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IMMUNOHISTOCHEMICAL EVALUATION OF SOX10 EXPRESSION IN TRIPLE NEGATIVE BREAST CANCER IN SAMPLE OF IRAQI PATIENTS (CLINICOPATHOLOGICAL STUDY)

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ABSTRACT

Background: Triple-negative breast cancer (TNBC) is an aggressive subtype characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression. Identifying reliable biomarkers for TNBC prognosis and progression remains a critical research focus. The SRY-related HMG-box 10 (SOX10) transcription factor has been implicated in various malignancies, including breast cancer, yet its role in TNBC in middle east remains understudied. **Aim of the study:** To evaluate SOX10 expression in TNBC cases using immunoreactivity score (IRS) and H-score methods and to assess its correlation with clinicopathological features, including tumor grade, tumor size, and nodal involvement. **Materials and methods:** A cross-sectional study was conducted on 50 TNBC cases at Al-Yarmouk Teaching Hospital and private laboratories in Baghdad from 1st of March 2024 to 1st of February 2025. Immunohistochemistry (IHC) was performed to assess SOX10 expression using both IRS and H-score systems. Statistical analysis was conducted using SPSS-27, with significance set at $p \leq 0.05$. **Results:** SOX10 expression was detected in 74.4% of cases based on H-score and 59.57% using IRS. Higher SOX10 expression was significantly associated with tumor grade ($p = 0.018$), tumor size ($p = 0.001$), T-stage ($p = 0.001$), and N-stage ($p= 0.002$), supporting its role in tumor aggressiveness, this is a part from age-related differences which were not statistically significant ($p = 0.380$). **Conclusion:** SOX10 is highly expressed in TNBC and correlates with tumor aggressiveness, making it a potential prognostic biomarker. Standardization of SOX10 scoring methods is necessary for its clinical utility.

KEYWORDS: TripleNegative Breast Cancer, SOX10 Immunohistochemistry expression, Prognostic Marker.

1. INTRODUCTION

Breast cancer is one of the most commonly diagnosed cancers among women worldwide.[1] In Iraq, it accounts for 19.7% of all cancer cases and contributes to 12.3% of cancer-related deaths.[2] Various biological pathways have been implicated in different subtypes of human breast cancer. While morphological characteristics remain the gold standard for tumor classification, advancements in molecular techniques have facilitated the categorization of breast cancer into four intrinsic subtypes, offering both prognostic and predictive insights. These subtypes, identified using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH), include luminal A (hormone receptor-positive [HR+] and human epidermal growth factor receptor 2-negative [HER2−]), luminal B (HR+/HER2+ or HR+/HER2−), basal-like (HR−/HER2−), and HER2-enriched (HR−/HER2+).[3]

Triple-negative breast cancer (TNBC) is a distinct subtype characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression. TNBC prevalence varies between 10% and 20% depending on the population studied.[4] It is considered highly aggressive, presenting with poorly differentiated tumors, increased rates of recurrence, metastasis, and a poorer overall prognosis compared to other breast cancer subtypes. Consequently, TNBC has become a focal point in breast cancer research.[5]

The SRY-related high-mobility-group box 10 (Sox10) protein, a transcription factor from the Sox family, plays

a pivotal role in the differentiation of neural crest cells into melanocytes, oligodendrocytes, and glial cells. It also promotes mesenchymal transition in mammary cells.[6] Sox10 is expressed in diverse cell types and tissues, including Schwann cells of peripheral nerves, epidermal melanocytes, oligodendrocytes in the cerebral cortex, mast cells, myoepithelial cells of submucosal bronchial glands, and acinar cells of mammary glands, as well as their respective tumors. Immunohistochemically, Sox10 expression has been observed in melanoma, peripheral nerve sheath tumors, and salivary gland myoepithelioma.[7]

Recent studies have reported that Sox10 is highly expressed in primary breast carcinomas, particularly in basal-like and unclassified TNBC subtypes.[8,9] While numerous international studies have explored Sox10 expression in breast cancer, there is a paucity of data on its expression within the Middle East, and specifically in Iraq. This study aims to address this gap.

Aim of the study

1. To evaluate SOX10 expression in TNBC cases using immunoreactivity score (IRS) and H-score methods
2. To assess its correlation with clinicopathological features, including tumor grade, tumor size, and nodal involvement.

