq, breast cancer is considered the most common cancer (Alwan *et al*., 2017). There were 4529 cases in 2013 considered 4422 females and 107 males, the percentage of total constitute around 18.84% with rate 12.9 for each 100000 populations(Annual Report, Iraqi Cancer Registry ,2017) .

Sporadic breast cancer incidence is well linked to an age rise. The average age of breast cancer is more than 55 years in Western countries, however,in Iran the mean age is about ten years less than other similar socioeconomic regions, (Harirchi *et al.,* 2000).

Although breast cancer is supposed to be a disease of the developed world with higher incidence rates, in less developed countries the rates are low but increasing mainly due to changes in lifestyle leading to nearly 58% of cancer deaths (WHO, 2018). Survival rate of breast cancer is fundamentally different all over the world, while it is ranged >80% in North America, Sweden and

Japan, it accounts for roughly 60% in middle-income countries and <40% in low-income countries (Coleman *et al.,*2008).These differences in survival rates among countries can be attributed to several factors,such as genetics, ethnicity, socio-economical and environmental factors including nutrition, lifestyle, reproduction, the use of exogenous hormones, mammographic screening, and cancer treatment possibilities (Bray *et al*., 2004; Jemal *et al*., 2010) .

2.2.2 **Risk factors of Breast Cancer**

Cancer Most women with one or more breast cancer risk factors never develop the disease, whereas many women with breast cancer have no known risk factors. Some risk factors, such as a person's age or race, are unchangeable, while others are linked to cancer-causing factors in the environment or to personal behaviors (American cancer society, 2014). The main factors involved:

2-2-2- 1-**Sex**

Women are 100 times more likely to be diagnosed with breast cancer than men. The incidence of male breast cancer is less than 1 % compared to female breast cancer risk (Hiler *et al.,* 2016).

2-2-2-2- Age

The incidence of breast cancer and death rates generally increase with age until the seventh decade (American cancer society, 2019), but breast cancer tends to be more aggressive in younger people (Sariego, 2010).

2-2-2-3- Race and Ethnicity

Race is a very important intrinsic factor elevating the risk of occurrence of breast cancer (Kaminska *et al*., 2015). Population data from the surveillance, epidemiology, and end results (SEER) showed a higher rate of breast cancer diagnoses in white women when compared to black women. It has been

demonstrated that Caucasian race is an independent risk factor for breast cancer (Hiler *et al*., 2016).

2-2-2-4- Family history

Women with a family history of breast cancer (two or more cases in women younger than 50 years or three or more cases at any age) who are negative in terms of BRCA mutations are approximately 11 times more likely to develop breast cancer (Momenimovahed and Salehiniya, 2019). The history of early-onset breast cancer in immediate relatives is a risk factor for the occurrence of breast cancer in BRCA1 and BRCA2 carriers (Nardo *et al*., 2014). In Iraq, it has been reported that 16% had a positive family history (Alwan, 2010).

2-2-2-5- Breast Density

The risk of breast cancer increases with increasing breast density (American cancer society, 2019).

2-2-2-6- Genetic predisposition

Inherited pathogenic genetic variations in BRCA1 and BRCA2, the most breast cancer susceptibility genes, account for 5% -10% of all female breast cancers and 15% -20% of all familial breast cancers (Turnbull and Rahman, 2008; Tung *et al*., 2016).

2-2-2-7- Menstrual cycles

The risk of breast cancer rises with earlier menstruation and later menopause (American cancer society, 2019).

2-2-2-8- Endogenous Hormone levels

Breast cancer is more common in postmenopausal women who naturally have high levels of certain endogenous sex hormones (estrogen and

testosterone) and prolactin (Tworoger *et al*., 2013; Brown and Hankinson, 2015; Sampson *et al*., 2017).

2-2-2-9- Pregnancy

Pregnancy has a dual effect on breast cancer risk (Nichols *et al*.,2019). Having a first child before age 35 and having a greater number of children is associated with decreased risk of HR+ breast cancer (Lambertini *et al*., 2016).

2-2-2-10- Breastfeeding

The breastfeeding for a year or more slightly reduces a women's overall risk of breast cancer, for every 12 months of breastfeeding, the risk of breast cancer was lowered by 4% (American cancer society, 2019). In the United Kingdom around 3% of breast cancers were related to women breastfeeding every child for less than six months (Parkin *et al*., 2011).

2-2-2-11- Hormonal birth control

Oral contraceptives (combined estrogen and progesterone) use is linked to a 20% increase in breast cancer risk, especially among women who start using them before their first pregnancy (Bassuk and Manson, 2015; Morch *et al.,* 2017).

2-2-2-12- Radiation

Radiation exposure increase breast cancer risk in females between the ages of 10 and 30 who received high-dose chest radiation therapy, breast cancer risk starts to rise about 8 years after radiation treatment and continues to be elevated for more than 35 years (Schaapveld *et al*., 2015).

2-2-2-13- Ovulation-stimulating drugs

Using ovulation-stimulating medications for more than 6 months increases the risk of developing breast cancer (Taheripanah *et al*., 2018).

2-2-2-14- Alcohol and Tobacco

Alcohol consumption raises the risk of breast cancer in women by about 7% to 10% for every 10 grams (roughly one drink) consumed per day on average (Liu *et al*., 2015).Smoking, especially heavy, long-term smoking may increase the risk of breast cancer by a small amount (Gaudet *et al*., 2013; Gram *et al*., 2019).

2.2.3 Signs and symptoms of breast cancer

Breast cancer typically has no symptoms when the tumor is small and most easily treated; the most common physical sign is a painless lump. Sometimes breast cancer spreads to underarm lymph nodes and causes a lump or swelling, even before the original breast tumor is large enough to be felt. Less common signs and symptoms include breast pain or heaviness; persistent changes, such as swelling, thickening, or redness of the skin; and nipple changes, such as spontaneous discharge (especially if bloody), scaliness, or retraction most of these signs and symptoms in malignant tumors was less visible unlike to benign tumors that was more prominent (American cancer society, 2017; American cancer society, 2019).

2.2.4 Classification of Breast Cancer

Breast cancer classification systems have been based on histopathological assessment including histological type and grade. Expression of estrogen receptor (ER), progesterone receptor (PR) and over-expression and/ or amplification of the human epidermal growth factor receptor2 (HER2) have been included to refine classification. Molecular data arising from a variety of techniques including comparative genomic hybridisation, gene expression profiling, Sanger sequencing and massively parallel sequencing have been used to refine breast cancer classification (Vuong *et al*., 2014)

2.2.4.1 Classification of breast tumors according tumors type

Breast tumors are classified into two types, benign tumors, tumors that do not contain the ability to spread in adjacent tissues, and the second category are malignant tumors, tumors that have the capability to spread in different tissues of the body (Muss, 2000). Breast diseases, both benign and malignant, are common. Typically, young women present with more benign pathologies; however, breast malignancies can occur in young women, especially in those harboring mutations in the breast related cancer antigen (BRCA) genes, other inherited genetic syndromes associated with increased risk of breast cancer, or familial predisposition for breast cancer. In all women aged 40 and over presenting with abnormalities of the breast, a primary breast cancer should be ruled out because it is the leading cancer among women in developed countries.(Meisner *et al*.,2008).

2.2.4.1.1 Benign breast tumors

Benign breast diseases are a heterogeneous group of breast lesions, including epithelial and stromal proliferation, developmental abnormalities, inflammatory lesions and tumors. Breast tumors may show a variety of symptoms or may appear incidentally. the prevalence of benign diseases is starting to rise, whereas at age forty and beyond, it is actually increasing instead of declining. (Guray and Sahin, 2006).

The benign tumor inculde fibrocystic change ,fibroadenoma , granulomatous mastitis ,and other disease (Lipoma and Fat necrosis) groups of benign breast tumors divided by (Guray and Sahin, 2006; Stachs *et al* .,2019) to these totais as well as other groups.

Nonproliferative benign breast lesions include (1) inflammatory fat necrosis, which follows surgical or blunt trauma, and generally resolves automatically; (2) lymphocytic mastitis, which can be seen in diabetic patients;

and (3) granulomatous mastitis, related with foreign body reactions (e.g.,silicone and paraffin for breast magnification and reconstruction after cancer surgery), sarcoid, or certain infections. Other nonproliferative benign breast lesions appear as tumor-like processes, including (4) fibroadenoma, a very prevalent (~25% of women), usually solitary, sharply demarcated,soft lesion in younger age groups; (5) phyllodes tumor (usually known as cystosarcoma phyllodes); (6) intraductal papilloma, solitary lesions that may be accompanied with bloody nipple discharge; (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 should be aspirated with ultrasound guidance and, when the liquid is not clear(Goldman ,2020).

2.2.4.1.2 Malignant Breast tumors

Breast cancer is usually classified primarily by its histological appearance (Makki, 2015). Based on the size, shape, and arrangement of breast cancer cells (American cancer society, 2019). Adenocarcinomas, which constitute more than 95% of breast cancer, are the most common breast malignancies (Vinay *et al.,* 2010).Breast carcinoma is divided into in situ carcinoma (ductal and lobular) and invasive disease (Vuong *et al*., 2014). Both tumor types arise from the same segment of the terminal duct lobular unit (TDLU) (Makki, 2015) Classified on the basis of whether or not they have penetrated the limiting basement membrane: Those that remain within this boundary are referred to as in situ carcinomas, and those that have spread beyond this boundary are referred to as invasive or infiltrating carcinomas. The main forms of breast carcinoma are as follows in this classification:

A. Noninvasive: - are not invading into lymph vascular channels or stroma, and limited by a basement membrane, includes two types, Usually, both types occur from cells in the terminal duct lobular unit.

- Ductal carcinoma in situ (DCIS): - It has a wide variety of histological appearances, tends to fill and distort duct-like spaces. Architectural patterns are often mixed and include papillary, micropapillary, solid and cribriform types

- Lobular carcinoma in situ (LCIS): -It has a uniform appearance. The cells are monomorphic with a soft, round nucleus and appear in loosely coherent clusters within the lobules. Intracellular mucin vacuoles (sometimes forming bookmark ring cells) are common (Kumar *et al.,* 2013).

B. Invasive (infiltrating): - the tumor cells invade the breast stroma and have the potential to spread beyond the ducts or lobules (Li *et al.,* 2005). The distinctive histological patterns of invasive carcinoma subtypes are

- Invasive ductal carcinoma (IDC): - This type of cancer is usually associated with DCIS and rarely with LCIS. Most ductal carcinomas produce a demoplastic response that removes normal breast fat and forms a hard, palpable mass. (Kumar *et al,* 2013). It is the main histological type in Iraq (Alwan, 2010).

- Invasive lobular carcinoma (ILC):- Appear in the milk producing glands at the terminal ends of the ducts, representing around 10% of all breast cancer cases. It consists of cells identical in morphology to those of LCIS cells. Two thirds of the cases are related to the adjacent LCIS. Cells invade stroma individually and are often aligned in single-strands or chains. This growth pattern is correlated with the presence of mutations that abrogate the function of E-cadherin, a surface protein that contributes to the cohesion of normal epithelial breast cells (Arpino *et al*., 2004). Other types : Medullary carcinoma,

Inflammatory carcinoma, mucinous carcinoma and Tubular carcinoma (Kumar *et al.,* 2013).

2.2.5 Breast tumors Diagnosis

The primary issues in the diagnosis of breast cancer in women are detection, the staging and monitoring of cancer, and accurate detection of the disease (Ng *et al*., 2013). Diagnosis of breast cancer involves: clinical breast examination, mammography, magnetic resonance imaging, meedle core biopsy, surgical (open biopsy), prognostic tumor markers.

2.2.5.1 Clinical breast examination

To detect breast abnormalities, clinical breast examination is used (CBE), particularly palpation, it remains an extremely useful and practical technique contribution to the early detection of breast cancer, whether carried out by the physician or by the patient herself (Saslow *et al*., 2004).

2.2.5.2 Mammography

Mammography is a method of examining the internal structure of the breast which uses a low amount of radiation to produce detailed images (American Cancer Society , 2018).

2.2.5.3 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) to create cross-sectional, very detailed images of the body by using magnetic fields instead of x-rays. MRI it used to tests for breast imaging, material is injected into a small seam in the arm during or before (American Cancer Society , 2012).

2.2.5.4 Core Needle Biopsy

A core needle biopsy provides accurate diagnostic information for malignancy and grade and can be used for rational treatment planning. Largeneedle used for a core biopsy to sample breast changes which are felt by the pinpointed ultrasound or mammogram or doctor (Sauer *et al*., 2005).

2.2.5.5 Surgical (open biopsy)

Rarely, surgery needs to remove part or all of the lump for microscopic examination, this is referred to as an open biopsy or a surgical biopsy. Most often, the surgeon removes the abnormal area or entire mass, also the close margin of normal breast tissue, this is called an excision biopsy. Only part of the mass may be removed, if it is too big to be removed easily, is called an incisional biopsy (American Cancer Society , 2012).

2.2.5.6 Prognostic tumor markers

There are certain prognostic (predictive) tumor markers for diagnosis of breast cancer that have been generally accepted as most significant, including: the primary human ER2, human fibroblast growth factor, and TGF-beta receptor homodimers (HER2) (Colombo *et al*., 2010), β-Catenin (Li *et al*., 2013), Kiel-67 (Ki-67) protein (Pavlakis *et al*., 2012), Vascular endothelial growth factor (VEGF) (Rachner *et al*., 2013), Carcinoembryonic antigen (CEA) and cancer antigens (CA15-3, CA27, CA 29, and CA549) (Dalamaga *et al*., 2013).

2.2.6. Microorganisms and cancer

The microbiome that inhabits human gastrointestinal tract and other anatomic sites can be considered environmental factors to which they are constantly exposed at high doses throughout their life (Weinstock, 2012; Goodrich *et al*., 2014).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). Regardless of its protective role against diseases, the microbiota has been associated with the

development and progression of cancer at different body regions including lung, liver, stomach, colon, and skin (Wang *et al*., 2016).

The microbiota could boost cancer by many mechanisms inducing chronic inflammation, changing the balance of host cell proliferation and apoptosis, and activating uncontrolled innate and adaptive immune responses (Schwabe and Jobin, 2013). Cancer was shown to originate from an infectious agent by injecting, for instance, cell-free filtrate taken from chicken sarcoma into a second fowl, which in turn produced a fresh tumor after injection (Rubin,2011). Nearly in the last three decades, substantial evidence has linked human cancer to several infectious agents, especially viruses (Parkin, 2006). It is estimated that 15-20% of cancers are caused by infectious agents (De Martel *et al.,* 2013) .

According to the International Agency for Research on Cancer (IARC), certain microorganisms have been proved to be capable of causing cancer development, these organisms include at least 6 virus species, 4 helminthes species and 1 bacterium species. The viruses involve: human papillomavirus (HPV), Epstein-Barr virus (EBV), human herpes virus 8/Kaposi's sarcoma herpes virus (HHV-8/KSHV), human T-cell lymphotropic virus type I (HTLV-1), hepatitis B virus (HBV) and hepatitis C virus (HCV). The helminthes species include *Schistosoma haematobium* and *S. japonicum*, Opisthorchis viverrini and Clonorchis sinensis. Finally, the bacterium was *H. pylori* (Table 2.1) (Kutikhin *et al.,* 2012). Approximately 15% of cancers registered in 2012 were caused by carcinogenic infections, e.g. *H. pylori*, HPV, HBV, HCV, and EBV (Plummer *et al*., 2016; WHO, 2018).

Table (2.1) Organisms confirmed to causes infection –associated malignancies in human(Kutikhin *et al.,* 2012)

Agent	Cancer	% of all cancers
HPV	Cervix	5.2
	Ango-gential	
	Mouth,pharynx	
HBVand HCV	Liver	4.9
EBV lymphoma	Nasopharynx	1.0
	Hodgkin lymphoma	
	Burkit	
HIV/HHV-8	Kaposi sarcoma	0.9
	Non-Hodgkin lymphoma	
Schistosomes	Bladder	0.1
HTLV-1	adult T-cell leukaemia/lymphoma (ATL)	0.03
Liver flukes	Liver	0.02
Total	/	17.8

2.2.7. Bacteria and cancer

Since 1890, when the pathologist William Russell illustrated "a characteristic organism of cancer," a small group of scientists have claimed that cancer is caused by bacteria, but not viruses only. Their reports demonstrated an uncommon microbe that could be seen microscopically in cancerous tissue and cultivated from cancer tissue and blood (Cantwell, 2012). , In 2013,Cummins found that certain bacteria were isolated constantly from cancer tissue (Cummins and Tangney ,2013). Associations between different bacteria and various tumours have been reported in patients for decades.

