SOCIODEMOGRAPHIC, DIETARY PATTERN, SERUM BIOMARKERS (INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 3 (IGFBP-3)) AND GENETIC POLYMORPHISMS IN BREAST CANCER AMONG PALESTINIAN WOMEN: A CASE CONTROL STUDY

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SOCIODEMOGRAPHIC, DIETARY PATTERN, SERUM BIOMARKERS (INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 3 (IGFBP-3)) AND GENETIC POLYMORPHISMS IN BREAST CANCER AMONG PALESTINIAN WOMEN: A CASE CONTROL STUDY

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

LIST OF SYMBOLS

α	Alpha
~	Approximately
β	Beta
°C	Celsius degree
χ^2	Chi square
÷	Division
=	Equal
<	Less than
≤	Less than or equal
δ	Mean Differences
µ	Micro sign
-	Minus
>	More than
≥	More than or equal
×	Multiplication
%	Percentage
+	Plus
±	Plus-minus
®	Registered sign
σ	Sigma
™	Trade mark sign
λ	Wavelength

LIST OF ABBREVIATIONS

1,25 (OH) D	1,25-Dihydroxyvitamin D
DAB	3,3'- Diaminobenzidine
A_{260}	Absorbance At 260 Nm
A_{280}	Absorbance At 280 Nm
A	Adenine
Adj. OR	Adjusted Odds Ratio
N-terminus	Amino Terminal Domains
ANOVA	Analysis of Variance
bp	Base Pair
BMI	Body Mass Index
BC	Breast Cancer
BRCA1	Breast Cancer Gene 1
BRCA2	Breast Cancer Gene 2
CA	Cancer Antigen
C-terminus	Carboxyl Terminal Domains
cm	Centimeter
CLIA	Chemiluminescence Immunoassay
CI	Confidence Interval
Corr. Coeff.	Correlation Coefficient
C	Cytosine
df	Degrees of Freedom
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate
EDTA	Ethylene Diamine Tetraacetic Acid
FBG	Fasting Blood Glucose
FFQ	Food Frequency Questionnaire

n	Frequency
G	Guanine
HR	Hazard Ratio
HC	Helsinki Committee
HRT	Hormone Replacement Therapy
HER-2	Human Epidermal Growth Factor-2
IHC	Immunohistochemistry
IGF-1R	Insulin-Like Growth Factor 1 Receptor
IGFBP-3	Insulin-Like Growth Factor Binding Protein-3
IGF-1	Insulin-Like Growth Factor-1
IBM	International Business Machines Corporation
IPAQ	International Physical Activity Questionnaire
JEPeM	Jawatankuasa Etika Penyelidikan Manusia
kDa	Kilodalton
kg	Kilogram
kg/m^2	Kilogram Per Square Meter
MDS	Mediterranean Diet Score
METs	Metabolic Equivalents
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
µg/ml	Microgram Per Milliliter
µl	Microliter
ml	Milliliter
mmHg	Millimeters of Mercury
mm	Millimeters
ng/ml	Nanograms Per Milliliter
NCBI	National Center for Biotechnology Information.
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
dbSNP	Single Nucleotide Polymorphism Database

OR	Odds Ratio
ER	Oestrogen Receptor
OD	Optical Density
OC	Oral Contraceptive
PHRC	Palestinian Health Research Council
PCR	Polymerase Chain Reaction
PR	Progesterone Receptor
ROC	Receiver Operating Characteristics Curve
RR	Relative Risk
RFLP	Restriction Fragment Length Polymorphism
rpm	Revolutions Per Minute
SNPs	Single Nucleotide Polymorphisms
SD	Standard Deviations
SPSS	Statistical Package For Social Sciences
T	Thymine
xg	Times Gravity
TAE	Tris- Acetate-EDTA
TP53	Tumour Protein P53
TNM	Tumour Node Metastasis
3'-UTR	Three Prime Untranslated Region
USM	Universiti Sains Malaysia
UCSC	University of California Santa Cruz
VIF	Variance Inflation Factor
Vs.	Versus
H_2O	Water
WHO	World Health Organization

LIST OF APPENDICES

SOSIODEMOGRAFIK, CORAK PEMAKANAN, BIOPENANDA SERUM (INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) DAN INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 3 (IGFBP-3)) DAN POLIMORFISMA GENETIK DALAM KALANGAN WANITA PALESTIN YANG MENGIDAP KANSER PAYUDARA: KAJIAN KAWALAN KES.

ABSTRAK

Kanser payudara (BC) kekal sebagai cabaran kesihatan awam global. Kajian kawalan kes ini meneroka hubungan antara protein pengikat faktor pertumbuhan seperti insulin 3 (IGFBP-3), faktor pertumbuhan seperti insulin 1 (IGF-1), polimorfisma genetik, corak pemakanan, dan risiko BC dalam kalangan wanita Palestin. Kajian ini melibatkan 112 kes BC yang baru didiagnosis melalui histopatologi dan 222 kawalan bebas kanser yang dipadankan dari segi umur. Pengumpulan data termasuk temu ramah secara bersemuka, analisis biokimia, genetik, imunohistokimia, dan bioinformatik. Sampel tisu tumor daripada kes BC dianalisis untuk status ER, PR, dan HER-2. Program SPSS versi 28 digunakan untuk semua analisis data. Analisis multivariat menunjukkan bahawa tahap IGF-1 serum yang tinggi dikaitkan dengan peningkatan risiko BC (OR=1.013; 95%CI 1.007, 1.019), serta status ER(+)/PR(+) (r_s = 0.232, $p \leq 0.001$) dan peringkat penyakit yang maju (III dan IV) (r_s = 0.191, $p \leq 0.001$; r_s = 0.119, p = 0.029). Risiko BC yang lebih tinggi juga dikaitkan dengan tidak pernah hamil (OR=2.122; 95%CI 1.022, 4.408), kelahiran pertama pada usia ≤18 tahun (OR=6.033; 95%CI 2.217, 16.413), dan senaman berintensiti rendah (OR=9.609; 95%CI 1.009, 91.471). Sebaliknya, wanita berumur 35–40 tahun menunjukkan risiko BC yang lebih rendah berbanding mereka yang berumur <35 tahun (OR=0.427; 95%CI 0.188, 0.971). Polimorfisma IGFBP-3 A-202C

