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EVALUATION OF SOME IMMUNOLOGICAL AND BIOCHEMICAL PARAMETERS IN FEMALE BREAST CANCER

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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EVALUATION OF SOME IMMUNOLOGICAL AND BIOCHEMICAL PARAMETERS IN FEMALE BREAST CANCER

By Ibtihal Najim Shihab ALTIMIMI

December 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

EVALUATION OF SOME IMMUNOLOGICAL AND BIOCHEMICAL PARAMETERS IN FEMALE BREAST CANCER

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Master of Science in Biology

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December 2023

A comprehensive investigation was undertaken at the Kirkuk Oncology Center in Iraq, utilizing a cross-sectional study design to delve into the immunological and biochemical profiles of female breast cancer patients. The cohort encompassed 270 women afflicted with breast cancer, juxtaposed with 50 healthy counterparts devoid of familial breast cancer history. The study meticulously dissected various facets, revealing intriguing insights into the disease spectrum. Predominantly, the study spotlighted the demographic landscape, unveiling the age bracket of 40-59 as the focal point of breast cancer incidence, with a notable concentration observed within the 40-49 age stratum. Moreover, a prevailing trend emerged among participants characterized by a primary level of education and marital status. Despite the prevalence of breast cancer, a noteworthy majority exhibited no familial predisposition to the ailment, underlining the significance of non-hereditary factors. Subsequent analyses delved into the biochemical intricacies, unraveling compelling differentials between breast cancer patients and the control cohort. Noteworthy disparities encompassed diminished hemoglobin (Hb) levels alongside elevated concentrations of CA 15-3, CEA, and IL-10 among breast cancer patients. Furthermore, an array of biomarkers including Ferritin, GSH, LDH, ALP, GGT, and SGOT demonstrated discernible distinctions between the cohorts, with Ferritin levels exhibiting a notable escalation corresponding to cancer advancement. Remarkably, the investigation also unveiled distinctive biochemical signatures across varying tumor-node-metastasis (TNM) stages, further accentuating the prognostic potential of these biomarkers. For instance, diminished Hb levels were particularly

conspicuous in T2N1M0 compared to other stages, whereas LDH levels exhibited significant elevation in T1N0M0 instances. These nuanced findings collectively underscore the diagnostic and prognostic utility of these biomarkers in the clinical management of breast cancer. The distribution analysis of breast cancer patients across TNM stages delineated a substantial proportion of early-stage manifestations juxtaposed with a cohort manifesting advanced characteristics, illuminating the heterogeneous nature of breast cancer progression and the imperative for tailored therapeutic approaches.

2023, 76 pages

Keywords: ALP, Breast cancer, CA 15-3, CEA, IL-10, GSH, LDH, GGT and SGOT

ÖZET

KADIN MEME KANSERİNDE BAZI İMMÜNOLOJİK VE BİYOKİMYASAL PARAMETRELERİN DEĞERLENDİRİLMESİ

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Irak'taki Kerkük Onkoloji Merkezi'nde, kadın meme kanseri hastalarının immünolojik ve biyokimyasal profillerini derinlemesine incelemek amacıyla kesitsel bir çalışma tasarımı kullanılarak kapsamlı bir araştırma yürütüldü. Kohort, ailesel meme kanseri öyküsü olmayan 50 sağlıklı meslektaşıyla birlikte meme kanserine yakalanmış 270 kadını kapsıyordu. Çalışma, çeşitli yönleri titizlikle inceleyerek hastalık spektrumuna dair ilgi çekici içgörüleri ortaya çıkardı. Çalışma ağırlıklı olarak demografik manzarayı ön plana çıkardı ve meme kanseri görülme sıklığının odak noktası olarak 40-59 yaş aralığını ortaya çıkardı; kayda değer bir yoğunluk 40-49 yaş grubunda gözlemlendi. Ayrıca, katılımcılar arasında ilköğretim düzeyi ve medeni durum ile karakterize edilen hakim bir eğilim ortaya çıktı. Meme kanserinin yaygınlığına rağmen, kayda değer bir çoğunluk, kalıtsal olmayan faktörlerin öneminin altını çizerek, rahatsızlığa ailesel bir yatkınlık göstermedi. Sonraki analizler biyokimyasal incelikleri araştırdı ve meme kanseri hastaları ile kontrol grubu arasındaki zorlayıcı farklılıkları ortaya çıkardı. Dikkate değer eşitsizlikler, meme kanseri hastaları arasında CA 15-3, CEA ve IL-10'un yüksek konsantrasyonlarının yanı sıra hemoglobin (Hb) seviyelerinin azalmasını da içeriyordu. Ayrıca, Ferritin, GSH, LDH, ALP, GGT ve SGOT'u içeren bir dizi biyobelirteç, kohortlar arasında fark edilebilir farklılıklar gösterdi; Ferritin seviyeleri, kanserin ilerlemesine karşılık gelen kayda değer bir artış sergiledi. Dikkat çekici bir şekilde, araştırma aynı zamanda değişen tümör düğümü metastazı (TNM) aşamalarında farklı biyokimyasal imzaları da ortaya çıkardı ve bu biyobelirteçlerin prognostik potansiyelini daha da vurguladı. Örneğin, diğer evrelerle karşılaştırıldığında

T2N1M0'da azalan Hb düzeyleri özellikle dikkat çekiciyken, T1N0M0 örneklerinde LDH düzeyleri anlamlı bir yükselme sergiledi. Bu incelikli bulgular, toplu olarak, bu biyobelirteçlerin meme kanserinin klinik yönetiminde tanısal ve prognostik faydasını vurgulamaktadır. Meme kanseri hastalarının TNM evreleri arasındaki dağılım analizi, erken evre belirtilerin önemli bir kısmını, gelişmiş özellikleri ortaya koyan bir grupla yan yana getirerek, meme kanseri ilerlemesinin heterojen doğasını ve kişiye özel terapötik yaklaşımların zorunluluğunu aydınlattı.

2023, 76 sayfa

Anahtar Kelimeler: Meme kanseri, CA 15-3, CEA, IL-10, GSH, LDH, ALP, GGT ve SGOT

PREFACE AND ACKNOWLEDGEMENTS

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Ibtihal Najim Shihab ALTIMIMI

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CONTENTS

LIST OF SYMBOLS

>	Bigger than
°C	Degree celsius
µL	Microliter
mL	Milliliter
%	Percentage
<	Smaller than

LIST OF ABBREVIATIONS

DTNB	5,5-Dithiobis(2-nitrobenzoic acid)
AP-1	Activator protein 1, a transcription factor
AAM	Age at menarche
BMI	Body mass index
BSA	Bovine serum albumin
BC	Breast cancer
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
CA 15-3	Cancer antigen 15-3
CEA	Carcinoembryonic antigen
CNB	Core needle biopsy, another type of breast biopsy
DCs	Dendritic cells, a type of immune cell
DNA	Deoxyribonucleic acid, a molecule
ELFA	Enzyme linked fluorescent assay
ER	Estrogen receptor, a hormone receptor
EDTA	Ethylenediaminetetraacetic acid
FNAC	Fine needle aspiration cytology, a type of breast biopsy
GOT	Glutamate oxaloacetate transaminase
HRT	Hormone replacement therapy
HER2	Human epidermal growth factor receptor 2
IL-10	Interleukin 10, a cytokine with anti-inflammatory properties
IDC-NST	Invasive ductal carcinoma no specific type
KLH	Keyhole limpet hemocyanin
LDH	Lactate dehydrogenase, an enzyme
MRI	Magnetic resonance imaging, an imaging technique
MHC-II	Major histocompatibility complex class II, a group of proteins
MLE	Master lot entry
NADH	Nicotinamide adenine dinucleotide, a coenzyme
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
PR	Progesterone receptor, another hormone receptor
PR	Prolactin receptor, a receptor involved in breast cancer
GSH	Reduced glutathione
RFV	Relative fluorescence value
RNA	Ribonucleic acid, a molecule
SPR	Solid phase receptacle
Th1	T-helper type 1 cells, a subset of T cells
Th2	T-helper type 2 cells, another subset of T cells
TNM	Tumor node metastasis

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1. INTRODUCTION

In the field of oncology, breast cancer is still a big problem because it affects women all over the world and has big health effects. It is the cancer that women are most likely to get and the main reason they die from cancer (Arbyn *et al.* 2020). Breast cancer is a significant health problem in Iraq that affects women in many different areas, including Kirkuk and Erbil. Gaining a thorough grasp of the immunological and biochemical factors linked to breast cancer is essential to furthering our understanding of the condition, enhancing the precision of diagnosis, and creating efficient treatment plans (Zubair 2021).

Because ferritin may play a role in the initiation and spread of cancer, this intracellular protein that stores iron has attracted a lot of attention in the field of cancer research. Since tumor growth and proliferation have been linked to changes in iron metabolism, ferritin is an interesting characteristic to look into in breast cancer (Lelièvre *et al* 2018). Serum ferritin levels have been found to be higher in breast cancer patients than in healthy controls,suggesting that ferritin may be used as a prognostic indicator (Khan 2018). Moreover, poorer prognoses and advanced tumor stages have been linked to elevated ferritin levels in breast cancer patients.According to these results,ferritin may be a useful marker for determining the prognosis and degree of the disease in breast cancer patients.Changes in redox status and GSH levels have been connected to the start and spread of cancer.GSH dysregulation in breast cancer has been linked to resistant to treatment and aggressive tumor behavior (Sun 2022).

In individuals with breast cancer, decreased GSH levels raise oxidative stress,which may have an impact on the course of the disease and how well treatment works.Knowing the connection between GSH levels and breast cancer may help develop new therapy modalities and individualized regimens. Enzymes LDH, ALP, and GGT are involved in a number of metabolic activities and have been linked to the metabolism,proliferation,and metastasis of cancer cells.LDH is connected to the energy metabolism and glycolytic pathway in cancer cells,whereas ALP and GGT are

connected to the advancement of cancer and a poor prognosis. In patients with breast cancer, elevated LDH levels have been linked to later stages of the disease and a lower prognosis (Liu 2019).

In a similar vein,higher levels of ALP and GGT have been linked to a poorer prognosis and an increased chance of metastasis in cases of breast cancer (Wang *et al* 2020).Breast cancer patients may benefit from using these enzymes as prognostic indicators to help with risk assessment and treatment selection.Research has indicated that patients with breast cancer who have low preoperative hemoglobin levels tend to have worse survival results.(Lee *et al.* 2017).Low hemoglobin levels,or anemia, have also been linked to a higher death rate in breast cancer patients (Fischer *et al.* 2019).The biology of breast cancer is significantly influenced by the immune system and its constituents. The immunomodulatory cytokine IL-10 has drawn a lot of attention because of its possible role in host immune response modulation and tumor immune escape mechanisms. Tumor microenvironment effects, immune effector function suppression, and tumor immune evasion are all possible with IL-10 (Vitale *et al.* 2019). Elevated serum IL-10 levels have been associated with worse overall survival and disease-free survival in breast cancer patients (Chang *et al.* 2021).

Variants in the IL-10 gene have also been linked to the likelihood and prognosis of breast cancer (Dincă *et al.* 2022).Knowing how IL-10 functions in breast cancer can help with understanding the therapeutic target's potential as well as how it affects immunotherapy tactics (Morales *et al.* 2020) The interplay between IL-10 and HER1/HER2 signaling pathways in breast cancer has gained attention as a potential mechanism influencing tumor growth and therapeutic response.Elevated IL-10 levels have been associated with HER2 overexpression in breast cancer patients (Chang 2021). Activation of HER1 signaling by IL-10 has also been implicated in breast cancer cell proliferation and survival (Bozorgi 2022).

The aim of this study was to assess and investigate potential associations of serum Ferritin, GSH, LDH, ALP, GGT, SGOT, CA15-3, CEA, and IL-10 in Female Breast Cancer.

2. LITERATURE REVIEW

2.1 Anatomy of Breast

The breast is a glandular organ made up of lobes. It is found in the superficial fascia of the front of the thorax (Chavez‐MacGregor *et al.* 2017). It contains fifteen to twenty lobes. A unique type of breast lobe consists of lobules, which are collections of tiny glands with the ability to produce milk. Via tiny lactiferous tubes known as ducts, milk travels from the lobules to the nipple. The nipple is a cylinder-shaped portion of the breast that contains tissue that helps in erections. Usually, it occurs in the center of the areola, a dark area on the skin. The breast's size, shape, and composition are all determined by the fat that fills the area between the lobes and ducts. Lobules within their own fatty coats are separated by the ligaments connecting the pectoralis muscle to the breast surface (Watkins *et al.* 2019).

We refer to these as Cooper's ligaments. They maintain the contour of the breast fat and provide support for it. The breast contains both lymph and blood arteries. A collection of lymph nodes is located in the axilla next to the breast (underarm), and the lymph vessels convey a clear fluid known as lymph node (Kim and Lim 2021).