Studies involving characterisation of bacteria within tumour tissues have traditionally been in the context of tumourigenesis as a result of bacterial presence within healthy tissues, and in general, dogma holds that such bacteria are causative agents of malignancy (directly or indirectly). While

evidence suggests that this may be the case for certain tumour types and bacterial species, it is plausible that in many cases, clinical observations of bacteria within tumours arise from spontaneous infection of established tumours. Indeed, growth of bacteria specifically within tumours following deliberate systemic administration has been demonstrated for numerous bacterial species at preclinical and clinical levels. The available data on links between bacteria and tumours, and propose that besides the few instances in which pathogens are playing a pathogenic role in cancer, in many instances, the prevalent relationship between solid tumours and bacteria is opportunistic rather than causative, and discuss opportunities for exploiting tumour-specific bacterial growth for cancer treatment . (Cummins and Tangney ,2013).

Previous studies found the bacteria associated with different parts of the body under different health condition. It has been shown that bacteria are able to reside in tumors upon systemic administration, causing increased replication locally ((Hooper ,2004; Morrissey *et al*., 2010 ; Zhu *et al* .,2013).

This was initially proved after intravenous administration of *Clostridium spp*. and has established with many other bacteria, such as *Salmonella bifidobacterium*, *E. coli, Vibrio cholera* and *Listeria monocytogenes* (Toso *et al*., 2002; Baban *et al*.,2010). It has been suggested that the presence of bacteria within tumors might be because of infection coming from the vascular system and due to their capability of survival and growth in the presence of nutrients within the tumor hypoxic area at later stages of cancer. It has been proposed that translocation of bacteria from the gastrointestinal tract may be an event that takes place in healthy individuals indicating a normal physiological occasion with no detrimental results (Duong *et al*.,2019).

Now, it is well-known that human indigenous microbes (microbiota) which contain mutualistic or pathogenic, transient or residential microbes (mainly bacteria) that normally inhabit human tissue surfaces (e.g., the

gastrointestinal tract lumen) may play an essential role in cancer risk (Blaser, 2006; Dethlefsen *et al*., 2007). In addition, it has been shown that many pathogenic microbes either indigenous or exogenous (foreign) can induce cancer development (Butel, 2000).

According to IARC*, H. pylori* is one among several microorganisms that have been proved to be able to cause cancer. In addition, other bacterial spp. that can also be potential etiological agents of cancer include: *H. bilis, H.hepaticus, Campylobacter jejuni, Fusobacterium varium, enteropathogenic E. coli, enterotoxigenic Bacteroides fragilis, B.vulgatus, Prevotella.spp., Streptococcus bovis and S. anginosus, Treponema denticola, S.Typhi, S. Paratyphi, S.Typhimurium, Borrelia burgdorferi, Bartonella spp.,Mycobacterium tuberculosis, Chlamydia pneumoniae, C. trachomatis, C. psittaci, Neisseria gonorrhoeae, Propionibacterium acnes and Tropheryma whippelii* (Kutikhin *et al*., 2012).

2.2.8. Bacteria and Breast cancer

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 considering that skin and oral bacteria have access to the mammary ducts through the nipple (Ramsay *et al*.,2004),in some studies suggesting their source to be from the mother's gastrointestinal tract (Donnet-Hughe *et al*.,2010). Looked at the nutrient-rich fatty composition of the female breast, the widespread vasculature and lymphatics, and the diffuse location of the lobules and ducts leading from the nipple, bacteria would be widespread within the mammary glands, irrespective of lactation. Urbaniak *et al.*, 2014 confirmed that the bacteria in breast tissue are unique. Concerning breast tumor, either benign or malignant, different bacterial strain were identified,such as *Bacillus spp., Micrococcus luteus, Propionibacterium acnes,P.granulosum, Staphylococcus spp., S. saprophyticus,Streptococcus oralis, and S. agalactiae.*

In addition, *Enterobacteriaceae,Listeria welshimeri,* and *Pseudomonas spp.* were also found in breast tumor tissues taken from other women (Urbaniak *et al.,* 2014). In spite of what mentioned above, the presence of bacteria at the site of tumor is not always implicated as a cause of cancer, such as in the case of cystic fibrosis patients with bacterial infection, which might not be the cause of this disease. On the contrary, the original cell transformation can arise many years before the appearance of cancer and the infection might be disappeared long before its effects were observed (Lax and Thomas, 2002) .

2.2.9. Mechanisms of bacterial replication within tumors

It has been suggested that bacteria might leave the gastrointestinal tract at very low numbers, and are normally and promptly eradicated by the immune system. Nevertheless, the replication of bacteria within tumors leads to dramatic rise in bacterial numbers within a limited area. While presence of bacteria in specific tumors is related to the development of that cancer, in many other cases, bacterial presence within tumors could suggest regional infections of already existing cancerous tissue (Mager, 2006; Alzahrani *et al.*, 2014).There are many factors suggested to be involved in the replication and survival of bacteria within tumors, these include:

1. The hypoxic nature of many solid tumors, this is considered as the key mechanism. This mechanism causes reduction in oxygen levels in tumor tissues relative to normal ones, offering a unique environment for growth of anaerobes and facultative anaerobic bacteria (Wei *et al*., 2008;Morgun *et al*., 2015; Dzutsev *et al*., 2017).

2. The availability of nutrients, e.g. purines, for bacteria within the necrotic region. This factor contributes to bacterial replication in the tumor (Baban *et al*., 2010; Cummins and Tangney ,2013).

3. Bacterial chemotaxis towards chemo-attractant compounds (e.g. citrate, aspartate, serine, galactose or ribose) found in necrotic regions and produced by

quiescent cancerous cells. This has also been proposed as a contributing factor (Baban *et al*., 2010; Bavle and Hosthor,2016).

4. Aberrant neovasculature and local immune suppression cancer cells can secrete anti –inflammatory such as IL10 and TGF-B that suppres immune response , these are elements thought to be important for tumor-specific bacterial replication (Baban *et al*., 2010; Cummins and Tangney ,2013).

5. Different mechanisms are utilized by tumor cells to evade recognition by the immune system the antigenicity of tumors cells was lower,therefore can not induce strength immune response againt them ,in addition the similarity between autigens of tumor cells ,and self-antigens of the human body cells ,as well as the tumor cells can secrete factors or proteins acts as aphysical barrier that minimum or prevent their recognition by immune cells (Kenneth *et al*., 2012) leading to inadequate immune response within the tumor, possibly providing a protection for bacteria against immune clearance, not present elsewhere in the body (Bermudes *et al*., 2002; Rea *et al*., 2018).

2.2.10. Effects of bacteria on cancer

2.2.10.1 Protective role against cancer

Microbiota has been suggested to have a protective role against breast cancer, and are essential for sustaining the healthy growth of breast and its immune development (Donnet-Hughes *et al*., 2010; Urbaniak *et al*., 2012). In addition, bacteria inhabiting the breast tissue have been shown to produce antioxidants that neutralize free radicals (Hieken *et al*., 2016; Urbaniak *et al*., 2016). These advantages of bacteria to breast health have been approved by many authors who correlated the antibiotics use with increased breast cancer risk (Velicer *et al*., 2004; Sergentanis *et al*., 2010). A number of studies have shown an association between tumor regression and bacterial infection. It was found that lung cancer patients who later acquired empyema had substantially improved survival rate after 5 years in comparison with uninfected patients

(Matsutani *et al.,* 2018). Microbiota of gastrointestinal tract has been suggested to prevent carcinogenesis through the biological activities (Meng *et al*., 2018).

In many cases, the predominant correlation between bacteria and solid tumors is opportunistic instead of causative. Many chances are available for cancer treatment by exploiting tumor-specific bacterial growth (Cummins and Tangney, 2013). Numerous natural and genetically modified non-pathogenic bacterial spp. Have been exploited as possible anti-cancerous agents since Coley's work. These bacteria work either directly as tumoricidal or indirectly through delivering tumoricidal molecules.

Importantly, live attenuated or genetically modified non-pathogenic bacteria have been shown to multiply selectively in tumors inhibiting their growth , bacterial toxins and enzymes have played a role in tumor treatment policy (Patyar *et al*., 2010).

The important mechanisms by which bacteria or their products could induce carcinogenesis involve: chronic infection, immune evasion and immune suppression (Kuper *et al*., 2000; Al-Hilu and Al-Shujairi 2020).

1. Chronic infection: Several bacteria have been shown to cause chronic infections or produce toxins that disrupt the cell cycle leading to cell growth alterations (Koyi *et al*., 2001; Kocazeybek, 2003; Parimon *et al.,* 2007). Chronic inflammations have been confirmed by many researchers to induce DNA replication and cell proliferation via activation of cyclin D1 and mitogen activated protein kinase (MAPK) pathways, and also increase transformation of cells as well as the rate of tumor development due to increasing the rate of genetic mutations (Coussens and Werb, 2002).

Chronic infection is frequently accompanied by the formation of reactive oxygen species (ROS) or reactive oxygen and nitrogen species (RNOS) by phagocytes at the infection site. The ROS and RNOS possibly cause damage to DNA, proteins and cell membranes, and alter gene expression and enzyme activities promoting carcinogenesis (Lee *et al .,* 2009).

It has been proposed that the inflammatory environment might act as a tumor initiator through oxidative stress induction, which encourages DNA damage (Touati, 2010). Some bacterial toxins, such as cytotoxic necrotizing factor 1(CNF1), cytotoxin- associated gene (CagA) and *Bacteroides fragilis* toxin (BFT), affect on important eukaryotic processes, e.g. cellular signaling and cell death. Therefore, these toxins and other not well-known toxins imitate tumor promoters and carcinogens (Dobrindt *et al*., 2015).

In addition, many pathogenic intracellular bacteria causing chronic infection have been demonstrated to impose a threat to host cell signaling pathways, increasing pathogen survival. The control of these signaling factors is fundamental to the inhibition or development of tumor formation (Lax, 2005).

2. Immune evasion: The immune system is the fundamental line of defense against cancer formation (Schoppmann *et al.,* 2002). Many strategies are developed by tumor cells to evade the immune system of the host, and different cellular and molecular means in charge of tumor evasion have been recognized. Some of these mechanisms affect immune anti-tumor effector cells, and dysfunction and apoptosis of these cells generates an immune imbalance, which is unable to be cured using immunotherapies that just activate protein immune responses against tumor (Whiteside, 2006).

Numerous infections caused by intracellular pathogens can suppress apoptosis mainly via altering the Bcl-2 family proteins expression or through inactivation of retinoblastoma protein (pRb) (Lara-Tejero and Galán, 2000; Nougayrede *et al.*, 2005).This action provides a place where intracellular pathogen can survive despite the efforts of the host immune system to destroy the infected cells by apoptosis. Consequently, it permits the incompletely transformed cells to avoid apoptosis process and, therefore, proceed to a higher level of transformation, turning into tumorigenic in the end (Parrino *et al.*, 2007).

3. Immune suppression: There are many mechanisms by which tumor cells can directly induce immune response suppression, including cytokines

secretion, modulating the function or killing immune cells via surface receptors of tumor cell. In addition, immune suppression can occur when the tumor develops from the hematopoietic tissues and disrupts the normal function of the bone marrow (Pardoll,2012). Pathogenic microorganisms including bacteria could induce immunosuppression, and subsequently reduce immune surveillance (Magnusson *et al*.,2001). Infectious agents have been suggested to inhibit tumor suppressors directly leading to cell transformation (Kuper *et al*., 2000).

2-3. Immunological parameters

2-3-1 Cancer antigen 15-3

Cancer antigen 15-3 (CA15-3) is a protein made by a variety of cells, particularly breast cancer cells. The protein moves into the blood, where it can be measured. CA15-3 levels are higher than normal in most women with breast cancer that has spread to other parts of the body (called metastatic breast cancer) (American Association for Clinical Chemistry.2013) . Cancer antigen 15-3 is an antigen expressed in benign and malignant breast ductal epithelium. It is a mucin belonging to a large family of glycoproteins encoded by the MUC 1gene, that are heterogeneously expressed on the apical surface of normal epithelial cell types, including those of the breast (Prabasheela and Arivazhagan,2011).

Not all types of breast cancer will cause CA 15-3 levels to rise, as some types of cancer cells don't over-produce the antigen.CA 15-3 is a tumour marker that is used to check how breast cancer treatment is working and look for cancer that has come back, or recurred, after treatment. If you are diagnosed with breast cancer that has spread to other parts of the body, or metastasized, you may have this test, along with other tests such as hormone receptor testing and HER2 status testing. CA15-3 is not measured for early stage breast cancer

because the levels of this protein are rarely higher than normal at this stage. (American Association for Clinical Chemistry.2013).

Cancer antigen15-3 which detects soluble forms of MUC-1 protein is the most widely used serum marker in patients with breast cancer. Its main use is for monitoring therapy in patients with metastatic disease. For monitoring therapy, CA 15-3 should be used in conjunction with diagnostic imaging, clinical history and physical examination. CA 15-3 is particularly valuable for treatment monitoring in patients that have disease that cannot be evaluated using existing radiological procedures(Duffy *et al.*,2010) .

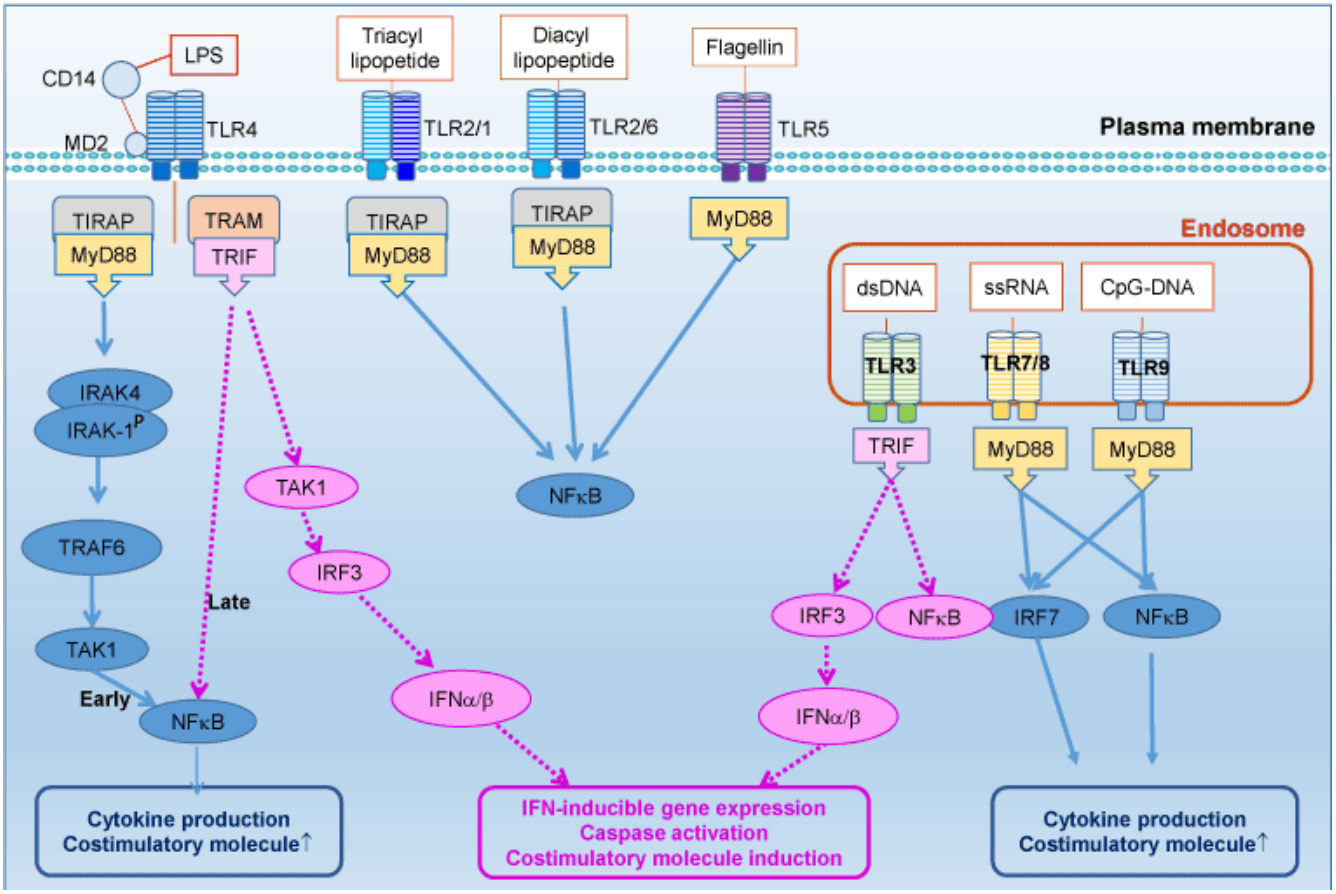
Healthy women are expected to have CA 15-3 assay values below 30U/ml (Nikhil *et al.*,2014). The upper limit of the range varies depending on the laboratory and kit used for the test. The elevation of CA 15-3 (values over 120kU/L) is found in over 30% of breast cancer patients with advanced disease(Bast *et al.*,2001). Serum CA 15-3 has been extensively studied mainly to monitor the response of breast cancer to the treatment or to detect early relapse in breast cancer follow-up (Chu and Ryu,2016).