menunjukkan hubungan yang signifikan dengan risiko BC. Alel homozigot CC lebih kerap berlaku dalam kes (70.5%) berbanding kawalan (20.7%) dan dikaitkan dengan peningkatan tahap IGFBP-3 ($r_s = 0.164$, $p = 0.003$), tahap IGF-1 ($r_s = 0.175$, $p \leq 0.001$), serta peningkatan risiko BC (OR=16.237; 95%CI 7.904, 33.356, $p \leq 0.001$). Genotip IGFBP-3 A-202C juga berkait rapat dengan status PR dan penerima hormon berganda positif ER/PR ($p = 0.020$). Analisis bioinformatik pula mengenal pasti varian IGF-1 (rs1520220, rs6214, rs7136446, rs6220) yang dikaitkan secara signifikan dengan karsinoma payudara dan neoplasma malignan payudara (p-value=6.29E−0.6 dan 2.84E−0.6). Tambahan pula, analisis pemakanan menunjukkan bahawa lapan daripada 14 kumpulan makanan yang dikaji, dikaitkan dengan pengurangan risiko BC, termasuk buah-buahan segar dan jusnya (OR=0.966; 95%CI 0.947, 0.985), produk daging (OR=0.963; 95%CI 0.934, 0.993), bijirin (OR=0.944; 95%CI 0.912, 0.977), produk tenusu rendah lemak (OR=0.970; 95%CI 0.945, 0.995), kacang (OR=0.835; 95%CI 0.745, 0.936), snek dan manisan (OR=0.967; 95%CI 0.937, 0.998), serta sup dan sos (OR=0.945; 95%CI 0.911, 0.979). Sebaliknya, telur dikaitkan dengan peningkatan risiko BC (OR=1.125; 95%CI 1.019, 1.243) dengan p-value < 0.05. Minuman pula menunjukkan korelasi positif dengan tahap IGF-1 ($r_p = 0.121$, $p = 0.027$). Penemuan ini menekankan kepentingan IGF-1 dan IGFBP-3 sebagai biomarker potensi risiko BC. Integrasi antara genetik, biokimia, pemakanan, dan faktor gaya hidup memberikan pemahaman baru untuk strategi pencegahan dan rawatan yang diperibadikan, terutamanya dalam keadaan sumber yang terhad seperti Semenanjung Gaza.

SOCIODEMOGRAPHIC, DIETARY PATTERN, SERUM BIOMARKERS (INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 3 (IGFBP-3)) AND GENETIC POLYMORPHISMS IN BREAST CANCER AMONG PALESTINIAN WOMEN: A CASE CONTROL STUDY.

ABSTRACT

Breast cancer (BC) remains a global public health challenge. This case-control study explores the relationships between serum insulin-like growth factor binding protein 3 (IGFBP-3), insulin-like growth factor 1 (IGF-1), genetic polymorphisms, dietary patterns, and BC risk in Palestinian women. The study involved 112 newly diagnosed BC confirmed by histopathology and 222 age-matched, cancer-free controls. Data collection included face-to-face interviews, biochemical, genetic, and immunohistochemical analyses, and bioinformatics. Tumour tissue samples from BC cases were analysed for ER, PR, and HER-2 status. SPSS program version 28 was used for all data analysis. Multivariate analysis revealed that elevated serum IGF-1 levels were associated with increased BC risk (OR=1.013; 95%CI 1.007, 1.019), as well as ER(+)/PR(+) status (r_s= 0.232, $p \leq 0.001$) and advanced disease stages (III and IV) (r_s= 0.191, p ≤ 0.001; r_s= 0.119, p = 0.029) respectively. Higher BC odds were linked to never being pregnant (OR=2.122; 95%CI 1.022, 4.408), first delivery at ≤18 years (OR=6.033; 95%CI 2.217, 16.413), and low-intensity exercise (OR=9.609; 95%CI 1.009, 91.471). Conversely, women aged 35–40 years had a reduced BC risk compared to those <35 years (OR=0.427; 95%CI 0.188, 0.971). The IGFBP-3 A-202C polymorphism showed significant associations with BC risk. The homozygous CC allele was more frequent in cases (70.5%) compared to controls (20.7%) and linked to

elevated IGFBP-3 (r_s = 0.164; p = 0.003) and IGF-1 levels (r_s = 0.175; $p \leq 0.001$), and an increased risk of BC (OR=16.237; 95%CI 7.904, 33.356, $p \leq 0.001$). Moreover, the IGFBP-3 A-202C genotype was significantly associated with PR status and double positive hormone receptor ER/PR (p = 0.020). Furthermore, bioinformatics analysis identified IGF-1 variants (rs1520220, rs6214, rs7136446, rs6220) significantly associated with breast carcinoma and malignant neoplasm of the breast (p-value = 6.29E−0.6 and 2.84E−0.6) respectively. Additionally, the dietary analysis revealed that eight of the 14 food groups studied were linked to a decreased risk of BC, including fresh fruits and their juices (OR= 0.966; 95%CI 0.947, 0.985), meat products (OR= 0.963; 95%CI 0.934, 0.993), grains (OR=0.944; 95%CI 0.912, 0.977), low-fat dairy (OR=0.970; 95%CI 0.945, 0.995), nuts (OR=0.835; 95%CI 0.745, 0.936), snacks and sweets (OR=0.967, 95%CI 0.937, 0.998), and soups and sauces (OR=0.945; 95%CI 0.911, 0.979), were associated with a reduction in BC risk, while eggs were associated with increased risk (OR=1.125; 95%CI 1.019,1.243) with p-values < 0.05. Drinks and beverages was positively correlated with IGF-1 levels (r_p = 0.121, p = 0.027). These findings underscore the importance of IGF-1 and IGFBP-3 as potential biomarkers for BC risk. Integrating genetic, biochemical, dietary, and lifestyle factors provides novel insights for personalized prevention and treatment strategies, particularly in resource-limited settings such as the Gaza Strip.

CHAPTER 1

INTRODUCTION

1.1 Background

BC has now overtaken lung cancer as the world's most commonly diagnosed cancer, accounting for 1 in 8 cancer diagnoses and 2.3 million new cases of cancer in women, and 685,000 of them passed away (Arnold *et al.*, 2022; World Health Organization, 2023). About 25% of all new malignancies found in females are predicted to be related to this malignant tumour, which is thought to be the most commonly diagnosed neoplasm in women globally (Ibragimova *et al.*, 2023). Although BC was once thought to be a disease primarily seen in affluent nations, more than half of diagnoses and two-thirds of BC-related deaths took place in less developed parts of the world (Sung *et al.*, 2021). Even though the majority of patients have early and treatable diagnoses, metastatic disease occurs in up to 30% of BC patients (Soni *et al.*, 2015). Recent research indicates that up to 60–75% of individuals with metastatic BC may have bone metastases, 32–37% will develop lung metastases, 32–35% will develop liver metastases, and up to 10% will develop brain metastases (Ibragimova *et al.*, 2023).

In Palestine, BC is the most common cancer among the population. In 2022, the number of new BC cases was 934, with an incidence rate of 18.5 cases per 100, 000 total population. It is considered the most common type with an incidence rate of 37.4 cases per 100,000 female population. The West Bank registered 540 new cases of BC, representing 15.8%. Among females, it is the topmost common cancer with 38.0% of all types of cancer, and the top leading cause of death. However, it is considered the third cause of cancer death by 11.7%. In the Gaza Strip 394 newly registered cases of BC, representing 19.2 % of all newly diagnosed cancer cases. In

females, it ranked first as the most common cancer with 36.9% of all types of cancer (Ministry of Health, 2023), and the first leading cause of death (Ministry of Health, 2018).