2. Literature review

2.1 Breast Tissue, Anatomic and Physiologic characteristics

The breast is primarily composed of three types of tissue: glandular, connective, and adipose. Glandular tissue, responsible for milk production, consists of lobules and ducts arranged in lobes surrounding the nipple-areolar complex. Adipose tissue makes up 50-70% of the breast's volume, although its proportion varies between individuals. Connective tissue, including Cooper's ligaments, plays a supportive role by anchoring the breast to both the skin and underlying tissues. These ligaments connect the deep and superficial fascial layers, ensuring the breast remains in place. The pectoralis major muscle forms the base of the breast, and although male breast tissue shares similarities with female breast tissue, it lacks specialized lobules due to hormonal differences (figure 2.1).[10–12]

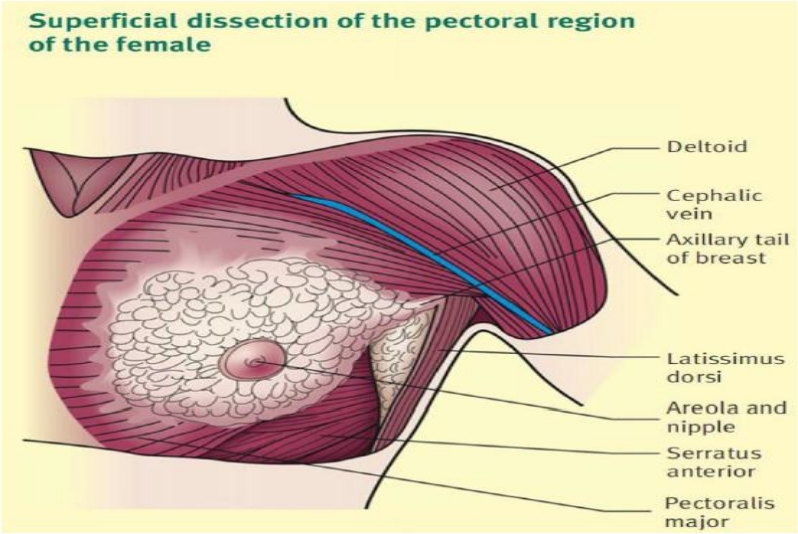

Figure 2.1: Anatomic demonstration of breast tissue.

The blood supply to the breast is primarily derived from the internal mammary artery, which provides approximately 60% of the circulation. This is supplemented by the lateral mammary branches of the lateral thoracic artery and the intercostal arteries. Venous and lymphatic drainage follow the same patterns as the arterial supply, with some variations in the connecting channels.[10,13]

2.2 Histology of the breast

Although the normal mammary epithelium comprises only a small portion of the overall breast tissue, the majority of breast diseases originate from it. The breast's morpho-functional unit is a complex branching structure organized into lobes, consisting of two primary components: the terminal duct-lobular unit (TDLU) and the larger ductal system.[14]

The TDLU consists of lobules, made up of acini, and terminal ductules, which form the gland's secretory portion. This unit connects to the subsegmental duct, which leads to the segmental duct, and ultimately to the lactiferous duct, which drains into the nipple. Beneath the nipple, there is a fusiform dilation called the lactiferous sinus, situated between the collecting and segmental ducts. The duct system is lined with a bilayered epithelium—an inner epithelial layer and an outer myoepithelial layer. Evaluating this dual-layered structure is crucial for distinguishing between benign and malignant breast tumors.[15]

The TDLU also comprises interlobular ducts and associated lobules, which contain multiple grape-like structures where milk is produced. The milk flows through the terminal ducts, interlobular ducts, excretory ducts, the lactiferous sinus, and lactiferous ducts, finally reaching the nipple.[16,17]