The axillary process of the breast rises and sides to meet the axilla; it is sometimes referred to as the "axillary tail of Spence." Breast tissue lies between the breast and the axilla. This area of the breast is significant from a medical perspective because the lymphatic drainage of the axillary process is prone to breast cancer. As illustrated in picture 2.1 (Alex *et al.* 2020). The tissue of the breasts can sense changes in hormone levels. throughout menstruation, pregnancy, and adolescence (Cserni 2020).

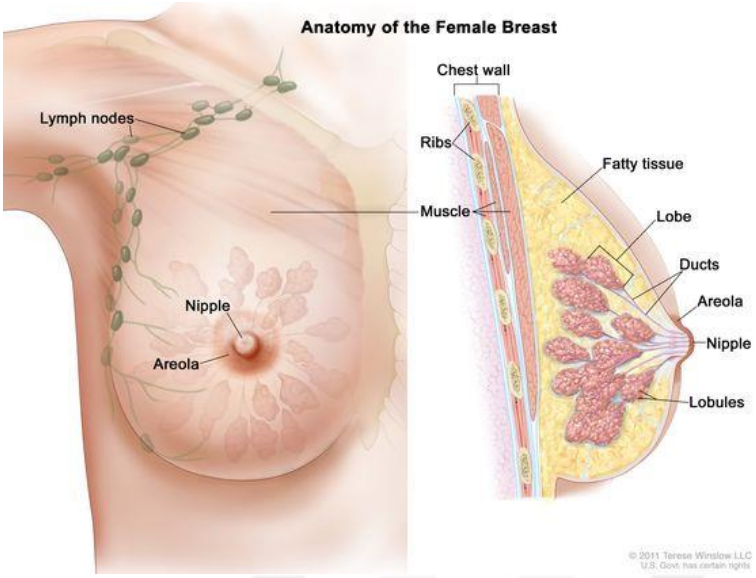

Figure 2.1 Anatomy of the female breast (Alex 2020)

2.2 Breast Cancer

Breast cancer (BC) is a potentially fatal malignant tumor. It is brought on by the breast tissue's unchecked cell development and its cells' propensity to infiltrate and destroy neighboring healthy tissue. The breast's ductal epithelial cell is where an initial tumor begins. However, if it becomes invasive, it may spread to the nearby lymph nodes. Both men and women are affected, however women are more susceptible to it due to their breasts and lifetime exposure to estrogen (Denton *et al.* 2017). Breast cancer exhibits a broad spectrum of clinical symptoms and therapy responses, as well as distinct symptoms resulting from alterations in genes, transcriptomes, and epigenomes. It is a biologically and histologically heterogeneous illness (Cserni 2020).

2.3 Epidemiology

More women die from breast cancer than from any other type of cancer, despite it being the most prevalent variety worldwide. Twenty-three percent of cancer-related deaths are caused by it.Despite the fact that cancer diagnoses and deaths are related, the incidence of the disease varies five times between high-incidence regions like the US and Western Europe and low-incidence regions like Africa and Asia (Johansson *et al.* 2021). In the

UK, breast cancer affects one in every twelve women between the ages of one and eighty-five. In women, breast cancer is common. A million new instances are discovered annually, accounting for 18% of all cancer cases worldwide. It is anticipated that by 2021, 85 women out of 100,000 will receive a breast cancer diagnosis (Starek-Świechowicz *et al.* 2021).

2.4 Breast Cancer Classification

The goal of classifying breast cancer is to separate the disease into various categories according to several criteria. The most crucial components of a complete categorization are histological type, grading, staging (tumor node metastasis),receptor status, and gene expression (Conti 2021).To identify the best approach to treat something is the aim of grouping.It has been demonstrated that a certain treatment is effective for a particular form of breast cancer (typically through randomized, controlled trials) (Nawaz *et al.* 2018). That treatment may not be effective if your breast cancer is not of the type described above.Certain types of breast cancer are extremely dangerous and may even be fatal, necessitating lengthy and painful therapies. Less intrusive treatments, such as lumpectomies, can be used to treat less threatening forms of breast cancer (Coleman 2017).

2.5 Breast Tumor Histological Classification

The majority of breast cancers, despite their wide variety, are classified as mammary ductal carcinomas and originate from the epithelium lining the ducts or lobules. When cancer cells proliferate within the epithelial tissue without spreading to neighboring tissues, this condition is known as carcinoma in situ. Invasive carcinoma, on the other hand, spreads to the surrounding tissue (Denton *et al.* 2017). Histopathology is categorized using light microscopy. Breast cancer is a condition that presents itself in a variety of ways. They are classified into many categories and subtypes by the World Health Organization according to their histological characteristics, prognosis, and bodily manifestations (Russnes 2017).

2.6 Invasive Ductal Carcinoma no Specific Type (IDC-NST)

IDC-NST is the most prevalent histological subtype, accounting for 40-75% of cases of invasive breast cancer. It typically exhibits a broad spectrum of clinical behaviors and physical characteristics. Tumor cells exhibit a wide variety of morphologies, protruding nucleoli, and many divisions. Necrosis and calcifications are seen in specific areas in over half of the cases (Tian *et al.* 2018).

2.7 Medullary Carcinoma

This particular subtype of invasive breast cancer accounts for roughly 5% of all instances. It is associated with reduced rates of spreading to the axillary lymph nodes and better clinical outcomes. Mutations in the BRCA1 germline (breast cancer gene 1) are frequently associated with it, and it typically affects individuals between the ages of 30 and 40 (Kim *et al.* 2021).

2.7.1 Metaplastic carcinoma

The most significant characteristic of this histological group is metaplastic differentiation. It primarily affects women after menopause and accounts for around 1% of all cases. These tumors behave aggressively and frequently spread to lymph nodes (Cserni 2020).

2.7.2 Apocrine carcinoma

Roughly 1–4% of all instances are of this type, and at least 90% of tumor cells exhibit unmistakable evidence of apocrine differentiation. This subtype typically has a poor prognosis and ranks highly on the histopathological scale. Although it can occur in individuals of any age, women who have had menopause are more likely to have it (Sauter 2018).

2.7.3 Mucinous carcinoma

Various names for this particular type of breast cancer include colloid, gelatinous, mucous, and mucoid carcinoma. It accounts for 2% of all newly diagnosed instances of breast cancer. Women over 60 years old are most likely to have this subtype, which has been associated with a favorable prognosis (Kim *et al.* 2021).

2.7.4 Cribriform carcinoma

This particular subtype of breast cancer typically affects individuals 50 years of age or older and has a favorable prognosis. It accounts for 1% to 3% of all cases of breast cancer. There is very little evidence of localized or distant metastases in cribriform cancer (Kim *et al.* 2021).

2.7.5 Tubular carcinoma

Well-defined subtype, accounting for around 2 percent of all newly diagnosed cases, primarily affects women between the ages of 50 and 60. The majority of tubular carcinomas are associated with a variety of precancerous growths that may develop into malignancy (Brenner *et al.* 2018).

2.7.6 Neuroendocrine carcinoma

It typically affects elderly adults and accounts for 0.5 to 5 percent of all instances of breast cancer. This kind of tumor is comparable to neuroendocrine tumors of the lungs and digestive system in that over half of the malignant cells consistently exhibit synaptophysin and chromogranin A expression (Cserni 2020).

2.7.7 Invasive lobular carcinoma

It ranks as the second most prevalent kind of cancer. It is responsible for 5–15 percent of all newly diagnosed cases, and older women make up the majority of those affected. Small tumor cells with few abnormalities that are uniformly distributed in a concentric pattern throughout the stroma make up the classic type of ILC (Hai *et al.*2019).

2.8 Breast Cancer Grade

The process of characterizing tumor cells or neoplasms under a microscope and their differences from normal cells is known as tumor grading. Predicting future events is the primary goal of grading. The tumor's aggressiveness and expected prognosis aid the physician in developing an effective treatment strategy (Hai *et al.* 2019). To determine the histologic grade of the tumor, the Scarff-Bloom-Richardson (SBR) technique for grading in Nottingham Modification is advised (Nawaz *et al.* 2018).

- The growth of tubules.
- Pleomorphism of the nucleus.
- Measured the number of mitoses.

Most of the time, doctors use the Nottingham system to grade breast cancer. (Kashyap *et al.* 2018). This system divides breast cancer into the following groups.

- Gx: No grade can be given
- G1 is also known as low grade or well-separated.
- G2: Middle school level or moderately different.
- G3: High grade or hard to tell apart.

2.9 Stages

The goal of breast cancer staging is to figure out the best way to treat it and give accurate information about how likely it is to come back (Bozorgi *et al.* 2022). Most people use the TNM (tumor node metastasis) staging system to figure out what stage their breast cancer is in. This system divides breast cancer into five stages, from 0 to IV, based on three important ways that cancer behaves (Santucci 2023).

- The size of the first cancerous growth (T).
- Whether or not there are regional lymph nodes and how big they are (N).
- Whether or not there are distant metastases (M).

2.9.1 Tumor size

Even after 20 years of follow-up, the size of the tumor is still a strong sign of what will happen. Even though some pathologists measure the tumor's macroscopic or microscopic size, which includes both the invasive and in situ parts, only the microscopic size of the invasive part is clinically important (Denton *et al.* 2017). Because bigger tumors tend to have more lymph nodes that are positive, the size of the tumor is a strong predictor of axillary lymph node involvement. But a worse prognosis is linked to a bigger tumor size, no matter what the lymph nodes look like (Nawaz *et al.* 2018). This is because patients without lymph nodes whose tumors were less than 1 cm had a much better chance of survival (80% vs. 65%) than those whose tumors were more than 2 cm. The primary (first) tumor is described by the tumor category (Lim *et al.*2018).

- TX means that the tumor can't be checked out.
- T0 means that there is no sign of the main tumor.

This means that the cancer is "in situ," which means that the tumor has not spread into healthy breast tissue.

T1, T2, T3, T4: These numbers depend on how big the tumor is and how much it has spread into nearby breast tissue. The bigger the tumor and/or the more it may have grown into the breast tissue, the higher the T number.

2.9.2 Axillary lymph node status

The amount of involvement of the axillary lymph nodes at the time of diagnosis is one of the most reliable independent signs of how breast cancer will progress (Cserni *et al.* 2018). Patients with lymph nodes that were clear of tumors had a much better prognosis than those with positive lymph nodes. Only 15–45% of node-negative patients got sick again, while 50–70% of node-positive patients did. Also, the chance of getting sick again and dying goes up as the number of lymph nodes affected goes up. When metastases are found in the sentinel lymph nodes, the status of the lymph nodes is checked with the sentinel node procedure, which is followed by an axillary node dissection (Angahar 2017).

The N category (lymph node involvement) tells if the cancer has spread to nearby lymph nodes or not (Rosen Sawaki *et al.* 2019).

NX means that the nearby lymph nodes can't be evaluated, like if they were already taken out (Denton *et al.* 2017).

N0 means that there is no cancer in nearby lymph nodes (Nawaz *et al.* 2018).

These numbers are based on how many lymph nodes are affected and how much cancer is found in those lymph nodes. The lymph nodes are involved more, the more the N number goes up, the more lymph nodes are involved (Guerrini *et al.* 2020).

2.9.3 Metastasis

Evidence of cancer spreading to other organs or regions of the body is shown by the M (metastasis) category (Klein 2020)

MX indicates that the spread of cancer cannot be determined.

When the distant metastases are not present, the result is M0.

M1 indicates the presence of metastases in distant locations.

Tumor node metastasis (TNM) staging assigns a numerical value from 0 to IV to the five stages of breast cancer (Li *et al.* 2018). Based on the research conducted by Mittendorf *et al.* in 2018.

Stage 0:

Lobular carcinoma in situ is another name for carcinoma in situ. The breast ducts and lobules contain abnormal cells at this early stage, but they have not yet metastasized (neoplasia) (Angahar 2017).

Stage I:

The tumor's size is less than 2 cm, and it hasn't reached the lymph nodes under the armpits (Angahar 2017).

Stage II:

Tumors that are less than 2 centimeters in width and have not metastasized to lymph nodes beneath the arm characterize this early stage of breast cancer. Another possibility

is that the tumor has spread to lymph nodes or is 2–5 cm wide. an arm tumor larger than 5 cm that has not metastasized (Hai *et al.* 2019).

Stage III:

Protects the breast and its surrounding tissues from harm and has a diameter larger than 5 centimeters (Hai *et al*, 2019).

Stage IV:

Metastatic breast cancer is a form of breast cancer that has spread to other parts of the body (Hai *et al.* 2019).

2.10 Symptoms

An individual with a breast lump may be oblivious to its presence. Depending on their cause, lumps in some women's breast tissue can be small or deep, while in others they are more noticeable. There are no outward symptoms of early breast cancer, but as the tumor expands and metastasizes, symptoms do appear. As shown in Figure 2. 2, this (Webber *et al.* 2017).