As a biomarker of the risk and mortality of chronic diseases, IGF-1 has been the subject of substantial research. It is postulated that IGF-1 stimulates the growth and multiplication of cells while suppressing apoptosis, hence promoting the development of tumours (Mukama *et al.*, 2023). The majority of the circulating IGF-1 is attached to insulin-like growth factor binding proteins (IGFBPs), mainly to IGFBP-3 in a ternary complex with an acid-labile subunit (Varma Shrivastav *et al.*, 2020). IGFBP-3 regulates the availability of circulating IGF-1 and its mitogenic and anti-apoptotic effects by binding to IGF-1 (Tas *et al.*, 2016).

The development of numerous human tissues is physiologically influenced by IGF-1 and its system-binding protein and receptor (Slepicka *et al.*, 2021). Normal mammary gland biology depends on the IGF pathway ligands, IGF-1 and IGF-2, and their receptors, mainly IGF-1R. Dysregulation of these ligands' expression and function increases the risk of BC and accelerates its progression by activating downstream signalling effectors, frequently in a subtype-dependent way (Lee *et al.*, 2022). The ability of IGF-1 to promote mitosis and inhibit programmed cell death makes it and its principal binding protein (IGFBP-3) recognized risk factors for BC. Research has demonstrated a relationship between women's IGF-1 and IGFBP-3 levels and both the onset and recurrence of BC (Monson *et al.*, 2020).

The associations between BC risk and IGFBP-3 and IGF-1 circulating levels have consistently been found in studies (Qian and Huo, 2020; Rodríguez-Valentín *et al.*, 2022). IGF-1 and BC have a substantial positive relationship ($p < 0.0001$), based on a combined analysis of 17 prospective studies, women in the quintile with the

highest IGF-1 had (Odds ratio (OR)=1.28; 95%Confidence interval (CI) 1.14, 1.44; *p*-< 0.0001). In this pooled study, moreover, higher IGFBP-3 was associated with a higher chance of developing BC, with an (OR=1.13; 95%CI 0.99, 1.28) at the highest IGFBP-3 quintile but the differences did not reach the statistical level ($p = 0.062$) (Key *et al.*, 2010).

Circulating IGF-1 levels and the development of BC have been found to be significantly correlated in numerous prospective and epidemiological investigations. There is compelling evidence linking the circulating levels of IGF-1 to the onset of cancer, specifically the incidence of BC. Higher circulating levels of IGF-1 were linked to a higher risk of BC incidence, according to a large prospective study by Mukama et al. (HR=1.25; 95%CI 1.06, 1.47) (Mukama *et al.*, 2023), Additionally, Werner and Laron discovered that the incidence of BC in premenopausal women was associated with circulating levels of IGF-1, but not among postmenopausal (Werner and Laron, 2020). It was clear from the analysis of combined data from 17 prospective studies comprising 4790 cases in 12 countries that women with substantially higher levels of serum IGF-1 were 30% more likely to develop BC than those with comparatively lower levels. Only oestrogen-receptor-positive ER(+) tumours showed this positive relationship (Key *et al.*, 2010). In addition, it was concluded through observational and Mendelian randomization analysis that circulating IGF-1 concentrations are associated with BC (Murphy *et al.*, 2020).

There is evidence that genetic variants, particularly single nucleotide polymorphisms (SNPs) with a rare allele frequency of more than 1%, may have a major impact on IGF-1 expression levels, despite the influence of environmental variables and lifestyle (Costa-Silva *et al.*, 2016; Vallejos-Vidal *et al.*, 2020). These genetic changes contribute to variations in BC susceptibility (Rodríguez-Valentín *et al.*, 2022).

SNPs can occur in either coding or noncoding sequences at the genomic level (Zhu *et al.*, 2019). SNPs are often regarded as the most valuable biomarker for illness diagnosis or prognosis because of their frequent occurrence, inexpensive genotyping, simplicity of analysis, and capacity to do association studies utilising statistical and bioinformatics methods (Vallejos-Vidal *et al.*, 2020). The identification and characterisation of biomarkers for the diagnosis of BC have resulted from the high throughput genotyping of BC samples. SNPs are the most often researched genetic variants. They can be used to uncover the underlying reasons for breast carcinogenesis as well as determine susceptibility to a variety of disorders (Bakshi *et al.*, 2020).

Increasing serum levels of IGF-1 have been associated with polymorphisms in the IGF-1 gene, as well as an increased risk of BC and aggressiveness of tumours (Ferreira-Fernandes *et al.*, 2015). Specifically, demonstrated in their research that IGF-1 polymorphisms, specifically, SNPs rs6220 (Adenine (A)[Guanine (G)) and rs7136446 (Thymine (T)[Cytosine (C)), have been linked to increased plasma levels of IGF-1, increased breast density, and an increased risk of BC. The two main alleles, A and T, were found to be normal, while the genotypes GG and CC were linked to an elevated BC risk (Verheus *et al.*, 2008; Smolarz *et al.*, 2021).

IGFBPs are groups of proteins that have high affinity and specificity for binding the mitogens IGF-1 and IGF-2. IGFBP-3 regulates the bioavailability and activity of IGF along with having IGF-independent effects such as cell growth inhibition and apoptosis induction (Baxter, 2023). IGFBPs with BC can interact in a stimulatory or inhibitory manner (Beattie *et al.*, 2015).

Evidence suggests that IGFBP family members have a role in the development and metastasis of tumours and may be useful prognostic markers for a variety of malignancies (Wang *et al.*, 2019a). Numerous connections between circulating

IGFBP-3 and the risk of cancer, especially BC, have been found through epidemiological researches. Some studies found a slight drop in hazard with increased IGFBP-3 serum levels, but other studies found an increase in risk or no connection (Cui *et al.*, 2020; Rodríguez-Valentín *et al.*, 2022). A published study investigating the relationship between serum levels of IGFBP-3 and the hazard of BC revealed that women in the highest tertile had a statistically significant increased risk of BC, according to the unadjusted analysis of the association between IGFBP-3 serum concentration and the risk of BC. Overall, the risk of BC increases with increasing blood levels of IGFBP-3 (OR=5.55; 95%CI 3.55, 8.68) compared to individuals in the lowest tertile (Rodríguez-Valentín *et al.*, 2022). Rinaldi et al.'s investigation revealed no relationship between young women and the risk of BC (OR=0.92; 95%CI 0.50, 1.70), p = 0.69) (Rinaldi *et al.*, 2006). This contrasts with other research that found increased IGFBP-3 concentrations to be unrelated to BC risk.