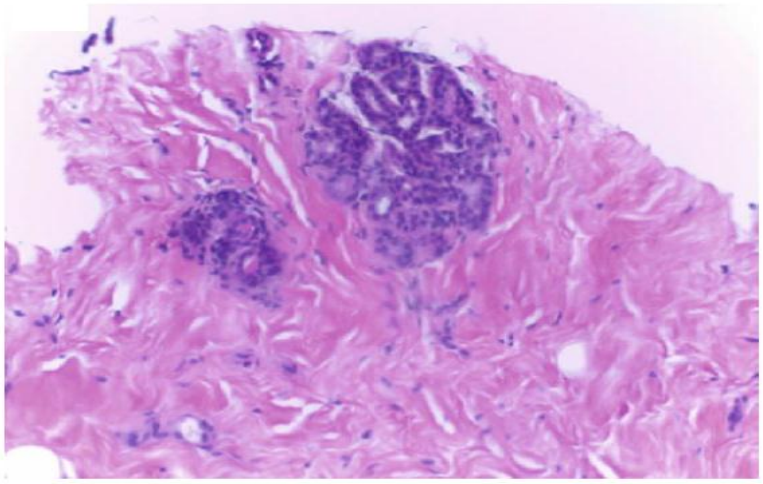

Figure 2.2: Normal breast tissue histology have large regions of cytoplasm (pinkish regions) with a dense cluster of nuclei forming glands in H&E-stained slides.[15]

2.3 Breast cancer

Breast cancer is not a single disease but rather a collection of diverse tumors, all characterized by the uncontrolled growth of malignant cells within the mammary epithelium. These tumors vary significantly in terms of clinical behavior, progression, and response to treatment. The classification of breast cancer is informed by both traditional immunopathological and histological methods, as well as modern molecular subtyping techniques, which help categorize these tumors into distinct groups based on their unique characteristics.[18]

Many breast cancer patients are asymptomatic, with their condition often discovered through routine screening. However, some may present with symptoms such as a palpable lump, changes in the breast's size or shape, nipple discharge, or breast pain (mastalgia). To confirm a diagnosis, a comprehensive approach is needed, including physical examination, imaging techniques like mammography, fine-needle aspiration (FNA), and tissue biopsy. Early detection plays a critical role in improving survival outcomes. Breast cancer typically spreads via lymphatic and hematologic pathways, which heightens the risk of distant metastasis and worsens the overall prognosis.[19,20]

2.3.1 Epidemiology of breast cancer

According to the 2022 statistics from the World Health Organization's Global Cancer Observatory (GLOBOCAN), breast cancer is the most common type of cancer affecting women worldwide. It currently impacts more than one in ten women globally. In 2022 alone, 2.3 million women were diagnosed with breast cancer, resulting in 666,103 deaths worldwide. By the end of that year, 8.1 million women who had been diagnosed within the previous five years were still alive, making breast cancer the most prevalent cancer in the world, surpassing lung cancer. This disease can affect women of any age post-puberty, though the incidence increases with age.[21]

While breast cancer incidence rates are generally higher in developed countries, it's important to note that 63% of global breast cancer deaths in 2020 occurred in Asian and African countries. Survival rates for breast cancer vary widely depending on the healthcare infrastructure of a given region.[22,23] For example, in countries with advanced healthcare systems such as Hong Kong, Singapore, and Turkey, the 5-year survival rate for localized breast cancer was reported at 89.6%, while regional cancer had a survival rate of 75.4%. In contrast, countries with less developed healthcare systems, including Costa Rica, India, the Philippines, Saudi Arabia, and Thailand, reported significantly lower survival rates, with 76.3% for localized cases and 47.4% for regional cases.[24]

By 2030, breast cancer cases are projected to rise substantially, with an estimated 2.7 million new diagnoses annually and around 870,000 deaths. This surge is expected to be most pronounced in low- and middle-income countries, where factors such as the adoption of Western lifestyles—characterized by delayed childbearing, reduced breastfeeding, earlier onset of menstruation, sedentary habits, and poor diet—are contributing to the increasing incidence of breast cancer.[25]

In Iraq, breast cancer is similarly the most frequently diagnosed malignancy among women. The Iraqi Cancer Registry's 2023 annual report, published by the Ministry of Health in 2024, documented 8,849 cases of breast cancer. This accounted for 20.23% of all cancer cases in the country, with the overwhelming majority (8,708 cases) being female patients, while only 141 male patients were

diagnosed that year.[26]

2.3.2 Risk factors

Breast cancer risk is influenced by a variety of factors, categorized into non- modifiable and modifiable risk factors, as identified through numerous epidemiologic studies.[27]

Non-Modifiable risk factors

1. Sex

The predominant risk for breast cancer is among females, primarily due to increased hormonal stimulation and circulating estrogens, which are linked with a higher risk of the disease. Conversely, breast cancer prevalence in males is about 1%.[28]