- Differences in breast size or shape.
- An enlarged or lumpy breast, typically affecting just one breast.
- Alterations to the nipple's form, including sores, ulcers, redness, or an inverted nipple, as well as clear or bloody discharge.
- Rashes, a scaly appearance, and unexplained redness are all changes that can occur on the skin of the breasts.
- Strange pain that persists and is unrelated to the typical menstrual cycle 10.

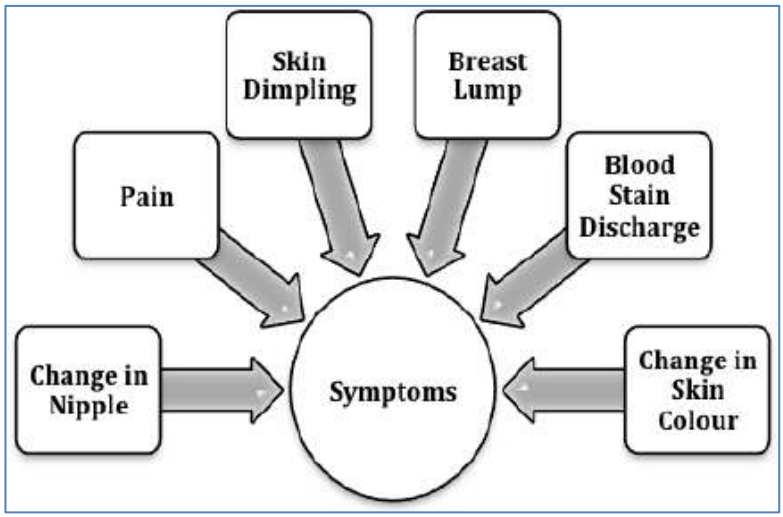

Figure 2.2 Symptoms of breast cancer (Webber *et al.* 2017)

2.11 Risk Factor for Breast Cancer

A risk factor is anything that makes it more likely that someone will get sick (Shetty *et al.* 2021). Disease occurrence, prognosis, and prevention all depend on risk factors.

2.11.1 Age and gender

Age and gender are the two main risk factors for breast cancer. Women are more prone than males to develop breast cancer because estrogen is more prevalent in women than in men (Angahar 2017). Aging may contribute to an increased risk of breast cancer, as environmental influences accumulate over a person's lifespan. Changes in methyl group addition or removal result in modifications to the methylation of DNA at regulatory areas. (Johnson *et al.* 2017).

2.11.2 Family history

The risk for women who have a family history of breast cancer is about twice that of women who do not have a family history of the disease. Particularly for first-degree relatives, this is accurate (mother, sister, daughter) (Reiner *et al.* 2018)

2.11.3 Genetic mutation

Hereditary variations in the BRCA1 and BRCA2 genes, as well as HER2, have been associated with an increased risk of breast cancer (Reiner *et al.* 2018, Sun *et al.* 2017).

2.11.4 Benign breast disease

There is a high correlation, according to numerous research, between benign breast diseases and the risk of breast cancer. The histological pattern of benign breast disease, in addition to other factors like hormonal condition, psychological stress, and family history, all influence this chance (Stachs *et al.* 2019).

2.11.5 Reproductive factors

There is an increased risk of breast cancer associated with endogenous hormones, early menarche, nulliparity, late menopause, and late age at first pregnancy, according to multiple epidemiological studies (Kumari *et al.* 2019, Khalis 2018).

Menarche, or a young woman's first period, is an important step in her development as a teenager. A woman's age at menarche (AAM) is an important clinical measure of her physical, nutritional, and reproductive health as she moves from childhood to young adulthood. It is known that young girls who go through puberty and menarche early are more likely to get breast cancer because they are exposed to high levels of hormones for a longer time when their ovaries are working (Kim and Lim 2021, Bjelland *et al.* 2018).

Women who have never given birth have a higher risk of breast cancer than women who have given birth more than once. Having a full-term pregnancy and giving birth with more than one person help lower the risk of breast cancer (Nawaz *et al.* 2018). Higher levels of the sex hormones estrogen and progesterone. Binding globulin is a protein that sticks to other proteins. Changes in hormones that happen during pregnancy

Differentiation and growth of mammary cells are signs of cancer (Johansson *et al.* 2021).

old age at menopause: Menopause is when a woman stops having her periods. It's called infertility when a woman's reproductive hormones change and her ovaries stop making eggs. Women who went through menopause later were more likely to get breast cancer. This could be because they were exposed to estrogen and progesterone for longer periods of time and in higher amounts. (Dall *et al.* 2017, Balekouzou *et al.* 2017).

Early age at pregnancy: Every full-term pregnancy in women younger than 20 years old lowers the risk of breast cancer by 7%. Estrogen, progesterone, and human chorionic gonadotropin all play a role in the normal progression of pregnancy, as well as the physical changes that happen in the breasts during this time. These changes caused by hormones could be important in keeping the mammary gland from turning cancerous (Meier 2014, Khalis 2018).

2.11.6 Hormonal factors

Oral contraceptives: The estrogen content of oral contraceptives is likely to blame for the small increased risk of breast cancer in reproductive-aged women who use or have used these medications (Hunte 2017).

endogenous estrogen: Breast cancer is more common in postmenopausal women with elevated levels of endogenous estrogen (de Roon 2018). Adipose tissue converts androstenedione to estrone, which is the principal estrogen source for postmenopausal women. According to Starek-Świechowicz *et al.* (2021), this results in an increase in the levels of free and albumin-bound estradiol, two forms of biologically active estrogens. The breakdown of estrogen into its genotoxic and mutagenic metabolites, along with the subsequent stimulation of tissue growth, can lead to cancer (Johnson *et al.* 2017).

2.11.7 Hormone replacement therapy (HRT)

Combining estrogens and/or progesterone to treat menopausal symptoms or osteoporosis has been shown to raise the risk of breast cancer in about a third of women (1.6 to 2 fold). When estrogen and progestin are used together, the effect is stronger than when estrogen or progesterone are used alone (Azam *et al.* 2020).

2.11.8 Environmental and lifestyle related factors

Breast cancer is one of numerous cancers made more likely by exposure to radiation. Dosage and duration of exposure both increase the risk (Sauter 2018). Breast cancer risk is three times higher in women who had diagnostic or therapeutic chest x-rays when they were teenagers or young adults. One possible explanation is that at that age, breasts are still developing (Momenimovahed *et al.* 2019). Contrarily, it appears that radiation exposure beyond the age of 40 does not raise the likelihood of breast cancer (Brenner 2018).

The definition of obesity was defined as a gain of 20% or more of body weight, while the definition of overweight was defined as a gain of 15% to 20% of body weight. According to the World Health Organization and the National Institutes of Health, a body mass index (BMI) of 30 kg/m2 or higher is considered obese, while a BMI of 25 kg/m2 or higher is considered overweight (Atoum *et al.* 2020). Although the risk of breast cancer in overweight women increases after menopause, it appears to be around 20-40% lower in overweight women before menopause. Obese women have an increased risk of postmenopausal breast cancer due to lower levels of sex hormone-binding globulin, which increases free estrogens, and higher levels of testosterone, estradiol, and estrone in the blood (Atoum *et al.* 2020). Hormones have an indirect effect on breast tissue growth and an indirect effect on DNA, increasing the risk of mutations in genes involved in vital cellular processes such as apoptosis, DNA replication, and DNA repair. As a result, tumor formation is enhanced in cells harboring these mutations (Williams *et al.* 2019).

Regarding nutrition and diet, research has shown that consuming more processed and fast food doubles the risk of breast cancer. Eating more unhealthy foods in larger portions has also been associated with this risk. Breast cancer rates are higher among American women due to the high cost of living, and this disparity is even more pronounced compared to women from Mediterranean countries (Krusinska *et al.* 2018). they consumed a significant amount of saturated fat and processed meat (Krusinska *et al.* 2018).

Homeostasis is jeopardized by stress, which can have numerous sources. Mental and physical stress may contribute to breast cancer development and progression (Chiriac *et al.* 2018). The body produces stress hormones such as cortisol and catacholamins in reaction to both mental and physical stress (adernaline and noradernaline). Carcinogenesis results from these hormones' effects on DNA (Yasuda *et al.* 2017).

2.11.9 Smoking

There is a small link between smoking and a higher risk of breast cancer, and this link gets stronger the more you smoke (Takada *et al.* 2020).

2.12 Diagnosis

A mammogram is an image of the breast obtained by X-ray. It has several advantages, including high sensitivity and specificity, and is regarded as the most effective method for identifying breast cancer. Please provide an example of a simple, low-cost, highly-specific approach. Evidence suggests that mammography can lower the breast cancer mortality rate by 19%. Aside from that, there are many good things about it (Jafari 2018).

Ultrasound is another important imaging modality for the diagnosis of breast cancer and evaluation of treatment effectiveness. Ultrasonography, in conjunction with mammography, is necessary for the diagnosis and treatment of breast issues. Ultrasound

can identify breast tumors in young women, expectant moms, and nursing mothers (Cserni *et al.* 2018). It is a useful diagnostic tool because it is highly sensitive and does not use ionizing radiation. Breast augmentation procedures and harpoon insertions both make use of ultrasound imaging modalities such as ultrasound contrast and elastography (Yuan *et al.* 2020, Liu *et al.* 2018).

Many believe that a breast biopsy is the gold standard for determining whether a woman has breast cancer. Core needle aspiration (CNA) and fine needle aspiration cytology (FNAC) are the two main options for breast biopsies (Sharma *et al.* 2020). A fast and inexpensive method to determine the nature of palpable breast lesions is FNAC, which examines the cytopathology of the fluid collected from the lesions prior to surgery or treatment. The results of FNAC are suggestive but not conclusive, so a vacuum-assisted breast biopsy (CNB) guided by ultrasound of the breast is recommended to confirm the diagnosis. Only a breast biopsy can confirm whether the suspicious area is actually cancerous (Shobha and Rajashekar 2017).

Magnetic resonance imaging (MRI) is an effective imaging method that produces clear pictures without the use of harmful radiation. The same way that mammography can detect breast cancer, this technique can as well (Kuhl 2019).

Chemicals or compounds released into the bloodstream by a tumor are known as serum tumor indicators. These substances can be detected in the blood and quantified (Bosch *et al.* 2018). Carcinoembryonic antigen (CEA) and CA15-3 were the most prevalent serum markers of breast cancer. One popular biomarker for advanced breast cancer is CA15-3, which stands for carbohydrate antigen 15-3. However, it does a poor job of detecting early-stage breast cancer. There has been extensive use of CA15-3 in the detection of recurrence and evaluation of treatment efficacy in metastatic breast cancer (Wang *et al.* 2017).

More precise prognostic information and sensitivity predictions made possible by multigene panels, genomic profiles, and signature scores have led to their increased use

in clinical practice, where they aid in the development of effective treatment plans (Colomer 2018).

Before recommending endocrine chemotherapy for every new case of breast cancer, it is important to check for hormone receptors in the tissue, such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal receptor (HER2) (Bosch *et al.* 2018).

2.13 Treatment

For patients with advanced breast cancer or localized breast cancer, chemotherapy can alleviate symptoms and reduce the likelihood of recurrence. It was previously believed that chemotherapy drugs could only kill cancer cells, but recent research has shown that they can also damage normal cells. Fatigue, queasy stomach, baldness, vomiting, and, in the worst-case scenario, death, are some of the side effects that may occur. To eliminate all dividing cells, one treatment option is to use chemicals that inhibit DNA synthesis. The fastest-dividing cells are the ones that benefit most from the medication (Momenimovahed *et al.* 2019).

- Anthracyclines are:. Anthracyclines, which include doxorubicin and epirubicin, are the type of drugs that are most often used to treat breast cancer (Cai *et al.* 2019).
- Taxoter is a semisynthetic taxane that has been shown to be effective in treating a number of solid cancers. Taxanes include Paclitaxel (Taxol) and docetaxel (Taxotere) (Waks *et al.* 2019).
- *5-deoxyfluorouridine (5-FU).
- Cyclophosphamide (Cytoxan) and Carboplatin (Paraplatin) (Alhareeri *et al.* 2020).

Radiation therapy is typically administered either prior to or following surgical procedures. The gold standard for treating breast cancer has been radiation therapy, both systemic and localized. Radiation treatment typically lasts between four and seven weeks and is administered to women five times weekly. Before surgery, patients with cancers that have progressed to stage T2 or higher are frequently given radiation

therapy. Several factors are considered before deciding to administer radiation therapy (Watkins *et al.* 2019).

When it comes to breast cancer, surgery is the gold standard. Staging often involves excision of the primary tumor as well as axillary lymph nodes. Determining the optimal course of treatment for the breast can be challenging for both the surgeon and the patient due to the wide variety of surgical options available, including breast-saving procedures such as lumpectomy and reconstruction. Breast reconstruction can be done either immediately following a mastectomy or at a later date (Yuan *et al.* 2020, Liu *et al.* 2018).