IGFPB-3 genetic variants have been demonstrated to change the amount of circulating IGFBP-3 and affect a person's vulnerability to several carcinomas, including BC. Although IGFBP-3 has a number of potentially useful polymorphisms, the promoter rs2854744 polymorphism (also known as A-202C) is the most significant and has been thoroughly investigated at the Deoxyribonucleic acid (DNA) level (Ma *et al.*, 2015). The findings demonstrated a relationship between elevated levels of IGFBP-3 in circulation and the A-202C polymorphism. It is hypothesised that the association results from elevated promoter activity, indicating cis regulation (Christopoulos *et al.*, 2015; Dj *et al.*, 2024). According to a study by Su et al. proliferative benign breast disease (proliferative BC) and IGFBP-3 levels were positively correlated with the uncommon allele of the well-studied IGFBP-3 rs2854744 A-202C (Su *et al.*, 2010). In a meta-analysis conducted by Qiu et al. the

polymorphism has been identified as a low-penetrant risk factor for BC associated with IGFBP-3A-202C polymorphism (Qiu *et al.,* 2010). The best way to manage this malignancy depends in large part on other tumours characteristics. ER, progesterone receptor (PR), and human epidermal growth factor-2 (HER-2) have become essential prognostic and therapeutic indicators for diagnosis and management. For the best possible treatment, all cases of breast carcinoma are screened for these indicators. Currently, immunohistochemistry (IHC) is the preferred method of testing (Fakhri *et al.*, 2018). This method is relatively less expensive, more accessible, and offers both predictive and therapeutic information (Gore *et al.*, 2018).

1.2 Problem statement and study rationale

BC is a significant global public health concern, representing 25.1% of all malignancies and surpassing lung cancer as the most diagnosed worldwide (Bashar and Begam, 2022; Elsous *et al.,* 2023). In Palestine, it has a high incidence rate, with 934 new cases, including 394 in the Gaza Strip, making up 19.2% of newly diagnosed patients (Ministry of Health, 2023). Over 60% of Palestinian patients receive late diagnoses, leading to a high mortality rate, often within five years of diagnosis (Abu-Odah *et al.*, 2022), which is exacerbated by a lack of awareness about warning signs and risk factors, underscoring the critical need for enhanced education and early detection efforts (Elshami *et al.,* 2022). Even though mammography screenings are either free or inexpensive for women starting at age 40, awareness and utilization of these programs remain low (Elshami *et al.*, 2018; AlWaheidi *et al.*, 2020; Jubran *et al.*, 2022). The diagnostic facilities in the Gaza Strip are limited, leading to delays in appointments and hindrances in conducting necessary tests like IHC testing due to shortages of slides and reagents. Furthermore, consumables, laboratory reagents such

as IGF-1 and IGFBP-3, diagnostic equipment, and gene labs to perform the genetic polymorphism tests cause delays in the diagnosis (Medical Aid for Palestinians, 2021). Fragmented pathology care and missed appointments also contribute to delayed diagnoses, contributing to low survival rates (AlWaheidi, 2019; Al Shiekh and Alajerami, 2020). BC diagnosis in women under 40 poses significant challenges for both patients and healthcare providers, often resulting in more aggressive forms of the disease and necessitating extensive treatments such as chemotherapy and mastectomy (Hironaka-Mitsuhashi *et al.*, 2019; Davey *et al.*, 2020). Despite being less common in this age group, the impact can be more profound due to later-stage detection and aggressive tumour features (Murphy *et al.*, 2019).

The healthcare system in the Gaza Strip faces notable deficiencies, with limited cancer services, medication shortages, and a lack of essential equipment and facilities for proper treatment and support (Hass, 2018; Eid *et al.*, 2022). Numerous SNPs such as the genetic polymorphisms of IGF-1 and IGFBP-3 and their serum levels have been associated with the risk of BC, making them promising biomarkers for the early diagnosis and treatment of BC. In the Gaza Strip, the evidence relating serum IGF-1 and IGFBP-3 levels in the circulation to BC risk has not been consistent and understood. Nevertheless, the participation of their gene's polymorphism has not clarified the association of BC risk. According to our knowledge, this is the first study that associates the serum levels of IGF-1 and IGFBP-3 with the risk of BC, elucidation the patterns of IGFBP-3 and IGF-1 gene polymorphism with BC risk, and ER/PR and HER-2 statuses as biomarkers for early detection of BC.

Understanding the relationship between IGF-1 and IGFBP-3 serum levels and BC can inform appropriate treatment strategies to mitigate BC risk. Furthermore, elucidating patterns of gene polymorphism in IGFBP-3 and IGF-1 may identify

women who are more susceptible to BC, aiding in the creation of strategies for early diagnosis and successful therapies for treating the illness.

Testing for ER, PR, and HER-2 status is crucial in the treatment of BC, serving as prognostic and predictive markers that influence treatment decisions and patient outcomes (Chand *et al.*, 2018).

Overall, addressing these multifaceted challenges and advancing our understanding of BC biology and risk factors are essential for improving outcomes and reducing the burden of the disease in affected populations.

1.3 Objectives

1.3.1 General objective

To investigate the interplay between sociodemographic factors, dietary patterns, serum biomarkers (IGF-1 and IGFBP-3), and genetic polymorphisms, in BC among Palestinian women in the Gaza Strip

1.3.2 Specific objectives

a. Objective 1

To determine the proportion of (A-202C) polymorphism of IGFBP-3 among women diagnosed with BC and women without BC

b. Objective 2

To compare the mean serum levels of IGF-1 and IGFBP-3 among women with BC (cases) and women without BC (controls).

c. Objective 3

To determine the association between the participants' clinical characteristics (sociodemographic, anthropometric, reproductive, medical, and lifestyle factors) with the serum levels of IGF-1 and IGFBP-3.

d. Objective 4

To determine the association between the IGFBP-3 (A-202C) polymorphism and the serum levels of IGF-1 and IGFBP-3.

e. Objective 5

To determine the association of the serum IGF-1 and IGFBP-3 levels, and IGFBP-3 (A-202C) polymorphism with hormone receptor (ER, PR, and HER-2) status, to understand the interactions between these biomarkers in BC women.

f. Objective 6

To elucidate the genetic linkage between IGF-1 and BC through the use of bioinformatics approaches.

CHAPTER 2

LITERATURE REVIEW

2.1 Breast cancer (BC)

Cancer is a condition that arises as a result of external factors (such as radiation, chemical, and viruses) as well as internal factors (including immune conditions, hormones, and inherited genetic mutations) that promote uncontrolled cell growth and the presence of abnormal cells, which eventually result in death following uncontrolled dispersion (American Cancer Society, 2019). This disease fundamentally involves genetic alterations, whether inherited or acquired, that initiate the activation of oncogenes or the deactivation of suppressor genes. Notably, many genes associated with BC are tumour suppressors, pivotal in regulating DNA repair and cell cycle mechanisms, such as BReast CAncer gene 1 (BRCA1), BReast CAncer gene 2 (BRCA2), Serine/threonine kinase 11, Phosphatase and TENsin homolog, Tumour protein P53 (TP53), Checkpoint kinase 2, Cadherin-1, ataxia-telangiectasis mutated, BReast CAncer gene 1 Interacting Protein 1, and Partner and Localizer of Breast Cancer 2. Mutations within these genes result in a loss of function, prompting genomic instability and unregulated cell cycling, ultimately fostering the uncontrolled proliferation of tumour cells (Haddad, 2020). Eventually, these cancerous cells coalesce into a mass or lump, commonly known as a tumour, named after the specific body part from which it originates (Godet and Gilkes, 2017).