Cancer antigen 15-3 may also be used in the postoperative surveillance of asymptomatic women who have undergone surgery for invasive breast cancer. In this setting, serial determination can provide median lead-times of 5-6 months in the early detection of recurrent/metastatic breast cancer. It is unclear however, whether administering systemic therapy based on this lead-time improves patient outcome. Serum markers in breast cancer are helpful for clinicians in providing more effective management of the disease. To this end, different markers have been proposed in the last years. In particular, Carcinoembryonic antigen (CEA) and MUC1 (CA 15-3) are the most widely used and investigated in the breast cancer follow-up period. Initial studies indicate that CA 15-3 is abnormal in the majority of patients with metastatic breast cancer and the antigen levels are correlated with changes in the clinical

status of breast cancer patients. However, CA 15-3 is not recommended as screening tool in early detection for breast cancer, even though it remains an important asset to monitor the efficacy of medical therapies after surgery(Shao *et al*,2015) .

2.3.2 Toll Like Receptors (TLR)

Toll-like receptors (TLRs), a family of pattern-recognition receptors of innate immunity system, are Type I transmembrane receptors that protect the host against pathogen infections (Wieck *et al* .,2016). Immune cells (macrophages, dendritic cells, mast cells, and eosinophils) as well as some epithelial cells express toll-like receptors (Ayala-Cuellar *et al*. ,2019).TLRs are defined by a cytoplasmic Toll/IL-1 receptor (TIR) domain and an extracellular leucine-rich repeat (LRR) domain. Toll-like receptors (TLRs) are one of the pattern recognition receptors(PRRs) expressed by various immune cells. However, they were first discovered in Drosophila common (Drosophila common) as important genes/proteins in embryonic development and ventral dorsal/polar body modeling, to date, 13 different types of TLRs (TLR1-TLR13) have been discovered and described in mammals since the first discovery of TLR4 in humans in late 1997. The discovery of TLR4 in humans has revolutionized the field of innate immunity and thus host and immunology and host- pathogen reaction (Vijay , 2018). , which can be classified into two subgroups according to their subcellular localization. TLR-3, 7, 8, and 9 are located mainly in intracellular endosome, whereas the others are distributed on the cell surface(Sun *et al*.,2016) (Figure 2-2).



Figure(2.2.) TLRs and their signaling pathways (Sun *et al*.,2016)

Toll like receptors recognized pathogen-associated molecular patterns (PAMPs), highly conserved components derived from bacteria, fungi, parasites and viruses, to prevent pathogens invading. Each TLR can recognize specific ligand, TLRs can also recognize endogenous danger-associated molecular patterns (DAMPs), which might be released in cancer development to activate inflammatory pathways (Zhao *et al*.,2014).

2.3.2.1 TLRs and cancer

Inflammation, as a new "hallmark of cancer", has been implicated in cancer progressions (Schwertfeger *et al* .,2015). TLRs regulate tumor immune responses by controlling the suppressive function of regulatory T (Treg) cells and through innate immune responses mediated by other immune cells. TLR signaling plays an important role in tumor cell proliferation, local invasion,

immune evasion, and distant metastasis. Increasing evidence shows that engagement of TLRs can enhance cancer cell progression, induce evasion of immune surveillance, and induce tumor chemoresistance and metastasis. Surprisingly, the activation of some TLRs, however, can induce the tumor cell apoptosis, or even inhibition of tumor growth. therefore, the role of TLRs in cancer development is complex.(Yang *et al*.,2014) .

The activation of TLRs results in the production of biological factors which drive inflammatory responses and activate the adaptive immune system. Several studies indicated that persistent inflammatory environment conditions can cause cancer formation and metastasis, due to production of chemokines and cytokines (Takeuchi *et al* .,2015). TLR signaling is through two pathways: MyD88-dependent and MyD88-independent pathways. The transcription factor NF-kB is an important tumor-promoting signaling pathway. TLRs signaling can intrinsically and extrinsically upregulate the well-known tumor-promoting inflammatory cytokines through NF-kB-dependent pathways including interleukin-6(IL-6), IL-1b and tumor necrosis factor a (TNF-) (Dabagh-Gorjani *et al*.,2014). These cytokines promote cancers in the intestine, stomach, skin and liver. TLR-induced NF-kβ activation promotes tumor cell survival in colon cancer (Luddy *et al* .,2014), lung cancer and prostate cancer. Tumor cells bearing TLRs and TLR signaling can promote tumor progression and immune evasion (Bhattacharya *et al*.,2012). It's considered as one of the chronic inflammation mechanisms in tumorigenesis and progression. In addition, in tumor cells, TLR4 mediates resistance of tumor cells to cell death induced by cytotoxic T lymphocytes and leads to tumor progression in vivo.(Sun *et al* .,2016).

2.3.2.2 TLRS expression and function in breast cancer

Toll like receptors play a role in both breast cancer cells and the microenvironment. TLRs are mainly expressed in macrophages, dendritic cells and other innate immune cells. Intriguingly, others have found that some TLRs are highly expressed in breast cancer cells (Bhatelia *et al*.,2014). TLR4 plays important roles in the migration of cancer cells (Mehmeti *et al* .,2015). TLR4 prompts human breast cancer cells invasiveness and leads to induction of pro-inflammatory and chemokine genes. Clinical studies showed a significant association of high TLR4 expression with lymph node metastasis and local cancer proliferation (Yang *et al*.,2014). The activation of TLR4 has been reported to regulate the expression of integrin αvβ3, and hence to promote the αvβ3-mediated adhesion and invasiveness of metastatic breast cancer cells (Liao *et al*.,2012).

Toll like receptor 4 Knockdown was impairs the proliferation and survival of breast cancer cells and reduces IL-6 and IL-8(CXCL8) , TLR4 antagonist can block invasiveness and migration of breast cancer cells (Yang *et al*.,2014). In contrast, HMGB1 acts as an endogenous TLR4 ligand to induce carcinogenesis (Chen *et al*.,2015). The toll-like receptor (TLR) pathways play a crucial role in breast cancer development. In particular, TLR signaling pathways promote survival, apoptosis and proliferation, as well as interferon (IFN), cytokine, and chemokine production. There is strong evidence showing that infectious agents activate TLRs to promote the progression of breast cancer and other cancers (Jouhi *et al* .,2014).

Signaling has also been shown to regulate cell death and increase expression of the anti-apoptotic proteins. The specific role of TLR mediated cellular process like growth, migration and resistance/sensitivity to death is emerging in the breast cancer. Increasing evidence suggests that the neoplastic process may

impede TLR signaling pathways to favor cancer progression (Matijevic and Pavelic . 2010).

2.3.2.3 Toll Like Receptor 4 (TLR4)

Toll like receptor 4 was one of the family of Toll like receptors on the cell surface and effectively recognizes LPS, or endotoxin, expressing on gram-negative bacteria with the help of CD14, MD-2 and the accessory protein LBP (LPS-binding protein) and then can be recognized by the body of the innate immune system. Then causing downstream signal transduction pathways and stimulating the body to generate an immune response cause a strong inflammatory response and metabolic changes (Zhao *et al*.,2014 and Lee *et al*.,2016).

It is the first TLR to be discovered in humans, and is one of the most conspicuous members of the TLR family, expressed by both immune and non-immune cells (Kawai and Akira ,2010) .TLR4 plays an important role in the innate immune system; as a switch of the inflammatory response, TLR4 can maintain the body's normal defense function of foreign pathogens. However, its over expression will cause a series of inflammatory response, resulting in the damage of tissue and organs (Wang *et al*.,2015).

Lipopolysaccharide (LPS) which can activate TLR4 is a main toxic component in gram-negative bacterial cell wall. It has extensive biological effects on the body and it also can induce inflammation (Moniruzzaman *et al* .,2016).

Toll like receptor 4 signaling can help tumor not only escape immune surveillance but also enhance tumor cell metastasis (Ma *et al* .,2014).in addition TLR4 is abundantly expressed by both cancer cells and immune cells in the tumor microenvironment (Ahmed *et al* .,2013b).

2.3.2.4 Toll Like Receptors 6(TLR 6)

Toll like receptor 6 is one of the Toll like receptors family are located mainly on the cell surface ,was enhances its capacity of recognizing various bacterial components including lipopeptides, peptidoglycan and lipoproteins of gram-positive bacteria and mycoplasma in conjunction with TLR2 (Wang *et al* ., 2015). TLR1 and TLR6 form heterodimers with TLR2, which are crucial in mucosal immune response regulation (Morgan *et al*.,2014) .TLR6 functionally interacts with TLR2 to mediate the cellular response to bacterial lipoproteins and activate the NF-κB pathway and inflammatory events, and consequently, may contribute to tumor development and progression (Hong *et al*.,2010).

Chan *et al*.,(2012) shown that a variant of TLR6 rs13281615 was associated with increased BC risk in the Chinese population . The TLR6 SNPs rs3796508 and rs5743810 have been reported in different types of diseases, but not in any malignancies. SemlaliI *et al*.2018 suggested a strong association between rs5743810 and protection against breast cancer risk in Saudi Arabian women. Importantly, the rs5743810 Pro allele could be a potential breast cancer diagnostic biomarker in this ethnic population .

2.3.3 Cytokines

Cytokines are low-molecular-weight proteins that mediate cell-to-cell communication. Immune and stromal cells, such as fibroblasts and endothelial cells, can synthesize them and they regulate proliferation, cell survival, differentiation, immune cell activation, cell migration, and death (Zamarron and Chen, 2011). Cytokines can contribute to carcinogenesis, depending on the stage of tumor development and the balance of different inflammatory mediator. cytokine have pro- or anti-tumor roles (Landskron *et al*., 2014).

2.3.3.1 Cytokines and cancer

Cytokines were discovered as secreted proteins, that regulates different immune functions, these functions extend to many other aspects of biology, including cancer (Arango Duque and Descoteaux., 2014). Mechanistically connection has been established between cancer and inflammation. At least 20% of all cancer developed in related with infection and chronic inflammation even those cancers that do not arise as consequence of chronic inflammation exhibit extensive inflammatory infiltrates with high levels of cytokine expression in the tumor microenvironment are several such cytokines were found to serve as growth and survival factors that act on premalignant cells, stimulate angiogenesis, tumer progression and metastasis, and also maintain tumor-promoting inflammation (Grivennikov *et al.,* 2010).

The term interleukin (IL) has been used to describe a number of secreted molecules produced by leukocytes that initiate a response by binding to high affinity receptors located on the surface of cells, act in a paracrine or autocrine fashion rather than as an endocrine signal. The response of a particular cell to these cytokines depends on the ligands involved specific receptors expressed on the cell surface and the particular signaling cascades that are activated, also ILs can exert both inflammatory and anti-inflammatory actions (Brocker *et al*., 2010).

Interleukins are a large group of immunomodulatory proteins that elicit a wide variety of responses in cells and tissues. These cytokines comprise a large number of the known immunological 'second-messenger' molecules within mammals , Interleukins initiate a response by binding to high-affinity receptors located on the surface of cells; ILs act in a paracrine or autocrine fashion, rather than as an endocrine signal, which is more common with steroidal and amino acid-derived hormones. The response of a particular cell to these cytokines depends on the ligands involved, specific receptors expressed on the cell

surface and the particular signalling cascades that are activated. ILs modulate growth, differentiation and activation during an immune response therefore these distinguishes them from chemokines -- the main function of which is to direct immune cells to the site of inflammation via chemotaxis -- and interferons (IFNs), which predominantly mediate cellular response to viral infection (Commins *et al*.,2010).

2.3.3.2. Interleukin 12 (IL12)

Interleukin-12 is a heterodimeric cytokine having many family members IL-23, IL-27, IL-35, and recently discovered IL-39. IL-12 is an effector cytokine and engages in anti-tumor and many immunotherapies as IL-12 DNA expression, Th1 helper cells, and natural killer cells. IL-12 stimulates interferon γ (INF-γ) synthesis by the activation of STAT4 which then differentiates the Th1 helper cells with the T-bet transcription, playing an important role in cancer treatment(Um eHabiba *et al*.,2022).

Interleukins12(IL-12) mainly interfere with cell-mediated immunity response. IL-12, a key member of the IL-12 family of cytokines, emerged as a potent inducer of antitumor immunity, IL-12 was originally identified in 1989 as a natural killer (NK) cell-stimulatory factor with multiple biologic effects on peripheral blood lymphocytes (Sun *et al* ., 2015). It is mainly produced by antigen-presenting cells (APCs) such as dendritic cells (DCs), monocytes, macrophages and B cells upon Toll-like receptor engagement (Ma *et al*.,2015). Thus, IL-12 is secreted as an early pro-inflammatory cytokine in response to infections (Medzhitov ,2001).

The principal role of IL-12 in breast cancer is that, breast cancer induces a local IL12-dependent type I immune response likely directed towards tumor associated antigens (Carpi *et al*.,2009). IL-12 plays a key role in the transition between innate immunity and adaptive immunity. IL12 stimulates NK cells and

T cells to produce IFN-γ, which activates macrophages to kill phagocytosed foreign substances including microbes. It also increases cytolytic activity by stimulating CD8 cells(Hamza *et al*.,2010) .

CHAPTER THREE

MATERIALS AND METHODS

3. Materials and Methods

3.1 Materials

3.1.1 Equipments and Instruments

Instrument that used in this study were illustrated in table (3-1)

Table (3.1): Laboratory equipments and devices

NO.	Instruments	Company	Country origin
1	Autoclave	Tripod	UK
2	Burner	Amal	Turkey
3	Centrifuge	Memmert	Germany
4	Disposable (Gel tube ,Surgical blade , Syringe)	Citro	China
5	Eppendorf tubes	Eppendorf	Germany
6	ELISA system	Biotech	USA
7	Filter paper	Hettich	Uk
8	Glassware	Hettich	UK
9	Hood	Bio LAB	Korea
10	Incubator	Selecta	Spain
11	Light microscope	Olympus	Japan
12	Micropipettes size (5-50 µl, 100-1000 µl , 0.5 – 10 µl)	Eppendorf	Germany
13	Oven	Olympus	Japan
14	Para film	BDH	Ergland
15	Petri dish	Sterilin	England
16	PH-meter	WTW	Germany

17	Plain tubes	Citro	Chain
18	Pasteur pipettes	Afco	Jordan
19	Refrigerator	Kiriazi	Egypt
20	Sensitive electronic balance	A and D	Japan
21	Slide	Sail Brand	China
22	Vitek 2 system	Biomerieux	France
23	Vortex mixer	Griffin	Germany

3.1.2 Chemical materials

Chemical materials, reagents, stains and solutions used in the present study illustrated in table (3.2)

Table (3-2): Chemical materials

NO.	Type of chemical	Company/origin
1	Ethanol 99%	Merck-England
2	Glycerol ($C_3H_8O_3$)	Merck-England
3	Gram stain set	BDH, England
4	Methyl red , Kovac's reagent	Sigma, USA
5	Sterile urea, Sodium chloride, KOH,	Sigma, USA
6	α-naphthol ,Hydrogen peroxide	BDH, England
7	Oxidase reagent	CDH (India)

3.1.3: Culture media

Culture media were used in the present study were illustrated in Table (3-3):-

Table (3.3): Culture media that used in current study

No.	Type of media	Manufacturing company	Origin
1	Blood agar base	Himedia	India
2	Brain heart infusion broth	Himedia	India
3	Eosin methylene blue agar	Himedia	India
4	Kligler Iron agar	Diffco-Michigan	USA
5	Mannitol salt agar	Himedia	India
6	MacConkey agar	Himedia	India
7	Peptone water	Himedia	India
8	Simmon's citrate agar	Himedia	India

3.1.4: Commercial kits

Commercial Kits were used in the present study were illustrated in table (3-4):

Table (3.4): Commercial kits that used in current study

NO.	Type of Kit	Company	Country
1	Cancer Antegin 15-3	Human	Germany
2	Interleukin 12	BT LAB	China
3	Toll Like Receptor Human(TLR4,TLR6)	BT LAB	China
5	Vitek 2 system kit	Biomerieux	France

3.2 : Preparation of culture media

The culture media were prepared according to the instruction of the manufacture company and sterilized by autoclave at 121ċ and 15 pound (Macfadden, 2000).