Endocrine therapy is used to treat tumors that have estrogen receptors (ER) and progesterone receptors (PR), which are involved in regulatory pathways that control cell growth. Two examples of medications used in endocrine therapy are aromatase inhibitors and tamoxifen (Cserni *et al.* 2018). The production of estrogen relies on the presence of aromatase, an enzyme. By blocking aromatase's action, aromatase inhibitors reduce tumor growth in postmenopausal women. There is substantial evidence that tamoxifen, when administered as an endocrine therapy subsequent to other treatments, is effective in treating early-stage breast cancer. By blocking estrogen's ability to bind to cancer cell receptors, tamoxifen reduced tumor growth (Momenimovahed *et al.* 2019).

2.14 Screening and Detection of Breast Tumor

One potentially important strategy in reducing breast carcinoma is to reduce the mortality by screening for breast cancer to achieve earlier detection of cancer. Large numbers of investigations are used to diagnose breast carcinoma including (Kuhl 2019).

2.15 Biochemical Changes Related to Breast Tumor

The biochemical profiles of transformed cells into cancer cells must be distinguished. In general, these cells primarily focus on growth by increasing anabolic processes and

decreasing catabolic functions, which is different from normal cells (Lee *et al.* 2018). Enzymatic changes in cancerous tissues may be the result of genetic reprogramming to cancerous behavior, a probable tumor survival mechanism. It is well-known that cancer cells undergo alterations in protein and nucleic acid metabolism. It was previously noted that cancer cells often displayed (Mishra *et al.* 2004).

an increase in the synthesis of RNA and DNA. increased glycolysis rates, both aerobic and anaerobic. Inconsistencies in cellular surface glycoprotein and glycosphingolipid composition lead to changes in surface charge and permeability.enzyme activity increased and pyrimidine catabolism decreased. proteins that are secreted, including proteases and kinases.enzyme patterns. The production of lytic enzymes, including proteases and glycosidase, as well as enzymes involved in nucleic acid synthesis, is elevated in cancer cells.The emergence of new antigens and the disappearance of well-known ones.the wrong hormones and growth factors being produced.

Depending on their needs and methods of follow-up, various oncology fields use different tumor markers (Yuan *et al.* 2020, Liu *et al.* 2018).Theoretically, tumor markers have a lot of potential in oncology; however, their actual use will depend on how sensitive and specific the marker is, as well as how reliable existing methods are for the same goal (Cummings *et al.* 2021).

2.16 Carcinoma Antigen 15-3 (CA 15-3)

Breast epithelium displays an antigen known as carcinoma antigen 15-3 on its luminal side. In patients with advanced disease, it shows greater sensitivity and is more specific for breast cancer (Cserni *et al.* 2018). A member of the MUC1gene family, mucin (MUC) is a carbohydrate-containing protein antigen (CA 15-3). The MUC1 gene is found in many different types of tissues. The most commonly used serum marker for breast cancer is mucins, which are large transmembrane glycoproteins with extracellular domains made of a highly O-linked glycosylated protein core composed of a variable number of highly conserved 20-amino acid repeat units. According to their genetic and

bimolecular features, mucins are grouped into seven families, denoted as MUC1–MUC7 (Cserni *et al.* 2018).

When diagnosing breast cancer, CA 15-3 is the serum biochemical tumor marker most often used. Additionally, benign breast and liver diseases have been found to have elevated levels. Given that both the percentage of patients with elevated values of this marker and the serum concentration of the marker tend to rise with tumor size and disease severity, it is more useful for evaluating breast cancer prognoses and monitoring treatment efficacy. At present, CA 15-3 is mainly used for two purposes: first, in preclinical settings to detect recurrences of breast cancer, and second, to monitor therapy in patients with advanced breast cancer. Multivariate analysis showed that tumor size and nodal status had no effect on the prognostic marker CA 15-3. Furthermore, CA 15-3 had a prognostic significance in multivariate analysis that was stronger than tumor size and nodal status combined (Momenimovahed *et al.* 2019).

2.17 Lactate Dehydrogenase

Almost every cell in your body contains the enzyme lactate dehydrogenase. This includes your kidneys, pancreas, brain, muscles, and blood. Enzymes are released into the blood's fluid portion upon cell breakdown or destruction; they convert sugar into energy. Lactate dehydrogenase (LDH) is an enzyme that speeds up the conversion of pyruvate and nicotinamide adenine dinucleotide (NADH) to lactate and NAD. LDH is a tetrameric member of the 2-hydroxy acid oxidoreductase family (Cserni *et al.* 2018).

Cells frequently employ this process for anaerobic respiration, which entails the transfer of a hydride ion from NADH to the pyruvate. This pair of subunits has the potential to combine in five distinct tetramers, or isoenzymes: 4H, 4M, and three hybrid tetramers (3H1M, 2H2M, 1H3M). Despite their shared enzymatic activity, the five isoforms exhibit distinct patterns of tissue distribution (Rosen Sawaki *et al.* 2019).

Despite their structural similarity, the LDH isoenzymes exhibit distinct kinetic properties. Research suggests that these variations are due to variations in the charged surface residues surrounding the active site. Renal disease, liver disease, anemia, pulmonary embolism, cancer, and muscle lesions or diseases can all cause an elevation of LDH activity. For anaerobic glycolysis to take place, the serum LDH enzyme is necessary. The level of LDH in the cytoplasm of cells is regulated by an increase in its gene as the number of cancer cells rapidly increases (Rosen Sawaki *et al.* 2019).

2.18 Interleukin 10 and Breast Cancer

IL-10, or cytokine synthesis inhibitory factor as it was previously called, is a potent anti-inflammatory cytokine that inhibits gene expression, cytokine synthesis in macrophages and T cells, and the antigen delivery capabilities of these cells. It suppresses a number of factors, including leukemia inhibitory factor, IL-10, GM-CSF, MIP-1a, RANTES (Regulated upon activation, normal T cell produced and released), IL-1a, IL-1b, TNF-a, IL-6, IL-8, IL-12, and IL-18. In addition to inhibiting IFN-c synthesis by activated Th cells and peripheral blood mononuclear cells (PBMC), IL-10 encourages mast cell growth. Furthermore, IL-10 is an effective regulator of B cell maturation and immunoglobulin secretion (Cserni *et al.* 2018).

In most cases, the interaction between IL-10 and other cytokines results in suppression. Research by Ouyang and O'Garra (2019) indicates that IL-10 mainly inhibits antigen presentation by DCs and macrophages. tested the possible signaling pathway and IL-10's inhibitory effect on various cytokines. Figure 1 shows some of the known interactions between IL-10 and various immune and tumor cells. Interleukin-10 (IL-10) suppresses MHC-II expression while upregulating CD80 and CD86, which are co-stimulatory molecules. The interleukin-10 (IL-10) pathway is obstructed during DC maturation and monocyte precursor differentiation. Thus, IL-10's capacity to inhibit antigen-presenting cells' production of Th1 and Th2-associated cytokines is the principal source of its immuno-inhibitory properties (APC). Th1 and Th2 production of IL-2 and IFN-c, IL-4 and IL-5, and CD4 and T cell growth are all inhibited by IL-10 (Hamidullah and Konwar 2012)

2.19 IL-10 as Prognostic Indicator for Breast Cancer

The progression of tumors is regulated by the intricate interplay of tumor cells, stromal cells, and T lymphocytes. Released by both immune cells and tumor cells, interleukin (IL-10) has a substantial impact on tumor cell growth and proliferation within the tumor microenvironment. Serum IL-10 levels are frequently higher in breast cancer patients. Metastatic cancer cells may secrete IL-10 more often to dampen the immune system's inflammatory response, according to one theory (Lee *et al.* 2018).

The inhibition of IL-6 production by increased IL-10 suggests that it may inhibit tumor growth by blocking anti-apoptotic and angiogenic proteins in tumor cells. It is well-documented that IL-6 stimulates tumor growth through these pathways. Supporting this idea is the observation that IL-10 and IL-6 levels were inversely related in breast cancer patients. On the other hand, IL-6 levels can both positively and negatively indicate the likelihood of breast cancer. These contradictory correlations with IL-6 level raise the question of whether IL-10 plays a definitive role in breast cancer prognosis. Lacking the estrogen receptor (ER), breast cancers overexpress IL-10 in comparison to ER-positive tumors (Rosen Sawaki *et al.* 2019)

Patients whose breast cancer tumors tested positive for the prolactin receptor (PR) exhibited lower levels of interleukin (IL)-10. An increase in IL-10 levels is associated with an increase in AP-1 expression in ER-negative tumors, which is higher than in ER-positive tumors. The cytokine IL-10 was discovered to be significantly different in the breast cancer group and the group whose biopsies came back negative. In fact, over half of the cases in both groups showed differences in IL-10. The role of IL-10 in hereditary disease risk assessment Recent years have seen increased interest in genetic polymorphism variations as a possible risk factor for complex and common diseases like breast cancer. A large number of genes have been identified that may be associated with an increased risk of breast cancer or its advanced stages. Breast cancer patients have been studied to identify any IL-10 polymorphism sites that may be associated with an increased risk of the disease (Denton *et al.* 2017).

3. MATERIALS AND METHODS

3.1 Study Design

This is a cross-sectional, hospital-based study. The Kirkuk Oncology Center's staff and the Kirkuk Health Directorate's scientific committee both gave their stamp of approval to the study's protocol before patient samples were collected. Research for this study began on November 1, 2022, and continued until the end of June 20, 2023, at the Oncology Center in Kirkuk City, Iraq. All women included in this study, whether they were considered a case or a control, verbally consented.

3.2 Study Population

3.2.1 Patients

In this study, 270 women who had been diagnosed with breast cancer were invited to take part. This study included women who went to the Oncology Center for treatment of breast cancer. Each woman in this study had a surgically removed breast mass that was then histopathologically examined to confirm a diagnosis of breast cancer. Consultation with a histopathologist verified the presence of breast cancer. The women, all hailing from Kirkuk City, ranged in age from thirty to fifty-nine.

3.2.2 Control

A number of 50 apparently healthy women without breast cancer and with negative family history for the first and second-degree relatives of breast cancer were considered a control group; their ages ranged from 30 to 59 years.

3.2.3 General information

A questionnaire form was prepared and filled by direct interview with each woman enrolled in this study and included: age, residence, family history of breast cancer… etc (Appendix1).

3.3 Materials

The following Table 3.1 shows the kits used in this work and their sources.

Table 3.1 Chemicals used in this study

No.	Biochemical parameters and chemicals	Company, Origin
1	IL-10 ELISA kit	OmniKine-Assay Biotechnology , USA
2	Alkaline phosphatase	Randox United Kingdom
3	Ferritin immunofluorescent kit	i-chroma, Korae
4	SGOT (AST)	Randox United Kingdom
5	Gamma-glutamyltransferase	Linear biochemistry, Spain
6	CEA immunofluorescent kit	Biomerieux SA Etoile-France
7	CA15-3 immunofluorescent kit	Biomerieux SA Etoile-France
8	GSH ELISA kit	Linear biochemistry, Spain
9	Lactate dehydrogenase by Spectrophotometer	Randox United Kingdom

Table 3.2 Shows the iinstruments used in this study

No.	Instruments	Source
1.	ELISA washer and reader	Biotek - U.S.A.
2.	Mini Vidas systems	Biomerieux SA Etoile - France
3.	Centrifuge	Kuksan - Japan
4.	Incubator	Fisher - USA
5.	Refrigerator	Marshal - Japan
6.	Spectrophotometer	CECIL - England
7.	I-chroma immunofluorescence	Korea

3.4 Methods

Using a sterile disposable syringe, a 5 ml venous blood sample was collected from each woman in the morning and placed in a plain tube. The samples were allowed to clot at room temperature before being subjected to a centrifugation at 6000 rpm to separate the serum. Subsequently, the obtained serum was aspirated and divided into aliquots, which were then stored at -20°C until the time of analysis. The serum samples from both patients and controls were subjected to assays for the following markers: Ferritin, GSH, LDH, ALP, GGT, SGOT, CA15-3, CEA, and IL-10.

3.4.1 Estimation of Serum CA 15-3

Principle

A fluorescent detection result is obtained at the end of the assay by combining the 2-strip enzyme immunoassay sandwich method with the Enzyme Linked Fluorescent Assay (ELFA) technology. The basic idea of the test is to combine the two-step sandwich method of enzyme immunoassay with the last step of fluorescence detection (ELFA). The Solid Phase Receptacle (SPR) serves as the solid phase and pipetting tool for the test. Inside the sealed reagent strips are the reagents that are ready to be used in the assay. Every single assay step is executed automatically by the device. There is a continuous pulsing of the reaction medium into and out of the SPR.

The SPR is used to repeatedly cycle the sample in and out. This procedure enables the collection of the sample's reactive antigenic determinants by the 115D8 attached to the interior wall of the SPR. When you wash something, any unbound components get washed away. Once the DF3 antibody has been tagged with alkaline phosphatase, it is incubated in the SPR. There, it binds with the DF3 reactive antigenic determinants. The cleaning procedures then remove the unbound conjugate. During the final detection stage, the 4-methylbuterylphosphorylryl substrate is cycled in and out of the SPR.