2.1.1 The epidemiology of BC

As cancer registries only keep track of incidence and death, the information provided is insufficient to determine the number of women with metastatic cancer or to assess which cases are currently treatable (Jakesz, 2008; Francies et al., 2020).

Global differences in BC incidence are attributed to wide ranges of differences in the levels of education, environmental circumstances, economic status, dietary practices (such as varying amounts of red meat in non-vegetarian diets), lifestyle variables such as alcohol consumption, tobacco consumption, and other cultural practices. The prevalence of BC may rise in both developed (32% to 56%) and developing (64% to 95%) nations by 2040 as a result of globalisation and the expanding economy (Anderson and Jakesz, 2008; Gandhi et al., 2017; Sung et al., 2021).

One hundred and fifty-seven out of 185 nations identified BC as the most prevalent cancer among women, followed by cervical cancer in 23 countries, predominantly in sub-Saharan Africa, it was also the leading cause of cancer-related deaths in 110 of those countries, with lung cancer coming in 25 countries and cervical cancer in 36 countries. However, there is a significant geographic variance in the rates of incidence and death (Figure 2.1) (Arnold *et al.*, 2022).

The International Agency for Research on Cancer's recent GLOBOCAN 2020 report revealed BC had the highest incidence and prevalence rates across 185 countries (Figure 2.2) (Sung *et al.*, 2021). Approximately one woman is given a BC diagnosis worldwide every 14 seconds. In 2020, there were 2.3 million new cases of BC among women worldwide and 685,000 fatalities. By the end of 2020, 7.8 million women who had been diagnosed with BC within the previous five years were still alive, establishing BC as the most common cancer globally (World Health Organization, 2023). In 2040, the American Cancer Society estimates that 28.4 million cancer cases will exist globally, representing an approximate 47% increase from the 2020 (Stephens, 2021).

According to reports, countries with a high human development index (HDI) experience the highest rates of premenopausal (30.6/100,000) and postmenopausal (253.6/100,000) BC incidence (Heer et al., 2020). In contrast, the lowest

premenopausal (8.5/100,000) and postmenopausal (53.3/100,000) BC mortality rates were observed in low- and medium-HDI countries (Oluwasanu and Olopade, 2020). Limited access to early detection and appropriate treatment options contributes to a higher BC mortality rate in poor nations (Heer et al., 2020).

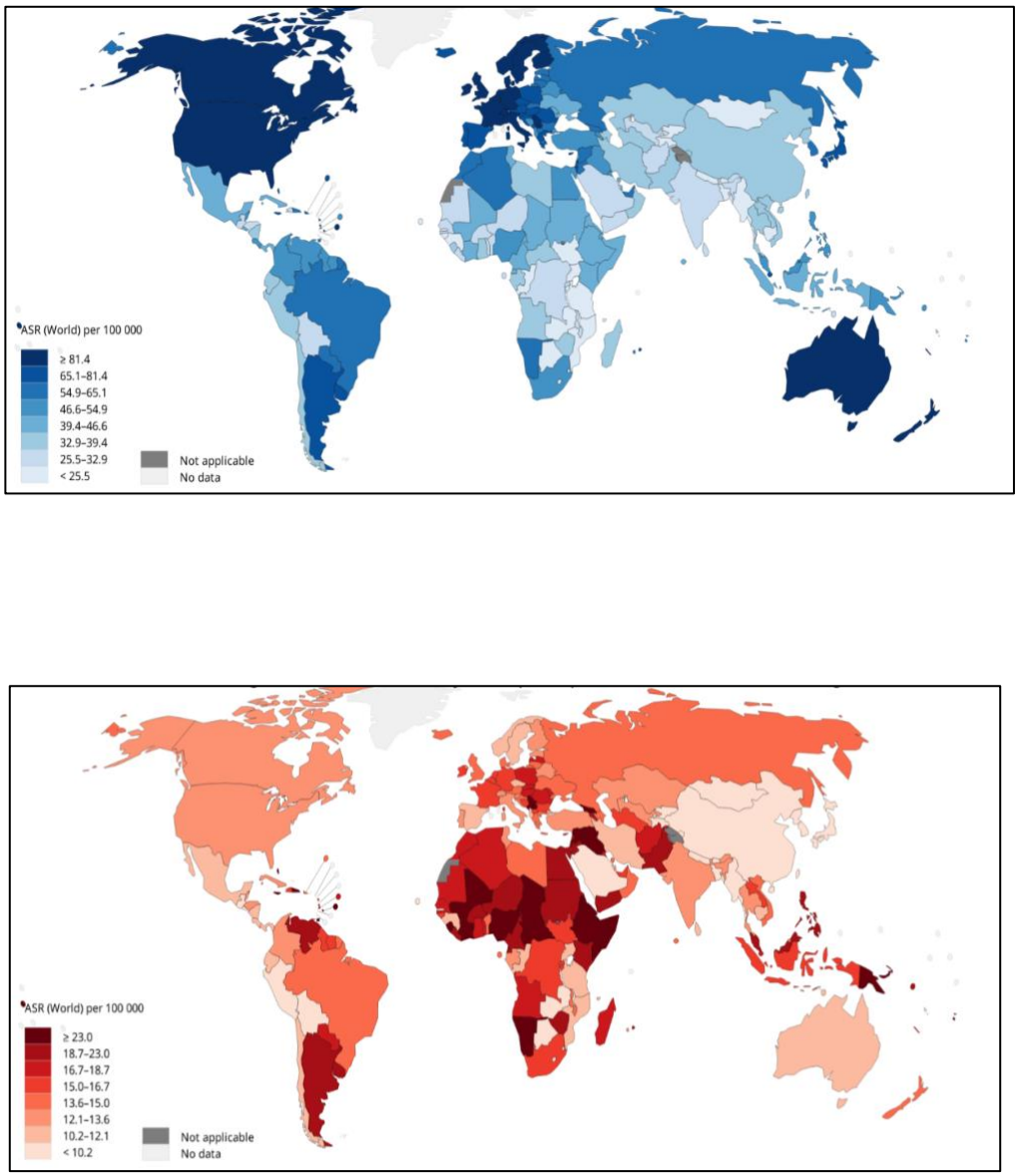

Figure 2.1: Age-standardized BC incidence (top, blue) and mortality (bottom, red) rates per 100,000 females across various countries (Adapted from (Arnold et al., 2022)).