3.2.1-: Blood agar medium

Blood agar medium was prepared according to manufacturer instructions by dissolving 40 g of blood agar base in 1000 ml D.W. The medium was autoclaved at 121 ºC for 15 min and pressure 15 pounds per square (psi), left cooled to 50 ºC and 5% of fresh human blood was added and mixed well inside sterilized hood . This medium was used as enrichment medium for the cultivation of the bacterial isolates and to determine their ability of blood hemolysis (Forbes *et al*., 2007).

3.2.2 MacConkey agar medium

It was made by dissolving 51.5 gm into 1000 ml of DW. it was used for the primary isolation of most Gram-negative bacteria and to differentiate lactose fermenters from non-lactose fermenters (Winn *et al*., 2006).

3-2-3 : Brain heart infusion broth

The broth was prepared by dissoliving (37gm)in (1000ml) ofD.W .It used for activation the bacterial isolates (Forbes *et al*., 2007)

3-2-4-: Brain heart infusion broth with 15% glycerol

This medium was prepared by adding 15 ml of glycerol to 85 ml of BHI broth before autoclaving at 121 ºC for 15 min and pressure 15 pounds per square (psi). The medium was used to save of bacteria (MacFadden, 2000).

3-2-5: Mannitol salt agar

This medium was prepared according to the manufacture company by dissolving 111 gm into 1000 ml of DW . It was used as a selective medium for diffrenation and isolation of *Staphylococcus* species contain 7.5-10 NaCl ,selective for *Staphylococcus* and *Micrococcus* and differential for *Staphylococcus* (Macfaddin, 2000).

3-2-6 : Chocolate agar

The medium was especially used to cultivation and isolation of fastidious bacteria .It was prepared according to manufacture .to similar to the process of preparing blood agar medium, but it differs in the time of adding the amount of blood , the medium was hot not cooled .

3-2-7-Eosin methylene blue agar

This medium was prepared based on the manufacturer company by dissolving 40 gm of its powder into 1000 ml DW.after complete mixing and dissolving, it was autoclaved at 121 ºC for 15 min and pressure 15 pounds per square (MacFaddin, 2000).

3-2-8- : Peptone water medium

This medium was prepared according to manufacturer instructions by dissolving 8(g) peptone in 1000 ml of distilled water, The medium was autoclaved at 121 ºC for 15 min and pressure 15 pounds per square(psi) ,then distributed into test tubes .It was used for the demonstration of the bacterial ability to decompose the amino acid tryptophan to indole (MacFaddin, 2000).

3-2-9-: Methyl red – Vogas-Proskauer medium (MR-VP)

MR-VP medium has been prepared and used to detect the partial and complete hydrolysis of glucose (MacFaddin, 2000).

3-2-10: Simmon's citrate medium

Simmon's citrate medium has been used for determining the ability of bacteria to utilize citrate as the sole carbon source (MacFaddin, 2000) **.**

3-2-11 : Kligler iron agar (KIA)

Kligler iron agar was used for detection carbohydrate fermentation and possible hydrogen sulfide (H_2S) production as a first step in the identification of Gram negative bacilli (McFaddin, 2000).

3-3-: Reagents and solutions

3-3-1: Oxidase reagent

The reagent was prepared directly by dissolving 1(gm) of tetra methyl-ρ-paraphenylene diamine di hydrochloride in 100 ml of distilled water, and then stored in a dark bottle. The reagent has been freshly prepared, the reagent was used to recognize bacterial capability to produce Oxidase enzyme (Forbes *et al*., 2007).

3-3-2 : Catalase reagent

The reagent was prepared by dissolving 3(ml) of H_2O_2 to 100(ml) of distilled water, and then stored in a dark container . The reagent was used to recognize bacterial capability to produce catalase enzyme (Forbes *et al*., 2007)

3-3-3- : Meth yl red reagent

The reagent was prepared by dissolving 0.1(gm) of methyl red in 300 ml of 99% ethanol and then, the volume was completed to 500 ml by distilled water. This reagent was used to identify the complete glucose hydrolysis (MacFaddin, 2000).

3-3-4 : Gram stain solution

Gram Stain solution was supplied from Syrbio company. The stain included ,four solution ,crystal violate ,iodine ,absolute alcohol ,and safranine .The solution was used to study differentiate between Gram positive and Gram negative bacterial cells ,morphology and their arrangement (Forbes *et al.,* 2007).

3-3-5- : Kovac´s reagent

It was prepared by dissolving 5g of (P-dimethyl aminebenzaldehyde) in 75 ml of amyl alcohol and then 25 ml of concentrated HCl was added to the mixture . The reagent used for the detection of indole production (MacFaddin, 2000).

3-3-6: Barrett's reagent (Voges-Proskauer reagents)

The reagent was prepared according to the method reported by (Winn *et al*., 2006), as follows:-Dissolved

A- Five (gm) of α-naphthol was dissolved in 100 ml of 99% ethanol alcohol, and then stored in a dark bottle in cool place.

B- Forty(gm) of KOH was dissolved in 100 ml of distilled water. It was used to detect the acetone formation in the culture media so this test improves the partial fermentation that lead to butylene glycol formation.

3.4 Methods

3.4.1 :Patients

3.4.1.1: Specimens collection

A hundred of women with breast tumors were involved in current study .only (50 biopies and 100 blood speciemen) it was taken from women with breast tumors(18 malignant and 82 benign) , while 50 blood specimens were collected from 50 appearantly healthy control group as control group ,involved 1.biopsies for cultivation. the tissue obtained was collected outside the marginal zone, approximately 5 cm away from the tumor. After excision, the fresh tissue was immediately placed in a sterile plane tube .it contain a normal saline solution (Urbaniak *et al*.,2016) . 2. biopsies for measure the mucosal immune parameters (TLR4,TLR6,IL12). 3.Serum separated from blood specimens by using gel tube were collected from patients women who were suffering from breast tumors and healthy group to measure the systemic immune parameters (CA15-3,TLR4,TLR6,IL12) with age ranging from (14-60) years in AL-Hilla-Teaching hospital and Imam Sadiq Hospital and a period from November 2021 through March 2022.

3-4-1-2:Ethical approval

1-The study was done and the cases were collected after getting the agreement of the patients (verbal acceptance) .

2-Approval of Babylon Science Collage Ethical committee.

3- Before starting the study,permission were taken from Babylon health presidency .

3-4-1-3 ;Study design

The specimens were proceed according to study design that showed in Figure (3-1) .

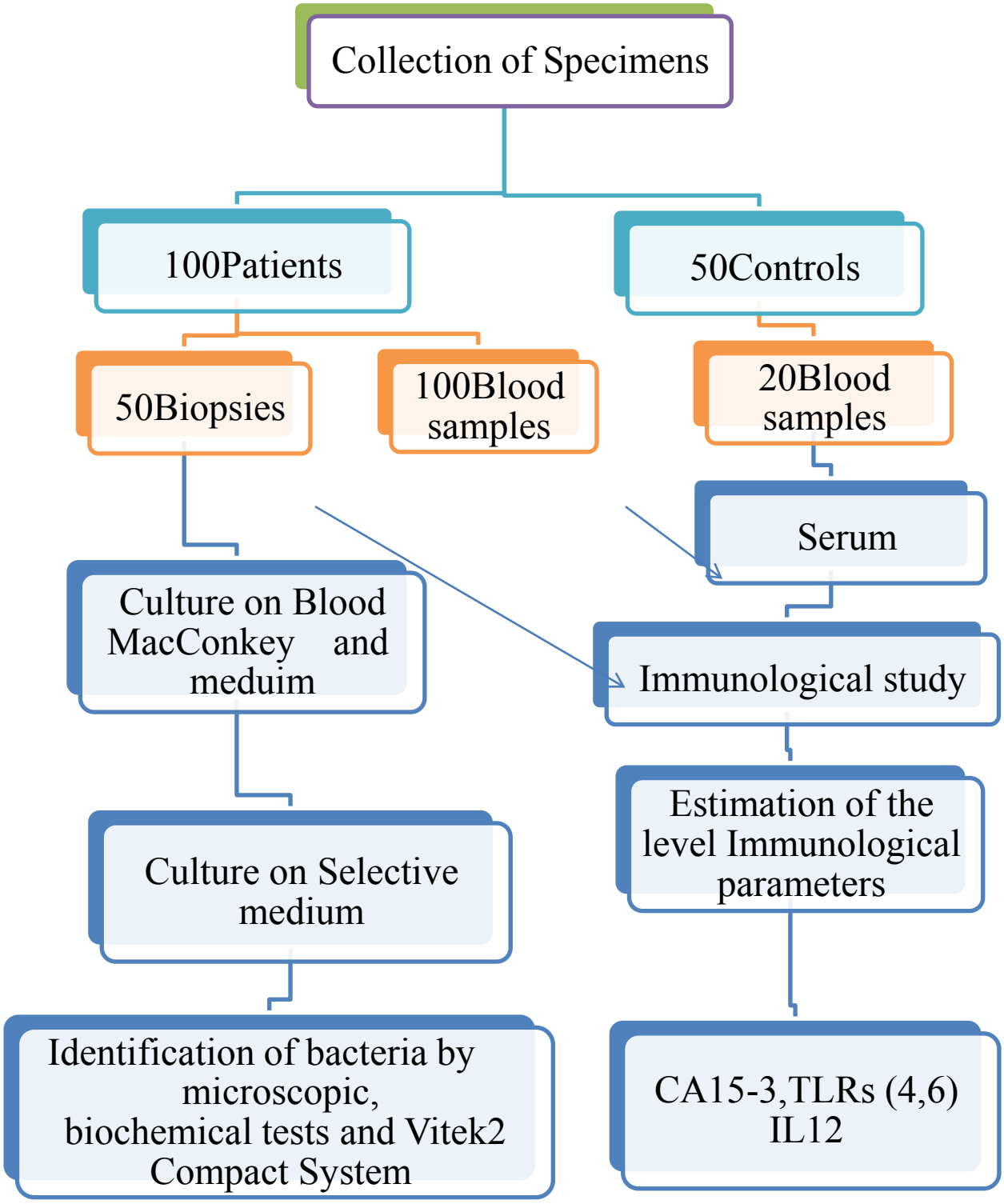


Figure (3-1):The Study Design

3-4-2: . Bacterial isolation and identification

For bacterial isolation, women breast tissue biopsies were minced with a sterile scalpel into smaller pieces, which were then grinded further by wooden sticks to make a homogenized mixture. Later, a loopful of the homogenate was inoculated onto blood agar, MacConkey agar, mannitol agar,eosin methylene blue agar media and incubated aerobically at 37°C for 24-48 hr and inoculated on the chocolate agar an aerobic. (Markey *et al.,* 2014). A single colony was taken from each positive culture, and its identification depended on the morphology properties (colony size, shape, color and nature of pigments, translucency, edge, elevation and texture). Then, colonies were stained by gram stain to observe a specific shape, type of reaction, aggregation and specific intracellular compounds. (Winn *et al*., 2006).

3-4-3-: Biochemical tests

3-4-3-1-: Catalase test

Catalase is an enzyme that catalyses the release of oxygen from hydrogen peroxide. A small amount of bacterial growth was transferred by a sterile wooden stick onto the surface of a clean dry glass slide, one drop of 3% H_2O_2 was added to it. The formation of gas bubbles indicated the positive result (Forbes *et al.*, 2007).

3-4-3-2-Coagulase test

This test was used to detect the ability of tested bacteria to produce the coagulase which is an enzyme-like protein that clots oxalated or citrated plasma. The test was performed as the following: Citrated human plasma were diluted 1:5 then mixed with an equal volume of an overnight bacterial broth culture, incubated at 37ºC. If clots form in 1-4 hours, the test was positive. A tube of plasma mixed with sterile broth was included as control (Brooks *et al*., 2007).

3-4-3-3-: Oxidase test

The test depends on the existence of certain bacterial oxidases that would catalyze the transport of electrons between electron donors in the bacteria and a redox dye (tetramethyl-ρ-phenylenediamine dihydrochloride); the dye was reduced to a deep purple color. A piece of filter paper was saturated in a petri dish with oxidase reagent (freshly prepared); and asingle cell of the bacterial colonies was spread on the filter paper by a wooden stick. The turning of the colour of the smear from rose to purple within 10 sec indicated a positive result (Forbes *et al*., 2007).

3-4-3-4-: Methyl –red test

The tubes of the MR-VP broth were inoculated with young colony of bacterial and were incubated at 37 ^{o}C for 24-48 hr. Five drops of methyl red reagent were then added to it. Immediately the result was read . The appearance and observation of red color means that a positive result and a complete hydrolysis of glucose (MacFaddin, 2000).

3-4-3-5-: Indole test

This test was performed by inoculating peptone water medium with fresh colony of bacterial growth by the loop, and it was incubated for 24-48 hr at 37 ^{o}C. Indole test was done by adding 6-8 drops of Kovac's reagent (p-dimethyl amino benzaldehyde in amyl alcohol). The positive reaction was characterized by the formation of red or pink color ring at the top of the broth (MacFaddin, 2000).

3-4-3-6-: Citrate utilization test

After sterilization of Simmon's citrate medium by autoclaving, the fresh bacterial colonies were inoculated and incubated for 24 hr at 37 ^{o}C, the change of the color of medium from green to blue with streaks of growth indicated positive results, while unchanged original green colour with no growth indicated negative results (Winn *et al*., 2006).

3-4-3-7-: Vogues –Proskauer test

The tubes of the MR-VP broth were inoculated with selected bacterial colonies and were incubated at 37 ^{o}C for 24 hr. Then, the result was read by adding 0.6 ml of alpha nephthol (reagent A) and 0.2 ml of 40 % KOH solutions (reagent B). The appearance of red colour after 15 min ment a positive result due to the partial hydrolysis of glucose, which produces acetone or acetyl - methyl - carbinol (MacFaddin, 2000).

3-4-3-8-: Vitek 2 system

The Vitek 2 system was used to confirm the biochemical test according to the manufacturer's instructions. This system consists of personal computer, reader incubator that prepared up of many inner constituents including: card filler mechanism, card cassette, bar code reader ,cassette loading processing mechanism, card sealer cassette carousel and incubator. In addition to transmittance optics, instruments control electronics, , waste processing and firm ware. The system was equipped with an extended identification data base for all routine identification tests that provide an improved efficiency in microbial diagnosis which reduce the need to perform any additional tests, so that will increase safety for both the test and the users.

All the following steps were prepared according to the manufacturer's instructions. Three ml of normal saline were placed in plane test tube and inoculated with a loop full of single colony. The colony must be aged 24 hr. the test tube was inserted into a dens check machine for standardization of colony to McFarland's standard solution (1.5×10^{8}cell/ml). The standardized inoculums are placed into the cassette and a sample identification number was entered into the computer software via barcode. Thus the VITEK 2 card connected to the sample ID number. Then, the cassette was placed in the filler module, when the cards are filled, transferred the cassette to the reader incubator module All following steps handled by the instrument, the instrument controls the incubation temperature,

the optical reading of the cards and continually monitors and transfers test data to the computer for analysis.

A. Standardization

After primary isolation, handling was minimized in a simple inoculum preparation, standardization and dilution step,. The standardized inoculum was placed into the cassette and a sample identification number was entered into the computer software via barcode.

B. Traceability

The VITEK 2 card type was then read from the barcode placed on the card during manufacturing and the card is thus connected to the sample ID,. Manufacturer barcodes link the card to patient information in this one easy barcode reading step.