4-Methylumbelliferone, whose fluorescence is measured at 450 nm, is produced when the conjugate enzyme hydrolyzes this substrate. The strength of the fluorescence is proportional to the concentration of CA 15-3 in the sample. After the test is over, the device prints out the results using the calibration curve it has stored in memory.

Table 3.3 Shows content of the kit

Component	Type	Description
CA 15-3 Strips	STR	Ready-to-use strips designed for the assay.
CA 15-3 SPRs	STR	Preparation of SPRs (Surface Plasmon Resonance) intended for test purposes. The interior of the SPRs is coated with monoclonal 115D8 antibodies derived from mice.
CA 15-3 Control	C1	Ready-to-use control solution containing Bovine albumin, DF3 reactive antigenic determinants sourced from humans, and 0.9 g/l sodium azide. MLE (Maximum Likelihood Estimate) data indicate the confidence interval in U/mL for the "Control C1 Dose Value Range."
CA 15-3 Calibrator	S1	Ready-to-use calibrator solution containing Bovine albumin, DF3 reactive antigenic determinants sourced from humans, and 0.9 g/l sodium azide. MLE data indicate the concentration in U/mL for the "Calibrator (S1) Dose Value" and the confidence interval in "Relative Fluorescence Value" for the "Calibrator (S1) RFV Range."
CA 15-3 Diluent	R1	Ready-to-use diluent solution containing Calf serum and 0.9 g/l sodium azide.
Specifications for Calibration Data	-	Factory master data for calibration can be obtained from MLE data (Master Lot Entry) provided in the kit or MLE barcodes printed on the box label.

Figure 3.1 CA 15-3 mini Vidas ELISA kit

Procedure:

- The necessary reagent was taken out of the refrigerator and given at least thirty minutes to reach room temperature.
- To test each sample, control, or calibrator, one "15-3" SPR and a "15-3" strip from the kit were used. With care, the storage pouch had been taken out.
- The instrument's "15-3" code served as the test's identification. "S1" had to be used to identify the calibrator, and duplicate testing was required. "C1" was to be used to identify the control if it was to be tested.
- Using a vortex-style mixer, the calibrator, control, and samples were combined (for serum or plasma separated from the pellet).
- The calibrator, control, and sample tested portions for this test were all 100 μL.
- The instrument was filled with the "15-3" SRPs and "15-3" strips. The reagent strips machine and the SPRs' assay code were compared to the color labels.
- The user's manual's instructions were followed to start the test. The device carried out every assay step automatically.
- After pipetting, the vials were capped again and cooled to between 2 and 8 0C.
- The assay took about sixty minutes to finish. Following the completion of the assay, the SPRs and strips were taken out of the apparatus.
- The used SPRs and strips were thrown away at the proper place.
- Calculation: The computer automatically assessed the test results after it was finished. For every sample that was evaluated, the fluorescence was measured twice in the reagent strip reading cuvette. Prior to the SPR being inserted into the substrate, the initial reading was a background reading from the substrate cuvette. After incubating the substrate with the enzyme still present inside the SPR, the second reading was obtained. The background measurement was subtracted from the result sheet to determine the RFV (Relative Fluorescence Value). The device used calibration curves that it had saved to automatically calculate the results (4-parameter logistic model). Concentrations having CA 15-3 titers more than 400 U/ml ought to have undergone a reassay following a maximum dilution of 1/10 in CA 15-3 diluent. The micro vides CA 15-3 kit's measurement range was expanded to include (2 to 400U/ml).

Figure 3.2 Mini vidas full automated system (Biomerieux SA Etoile-France)

3.4.2 Estimation of CEA by immunofluorescence

Principle

Using a fluorescent detection endpoint, the assay method utilizes a two-step enzyme immunoassay sandwich technique (ELFA). Coated with monoclonal immunoglobulins derived from mice that target CEA, the Solid Phase Receptacle (SPR®) serves as both a solid substrate and a pipetting device (Carcinoembryonic Antigen). All of the necessary assay components are already included in the strip. The apparatus runs the whole assay mechanically, with the reaction medium cycling in and out of the SPR.

After the sample is diluted, it is incubated with the SPR to help it bind to any CEA antigen that may be present. The unbound components are successfully removed during the first wash step. The next step is a second round of incubation with goat polyclonal antibodies against CEA that have been labeled with alkaline phosphatase. Subsequently, washing procedures remove unbound conjugates. The final detection step involves cyclic interactions between the SPR and the substrate (4-Methylumbelliferyl phosphate). The fluorescent product known as 4-Methylumbelliferone is produced when the substrate hydrolyzes by means of the conjugated enzyme. Its fluorescence is measured at 450 nm. One can tell the concentration of antigens in a sample by looking

at the fluorescence intensity. After the test is over, the device prints out the results using the calibration curve it has saved in memory.

Kit contents

The kit composition for 60 tests, which is used for the CEA (Carcinoembryonic Antigen) detection assay, consists of the following components:

- Ready-to-use CEAS Strips: 60 strips total, with each strip optimized for a single test.
- Designed to be used in conjunction with the strips, the CEAS SPRs (Solid Phase Receptacles) are 60 in number. Monoclonal immunoglobulins that target CEA are coated on the inside of every SPR (mouse).
- LYophilized control material (2 mL) in 1 vial for CEAS (C1). Before using, dilute with 2 milliliters of distilled water, let stand for 5 to 10 minutes, and then stir.
- Three vials of lyophilized calibrator material, 2 mL each, make up the CEAS Calibrator (S1). After waiting 5 to 10 minutes, mix with 2 mL of distilled water to reconstitute before use. Once reconstituted, the calibrator maintains its stability for a month at 2-8°C or until its expiration date at -25 ± 6°C. Up to three freeze-thaw cycles shouldn't damage it. A preservative, bovine albumin, and human CEA make up the calibrator, just as they do in the control. It gives you the concentration in nanograms per milliliter ("Calibrator (S1) Dose Value") and a range of values for the relative fluorescence to establish a confidence interval ("Calibrator (S1) RFV Range").
- CEAS Diluting Agent: Three vials, with 6.7 mL of liquid diluent in each vial (R1). Sodium azide (1 g/L) and bovine albumin make up this ready-to-use diluent.

Procedure

- We took the reagents out of the fridge when we needed them.

- A single "CEAS" strip and a single "CEAS" SPR® were utilized for the testing of each sample, control, or calibrator. After the required SPRs were removed, great care was taken to reseal the storage pouch.
- The instrument's "CEAS" code served as a means of identifying the test. "S1" was used to identify the calibrator, and it was tested twice. The control was designated as "C1" if it was to be tested.
- The samples, control, and calibrator were combined in a vortex-type mixer (for serum separated from the pellet).
- The 200 μL portion of the test included the calibrator, control, and sample.
- I positioned the "CEAS" SPRs and "CEAS" strips on the instrument according to their respective roles. The color labels on the SPRs and Reagent Strips were double-checked to match the assay code.
- I followed the instructions in the operator's manual to start the assay, and the instrument took care of the rest.
- After pipetting, the vials were sealed and brought back to the proper temperature.
- About sixty minutes passed before the test was finished. After they were done, the SPRs and strips were taken out of the device.
- An acceptable container was used for the disposal of used SPRs and strips.

3.4.3 Determination of gamma-glutamyltransferase (GGT)

In order to create L-γ-glutamyl-glycylglycine and 5 amino-2-nitro-benzoate, the enzyme gamma-glutamyltransferase (γ-GT) helps move a γ-glutamyl group from γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine.

A direct correlation between the enzyme activity detected in the sample and the quantification of 5-amino-2-nitro-benzoate formed can be drawn from the kinetic tracking at 405 nm.

Reagents compositions

R1:

- Buffer: TRIS at a concentration of 133 mmol/L, with a pH of 8.2.
- Additional Component: Glycylglycine at a concentration of 138 mmol/L.

R2:

- Substrate: Glupa-C containing L-γ-glutamyl-3-carboxy-4-nitroanilide at a concentration of 23 mmol/L.

Procedure:

- Bring the reaction temperature up to room temperature before incubating the samples, controls, and working reagent.
- Dampen the photometer with distilled water until the absorbance reads zero.
- Transfer the following amounts to a cuvette using a pipette:
- Reagent for functionality: 1 milliliter
- A 100 μL sample
- Toss gently to combine.
- Before beginning the timer, place the cuvette in the cell holder.
- After one minute of incubation, take note of the first absorbance reading.
- At the 1, 2, and 3-minute marks, take the absorbance readings again precisely.
- The difference between the absorbances should be calculated.
- Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

Calculations:

- Calculate enzyme activity in Units per Liter (U/L) using the formula: U/L = ΔA/min x 1111

Note: For samples with ΔA/min values exceeding 0.200 at 405 nm, follow these additional steps:

- Dilute the sample 1:10 with saline.
- Assay the diluted sample again.
- Multiply the results by 10 to correct for the dilution factor.

3.4.4 Estimation of lactate dehydrogenase

Principle

The randox GOD\APA-USA kit measures the specimen's LDH activity at 340 nm, which is directly proportional to the decrease in absorbance caused by the conversion of NADH to NAD+..

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^{+} \qquad (3.1)$$

Reagents

Vial R1 (substrate-buffer) ---------- Buffer Tris pH 7.2 80 mmol/L

Pyruvate 1.6 mmol/L

Preservative Vial R2 (Standard - coenzyme) NADH > 0.20 mmol/L NaCl 200 mmol/L.

Table 3.4 Estimation of lactate dehydrogenase reagents

	Sample	**Standard**	**Blank**
Reagent 1	1 ml	1 ml	1 ml
placed at 37ºC for 5 minutes			
Sample	0.02 ml		
Standard		0.02 ml	
Distilled water			0.02 ml

Mix. Start a timer. After 30 seconds, record initial absorbance at 340 nm (or 334 nm)Record the absorbance again after 1 minute and 2 minutes (Δ Calculate absorbance change per minute (ΔAbs/min.).

Calculation:

LDH (IU/L)=(ΔAbs/min) Assay/(Δ Abs/min) Calbiration)×calbirator Activity

3.4.5 Detection of IL 10 by enzyme linked immunosorbent assay

Principle:

If you want to know how much recombinant or natural IL-10 is in any kind of experimental material—cell lysates, serum, plasma—the OmniKine Human IL-10 ELISA Kit has you covered. As a quantitative method, this immunoassay makes use of a "Sandwich" Enzyme-Linked Immunosorbent Assay (ELISA), where the investigator coats the bottom of each well with primary capture antibodies and then adds secondary detection antibodies. This format binds the target protein (antigen). In each well, the capture antibodies attach to a particular epitope on the human IL-10 cytokine, while the detection antibodies that the user has added bind to specific epitopes on the target protein that has been captured. To ensure that non-specific binding of proteins to other proteins or the solid phase is eliminated, a series of wash steps must be performed between each operation. After the target antigen is incubated and "sandwiched," the secondary antibody's constant heavy chain is covalently or Avidin/Streptavidin-Biotin interaction-bound to a peroxidase enzyme, enabling a colorimetric reaction to happen upon addition of the substrate. A peroxidase-catalyzed reaction yields a blue color that stands in for the antigen concentration upon addition of the substrate TMB (Tetramethylbenzidine). To stop the reaction after enough color has developed, add stop solution; this will turn the solution yellow. After that, IL-10 levels can be determined by reading the absorbance of each well with a spectrophotometer, which allows for the development of a standard curve.

Materials included:

- 96-well microplate or capture antibody-coated strips
- Detection antibody linked to biotin
- Avidin-hrp conjugate solution, ready to use
- Cytokine protein reference
- substrate that is ready to use
- Solution to Stop
- Plate sealers for adhesives
- Washing buffer (10X)
- Protein diluent standard
- Antibody detection diluent

Procedure:

All the steps were performed according to the instructions provide with kit**.**

a- Immunoassay protocol

- To equilibrate solutions when they were applied to the microplate wells, all incubation steps were conducted on an orbital shaker.
- Prior to usage, all given solutions were at room temperature.

b- Reconstitution standard and unknown sample to immunoassay

The standard curve for the immunoassay was determined by applying an unknown sample to the Sandwich ELISA after serially diluting a known standard sample. This is essential for deciphering the results of the unidentified samples.

Figure 3.3 OmniKine ELISA for detection of IL-10

- We diluted the known standard sample from 2 ng/ml to 0 ng/ml in a series of microfuge tubes.
- Each tube was carefully mixed by inverting multiple times or gently vortexing to ensure sufficient equilibration.
- The wells of a particular row or column of the 96-well microtiter plate were treated with 100 l of each serial dilution step in duplicate or triplicate, and then left to incubate at room temperature for 2 hours.
- The microplate was made airtight by using one of the adhesive seals provided with the microtiter plates.
- The protein standard solution was carefully removed from the microtiter plate wells by aspiration and blotting three to four times on a stack of paper towels. This process was repeated until the wells were completely dry.
- A 1X wash buffer was made from 10X wash buffer using pure water.
- Three hundred to four hundred liters of Wash Buffer was added to each well, and then they were gently shaken on an orbital shaker for five to seven minutes. This washing process was carried out consecutively four times.