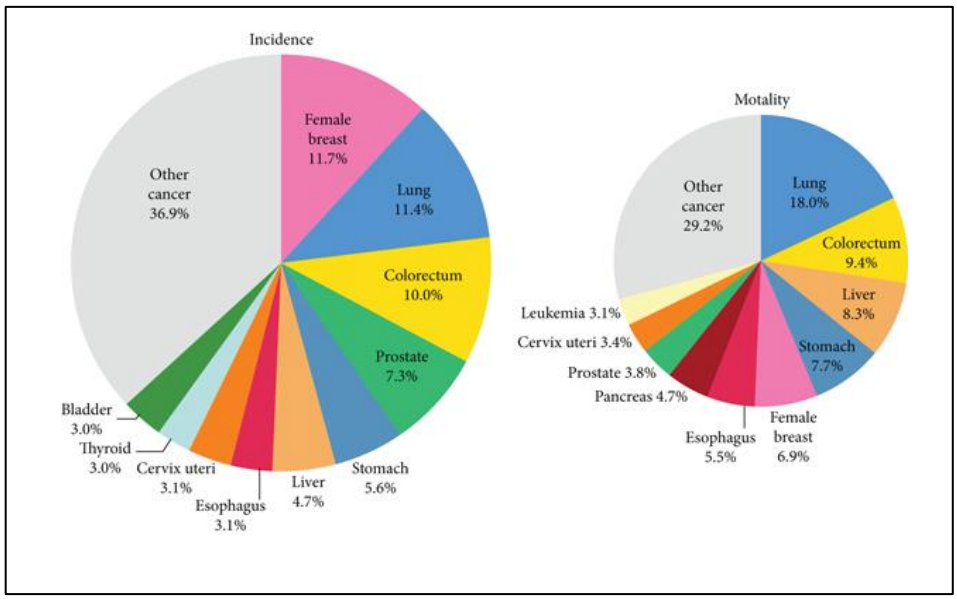

Figure 2.2: Distribution of cancer incidence and mortality across various cancer types (Adapted from (Kashyap et al., 2022)).

2.1.2 BC in Palestine

In Palestine, BC is consistently the most common malignancy among the population. In 2022, the number of new BC cases was 934 cases, resulting in an incidence rate of 18.5 cases per 100,000 individuals in the total population. The incidence rate of BC among females is 37.4 cases per 100,000 female population, making it the most prevalent kind of cancer. 540 new BC cases were registered in the West Bank, making about 15.8% of all new cases. It was the most common kind of cancer and the main leading cause of death for women, accounting for 38.0% of all cases. However, it is considered the third leading cause of mortality from cancer at 11.7%. In the Gaza Strip, 394 newly diagnosed cases of BC were reported, accounting for 19.2% of all newly diagnosed cancer cases and having an incidence rate of 18.2 cases per 100,000 people. In females, it ranked first as the most common cancer with 36.9% of all cancer types (Ministry of Health, 2023), and it was also the primary cause of cancer-related mortality (Ministry of Health, 2018).

2.1.3 Types of BC

It is categorized into invasive and non-invasive BCs, according to their location.

2.1.4 Non-invasive BC

This type of cancer is limited to the ducts and lobules in which it is located, without spreading to the surrounding fatty and connective tissues of the breast (West *et al.*, 2017).

2.1.4(a) Lobular carcinoma in situ

Breast lobules are formed by this type of BC (Inoue *et al.*, 2017). The BC has not penetrated outside the lobules into the breast tissue (Clauser *et al.*, 2016). Figure 2.3 shows the illustration of lobular carcinoma in situ.

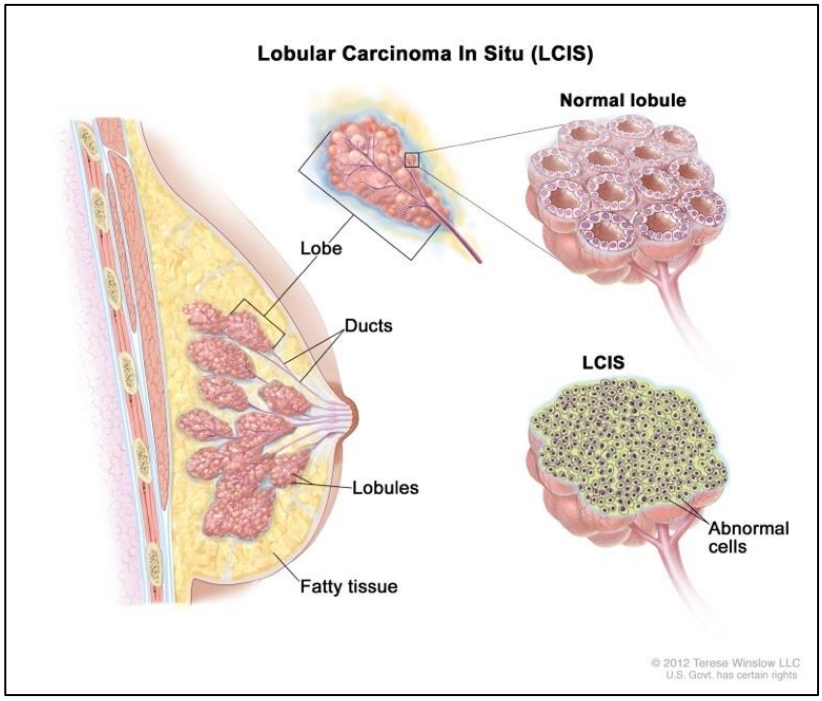

Figure 2.3: Illustration demonstrating lobular carcinoma in situ, where abnormal cells are located within the lobules of the breast (Adapted from (PDQ Adult Treatment Editorial Board, 2024)).

2.1.4(b) Ductal carcinoma in situ

Ductal carcinoma in situ, is one of the most prevalent forms of BC. It is a non-invasive BC that forms within of pre-existing normal ducts (Feng *et al.*, 2018). Figure 2.4 demonstrates the illustration of the ductal carcinoma in situ.