C. Load and Go

Place the cassette in the filler modu le. When the cards were filled, transfer the cassette to the reader/incubator module,. All subsequent steps are handled by the instrument.

3.4.4: Immunological Study

3.4.4.1 Human Cancer Antigen 15-3 (CA 15-3) ELISA Kit

The test used for serum of patient and AHC group to determine the concentration of CA15-3 this test achieved according to the company (Human) as follows:

1-The component of the kit and samples were equilibrated at room temperature before use

2-The components of the kit were show in table (3.5)

Table (3-5) Component of the CA15-3 kit

Component	Quantity
Human CA15-3Ag microplate	1plate 96well
Standard solution	6vial ×2ml
Conjugate B	13ml
Conjugate E	13ml
Wash Buffer	20ml
Substrate Reagent	13.5ml
Stope solution	8ml
Plate sealer	2 pieces

3-4-4-1-1-: Test principle

The ELISA kit uses sandwich- enzyme –linked immunosorbent assay (ELISA) as the method .The micro ELISA plate provided in this kit, has been pre-coated with an antibody specific to human CA 15-3 Ag . Standers or sample are added to the micro ELISA plate wells and combined with the specific antibody .Then a biotinylated detection antibody specific for human CA 15-3 and Avidin-Horseradish peroxidase (HRP) conjugate were added to each micro plate well successively and incubated .free components were washed away. The substrate solution was added to each well .Only those wells that contain human CA 15-3 , biotinylated detection antibody and Avidin-HRP conjugate will appear blue in colour. The enzyme substrate reaction was terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2nm .The OD value is proportional to the

concentration of human CA15-3Ag.You can calculate the concentration of human CA15-3Ag in the sample by comparing the OD of the samples to the standard curve.

3-4-4-1-2-: Reagent preparation

1- All reagents were brought at room temperature (18-25c) before use.

2-**Wash buffer:** Twenty ml of concentrated wash buffer was diluted with 980ml from distilled water to prepare 1000ml of wash buffer.(1ml wash buffer add to 49 ml from distilled water)

3-**Standard solution**: ready to use .

3-4-4-1-3-: Protocol of ELISA was included

1-Twenty five microliter of stander or sample was added to each well .

2- Hundred microliter of conjugate B was added each well and mix carefully then cover with adhesive strip and incubate for one hour in temperature 20-25°c.

3-The plate was washed for three time in 350µl wash buffer.

4- Hundred microliter was added of conjugate E , then cover with adhesive strip and incubate for one hour in temperature 20-25°c.

5- The plate washed for three time in 350µl wash buffer.

6-Hundred microliter of substrate reagent was added, then. Incubated for 20 minute at 20-25°c.

7-Fifty microliter of stop solution was added to each well and mix carefully. the OD value was Determined at 450nm immediately or within 30 minutes . Recorded the result by using fit equation.

3-4-4-2 Toll like receptor 4 and 6

3-4-4-2-1 Toll like receptor 4 (TLR4) ELISA Kit

The test used for serum and biopsy of patients and AHC group was determine the level of serum TLR4 achieved according to manual procedure of the company (BT LAB) as follows:

1-The component of the kit and samples were equilibrated at room temperature before use

2-The components of the kit were show in table (3-6)

Table (3-6) Component of the TLR4 kit

Component	Quantity
Human TLR4 microplate	1plate 96well
Standard Solution (16ng/ml)	0.5ml x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Biotinylated Human TLR4 Antibody	1ml x1
Plate sealer	2 pieces

3-4-4-2-1-1Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human TLR4 antibody. TLR4 present in the sample was added and binds to antibodies coated on the wells. And then biotinylated Human TLR4 Antibody was added and binds to TLR4 in the sample. Then Streptavidin-HRP was added and binds to the Biotinylated TLR4 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human TLR4. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm .

3-4-4-2-1-2 Reagent Preparation

1-All reagents should be brought to room temperature before use.

2- **Standard** 120µl was reconstituted of the standard (16ng/ml) with 120µl of standard diluent to generate a 8ng/ml standard stock solution. the standard was allowed to sit for 15 mins with gentle agitation prior to making dilutions. Prepared duplicate standard points by serially diluting the standard stock solution (8ng/ml) 1:2 with standard diluent to produce4ng/ml, 2ng/ml, 1ng/ml and0.5ng/ml solutions. Standard diluent serves as the zero standard(0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

a. Standard 8ng/ml No.5 120µl Original Standard + 120µl Standard Diluent
b. Standard 4ng/ml No.4 120µl Standard No.5 + 120µl Standard Diluent
c. Standard 2ng/ml No.3 120µl Standard No.4 + 120µl Standard Diluent
d. Standard 1ng/ml No.2 120µl Standard No.3 + 120µl Standard Diluent
e. Standard 0.5ng/ml No.1 120µl Standard No.2 + 120µl Standard Diluent

3-Wash Buffer Diluted 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer.

3-4-4-2-1-3 Assay Procedure

1. All reagents were prepared, standard solutions and samples as instructed. Brought all reagents to room temperature before use. The assay is performed at room temperature.

2. The number of strips were determined required for the assay. Inserted the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. Fifty µl standard was added to standard well.

4. Forty µl sample was added to sample wells and then added 10µl anti-TLR4 antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mixed well. Covered the plate with a sealer. Incubate 60 minutes at 37°C.

5. The sealer was removed and washed the plate 5 times with wash buffer. Soaked wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washed , aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Fifty µl substrate solution A was added to each well and then added 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. Fifty µl Stop Solution was added to each well, the blue color will change into yellow immediately.

8. The optical density (OD value) was determined of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution. Recorded the result by using fit equation

3-4-4-2-2 Toll Like Receptor 6 ELISA Kit

The test used for serum and biopsy level of patient and serum of AHC group to determine the concentration of TLR6 this test achieved according to the company (BT LAB) as follows:

1-The component of the kit and samples were equilibrated at room temperature before use

2-The components of the kit were show in table (3-7)

Table (3-7) Component of the TLR6 kit

Component	Quantity
Human TLR6 microplate	1plate 96well
Standard Solution (16ng/ml)	0.5ml x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Biotinylated Human TLR6 Antibody	1ml x1
Plate sealer	2 pieces

3-4-4-2-2-1 Assay Principle

This kit was an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human TLR6 antibody. TLR6 present in the sample was added and binds to antibodies coated on the wells. And then biotinylated Human TLR6 Antibody was added and binds to TLR6 in the sample. Then Streptavidin-HRP was added and binds to the Biotinylated TLR6 antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human TLR6. The reaction was terminated by addition of acidic stop solution and absorbance is measured at 450 nm .

3-4-4-2-2-2-Reagent Preparation

1- All reagents was brought to room temperature before use.

2- Standard 120µl reconstituted of the standard (12.8ng/ml) with 120µl of standard diluent to generate a 6.4ng/ml standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (6.4ng/ml) 1:2 with standard diluent to produce3.2ng/ml, 1.6ng/ml, 0.8ng/ml and0.4ng/ml solutions. Standard diluent serves as the zero standard(0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

a. Sandard 6.4ng/ml No.5 120µl Original Standard + 120µl Standard Diluent .

b. Standard 2ng/ml No.4 120µl Standard No.5 + 120µl Standard Diluent.

c. Standard 1.6ng/ml No.3 120µl Standard No.4 + 120µl Standard Diluent.

d. Standard 0.8ng/ml No.2 120µl Standard No.3 + 120µl Standard Diluent.

e. Standard 0.4ng/ml No.1 120µl Standard No.2 + 120µl Standard Diluent .

3- **Wash Buffer** Diluted 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer.

3-4-4-2-2-3 Assay Procedure

1. All reagents were prepared standard solutions and samples as instructed. Brought all reagents to room temperature before use. The assay was performed at room temperature.

2.The number of strips was determined required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. Fifty µl standard was added to standard well.

4. Forty µl sample was added to sample wells and then add 10µl anti-TLR6 antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mixed well. Cover the plate with a sealer. Incubated 60 minutes at 37°C.

5. The sealer was removed and washed the plate 5 times with wash buffer. Soaked wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and washed 5 times with wash buffer. Bloted the plate onto paper towels or other absorbent material. 6.50µl substrate solution A was added to each well and then added 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7.Fifty µl Stop Solution was added to each well, the blue color will change into yellow immediately.

8.The optical density (OD value) was determined of each well immediately using a microplate reader set to 450 nm within 10 minutes after added the stop solution. - Recorded the result by using fit equation.

3-4-4-2-3 Human Interleukin 12 ELISA Kit

The test used for serum and biopsy of patient and serum of control to determine the concentration of IL12 this test achieved according to the company (BT LAB) as follows:

1-The component of the kit and samples were equilibrated at room temperature before use

2-The components of the kit were show in table (3-8)

Table (3-8) Component of the IL12 kit

Component	Quantity
Human IL12 microplate	1plate 96well
Standard Solution (16ng/ml)	0.5ml x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Biotinylated Human IL12 Antibody	1ml x1
Plate sealer	2 pieces

3-4-4-2-3-1 Assay Principle

This kit was an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IL-12 antibody. IL-12 present in the sample was added and binds to antibodies coated on the wells. And then biotinylated Human IL-12 Antibody was added and binds to IL-12 in the sample. Then Streptavidin-HRP was added and binds to the Biotinylated IL-12 antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color develops in proportion to the amount of Human IL-12. The reaction was terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

3-4-4-2-3-2 Reagent Preparation

1- All reagents should be brought to room temperature before use.

2-**Standard** 120µl was reconstituted of standard (80ng/L) with 120µl of standard diluent to generate a 40ng/L standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation prior to making dilutions. Prepared duplicate standard points by serially diluting the standard stock solution (40ng/L) 1:2 with standard diluent to produce20ng/L, 10ng/L, 5ng/L and2.5ng/L solutions. Standard diluent serves as the zero standard(0 ng/L). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

a.Standard 40ng/L No.5 120µl Original Standard + 120µl Standard Diluent

b.Standard 20ng/L No.4 120µl Standard No.5 + 120µl Standard Diluent

c. Standard 10ng/L No.3 120µl Standard No.4 + 120µl Standard Diluent

d. Standard 5ng/L No.2 120µl Standard No.3 + 120µl Standard Diluent

e. Standard 2.5ng/L No.1 120µl Standard No.2 + 120µl Standard Diluent

3-Wash Buffer Diluted 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer.

3-4-4-2-3-3 Assay Procedure

1. All reagents were prepared standard solutions and samples as instructed. All reagents were brought to room temperature before use. The assay is performed at room temperature.

2. The number of strips was determined required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. Fifty µl standard was added to standard well.

4. Forty µl sample was added to sample wells and then add 10µl anti-IL-12 antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mixed well. Covered the plate with a sealer. Incubated 60 minutes at 37°C.

5. The sealer was removed and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washed, aspirated or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Fiftyµl substrate solution A was added to each well and then add 50µl substrate solution B to each well. Incubated plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. Fifty µl Stop Solution was added to each well, the blue color will change into yellow immediately.

8.The optical density (OD value) was determined of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution. Recorded the result by using fit equation

3-4 -5: Statistical Analysis

Data were processed and analyzed with the statistical package SPSS. Results are expressed as (mean±SD) paired t-test was used to analyze the differences between systemic and mucosal immune response of the breast tumors. Independent –samples T test was used to compare patient and control systemic . ANOVA test between groups.Correlation test between immunological markers .P-value below 0.05 were considered to be statistically significant. (Al-Rawi, 2000).

CHAPTER

FOUR

RESULTS

&

DISCUSSION

4-Results and Discussion

4-1: Patient's clinical characteristics and types of breast tumors

The women afflicted with breast tumors divided accorrding to the age . The higher percentage found in age group (41-60) years was 40(40%) While percentage patient with age group (21_40) years was 37(37%) and less than percentage with age group (≤ 20) was 23 (23%) . The ratio age female with breast tumor (malignant and benign tumors) showed in Table (4-1).

Table (4-1)Distribution of women with breast tumors according to the age group

Age group /year	Percentage %
≤20 (n=23)	23
21-40 (n=37)	37
41-60 (n=40)	40

The study was agreed with study of (Shakir, 2019) in Baghdad who showed was more common among breast tumor in women at age group (41-60) and lowest percentage at age group (≤20). Other study also found high percentage at age group 40-50 year women with breast tumors compared at other age group (Al-Saadi,2021). Age remains the number one risk factor associated with breast cancer (Hiler *et al*., 2016). Age was a risk factors for breast cancer evolution , the increasing incidence of cancer in women above the age of 40 years may bedue to increased chromosomal damages as a result of repeated divided progress the age , which lead to the accumulation of mutations in the DNA that bring about to cancer development and "the age-

related increase in chromosomal harm occurred hurry in women than in men" because the increasing level of aberrations, and rise in the level of X chromosome damage was the main contributor of aging in women (Wojda *et al*., 2006 and Orta and Günebakan, 2012). Also menstruation after the age of forty was one of factors that cause breast cancer (American cancer society, 2019).

4-1-1 Diagnosis of breast disease

The diagnosis of Breast diseases by histological examination the participating women suffered from malignant and benign tumor was divided into (18%) the malignant tumor and (82%) for benign tumor and this result was agreed with another study in the southern Thailand found bengin breast disease ratio was 72.9% of the women and (27.1%) women with malignant breast tumors (Kotepui *et al*,2014). but it didn,t agree with (shakir ,2019) and other studies in Baghdad Iraq where they found high rate of malignant tumors compared with benign tumors because of quality and quantity of samples taken the patients group with special oncology surgery centers such as Al –Amal oncology center and mediccal Education city in Baghdad was in place where sampled was collected and nature of nutrition ,housing density and increased pollution in addition to other factors that vary from place to place so was nature of 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) .

The malignant tumor involved Invasive ductal carcinoma(IDC) and invasive lobular carcinoma (ILC) (Makki, 2015) while the benign breast tumors and diseases inculde fibrocystic change ,fibroadenoma , granulomatous mastitis ,and other disease (Lipoma and Fat necrosis) groups of benign breast tumors divided by (Guray and Sahin, 2006 ;Stachs *et al* .,2019) to these as well as other groups.

The malignant tumor of the type disease of breast tissue distribution on percentage according age highest percentage was (30%) in age group 41-60year ,while age group 21-40year was (16.21%) and was zero in age group ≤20 year .while the benign tumor percentage was in age group ≤ 20 year was (100%) , while was in age group (21-40) year (83.78%) , and in age group (41-60)year (70%) . The was showed in table (4-2).

The malignant and benign breast tumors percentage differences according marital status and type of breastfeeding .In which the malignant tumor percentage of married women was (26.08%) while in the benign tumor was(73.91%). In addition the malignant tumor was zero of in unmarried women but the percentage of benign tumor was (100%) .in women who were breastfeeding the incidence of malignancy was (34.61) while the percentage of benign tumor was (65.38) and the percentage was in women with non-breastfeeding (zero) for benign and malignant tumors was (100%) , percentage of mixed breastfeeding in malignant tumors was (12.50%) in benign tumor was (87.50%) . as table (4-2)

Table 4.2: Socio-demographic characteristics and other risk factor for women the benign and malignant breast tumors

A variable	Number	Benign		Malignant	
		No	%	No	%
Total	100	82	82	18	18
Age group					
≤20	23	23	100	0	0
21-40	37	31	83.78	6	16.21
41-60	40	28	70	12	30
Married	69	51	73.91	18	26.08
Unmarried	31	31	100	0	0
Breastfeeding	26	17	65.38	9	34.61
Non-breastfeeding	34	0	0	34	100
Mixed feeding	40	35	87.50	5	12.50

These results of the present study were agreement with other studies (Carroll *et al*., 2008 and Shakir , 2019) who recorded that the age group of ≤20 years the highest benign tumor rate compared with other age.and higest ratio of malignant tumor at age group (41-60) compared with other age group . 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 did not agree with studyof (Salman ,2021) in Baghdad . while breastfeeding maintain breast health and menstruation rate decreases during breastfeeding thus the estrogen level decreases (American cancer society, 2019).