- At the end of the fourth wash step, the detection antibody solution was diluted 1:200 in detection antibody diluent until it reached a concentration of 0.5g/ml.
- Ensuring that there was sufficient detection antibody solution for all wells, the test tube was inverted multiple times or vortexed to achieve proper equilibration.
- Before being sealed and left to incubate at room temperature for 2 hours, each well was given 100 l of the diluted detection antibody solution.
- A vacuum-based aspirator or a paper towel was used to remove the detection antibody solution from the microplate wells. Light shaking was performed in between each of the four consecutive steps.
- Each well was then incubated at room temperature for 30 minutes after the addition of 100 l of Ready-to-Use Avidin-HRP Conjugate Solution, which followed the fourth wash step.
- The Avidin-HRP conjugate solution was removed from the microplate wells using paper towel blotting. To create the TMB substrate solution, the mixture was heated to room temperature in the absence of fluorescent or ultraviolet light, which would have destroyed the TMB. There were four separate washes, with light shaking in between each.
- Once the fourth wash step was completed, 100 l of TMB substrate solution was added to each well and left to incubate at room temperature for color development. Some wells could turn blue very fast depending on the concentrations of the analyte and/or detection antibody-HRP, so the color development was carefully watched.
- Instantly after the blue color had ceased to develop, 100 l of stop Solution was added to every well. In a flash, the blue hue of the wells turned yellow.
- Scanning the plate with a microplate reader set to 450 nm allowed us to assess the optical density (absorbance) of each well within 30 minutes of adding the Stop Solution.

Interpretation of data:

For every standard, the mean of the replicate readings was calculated. Microsoft Excel 2010 was used to create a standard curve. The curve had the average optical density

values in absorbance units (y-axis) and was plotted against the known standard concentrations in pg/ml (x-axis)

3.4.6 Aspartate aminotransferase

Principle:

$$\alpha - \text{oxoglutarate} + \text{L} - \text{aspartate} \xrightarrow{\text{GOT}} \text{L} - \text{glutamate} + \text{oxaloacetate} \qquad (3.2)$$

Checking the concentration of the oxaloacetate hydrazone formed with 2,4 dinitrophenyl-hydrazine is a way to measure aspartate aminotransferase.

Table 3.5 Aspartate aminotransferase reagent

1- R1(Buffer):	
Phosphate buffer	100 mmol/l, pH7.4
L-aspartate	100 mmol/l
α-oxoglutarate	2.0 mmol/l
2- R2:	
2,4 dinitrophenyl hydrazine	100 ml
3- R3:	
Sodium hydroxide	100 ml
4- Calibration:	
Pyruvate standard	10 ml

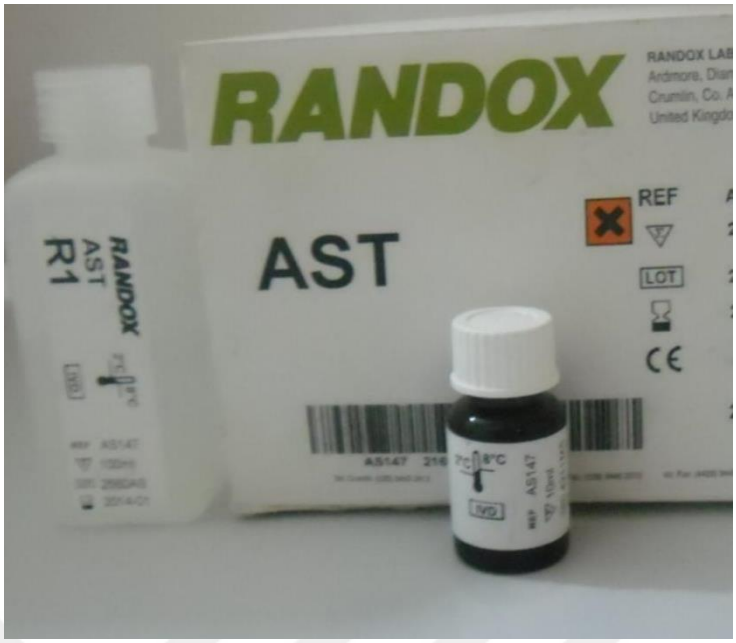

Figure 3.4 AST (GOT) kit

Table 3.6 Aspartate aminotransferase procedure

Wave length		546 nm (530-550 nm)
Cuvette		I cm light path
Incubation temperature		37°C
Measurement against reagent blank		
	Reagent Blank	Sample
Sample	---	0.1 ml
Buffer R1	0.5 ml	0.5 ml
Distilled water	0.1 ml	---
The tubes were mixed, incubated for exactly 30 min.at 37°C		
2,4dinitrophenyl hydrazine R2	0.5 ml	0.5 ml
The tubes were mixed, allowed to stand exactly 20 min.at 37°C		
Sodium hydroxide R3	5 ml	5 ml
Mixed, read the absorbance of sample against the reagent blank after 5 min		

Table 3.7 Aspartate aminotransferase calculation

Absorbance	U/l	Absorbance	U/l	Absorbance	U/l	Absorbance	U/l
0.020	7	0.060	19	0.100	36	0.140	59
0.030	10	0.070	23	0.110	41	0.150	67
0.040	13	0.080	27	0.120	47	0.160	76
0.050	16	0.090	31	0.130	52	0.170	89

Serum GOT was calculated according to the following values.[(110)]

3.4.7 Alkaline phosphatase

Principle:

Detecting alkaline phosphatase activity using colorimetry:

4-Aminoantipyrine and potassium ferricyanide are used to measure the liberated phenol. Enzymatic reactions are halted when sodium arsenate is present in the reagent.

$$\text{Phenylphosphate} \xrightarrow[\text{pH10}]{\text{alkaline phosphatase}} \text{phenol} + \text{phosphate} \quad (3.3)$$

Reagents:

- The first reagent, sodium phenyl phosphate, is a substrate buffer (2 x 56 ml).
- Carbonate-bicarbonate.
- The pH of the buffer is 10.
- Merthiolate sodium.
- Phenol is the second standard reagent (1 x 2.5 ml)
- Reagent 3: 4-aminoantipyrine (Blocking reagent, 1 x 27 ml).
- Metal arsenate, sodium.
- Fourth Reagent (Color Reagent): Potassium Ferricyanide (1 x 27 ml).

Table 3.8 Alkaline phosphatase procedure

Wave length:	510 nm			
Zero adjustment:	Reagent blank			
Incubation temperature:	37°C			
	Serum sample	**Serum blank**	**Standard**	**Reagent blank**
Reagent 1	2 ml	2 ml	2 ml	2 ml
The tubes were incubated for 5 min.at 37°C.				
Serum	50 µl	--	--	--
Reagent 2	--	--	50 µl	--
The tubes were mixed, incubated for exactly 15 min.at 37°C.				
Reagent 3	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Mixed with vortex				
Reagent 4	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Serum	--	50 µl	--	--
Distilled water	--	--	--	50 µl
Mixed, let stand for 10 min in the dark measure.The color intensity was stable for 45 min.				

Calculation:

$$Result = \frac{OD\ serum\ sample - OD\ serum\ blank}{OD\ standard} \times n \quad (3.4)$$

In U/l calculation unit, n = 142[111]

3.4.8 Estimation of serum ferritin

Principle

One immobilized antigen on the test strip catches the recombinant protein-antibody complexes that are generated when the sample's antibody reacts with the buffer's detector recombinant protein. The complexes then migrate onto the nitrocellulose matrix. A stronger fluorescence signal is observed on the recombinant protein detector, which is used by Instrument for ichromaTM assays to measure ferritin concentration, when the quantity of antibody in the sample is higher. This is because recombinant protein antibody complexes are formed in response to higher antibody quantities.

Kit components

"Cartridges," "Detection Buffer Tubes," and a "ID chip" comprise ichromaTM Ferritin. Contains usage instructions.

This test strip and film cartridge has a line that is coated with anti-human ferritin and another line that is coated with keyhole limpet hemocyanin (KLH).

2-The dehumidifier is packaged in an aluminum foil-wrapped cartridge. An ID card and twenty-five individually packaged cartridges are also included in the box.

The recognition buffer contains 3-anti-human ferritin fluorescent conjugate, anti-KLH fluorescence conjugate, phosphate-buffered saline (PBS) sodium azide, sucrose, and bovine serum albumin (BSA) for fixation.

The 4-a tube is already reserved for use as the detecting buffer. A styrofoam box with ice packs is used for delivery after packaging 25 detection buffer tubes in a box.

Assay procedure

1) Using a transfer pipette, 30 μL of the human serum sample was moved to a tube that had previously included the buffer for detection.

2) The sample was mixed thoroughly by shaking it about 10 times after the lid of the detection buffer tube was closed.

(3) A 75 μL mixture of the sample I transferred the liquid sample to the sample well of the assay cartridge upon pouring.

A 10-minute period was spent at room temperature with the test cartridge loaded with the sample.

5) The ichromaTM testing device was equipped with a cartridge holder, and the sample-loaded cartridge was inserted into it. Before being inserted into the cartridge holder, the cartridge was correctly oriented. This is why an arrow has been meticulously marked on the cartridge.

6) The scanning procedure was initiated by pressing the 'Select' button on the ichromaTM testing equipment.

7) The ichromaTM testing device will immediately start scanning the batche d'cartridge.

8) The instrument's display panel recorded the ichroma™testing result.

3.4.9 Estimation of serum reduced glutathione(GSH)

Principle

The activity of Elman's reagent [5,5-dithiobis(2-nitrobenzoic acid)-DTNB](1), which is dependent on the GSH sulfhydryl groups, was used in a modified procedure to gauge serum GSH. The disulfide chromogen DTNB was easily reduced to an extremely yellow compound by the sulfahydryl group of GSH. The reduced chromogen's absorbance at 412 nm is a good proxy for its GSH concentration.

Reagents:

0.1 M sodium phosphate, pH 8.0, with 1 M ethylene diamine tetraacetic acid is the reaction buffer (EDTA).

Blend 4 milligrams of Ellman's Reagent with 1 milliliter of reaction buffer to make a solution.

Table 3.9 Estimation of serum reduced glutathione(GSH) procedure

Pipette into well identified test tubes:	Test	standard
Reagent	2.5 ml	2.75 ml
Sample	250 µL	
Ellman`s reagent	50 µL	50 µL
Mix and incubate at 25 C in water bath for 15 minutes.		
The tubes were mixed by a vortex mixer, with a spectrophotometer set to 412nm, zero the instrument on the blank and then measure absorbance of each sample.		

Calculation:

Calculate the amount and concentration of sulfhydryls in the sample from the molar extinction coefficient of TNB (14,150$M^{-1}cm^{-1}$).

$$Glutathione\ Concertation\ (M) = \frac{A}{bE} \times \frac{Total\ volume}{Serum\ volume} \quad (3.5)$$

A=Absorbance

$b = 1cm$

E = 14,150M-1cm-1.

Total Volume=2.8 ml

Serum Volume=0.25 ml

4. RESULTS AND DISCUSSION

4.1 General Characteristics of Breast Cancer Women

The age distribution shows that the most common cases fall within the 40-49 years, accounting for 28.5% of the total, followed by the 50-54 age group at 11.5% and the 55-59 age group at 18.9%. In terms of educational level, 44.8% have primary education, while 28.9% are illiterate. Regarding marital status, 55.9% of these women were married, and 44.1% were single. Concerning the number of children, 46.7% have none, and 18.1% have three children. In the context of smoking, 83.3% do not smoke themselves, while 16.7% were regularly smoking. Remarkably, 83.0% have smoking spouses. Lastly, regarding menstrual cycle regularity, 52.6% experience irregular cycles, while 47.4% have regular cycles.

Table 4.1 General characteristics of breast cancer women

General characteristics	No.	%
Age range		
30-34	16	5.9
35-39	19	7.0
40-44	76	28.1
45-49	77	28.5
50-54	31	11.5
55-59	51	18.9
Total	270	100
Educational level		
Illiterate	78	28.9
Primary	121	44.8
Secondary	36	13.3
College	35	13.0
Total	270	100
Marital Status		
Married	151	55.9
Single	119	44.1
Total	270	100
Number of Children		
0	126	46.7
1	7	2.6
2	40	14.8
3	49	18.1
4	48	17.8
Total	270	100
Smoking		
No	225	83.3
Yes	45	16.7
Total	270	100
Smoking Spouse		
No	46	17.0
Yes	224	83.0
Total	270	100
Menstrual Cycle	Frequency	Percent
Irregular	142	52.6
Regular	128	47.4
Total	270	100

Our findings were consistent with studies done recently by Sonke *et al.* (2018) and Zaheer *et al.* 2019) who showed that women aged >48 were most affected by breast cancr

The present study aligns with a study conducted by Johnson *et al.* in 2018. Who suggested that the low cancer incidence in the age group of 0 to 40 is associated with obesity, as obesity leads to an increase in cancer incidence as individuals age. in addition,Wu *et al.* (2019) proposed that hormonal and genetic factors may contribute to the age-standardized incidence of breast cancer in women aged 50 to 60 in the UK.