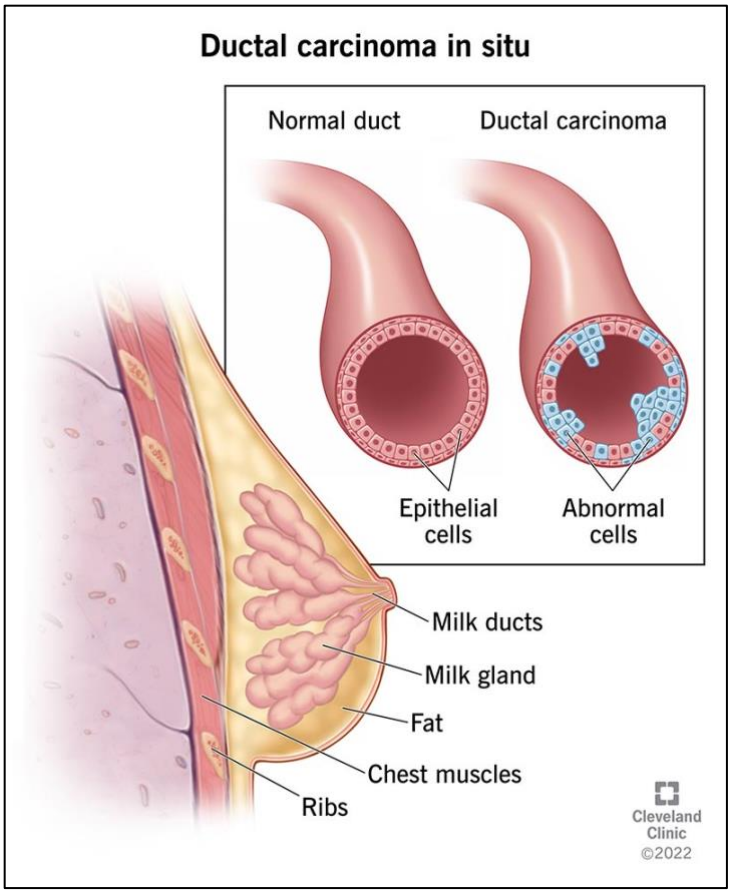

Figure 2.4: Illustration demonstrating ductal carcinoma in situ, precancerous cells have not spread beyond the milk ducts (Adapted from (ClevelandClinic, 2022)).

2.1.5 Invasive BC

BC occurs when abnormal milk duct or lobule cells spread and come into contact with breast tissue (Harris *et al.*, 2016). When BC spreads to multiple organs, it is known as metastatic or invasive BC, commonly affecting the brain, bones, lungs, and liver (Dissanayake *et al.*, 2023). Around 50% to 80% of newly diagnosed BC cases are invasive ductal carcinoma, with the remaining cases being invasive lobular carcinoma. Invasive ductal carcinomas may be categorized as "no specific type" or "special type" depending on their morphological characteristics (Masood, 2016; Henry and Cannon-Albright, 2019).

2.1.5(a) Invasive ductal carcinoma no specific type

The majority of invasive breast carcinomas are classified as invasive ductal carcinoma - no specific type, comprising 40% to 75% of all cases. These carcinomas exhibit diverse clinical behaviour and morphological variations, often featuring pleomorphic tumour cells with multiple mitoses and protruding nucleoli (Makki, 2015; Masood, 2016). Figure 2.5 shows the invasive ductal carcinoma of the breast.

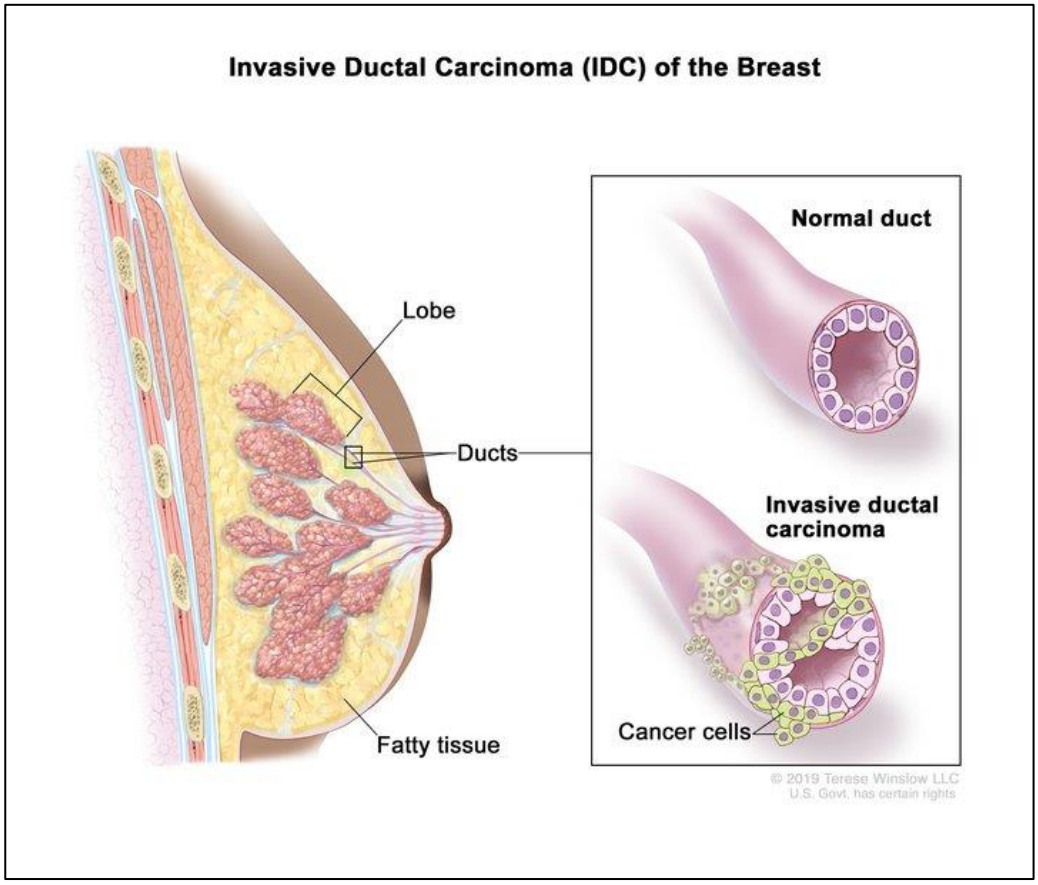

Figure 2.5: Invasive ductal carcinoma of the breast. The drawing depicts a cross-sectional view of a lobe, ducts, and fatty tissue. An inset compares a normal duct alongside a duct with invasive ductal carcinoma, showing cancer cells spreading outside the duct (Adapted from (Winslow, 2022))

2.1.5(b) Invasive ductal carcinoma special type

Medullary carcinoma, mucinous carcinoma, tubular carcinoma, metaplastic carcinoma, apocrine carcinoma, cribriform carcinoma, neuroendocrine carcinoma are the specific forms of BC that are most frequently detected (Masood, 2016).

A- Medullary carcinoma

A specific type of invasive BC, accounting for about 5% of cases, is linked to better clinical outcomes and less involvement of the axillary lymph nodes. It typically affects patients aged 30 to 40, and is often associated with germline BRCA1 mutations (Masood, 2016).

B- Mucinous carcinoma

Colloid carcinoma, which constitutes only 2% of all BC, typically affects postmenopausal women and is associated with a favourable prognosis (Anuradha and Lakshmi, 2014).

C- Tubular carcinoma

The prognosis for women with tubular carcinoma is often better than that of women with more prevalent invasive carcinomas (Priya and Prasaad, 2017).

D- Metaplastic carcinoma

A rare and aggressive subtype, accounting for less than 1% of cases. It is typically negative for ER, PR, and HER-2 and has a poorer prognosis than other triple-negative BC (Schwartz et al., 2013).

E- Apocrine carcinoma

It affects 1% to 4% of all cases, and typically has a high histological grade and poor prognosis. Postmenopausal women are more likely to develop this subtype (Vranic *et al.*, 2013; Makki, 2015).