The benign tumor divided to four group from total ratio in this study according to the histological examination involved Fibrocystic change was 36(43.90%). while percentage Fibroadenoma was 28 (34.14%), and granulomatous mastitis was 11 (13.41%) and other (Lipoma, fat necrosis) was 7 (8.53%). as in Figure (4-1) . And did no agree with (Kotepui *et al*,2014) who found highest proportions fibroadenoma and fibrocystic change respectively . 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 (15%) , granulomatous mastitis was (22%) and other benign disease (lipoma and fat necrosis)was percentage (23%).as in table (4-3)

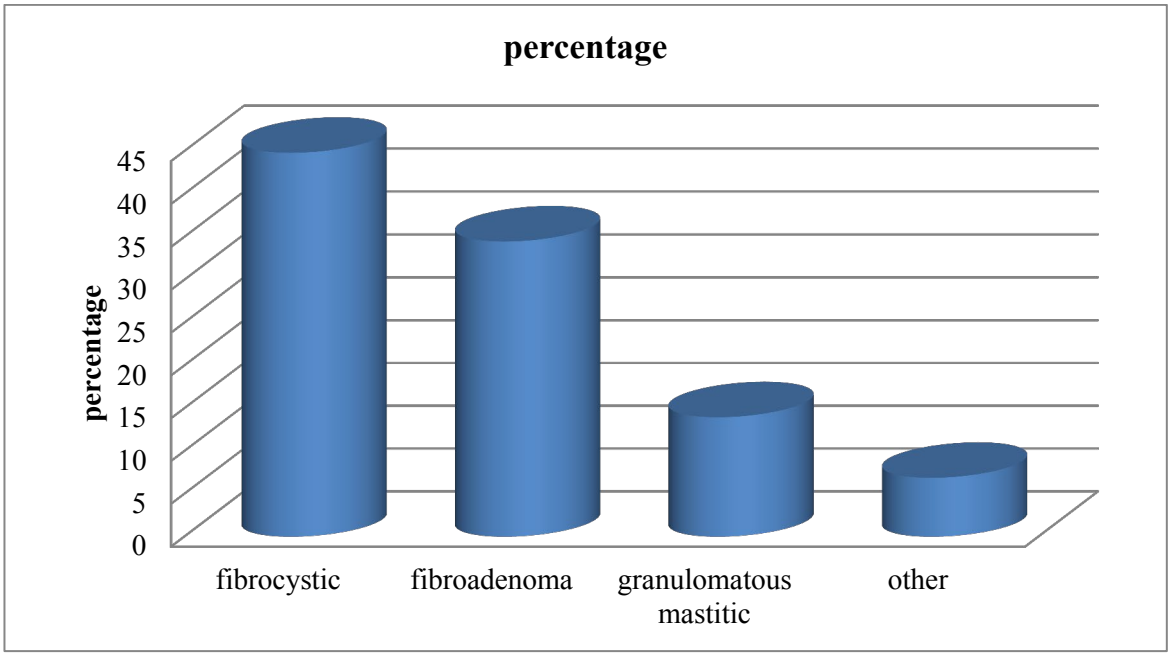


Figure (4-1) Distribution of type of benign breast tumors

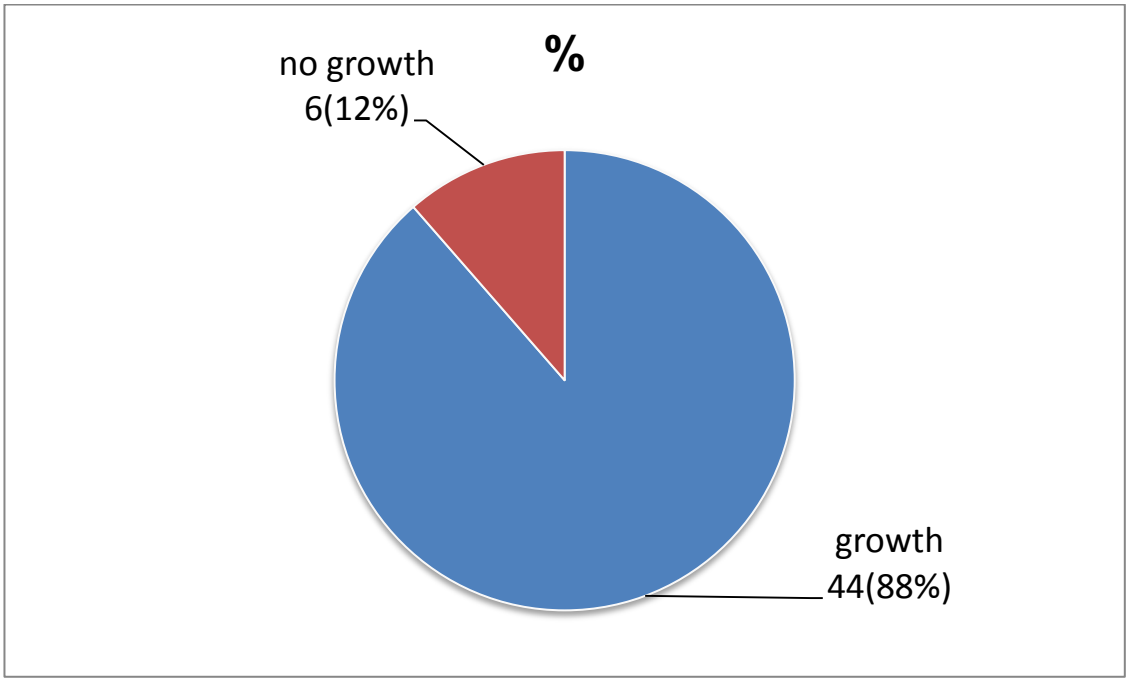
Table (4-3) distribution of benign breast tumors according to the age group of patients

Age/ year	Fibroadenoma %	Fibrocystic change %	Granulomatous mastitits %	Lipoma and fat necrosis %
≤ 20	50	50	0	0
21-40	41.66	33.33	25	0
41-60	15	40	22	23

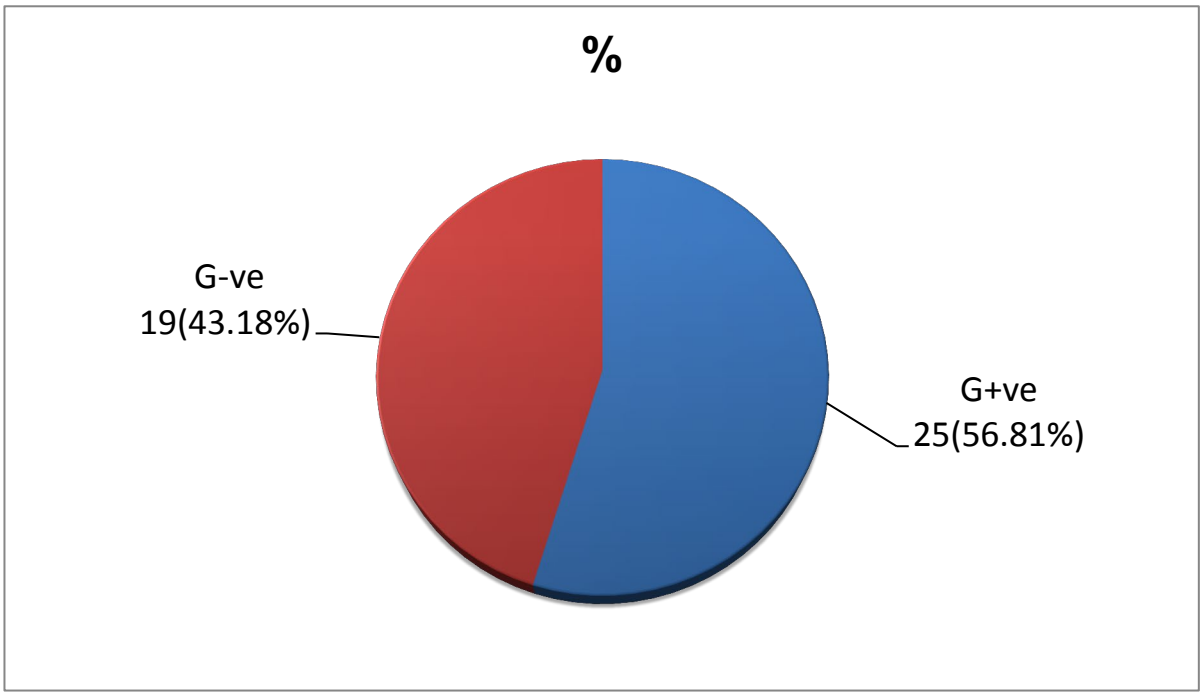
4-2. Bacteriology study

4-2-1. Identification and Isolation of bacteria in breast tumor tissue

The isolation and diagnostic of bacteria from Women with breast tumors (50 biopsies) adiagnosed to have either benign or malignant tumor, bacteria were isolated. The results growth 44 (88%) were detected as Gram-positive (G^{+ve}) and Gram-negative (G^{-ve}) bacteria, while 6 (12%) of the samples were showed no growth. as figure (4-2). On the other hand,the single isolates was (76%) and mixed isolate was(24%) .which distribution between 25 (56.81%) as G^{+ve} bacteria,while 19 (43.18%) were detected as negative (G^{-ve}) bacteria, as figure (4-3).



Figure(4-2) Distribution of the bacteria growth in breast tumors tissue



Figure(4-3) Distribution of bacterial growth (G+ve and G-ve)

The breast tissues has been suggested to have a variety collection of bacterial spp. (Kim *et al*., 2009). The current result was agreed with study in Najaf city that 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 (Khdear,2021)

Gram positive bacteria(G+ve) included Growth, *Staphylococcus aureus, S.epidermidis, S.warneri, Enterococcus faecalis,*while Gram negative bacteria included *Pseudomonas fluorescens and Acientobacter baumannii* were identified using enriched and differential media (Blood agar and MacConkey agar ,Eosin methylene blue, 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* was coagulase positive but

S.epidermidis and *S.werneri* were coagulase negative ,*Enterococcus.feacalis*,is catalase negative and grow on mannitol salt agar .Gram negative bacteria were diagnostic by Vitek 2 compact system and biochemical test such as indole, methyl red, Voges-Proskauer, Simmons' citrate test and Kligler iron agar test the result Positive. Result of indole formation of red ring after added Kovacs reagent, Simmon Citrate positive result convert of color media after incubation 24 hr from blue colour to green color negative result remain the blue color. Voges-Proskauer test positive result formation of red ring after added of reagent Table. (4-4) showed the biochemical test for identification gram negative bacteria and gram positive bacteria .

Table (4.4) Biochemical test for diagnosis of bacteria that isolated from breast tissue biopsy

A-Gram negative bacteria

Biochemical test / Bacteria	Indole	methyl red	Voges-Proskauer	Simmon citrate	Catalase	Oxidase
Acientobacter baumannii	-	+	-	+	+	-
Pseudomonaus flourescens	-	-	-	+	+	-

B-Gram positive bacteria

Biochemical tests / Bacteria	**Catalase test**	**Coagulase test**	**Oxidase Test**	**Mannitol fermentation**	**Simmon citrate**
Staphylococcus Aureus	+	+	–	+	+
Staphylococcus epidermidis	+	–	–	–	–
Staphylococcus warneri	+	–	–	–	–
Enterococcus feacales	–	–	–	+	–

The microorganism was diagnostic by different test such as macroscopic and microscopic, biochemical test, and Vitek 2 compact system results were mentioned in Table(4-5), probability of bacteria diagnostic by Vitek2 compact 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*12(27.26%), *S.epidermidis* 8(18.18%) ,*S.warneri* 3 (6.81%), *Enterococcus feacales* 2 (4.54%), *Acientobacter bumanni* 5(11.36%), and the higest percentage of bacteria was for *Pseudomonas florescences* was14 (31.81%) .the percentage of bacteria isolated from breast tumor biopsy listed in table (4-6).

Table (4-5) The probability of diagnostic bacterial isolates from breast tissue biopsy by vitek2compact system

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

Table (4-6) The percentage of bacteria isolated from breast tissue biopsy

NO.	Bacteria	NO	Percentage %
1	*Enterococcus feacales*	**2**	4.54
2	*Staphylococcus aureus*	12	27.26
3	*Staphylococcus warneri*	3	6.81
4	*Staphylococcus epidermidis*	8	18.18
5	*Acientobacter baumanni*	5	11.36
6	*Pseudomonas florescence*	14	31.81
Total		**44**	**99.96**

This current study found that the breast tissue from malignant and benign tumors containing many bacterial spp, and was not sterile, ,where was the growth percentage 89% while no growth 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. Bacterial isolates were obtained from tumors regardless of being malignant or benign, in which Gram positive and negative bacteria were cultivated, and the microbial profiles did not differ significantly between the benign and malignant samples. Consistent with these findings, it has been observed that the microbiome profile of normal adjacent tissue from patients with malignant cancers was similar to that of normal adjacent tissue from women with benign tumors, in comparison with normal tissue from entirely healthy women and current studies agree that the breast tissue has its own microorganisms (Urbaniak *et al*., 2014).

Though, in another study where breast tissues were collected from 43 women (aged 18-90) undergoing breast surgery, different facultative anaerobic bacteria were identified from breast tissue of women with malignant cancer in comparison with those with benign disease (Hieken *et al*., 2016). The microbiota composition differs from one person to another, and is determined by a groups of factors, such as lifestyle,diet, environment as well as the expression of genes included in the immune response of the host (Blekhman *et al*., 2015).

Proportion and types of bacteria in this study didn,t agree to study (Shakir,2019) but Partially similar where the higest percentage of bacteria was found *S.epidermidis and Bacillus spp*. In addition to the precense of other bacteria types involved,*P.fluorescens*, *K.pneumoniae E. faecalis, Microcoucuss spp, E.coli, Serratia marcescens, S. aureus, Aeromonas. spp*. While in this study where the higest percentage of bacteria was found *Pseudomonas florescence and S.aureus*.in other study in which *Enterobacteriaceae* and *Staphylococcus,* among other taxa, were molecularly detected within the breast tissue of Irish women. Whereas bacteria diagnosed in breast tissues of Canadian women included *Bacillus, Enterobacteriaceae, Staphylococcus, Pseudomonas* with other bacterial spp. (Urbaniak *et al*., 2016).

Microbiota are produce many metabolites, synthesizing, which, can result to unbalance in hormones. Levels of steroid hormones, especially estrogen, are dependent on the metabolic activity of the microbiota, which are the dominant including toxins, virulence factors, fatty acids, cofactors and vitamins, potent enough to trigger several signaling cascades. All of these factors can contribute to the overall risk of breast carcinogenesis (Parida and Sharma, 2020).

4-3:Immunological study

4-3-1 Cancer antigen 15-3

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 146.79U/ml compared to control (AHC) was 94.17U/ml with high significantly where p-value was (0.006) .as table (4-7) .

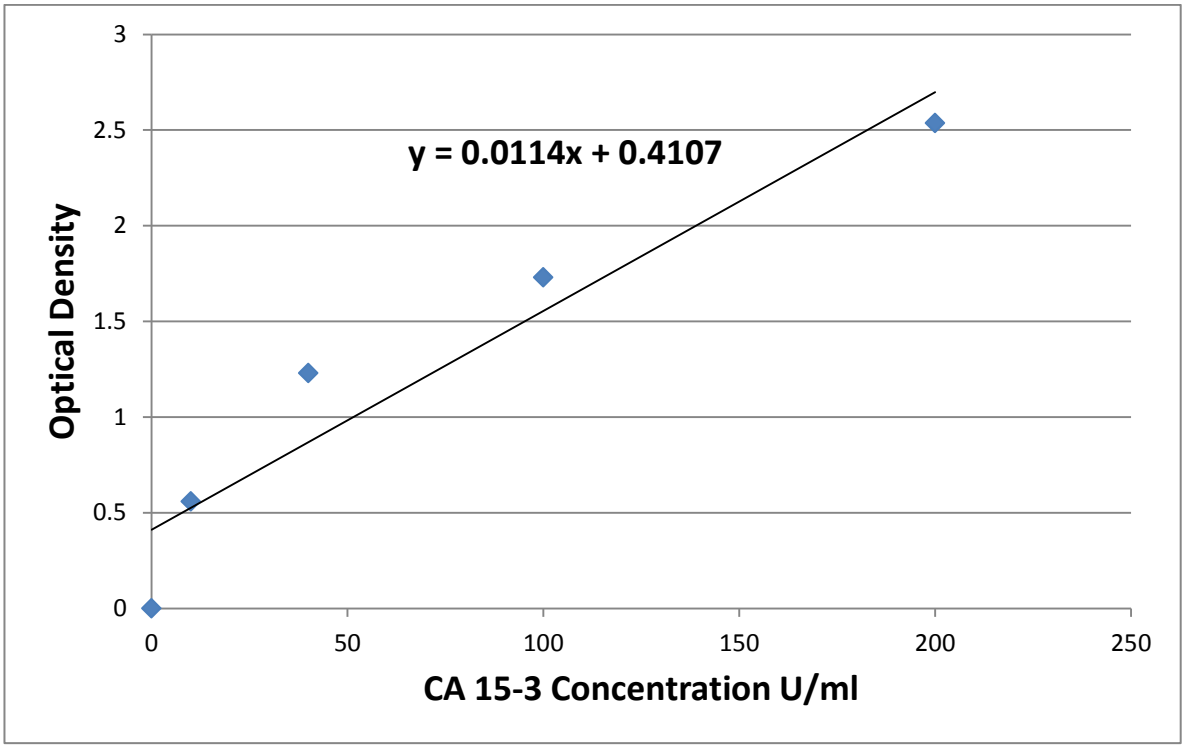


Figure (4-4) Standard Curve of CA 15-3

Table (4-7) Concentration of serum CA 15-3 in women with breast diseases and control group

Marker	Patient M±SD	Control M±SD	P_value
CA 15-3 serum	146.79±62.01 U/ml	94.17±11.24 U/ml	0.006

Cancer antigen 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 U/ml and 227.88 U/ml) while the mean less than in fibroadenoma was 104.58U/ml and fibrocystic change as 103.10 U/ml with no significant .as in table (4-8).