Higher levels of education are associated with better health outcomes and easier access to medical treatment. In this context, 44.8% of the people surveyed have only completed elementary school, and 28.9% are completely illiterate. These numbers are in line with what Lee *et al.* (2019) and Akoko *et al.* (2022). found when they looked at how education affected women's reproductive health. Reproductive health issues were less likely to be known about and fewer people with lower levels of education sought medical treatment for them, according to their study.

Many factors, such as a woman's marital status and the number of children she has, interact to impact her health choices. Among the female participants, 54.1% were unmarried and 55.9% were married. Miller *et al.* (2018) found a correlation between marital status and healthcare use, so this makes sense. They found that prenatal care and regular checkups were more commonly sought after by married women. When broken down by the total number of children, 18.1% have three and 46.7% are childless.

The results align with Bloom *et al.*'s (2014) study on the impact of family size on women's health, specifically focusing on family history, symptom recognition, and diagnostic procedures for breast cancer.

4.2 Breast Cancer Statistics: Family History, Awareness of Symptoms, and Diagnosis Methods

Table 4.2 provides a wealth of information regarding various breast cancer quality characteristics among research participants. The data illustrates the distribution of patients based on their family history of breast cancer; 8.1% of patients report a positive

family history, while 91.9% report no such history. The table also shows how patients found out they were sick; the most common response was that they felt a lump, which accounts for 21.9% of the total. The following symptoms were reported by 9.3% of participants: pain in the breast or nipple area, non-breast milky nipple discharge, and skin changes (8.9%). Lastly, the table delves into the methods of diagnosis, revealing that 20.4% of participants were diagnosed through biopsy, 20% through clinical examination, 20% through mammography, and MRI (19.6 percent).

Table 4.2 Breast cancer statistics: family history, awareness of symptoms, and diagnosis methods

Family History	No	%
No	248	91.9
Yes	22	8.1
Total	270	100
How they become aware of	No	%
Feeling lump	59	21.9
Growing veins on the breast	24	8.9
How they become aware of	1	0.4
Irritation around the nipple	13	4.8
Nipple crust	24	8.9
Nipple discharge that is not breast milk	25	9.3
Pain in breast	25	9.3
Pain in the nipple area	25	9.3
Skin changes	24	8.9
Swelling of part of the breast	25	9.3
Thickening of the skin	25	9.3
Total	270	100
Diagnosis Methods	No	%
Biopsy	55	20.4
Clinical Exam	54	20
Mammography	54	20
MRI	53	19.6
Ultrasound	54	2
Total	270	100

According to the findings, 8.1% of the participants reported a positive family history of breast cancer, compared to 91.9% who had no family history of the disease. This result agrees with the findings of Oeffinger *et al.* (2015), who conducted a comprehensive study on the impact of family history on the risk of breast cancer. They found evidence that suggests a higher risk of breast cancer in families where the disease has run in the

family. Understanding how people become aware of their symptoms is crucial for early detection and prompt treatment of breast cancer. The table shows that a lump was the first symptom that the majority of participants (21.9%) noticed when they were sick. This finding agrees with what Badakhsh *et al.* found in their investigation of breast cancer education and screening methods for early detection (2018). Important components of early detection and breast cancer awareness programs, according to their research, include self-examination and the capacity to detect palpable lumps. Additionally, 9.3% of participants reported breast or nipple area pain, 9.3% reported non-breast milky nipple discharge, and 8.9% reported skin changes as additional methods of awareness, according to the data. Arab *et al.* (2016) examined the spectrum of breast cancer symptoms, and these findings are in line with their findings. The importance of knowing the various breast cancer warning signs and symptoms was emphasized by their study. Breast cancer treatment plans are heavily influenced by the methods used for diagnosis. Based on the data, the diagnostic techniques that were most commonly used by the participants were MRI (20.4%), clinical examination (20%), mammography (20%), and biopsy (20.4%). (19.6 percent). Geczik *et al.* (2020) showed that a multidisciplinary approach, including imaging techniques like MRI and mammography, clinical evaluation, and biopsies to confirm the presence of malignant tissue, is valuable for diagnosing breast cancer, so this is in line with their recommendations.

4.3 Prevalence of Chronic Diseases and Treatment Methods in The Study Population

Table 4.3 shows important information about the co-occurring conditions and associated therapeutic approaches in individuals with breast cancer. Notably, 24.1% of the patients had diabetes, and 55.2% had hypertension, indicating how common these chronic illnesses were in the population under study. Additionally, the chart lists the medications used to treat these comorbidities. Metformin (12.2%) and Insulin (11.9%) are commonly used to manage diabetes, while amlodipine (10.7%), losartan (9.6%), and other medications were used to treat hypertension.

Table 4.3 Prevalence of chronic diseases and treatment methods in the study population

Chronic Disease	No.	%
Diabetes	65	24.1
Hypertension	149	55.2
None	56	20.7
Total	270	100
Chronic Disease Treatment	No	%
Amlodipine	29	10.7
Atenolol	22	8.1
Insulin	32	11.9
Lisinopril	21	7.8
Losartan	26	9.6
Metformin	33	12.2
Metoprolol	27	10.0
None	56	20.7
Valsartan	24	8.9
Total	270	100

The results show that a large percentage of the breast cancer patients who took part in the study also had other health issues; for example, 25.2% had diabetes and 55.2% had hypertension. Research on comorbidities in breast cancer patients has shown results that are in line with these findings. Research by Sgaard *et al.* (2013) on the prevalence of comorbidities in breast cancer patients found that hypertension and diabetes are two of the most common chronic illnesses in this group. Additionally, table 4 details the diabetes and hypertension treatments used for breast cancer patients. The two most common medications used to treat diabetes were metformin (12.2%) and insulin (11.9%). Wojciechowska *et al.* (2016) looked at diabetes treatment for cancer patients, and their results are in line with these findings. Based on their research, metformin was identified as potentially useful in cancer treatment. If metformin has antitumor effects, it might affect the effectiveness of cancer treatments in addition to helping with diabetes management. Patients were prescribed a plethora of medications, including the blood pressure medications amlodipine (10.7 percent) and losartan (9.6 percent) to manage their hypertension. Presented here are treatment options that are consistent with those suggested by the American Heart Association (AHA) and the American College of Cardiology (ACC) for the management of hypertension (Whelton *et al.* 2018). Because every patient is unique, medical providers must tailor treatment programs to address specific needs by considering factors like medication tolerance and co-occurring

disorders. 4.4. Biochemical parameter levels in the control group and the breast cancer women.

4.4 Levels of Biochemical Parameters in Breast Cancer Women and The Control Groups

Table 4.4 provides a striking comparison of the biochemical parameters of women with breast cancer (n = 270) and the control group (n = 50), with the results of each parameter presented as the mean ± SD. The differences between the two groups are statistically significant (p 0.001), which is a very high level of significance. In breast cancer women, the levels of Ferritin (222.67 ± 40.97 ng/mL), GSH (9.48 ± 1.31 units/mL), LDH (246.4 ± 32.02 U/L), ALP (102.03 ± 10.71 U/L), GGT (29.612 ± 2.57 U/L), and SGOT (37.49 ± 2.97 U/L) were notably distinct from those in the control group (Ferritin: 113.51 ± 15.72 ng/mL, GSH: 25.77 ± 4.66 units/mL, LDH: 90.33 ± 6.44 U/L, ALP: 67.55 ± 3.76 U/L, GGT: 39.52 ± 4.22 U/L, SGOT: 10.44 ± 1.17 U/L).

Table 4.4 Levels of biochemical parameters in breast cancer women and the control groups

Parameters (mean±SD)	Breast Cancer Women (n:270)	Control Group (n:50)	P-value
Ferritin (ng/mL)	222.67 ± 40.97	113.51 ± 15.72	0.001
GSH (units/mL)	9.48 ± 1.31	25.77 ± 4.66	0.001
LDH (U/L)	246.4 ± 32.02	90.33 ± 6.44	0.001
ALP (U/L)	102.03 ± 10.71	67.55 ± 3.76	0.017
GGT (U/L)	29.612 ± 2.57	39.52 ± 4.22	0.001
SGOT (U/L)	37.49 ± 2.97	10.44 ± 1.17	0.001

Normal ranges for these common blood tests:

- Ferritin (ng/mL): Female: 11 to 307 ng/mL
- LDH (U/L): 140 to 280 U/L (for adults)
- ALP (U/L): 20 to 140 U/L (for adults)
- GGT (U/L): 9 to 48 U/L (for adults)
- CA15-3 (U/mL): less than 30 U/mL

- CEA (ng/mL): less than 5 ng/mL

Table 4.5 shows that ferritin levels were significantly higher in the breast cancer group than in the control group in this study. Possible causes for this upregulation include a protein produced by tumors that interferes with iron metabolism or the fact that cancer has non-specific effects on reticuloendothelial iron metabolism (Shpyleva *et al.* 2011).Specifically, higher ferritin levels have been linked to breast cancers in their later stages (Mishra *et al.* 2004). Ali (2018) found similar results to ours and suggests that lactate dehydrogenase, alkaline phosphatase, and γ-glutamyl transferase are good indicators of breast cancer.

The induction of glycolysis by tumor cells is the primary mechanism by which elevated levels of LDH have been found in malignancies (Rajeswari *et al.* 2016). Consistent with the results of Agrawal *et al.*(2016), we also find that serum LDH levels are significantly higher in the breast cancer group than in the control group . It's important to remember that aging is accompanied by degenerative processes like tissue breakdown and necrosis, which could explain why LDH levels increase with age and postmenopausal status (Swetha *et al.* 2013). Compared to the control group, BC patients had significantly higher serum ALP levels, and postmenopausal women showed an even greater increase. The majority of the evidence points to the acceleration of de novo synthesis and subsequent release of the enzyme into the bloodstream as the cause of the increase in serum ALP (Shrivastava *et al.* 2016). Consistent with our finding, Mishra *et al.* (2004) and Rajeswari *et al.* (2016) found in their studies that women with breast cancer had significantly higher serum gamma-glutamyltransferase (GGT) levels

4.5 Levels of Hb, CA 15-3,CEA and IL-10 in Breast Cancer Women and The Control Group

The study showed that breast cancer women exhibited significantly lower Hb levels (10.96 ± 1.17 g/dL) compared to the control group (13.54 ± 2.44 g/dL), along with substantially elevated levels of CA 15-3 (22.88 ± 4.31 U/mL) compared to the control group (2.65 ± 0.17 U/mL), CEA (6.3 ± 1.75 ng/mL) compared to the control group

(0.82 ± 0.11 ng/mL), and IL-10 (30.12 ± 2.06 pg/mL) compared to the control group (7.37 ± 1.13 pg/mL).

Table 4.5 Levels of Hb, CA 15-3,CEA and IL-10 in breast cancer women and the control groups

Parameters (mean±SD)	Breast Cancer Women (n:270)	Control Group (n:50)	P-value
Hb (g/dL)	10.96 ± 1.17	13.54 ± 2.44	0.001
CA15-3 (U/mL)	22.88 ± 4.31	2.65 ± 0.17	0.001
CEA (ng/mL)	6.3 ± 1.75	0.82 ± 0.11	0.001
IL-10 (pg/mL)	30.12 ± 2.06	7.37 ± 1.13	0.001

Normal ranges for these common blood tests:

- HB (g/dL): Female: 12.1 to 15.1 g/dL
- CA15-3 (U/mL): less than 30 U/mL
- CEA (ng/mL): less than 5 ng/mL

Table 4.5 displays the lower mean hemoglobin value of breast cancer cases in our study. This was in line with the findings of Ali *et al.* (2018), who discovered a correlation between the prevalence of anemia in breast cancer patients and other known factors.

According to prognostic factors and research, patients with anemia had a lower chance of survival and a shorter overall survival time. Among the many potential causes of low hemoglobin levels in breast cancer patients is the disease itself, which can cause anemia via inflammatory cytokines and tumor-related bleeding, among other things. Cancer treatments like radiation and chemotherapy can also harm healthy red blood cells and hinder bone marrow function, which can result in anemia. Low hemoglobin levels can be worsened by iron deficiency, chronic inflammation, or bone marrow involvement caused by metastasis. There is a pressing need for effective management and intervention to address the impact of anemia on patient well-being and outcomes in breast cancer patients, as it has been linked to poorer prognostic factors and shorter overall survival (Lamy *et al.* 2014). Women with breast cancer had significantly higher

mean concentrations of CA 15-3 compared to healthy women who served as controls in this study. In a previous study conducted in Baghdad, Othman *et al.* (2018) found that CA15-3 levels were significantly higher in older breast cancer patients and those whose homes were located near incinerators or industrial facilities. Previous research has shown results that are consistent with the present investigation (Rack *et al.* 2010, Lumachi, 2010). Prabasheela and Arivazhagan discovered that breast cancer patients had significantly higher serum levels of CA 15-3. (2011). Consistent with earlier research, our results demonstrate a positive correlation between CA 15-3 levels and disease progression (Ismail *et al.* 2018).