F- Cribriform carcinoma

A rare type of invasive BC, resembles the histological features of cribriform ductal carcinoma in situ. Its frequency ranges from 0.3% to 6% of primary BC and is associated with a good prognosis (Adachi *et al.*, 2021; Demir *et al.*, 2021).

G- Neuroendocrine carcinoma

This uncommon subset of tumours, which accounts for 2% to 5% of all invasive BC, is identified by the presence of neuroendocrine markers and characteristics similar to those of gastrointestinal and pulmonary neuroendocrine tumours. A less favourable prognosis may arise from the fact that neuroendocrine BC is frequently hormone receptor-positive and HER-2-negative, in contrast to other invasive BC (Trevisi et al., 2020).

2.1.5(c) Invasive lobular carcinoma

As illustrated in Figure 2.6, invasive lobular carcinoma is the second most common type of BC primarily affects older women and makes up 5% to 15% of new occurrences (Akram *et al.*, 2017). It is distinguished by tiny tumour cells distributed in a concentric pattern throughout the stroma with little atypia. Tumour cells in pleomorphic invasive lobular carcinoma are apocrine, exhibit strong mitotic activity, and have hyperchromatic, eccentric nuclei. Histiocytic or signet ring cells may also be present in these tumours, and TP53 mutations are more frequently found in these tumours (Masood, 2016; Thomas *et al.*, 2019).

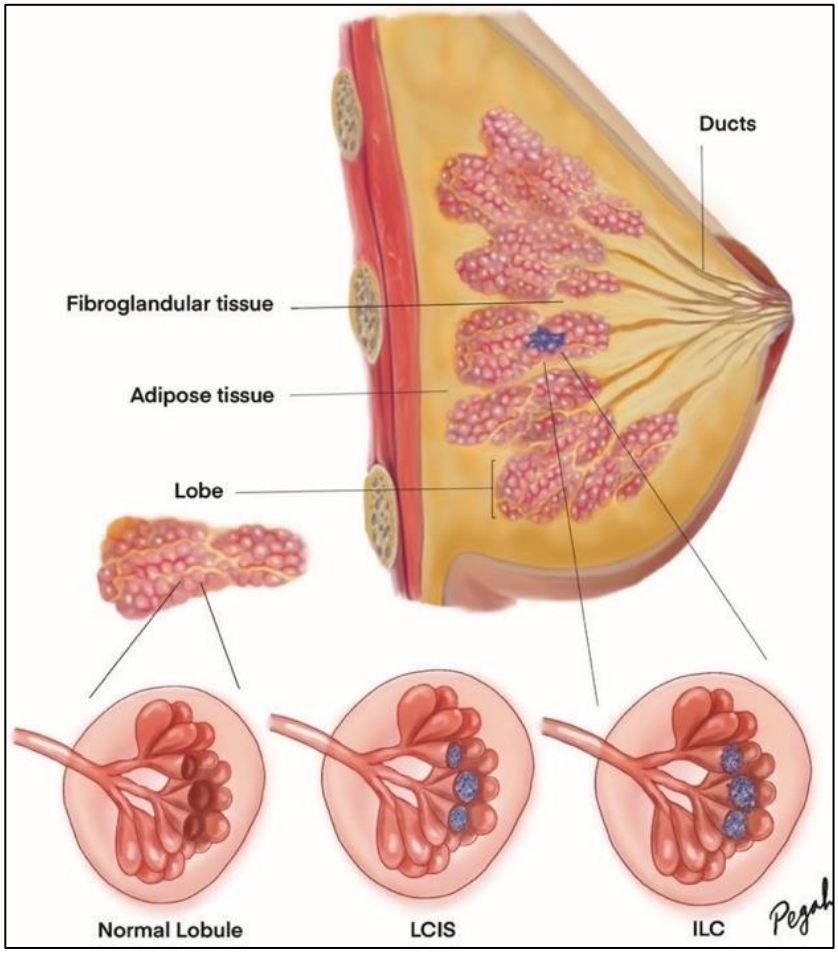

Figure 2.6: Anatomy of the breast and classification of lobular carcinoma types. The breast is comprising of adipose, fibrous, and glandular tissues, with lobules connected by ducts that drain towards the nipple. Each lobule contains multiple acini. Lobular carcinoma in situ is characterized by monomorphic cells that proliferate and expand within a lobule without breaching the basement membrane. Conversely, invasive lobular carcinoma involves the extension of these cells beyond the membrane, spreading and surrounding the ducts (Adapted from (Albasha et al., 2023)).

2.1.6 Molecular classification of BC

A variety of malignancies, including BC, exist with distinct biological traits, clinicopathological symptoms, and molecular markers (Yersal and Barutca, 2014). Examples of BC subtypes include luminal A (ER(+) and/or PR(+), but HER-2(-)), luminal B (ER(+) and/or PR(+), HER-2(+)), HER-2 overexpression (ER(-), PR(-), but HER-2(+)), and basal-like which is also called triple-negative BC (ER(-), PR(-), HER-2(-), cytokeratin positive and/or epidermal growth factor receptor 1), imitating normal breast epithelium (Badowska-Kozakiewicz and Budzik, 2016).

2.1.6(a) Oestrogen receptor (ER)

Since ER expression is markedly elevated in 70–75% of invasive breast carcinomas, ER is a crucial diagnostic determinant (Colomer *et al.*, 2018; Li *et al.*, 2020b). According to the current protocol, both initial invasive tumours and recurrent lesions have their ER expression evaluated. This approach is important to select the patients who will benefit most from the usage of endocrine medication, which mostly consists of third-generation aromatase inhibitors, pure oestrogen receptor down regulators, or selective oestrogen receptor modulators (Duffy *et al.*, 2017). ER expression may also be a predictive indicator, and patients with high ER expression typically have much better clinical outcomes, even though the diagnosis of changed ER expression is particularly important for choosing the appropriate therapy (Nasrazadani *et al.*, 2018).

2.1.6(b) Progesterone receptor (PR)

In the normal growth of the mammary gland as well as the origin and progression of BC, the PR is a crucial steroid hormone receptor. It is an increased target gene of ER that is dependent on oestrogen for expression, and it affects the activity of ERα in BC. PR is an important prognostic biomarker, particularly in

hormone-positive BC. It is also a biomarker that is frequently utilised during diagnosis to describe BC, and it has a role in molecular subtyping and treatment selection. When it comes to tumours, those with a more favourable baseline prognosis, such as the luminal A subtype, typically exhibit higher PR expression compared to tumours with a less favourable prognosis, such as the luminal B subtype (Li *et al.*, 2022). The current standard method for testing ER, PR, and HER-2 is IHC. IHC assessments of hormone receptors, such as (ER and PR), are determined by two key characteristics: the percentage of cells that are stained and the intensity of the staining. Currently, the Allred (Quick) scoring system is commonly used to evaluate these features. According to this system, a score of 0–1 is classified as negative, while a score of 2–8 is considered positive (Rai *et al.*, 2020). Table 2.1 describes the scoring system for evaluating ER and PR expression.