Table (4-8) Concentration of serum of CA15-3 in womem with breast tumors according type of disease and control group .

Types of breast diseases	CA 15-3 Serum U/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(AHC)	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 (Atoum *et al*.,2012).

As well as in other study (Wojtacki *et al*.,1994) 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 ($p < 0.01$). Previous researches demonstrated that the

CEA and CA15-3 levels are associated with tumor burden indicators including tumor size and lymph node status(and Soletormos *et al*,2004 and Park *et al* ,2008) 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).

There are other factors that cause an increase in CA15-3 in the blood, the most important of which are benign ovarian cysts, benign breast disease, cirrhosis of the liver, sarcoidosis, and lupus erythematosus(Lori *et al* .,2020).

4-3-2 Toll Like Receptor in breast tumor tissue and serum in women

4-3-2-1 .Toll like Receptor 4 (TLR4)

Toll like receptor 4 (TLR4) were measured by using Enzyme Linked Immunosorbent assay (ELISA) were used for quantification of human TLR4 . The result of this test were calculated by using standard curve fit equation Figure(4-5) .The mean level of TLR4 concentration in serum of patient was 4.58 ng/ml while control was 4.18 ng/ml with p value (0.28). as in table(4-9) .

The TLR4 level concentration of patients measured serum and tissue the mean of TLR4 differed between patients according to the types of breast tumor as well as between systemic and mucosal .TLR4systemic mean of fibroadenoma 5.14ng/ml ,fibrocystic change 3.98 ng/ml ,invasive carcinoma 4.91ng/ml and granulomatous mastitis 3.65 ng/ml , There was no significant .TLR4 mucosal mean of fibroadenoma 4.48ng/ml ,fibrocystic change 3.21ng/ml ,invasive carcinoma 3.12ng/ml and granulomatous mastitis was 3.51 ng/ml as well as no significant .as in table (4-10).

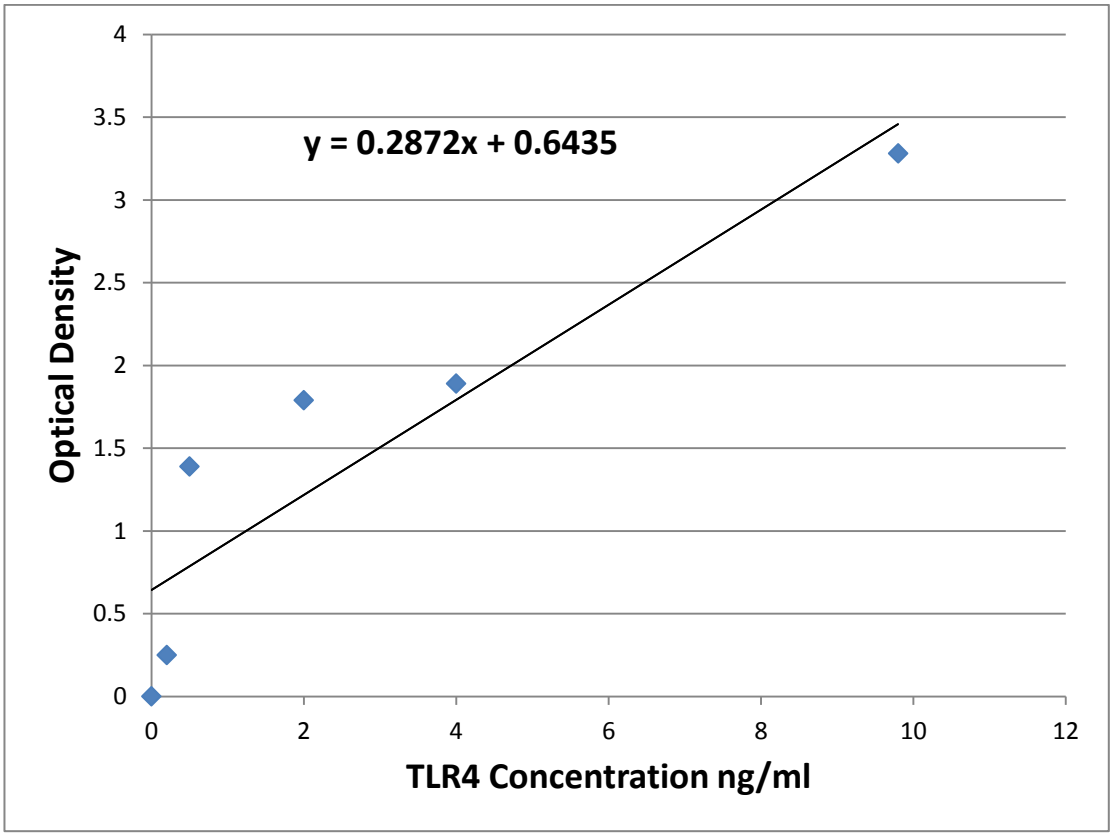


Figure (4-5) Standard Curve of TLR4

Table (4-9) Concentration of serum TLR4 in women with breast tumor and control group

Cytokine	Patient M±SD	Control M±SD	P_value
TLR4 serum	4.58±1.60 Ng/ml	4.18±0.72 Ng/ml	0.28

Table (4-10) Concentration of serum and tissue of TLR4 in women with breast tumors according type disease

Type of breast tumor	TLR4 ng/ml M±SD	
	TLR4 serum	TLR4 tissue
Fibrocystic change	3.98±0.64 (a)	3.21±1.31 (a)
Fibroadenoma	5.14±2.15 (a)	4.48±1.90 (a)
Invasive carcinoma (IDC,ILC)	4.91±1.80 (a)	3.12±0.73 (a)
Granulomatous mastitis	3.65±0.56 (a)	3.51±1.56 (a)

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

The results were appeared increased concentrations of TLR4 in patients than control this might be present microbial in breast tissue as previously mention. El-Kharshy *et al*.,2021) found TLR4 increased in breast patients compared with control ,these study result was a significant increase in serum TLR4 was also detected in patients with both non-metastatic (1,945.2±1,709.53 pg/ml) and metastatic breast cancer (7,800.1±13,041.28 pg/ml), compared with the control group (1,106.8±108.32 pg/ml; P=0.0001). Breast cancer cells possess high expres sion levels of TLR4, indicating that this receptor is critical to the development of breast cancer (Yang *et al*.,2013)

Toll-like receptors (TLRs) commonly recognize different molecules of microbial origin and trigger upregulation of inflammatory cytokines through cytoplasmic signaling (Janssens and Beyaert,2003). More

specifically for TLR4, its stimulation results in MyD88-dependent activation of NF-κB and MAPK among other pathways, thus, inducing a series of inflammatory cytokines and pro-survival factors (Lee *et al*.,2010).

Expression of TLR4 prevails among key cell subsets of innate immunity, such as monocytes, macrophages, neutrophils and dendritic cells (DCs), while it is identified at a lower level on T cells and B lymphocytes (Vijay ,2018). Table 4-10 appeared The concentrations of TLR4 in serum was non significantly increased compared with local (breast tissue). Rajput *et al* at found TLR4 is also expressed on tumor cells and holds a prominent role in inflammation-fueled cancer progression and metastasis. In particular, TLR4 promotes tumor cell proliferation, invasion, survival and migration, the induction of epithelial–mesenchymal transition (EMT), the expansion of cancer stem-cells (Rajput *etal*.,2013) .

4-3-2-2.. Toll Like Receptor 6 (TLR6)

Toll like receptor 6 (TLR6) were estimated by using Enzyme Linked Immunosorbent assay (ELISA) It used for quantification of human TLR6 . The results of this test were calculated by using standard curve fit equation Figure(4-6) .The mean level of TLR6 in serum of patient was 2.11 ng/ml while control was 1.58 ng/ml with found significant differences where p-value was (0.01) . as in table(4-11) .

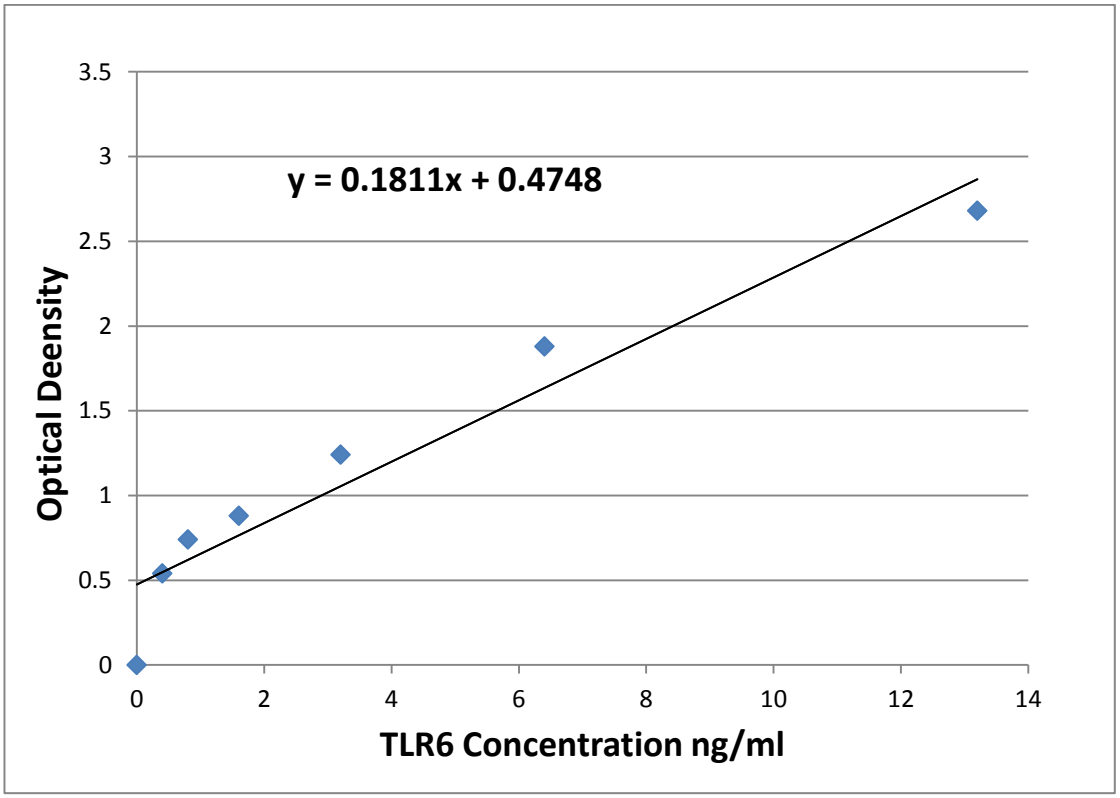


Figure (4-6) Standard Curve of TLR6

Table (4-11) Concentration of serum TLR6 in women with breast diseases and control group

Cytokine	Patient M±SD	Control M±SD	P_value
TLR6 serum	2.11±0.96 Ng/ml	1.58±0.42 Ng/ml	0.01

This result was agreed with other studies which were appeared high gene expression for TLR 6 with breast cancer (Mauldin *et al*.,2015). SemlaliI *et al*.2018 found the association between polymorphism of IL-6 genes with breast in the Saudi Arabian population .Previous studies have shown that a variant of TLR6 rs13281615 was associated with increased BC risk in the Chinese population (Chan *et al*.,2012).

The TLR6 mean in serum and tissue of patients were higher in than serum btween types of tumors where it was in fibrocystic change 3.33ng/ml ,fibroadenoma 2.59ng/ml ,invasive carcinoma 4.09ng/ml and granulomatous mastitis 3.35ng/ml without significant between tumors groups in mucosal .Compared with mean of TLR6 serum of the tumor types where was fibrocystic change 2.22 ng/ml ,fibroadenoma 2.64 ng/ml ,invasive carcinoma 1.91ng/ml and granulomastous mastitis 1.31 ng/ml. as in table (4-12) . The experimental of Morgan *et al*.,2014 expressed that TLR1 and TLR6 form heterodimers with TLR2, which are crucial in mucosal immune response regulation .

Table (4-12) Concentration of serum and tissue of TLR6 in women with breast tumors according type tumor

Type of breast disease	TLR6 ng/ml M±SD	
	TLR6 serum	TLR6 tissue
Fibrocystic change	2.22±0.66 (a b)	3.33±1.435 (a)
Fibroadenoma	2.64±0.90 (b)	2.59±1.34 (a)
Invasive carcinoma (IDC,ILC)	1.91±0.59 (a b)	4.09±1.74 (a)
granulomatous mastitis	1.31±0.32 (a)	3.35±0.97 (a)

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

4-3-3 Interleukin 12 (IL-12)

Interleukin 12 (IL-12) were measured by using Enzyme Linked Immunosorbent assay (ELISA) were used for quantification of human IL-12 the result of this test were calculated by using standard curve fit equation Figure (4-7).Mean of IL12 concentration in serum of patient was 16.57 ng/L while control was 22.02 ng/L with significant difference(0.0002) . as in Table(4-13) .

The mean of interleukin 12 was higher in breast tumor tissue compared with serum according to the types of tumors where it was mucosal as follows fibroadenoma 34.41 ng/L ,fibrocystic change 24.54 ng/L ,invasive carcinoma 35.07 ng/L and in granulomatous mastitis 25.35 ng/L with high significant . while mean in systemic of types of tumors as follows fibroadenoma 24.14 ng/L ,fibrocystic change 20.32 ng/L ,invasive carcinoma 24.68 ng/L and granulomatous mastitis 14.29 ng/L and no significant.as

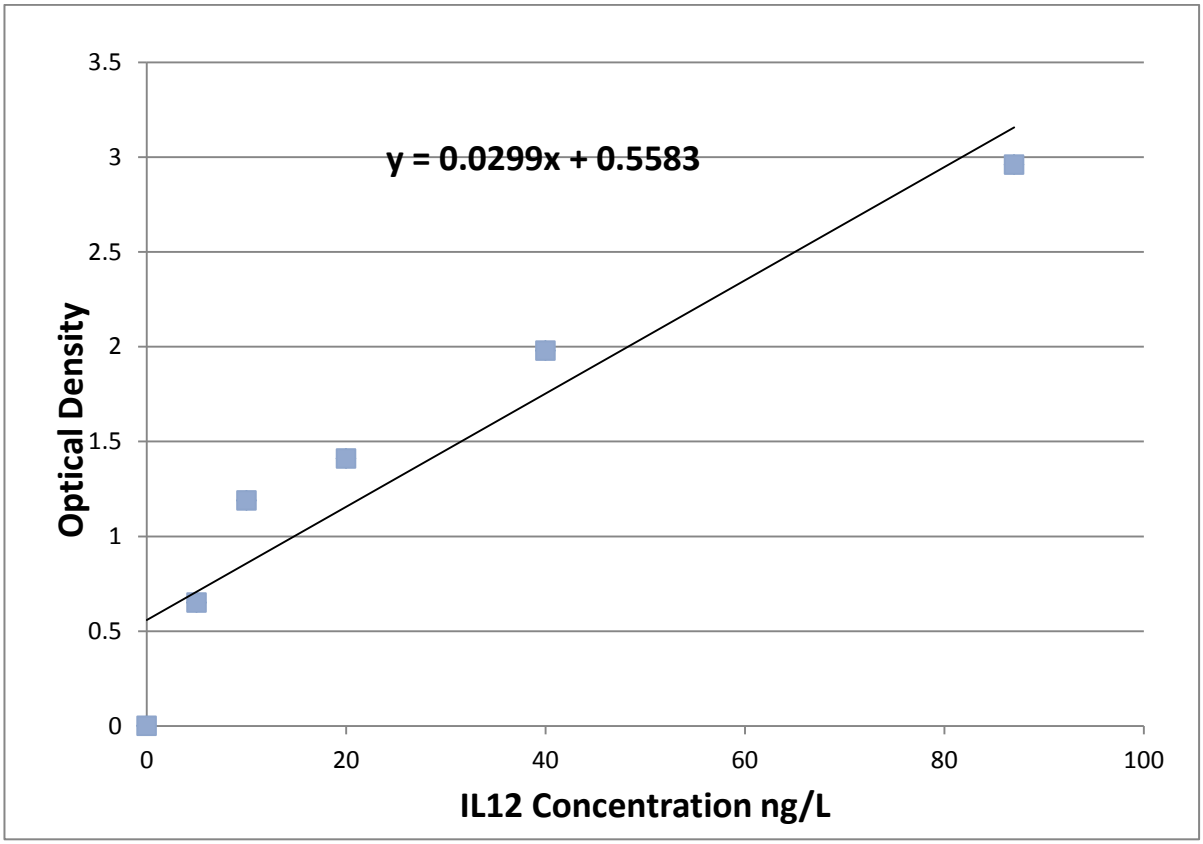


table (4-14) .