A strong correlation between increased levels of carcinoembryonic antigen (CEA) and breast cancer was found in this study. The results of this study are in line with a previous one that found a statistically significant association in CEA concentrations between the control group and breast cancer patients ($p<0.05$), as both groups showed higher levels of CEA than the control group. Also, a different study found that 140 breast cancer patients who had surgery and went to different Chinese hospitals had higher CEA levels than 280 control group females (DeSantis *et al.* 2014). Lee *et al.* (2017) and Wu *et al.* (2014), which compared healthy females as a control group with post-operative breast cancer patients, found higher CEA levels in the serum of the patients and a significant association between the two groups ($p<0.05$).

Our current study's findings are in line with these previous studies. Additional research is needed to establish the utility of these serum biomarkers in clinical practice, but they show promise as prognostic indicators for breast cancer patients after surgery. It should be noted that subclinical metastases are more likely to occur when CEA concentrations surpass 7.5 μg/L. (Molina *et al.* 2014). At the time of diagnosis, patients whose CEA levels are within the normal range have a much better prognosis than those whose levels are elevated (Sinha *et al.* 2014). Consistent with the work of Hussain *et al.* (2022) we find that increased IL-10 levels are linked to the development of breast cancer (BC) and tumors. Consistent with previous studies, our results demonstrate that IL-10 levels in the sera of patients suffering from a comparable illness or condition are substantially elevated. The concordance in results across research provides more proof that IL-10

plays a role in the related disorder by indicating a repeatable pattern (Ahmad *et al.* 2018, Sheikhpour *et al.* 2018). It is noteworthy that breast carcinoma and other tumor cells contain high amounts of IL-10. Prior studies have shown that IL-10 is the most abundant cytokine in the BC cell environment (Moghimi *et al.* 2018).

4.6 Distribution of Breast Cancer Patients Across Different Stages According to TNM (Tumor, Nodes, Metastasis) Classification System

Table 4.6 presents the distribution of breast cancer patients across various stages as percentages of the total study population of 270 individuals. It reveals that T1N0M0 constitutes 29.63% of the cases, indicating a significant proportion of early-stage breast cancer, while T2N1M0 represents 45.56%, signifying a larger group with more advanced disease characteristics, including regional lymph node involvement. T3N2M1 accounts for 24.81%, indicating an advanced stage characterized by significant tumor size, extensive lymph node involvement, and distant metastasis.

Table 4.6 Distribution of breast cancer patients across different stages according to TNM (Tumor, Nodes, Metastasis) classification system

Stage	Number of Patients (n)	Percentage (%)
T1N0M0	80	29.63
T2N1M0	123	45.56
T3N2M1	67	24.81
Total	270	100

Consistent with previous research and literature on breast cancer staging, the provided table classifies patients into various stages of the diseaseadd sörce??. This categorization sheds light on the distribution of breast cancer cases in different stages within the study population and helps us comprehend the severity of the disease.

The literature suggests that a large percentage of breast cancer cases are diagnosed at this more advanced stage owing to factors like delayed diagnosis or increased awareness and screening; the predominance of patients in the T2N1M0 stage (45.56 percent) is in line with these findings (Cserni *et al.* 2018). In order to decrease the number of patients

in advanced stages, researchers have stressed the significance of early detection and timely intervention (Al-Khafaji *et al.* 2014). On the flip side, because early-stage breast cancer is typically associated with a better prognosis and can be treated less aggressively, the lower percentage of patients in the T1N0M0 stage (29.63 percent) is consistent with this. Furthermore, substantial field research backs up the table's use of the TNM classification system. Brierley *et al.* (2017) and have shown that the TNM system has been used for a long time in clinical practice to stage breast cancer.It provides a thorough framework for evaluating the disease's extent by considering the primary tumor's size (T), the involvement of regional lymph nodes (N), and the presence of distant metastasis (M). Finally, the presence of T3N2M1 (24.81 percent) is in line with previous studies that have shown that this stage is associated with large tumors, widespread lymph node involvement, and distant metastasis. These factors contribute to a worse prognosis and faster disease progression, as demonstrated by authors like Sakamoto *et al.* (2005).

4.7 Biochemical and Hematological Parameters in Different Stages of Breast Cancer

In this analysis, we compared the mean values of various biomarkers among three distinct (Table 4.7) TNM stages: T1N0M0, T2N1M0, and T3N2M1, in a cohort of breast cancer patients. The p-values associated with each comparison provide insight into the statistical significance of these differences. Notably, Ferritin levels show a significant increase from T1N0M0 (223.3±30.2) to T3N2M1 (264.5±33.5), indicating a progressive rise in Ferritin with increasing cancer stage ($p=0.001$). Hemoglobin (HB) levels also significantly vary, with T2N1M0 (10.34±1.1) showing a lower value than both T1N0M0 (11.23±1.1) and T3N2M1 (11.76±0.9) ($p=0.001$). LDH levels are significantly higher in T1N0M0 (255.6±34.2) compared to T2N1M0 (243.3±30.2) and T3N2M1 (241.2±30.9) ($p=0.008$). However, for GSH, ALP, GGT, CA15-3, CEA, SGOT, and IL-10, there are no statistically significant differences among the three groups ($p>0.05$).

Table 4.7 Biochemical and hematological parameters in different stages of breast cancer

Parameters	T1N0M0 (n:80)	T2N1M0 (n:123)	T3N2M1 (n:67)	P-Value
Ferritin (ng/mL)	223.3±30.2	199.3± 31.4	264.5± 33.5	0.001
GSH (units/mL)	9.56±1.3	9.44± 1.2	9.45± 1.4	0.18
LDH (U/L)	255.6±34.2	243.3± 30.2	241.2±30.9	0.008
ALP (U/L)	101.7± 9.4	101.3± 11.1	103.6±11.1	0.36
GGT (U/L)	29.93±2.6	29.59± 2.5	29.26±2.4	0.28
HB (g/dL)	11.23±1.1	10.34± 1.1	11.76±0.9	0.001
CA15-3 (U/mL)	22.82±4.1	22.84± 4.3	23.044.4	0.94
CEA (ng/mL)	6.13±1.7	6.32± 1.7	6.44±1.7	0.54
SGOT (U/L)	37.32±3.1	37.49± 3.1	37.71±2.8	0.37
IL-10 (pg/mL)	30.2±1.7	30.1± 2.1	30.342.2	0.53

The study found that ferritin levels varied significantly (p=0.001) across the different breast cancer stages. According to T3N2M1, elevated ferritin levels are associated with advanced cancer stages and could indicate systemic inflammation and the progression of the disease (Hu *et al.* 2019). Also, the work of Yousif *et al* (2016) increased ferritin levels may signal systemic inflammation and disease progression, as shown by T3N2M1 which has been associated with advanced cancer stages. Yousif *et al.* (2016) found that elevated ferritin levels, such as those in T3N2M1, are indicative of systemic inflammation and the progression of cancer. An increase in serum ferritin levels could be due to the increased iron demands of cancer cells. These cells modify transferrin receptors and proliferate more rapidly, both of which require iron. Inflammation from metastases, hepatic necrosis, and decreased hepatic clearance of ferritin are other potential causes of excessive serum levels, alongside increased synthesis by malignant cells. The elevated ferritin levels observed in advanced-stage breast cancer compared to early stages may be caused by a combination of these factors (Ramirez-Carmona *et al.* 2022).

In this study, the levels of LDH in T1N0M0 (255.6±34.2) were significantly greater than in T2N1M0 (243.3±30.2) and T3N2M1 (241.2±30.9), with a p-value of 0.008. Anupama Sh *et al.* (2011), Abdalla *et al.* (2016), and Sandhya *et al.* (2011) also found similar results (1999). Rapid cell proliferation in tumors can lead to the exusion of lipoprotein hydrolyzed (LDH) from cell cytoplasm into extracellular fluid. The tumor microenvironment can be hypoxic, which can improve the conversion of pyruvic acid to

lactic acid. To ensure effective anaerobic glycolytic metabolism, dysregulated levels of lactate dehydrogenase (LDH) are present, leading to decreased oxygen reliance. On the flip side, these findings were linked to research conducted by Mishra *et al* (2004).

Which found that, with a p-value of 0.001, mean serum LDH levels were significantly higher in breast cancer patients compared to healthy controls. Elevated serum LDH levels are significantly associated with an increase in aggressiveness and fixity during growth. LDH levels may be an indicator of tumor growth and invasive potential, and they correlate with tumor burden. The diagnostic importance of lactate dehydrogenase (LDH) in breast cancer is supported by the fact that an elevated LDH level correlates with tumor mass or prognosis in early stages of breast cancer in humans (Abdolhassan *et al.* 2015). Consistent with these findings, other investigations have shown that LDH serum levels are significantly higher than control levels. As previously stated by Choudhari *et al.* (2013) and Amritpal *et al* (2015). Hemoglobin levels change significantly between the stages (p=0.001). A common symptom among cancer patients, reduced HB levels may indicate anemia, which can be caused by the disease or its treatment. This is seen in breast cancer patients with T2N1M0 (Lee *et al.* 2017). The substantial decrease in hemoglobin levels in women with breast cancer metastases is clinically significant because anemia is often associated with low hemoglobin levels, which can have a negative impact on a patient's overall health, energy levels, and quality of life (Rajizadeh *et al.* 2017). Anemia can make it harder for a patient to tolerate some cancer treatments, like chemotherapy, so this finding could also call for measures to manage anemia, which could affect treatment choices. A significant drop in hemoglobin levels may indicate a more advanced stage of breast cancer, which can affect a patient's prognosis and treatment options. Additionally, this finding has significant consequences for breast cancer research. The precipitous drop in hemoglobin levels in breast cancer patients necessitates further research into the causes of this phenomenon and the potential utility of hemoglobin as a disease progression indicator (Panis *et al.* 2012).

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

- Age distribution: Most cases fall within the 40-59 age range.

- Education and marital status: A significant portion of patients have primary education and are married.

- Family history: The majority had no family history of breast cancer.

- Awareness and diagnosis: Patients commonly found their condition through self-examination (feeling a lump), and diagnostic methods included biopsy, clinical examination, mammography, and MRI.

- Children, smoking, and menstrual cycle: Varied percentages regarding children, smoking habits, and menstrual cycle regularity.

- Chronic diseases: Many breast cancer patients had diabetes and hypertension as concurrent conditions.

- Diabetes management mostly included Metformin and Insulin, while hypertension was typically treated with Amlodipine, Losartan, and other medications.

- There were significant differences in biochemical parameters between breast cancer patients and control group. Notably, breast cancer patients had lower Hb levels and elevated levels of CA 15-3, CEA, and IL-10 compared to the control group. Ferritin, GSH, LDH, ALP, GGT, and SGOT levels were also notably distinct between the two groups.

- The distribution of breast cancer patients across various stages showed that T1N0M0 constituted 29.63% of cases, T2N1M0 represented 45.56%, and T3N2M1 accounted for 24.81%.

- When comparing the mean values of various biomarkers among different TNM stages, Ferritin levels significantly increased with advancing cancer stage. Hemoglobin (Hb) levels were lower in T2N1M0 compared to T1N0M0 and T3N2M1. LDH levels were significantly higher in T1N0M0. However, no statistically significant differences were observed for GSH, ALP, GGT, CA15-3, CEA, SGOT, and IL-10 among the three groups.

5.2 Recommendations

- Focus on breast cancer awareness and screenings, especially in the 40-59 age range.

- Develop targeted educational programs for patients with primary education and those who are married.

- Continue monitoring and research into the significance of family history in breast cancer cases.

- Promote self-examination for early detection and diagnostic methods like biopsy, clinical examination, mammography, and MRI.

- Tailor support and information to address the specific needs related to children, smoking habits, and menstrual cycle regularity in breast cancer patients.

- Enhance integrated care for patients with diabetes and hypertension alongside their breast cancer treatment.

- Ensure effective and personalized management of diabetes with a focus on Metformin and Insulin, and hypertension with Amlodipine, Losartan, and other relevant medications.

- Consider the diagnostic potential of biochemical parameters like Hb, CA 15-3,CEA, IL-10, Ferritin, GSH, LDH, ALP, GGT, and SGOT in breast cancer patients.

- Provide appropriate care and support for breast cancer patients at different TNM stages, with a particular emphasis on those in stage T2N1M0.

- Monitor and assess biomarkers closely in breast cancer patients at various TNM stages, particularly Ferritin, Hb, and LDH levels, to guide treatment and care decisions.

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