Figure (4-7) Standard curve of IL12

Table (4-13) Concentration of serum IL-12 in women with breast tumors and control group

Cytokine	Patient M±SD ng/l	Control M±SD ng/l	P_value
IL12 serum	16.57±4.61	22.02±3.56	0.0002

Table (4-14) Concentration serum and tissue of IL-12 in women with breast tumors according type disease

Type of breast disease	IL-12 ng/L M±SD	
	IL-12 serum	IL-12 tissue
Fibroadenoma	24.14±16.24 (a)	34.41±8.41(b)
Fibrocystic change	20.32±14.94(a)	24.54±5.72(a)
Invasive carcinoma(IDC,ILC)	24.68±7.58(a)	35.07±5.92(b)
granulomatous mastitis	14.29±1.72(a)	25.35±9.63(a)

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

Interleukin 12 concentration were decreased in patient with breast disease compared with control group .This Agree with study in Babylon city (Abd Razak *et al*.,2011) and as well as other study in Eygept country by(Youssef *et al,*2015) found significants and decrease in IL-12 expression in BC patients than benign tumor patients than healthy subjects.,in this study mean of IL12 in malignant tumors was equal with some groups of benign tumors and higher than the set of cystic neutrophilic granulomatous and fibrocystic change of totals of benign tumor in systemic concentration of IL12 mucosal in malignant tumors was higher compared with benign tumors . but this results disagree with Hussein *et al*.,2004 who reported higher levels of IL-12 in BC patients than control subjects, this conflict may be

attributed to the hormone receptor (ER, PgR and HER2) status, Several reports demonstrated direct down regulation of cytokines in different organs by ER (Salem,2004) and PR (Davies *et al*.,2004) .The inverse correlation between IL-12 and hormone receptors status may reflect the greater aggressiveness of this subtype of breast tumors, since the use of IL-12 as anti tumor is directed to induce or increase hormone sensitivity (Carpi *et al*.,2009)

4-4 Compare between the level of serum and tissue Immunological parameters in women with breast tumors (TLR4,TLR6,IL12)

According to the results of the comparison showed in table (4-15) It systemic TLR4 mean was 3.94 ng/ml with higher significantly while mucosal TLR4 mean lower than systemic 3.18ng/ml with p value (0.0004). mucosal TLR6 appaered significantly higher than the systemic (2.97 ,2.10) ng/ml respectively. Mucosal IL12 mean higher than the systemic IL12 the mean (24.59 ,16.57) ng/L respectively.

Table (4-15) Concentration of serum and tissue of TLR4,TLR6,IL12 in women with breast tumors

Parameters	Serum M±SD	Tissue M±SD	P value
TLR4	3.94±0.69	3.18±0.83	0.0004
TLR6	2.10±0.98	2.97±1.19	0.003
IL12	16.57±4.61	24.59±5.33	0.0004

4-5 Correlation between TLR4 ,TLR6 and IL12 in women with breast tumors

Correlation between IL-12 and TLR6 serum was positive correlation and p value was significant at p<0.05. as in table (4-16) ,while correlation between serum IL-12 and TLR4 was negative correlation and p value was significant at p>0.05 as in table (4-16) .Correlation between serum IL-12 and tissue IL-12 was negative correlation and P value was significant at p>0.05 as in table (4-20).

Correlation between tissue IL12 and TLR6 was positive correlation and p value was >0.05 ,also positive correlation between tissue IL12 and TLR4 and p value was>0.05. as in table (4-17). Correlation between serum TLR6 and TLR4 was positive correlation and p value was siginficant at p<0.05 .as in table (4-16).while correlation between tissue TLR6 and TLR4 was negative correlation and p value was >0.05.as in table (4-17). correlation between serumTLR6 and tissue TLR6 was negative correlation and p value <0.05 .as in table (4- 19). Correlation serum TLR4 and tissue TLR4 was positive correlalion and p value<0.05 as in table (4-18) .

Table (4-16) Correlation between serum IL12 ,TLR6, TLR4 in women with breast tumors

Parameters	IL12	TLR6	TLR4
IL12	1	0.345*	-0.028
Sig		0.046	0.875
TLR6	0.345*	1	0.353*
Sig	0.046		0.041
TLR4	-0.028	0.353*	1
Sig	0.875	0.041	

(*) Corrlation was significant at the 0.05 level, (-)Inverse relationship,(+) or without a sign it is positive relationship .

Table (4-17) Correlation between tissue IL12 ,TLR6, TLR4 in women with breast tumors

Parameters	IL12	TLR6	TLR4
IL12 sig	1	0.202 0.253	0.230 0.185
TLR6 Sig	0.202 0.253	1	-0.145 0.414
TLR4 Sig	0.230 0.185	-0.145 0.414	1

Table(4-18) Correlation between serum and tissueTLR4 in women with breast tumors

Parameters	TLR4 serum	TLR4 tissue
TLR4serum	1	0.346*
TLR4 tissue	0.346*	1

Significant (0.04)

Table(4-19) Correlation between serum and tissue TLR6 in women with breast tumors

Parameters	TLR6 serum	TLR6 tissue
TLR6serum	1	-0.416*
TLR6 tissue	-0.416*	1

Significant (0.01)

Table(4-20) Correlation between serum and tissue IL12 in women with breast tumors

Parameters	IL12 serum	IL12 tissue
IL12serum	1	-0.123
IL12 tissue	-0.123	1

Significant (0.4)

The general pattern of TLR expression in tumor cells suggests that TLR-mediated signaling plays a crucial role in cancer tumor development. It is possible that tumor cells express multiple TLRs to recognize various damage-associated molecular patterns (DAMPs) in their microenvironment. This may enhance the biological process mediated by TLR activation to produce favorable conditions for growth and survival. However, the significance of the expression of several TLRs in various cancer cells is not fully understood. Semlali et al. reported that different TLRs, specifically TLR 2, 6, and 9, are expressed in normal colon epithelial tissues, and their expression has been reported to be decreased in most colorectal cancer tissues

compared to normal matching tissues (Semlali *et al*.,2017) . Conversely, TLR4 expression increases in colon and breast cancer tissues compared to normal tissues (Semlali *et al* .,2016)

Shi *et al* .,2020 appeared the expression levels of TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10 were correlated with those of the inflammatory cytokines interleukin-1β and tumor necrosis factor-α.

The results of correlation was positive among TLR 4 and IL-12 this expressed by Toll-like receptor activation is the trigger that sets the immune system into action. The application of TLR ligands in cancer therapy is therefore an attractive possibility that has been intensively studied in the past years in the context of cancer treatment or prevention (as anti-tumor vaccine adjuvants). Macrophages stimulated by endotoxin respond by secretion of chemokines and proinflammatory cytokines, including TNFα and interleukin-1β, which coordinate local and systemic inflammatory responses. Dendritic cells, stimulated by endotoxin, secrete IL-12, which is important in anti-tumor immunity (Kadowaki *et al.*2001) .

CONCLUSIONS

AND

RECOMMENDATIOS

Conclusions

1-The incidence of benign breast tumors was greater than malignant.

2- The bacterial growth rate was very high in tissue culture and G+ve bacteria were higher than the G-ve bacteria .

3- Most common bacteria in breast tissue was *Staphylococcus . spp* followed by the bacteria *Pseudomonas fluorescens.*

4-The level of CA 15-3in the serum of women with malignant tumors was higher than that of women with benign tumors .

5-The level of IL12 in women patients was lower than in healthy women .

6- A positive relationship appeared between IL12 and TLR6 , an inverse relationship between IL12 and TLR4 ,and a positive relationship TLR4 and TLR6 in systemic breast .

7-A positive relationship was appeared between IL12 and TLR6 , and positive relationship between IL12 and TLR4 ,but an inverse relationship between TLR4 and TLR6 in mucosal breast.

8-Bacterial infection induced increased the concentration of toll like receptors in breast diseases.

Recommendations

1-Study of virulence genes in bacteria and their relationship to breast diseases.

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Appendix

الخلاصة

تعتبر أمراض الثدي واحدة من أكثر الأمراض شيوعًا بين النساء ، ويعد سرطان الثدي الخبيث هو السبب الرئيسي للوفيات المرتبطة بالسرطان في البلدان النامية وهو من أكثر أنواع السرطانات تشخيصًا وانتشارا بين النساء في جميع أنحاء العالم. يعتبر سرطان الثدي في العراق أكثر أنواع السرطانات شيوعاً مقارنة بأنواع السرطان الأخرى. قد يكون ورم الثدي خبيثًا وحميدًا ، لذا فإن هذه الأورام وأمراض الثدي تكون مصاحبة بالاخماج البكيتيرية، ولكن قد تختلف أنواع البكتيريا بين أورام الثدي ، لذلك هدفت الدراسة الحالية إلى الكشف عن أنوع البكتيريا في أنسجة الثدي. وتقدير تركيزالمستضد السرطاني CA15-3 كعلامة تشخيصية لسرطان الثدي. بالإضافة إلى تقدير تراكيزTLR4وTLR-6 و IL12 في النساء المصابات بأمراض الثدي عن طريق قياسها بواسطة جهاز المقايسة الامتصاصية المناعية للأنزيم المرتبط (ELISA). تم جمع عينات (أنسجة الثدي والدم) (50 عينة نسيج و100عينة دم) تم أخذها من النساء المصابات بأمراض الثدي50 عينة للسيطرة (50 عينة دم فقط) من النساء السويات وتراوحت أعمارهن بين (14 إلى 60 سنة). تم جمع العينات من مستشفى الحلة التعليمي ومستشفى ألامام الصادق في محافظة بابل خلال الفترة من تشرين الثاني 2021 - آذار 2022.

أن الأنسجة التي تم الحصول عليها كانت خارج المنطقة الهامشية ، على بعد حوالي ٥ سم من الورم. بعد الاستئصال ، ثم وضع النسيج الجديد على الفور في انبوب بلاستك معقم يحتوي على محلول ملحي عادي وتم تقطيعها وتجانسها وهرسها باستخدام مشرط جراحي معقم وعيدان خشبية في خلال 30 دقيقة من التجميع. تمت زراعته على أكار الدم ووسط ماكونكي أكار ، ثم وسط انتقائي ، وتم التعرف على البكتيريا عن طريق الفحوصات المجهرية والكيميائية الحيوية وتم تأكيدها بواسة جهاز الفايتك Vitec 2Compact System ، وظهرت نتائج الزرع النسيجي وكانت نسبة الأورام الخبيثة (18%) أما الحميدة (82%). أمرض الثدي الحميد مقسم إلى أربع مجاميع من النسبة الكلية في هذه الدراسة حسب الفحص النسيجي المتضمنة أولا التغيرات الكيسية الليفية كانت نسبتها (43.90%) بينما كانت نسبة الورم الغدي الليفي هي (34.14%) ثم التهاب الضرع الحبيبي (13.41%) والامراض الاخرى هي (الورم الشحمي، النخر الدهني) بنسبة (8.53%) .

كانت نسبة نمو البكتريا هي (88.57%) بينما لم يكن هناك نمو (11.42%) وتبين أن أكثر أنواع البكتريا النامية شيوعًا في الأنسجة هيPseudomonas florescence 32.25% ثم Staphylococcus aureus 29% بينما كانت أقل نسبةهي 3.22% بكتيريا Enterococcus feacales ، تم قياس مستضد السرطان CA15-3 في مصول مجموعة النساء المصابات بأورام الثدي وومجموعة النساء السويات (السيطرة) ،و ظهرت النتائج بأن تراكيز مستضد السرطان CA15-3 زادت بشكل ملحوظ في المريضات (146.79وحدة\مل) مقارنة بالسويات (94.17 وحدة\مل). أن تركيز المستضد CA 15-3 في مصول المريضات يختلف باختلاف نوع أ ورام الثدي حيث كانت أعلى تركيز في مصول مجموعة

النساء المصابات بسرطان القنوات(الاقنية) والسرطان الفصيصي كذلك في مجموعة التهاب الضرع الحبيبي مقارنة بأمراض الثدي الحميدة الأخرى .

تضمنت الدراسة الحالية قياس تركيز TLR4 و TLR6 المصلي والنسيجي . حيث أظهرت النتائج أن متوسط تراكيز TLR4 و TLR6 في مصول المريضات (2.11 , 4.58) نانوكرام \ مل على التوالي مقارنة بالسيطرة (1.58 , 4.18) نانوكرام \ مل ، مع وجود فرق معنوي في تركيز TLR6 كما ظهرت نتائج تراكيز TLR4 أعلى بالمصل مقارنة بالنسيج في مختلف ألاورام بينما كانت تراكيز TLR6 أعلى في النسيج منها في المصل في مختلف أنواع الأورام .

بلغ متوسط تركيز IL12 في مصول المريضات (16.57) نانو غرام /لتر بينما كان التركيز أعلى في النساء السويات(22.02 نانوغرام/لتر) مع فرقا معنوياً (0.0002).و كان متوسط تركيزالا نترلوكين 12 أعلى في النسيج مقارنة بالمصل حسب أنواع الأورام.

بينت نتائج هذه الدراسة ، وجود علاقة إيجابية بين تراكيز أنترلوكين IL-12 و TLR-6 جهازيا ، في حين كان الارتباط السلبي بين IL-12 و TLR-4 جهازيا عند مستوى معنوي (p> 0.05). وكذلك الارتباط بين IL-12 المصل والنسيج أرتباطا سلبيا.

كان الارتباط أيجابيا بين IL12 و TLR-6 موضعيا ، وكذلك ارتباط إيجابي بين IL12و TLR4 نسيجيا . أما الارتباط بين TLR-6 و TLR-4 مصليا فكان ارتباطًا إيجابيًا ، بينما كان الارتباط بين TLR-6 TLR-4 نسيجيا ارتباطًا سلبيًا. وكان الارتباط بين TLR-6 المصلي و TLR-6 النسيجي سلبيا. كذلك هناك ارتباط إيجابي بين TLR-4 مصليا و TLR-4 النسيجي .

نستنتج من نتائج هذه الدراسة إلى أن العدوى البكتيرية تزيد من تراكيز المستقبلات الشبيهة بالتول TLR-6 في أورام الثدي .

وزارة التعليم العالي والبحث العلمي

جامعة بابل

كلية العلوم

قسم علوم الحياة

العلاقة بين المحتوى البكتيري وبعض المعايير المناعية في النساء المصابات بأورام الثدي

رسالة مقدمة الى

مجلس كلية العلوم /جامعة بابل كجزء من متطلبات نيل درجة الماجستير

في العلوم /علوم الحياة

من قبل الطالب

ثامر شذر شهيد حمود

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١٤٤٤هـ **٢٠٢٢م**