2. Age

The risk of breast cancer escalates with age; approximately 80% of those diagnosed are over the age of 50, with more than 40% being older than 65. Additionally, younger age at menarche and older age at menopause increase lifetime exposure to hormones, thus raising breast cancer risk.[29,30]

3. Race/Ethnicity

Breast cancer presents more aggressively and with higher mortality rates among black women compared to white women, often characterized by higher occurrences of estrogen receptor-negative tumors.[31,32]

4. Genetic Mutation and Family history

Genetic mutations, particularly in the BRCA1 and BRCA2 genes, significantly elevate breast cancer risk. Women with a first-degree relative diagnosed with breast cancer have a twofold increased risk. The susceptibility to breast cancer increases with a younger age at diagnosis of the relative and the number of affected first-degree relatives.[33,34]

5. Exposure to ionizing radiation

Exposure to ionizing radiation, whether from nuclear explosions, diagnostic fluoroscopy, or radiotherapy during adolescence, is a recognized carcinogen for breast cancer, likely due to DNA mutation induction.[35]

6. Pregnancy History and Breast feeding

Parity, older age at first full-term pregnancy, and breastfeeding history influence breast cancer risk. Nulliparity increases risk in older women, while early age at first live birth is protective. Breastfeeding is notably beneficial, reducing breast cancer risk with longer durations associated with greater reductions.[36,37]

7. Density of the breast tissue

High breast density is a strong risk factor for breast cancer. Mammographic density, which is influenced by hormonal exposure and is also heritable, decreases with age, reflecting a reduction in glandular tissue and an increase in fatty tissue. Women with higher mammographic density face more than a fourfold increased risk of developing breast cancer.[38–41]

Modifiable risk factors

1. Use of oral Contraceptives and Hormone replacement therapy

Usage of oral contraceptives and hormone replacement therapy post-menopause has been linked to an increased risk of breast cancer. Studies, including those from the Women's Health Initiative, underscore the risks associated with these hormonal treatments.[42–44]

2. Diet and Alcohol intake

While studies have been inconsistent about many dietary factors, the link between alcohol consumption and breast cancer is well-established, showing a linear dose-response relationship with increased risk.[45–47]

3. Smoking

Both active and passive smoking are important risk factors for breast cancer, promoting carcinogenic processes through increased oxidative stress and mutations in oncogenes and tumor suppressor genes, notably p53.[48]

4. Physical activity

High levels of physical activity have been consistently associated with reduced breast cancer risk. This effect is attributed to changes in body composition, reduced insulin resistance, and altered levels of sex steroid hormones.[49–51]

5. Obesity

High body mass index and adiposity are correlated with an elevated risk of breast cancer.[52–54]

2.4 Diagnosis of breast cancer

Breast cancer is a heterogeneous disease, reflected in its diverse clinical manifestations. A significant proportion of breast cancers are now diagnosed during the preclinical phase through screening programs, before any symptoms develop.[55] The proportion of early-stage diagnoses varies across countries and depends on factors such as the availability and quality of organized mammographic screening, access to diagnostic imaging, the age of the population, and public awareness of breast cancer.[56,57]

2.4.1 Clinical presentation of breast cancer

Breast cancer symptoms and signs are varied, making it difficult to distinguish between malignant and benign tumors in some cases. Therefore, clinical assessment alone is insufficient for diagnosis. A "triple assessment" approach—clinical evaluation, breast imaging, and biopsy—is necessary. While biopsy confirms the diagnosis, the concordance between all three modalities is considered a quality measure to avoid diagnostic errors.[58,59]

The most common clinical presentation of breast cancer is a palpable breast mass. Other signs may include skin

retraction, nipple inversion, changes in breast size or shape, discoloration, breast pain, edema, skin redness, and regional nodal swelling. Clinical assessment involves a thorough history and physical examination to assess the breast.[60]

2.4.2 Imaging of breast cancer

Following clinical evaluation, breast imaging plays a key role in further characterizing tumors and assessing for bilaterality. Common imaging modalities include mammography, ultrasound, and sometimes MRI.

1. Mammography

Mammography is typically recommended for women over 35, except during pregnancy or in younger patients. It should ideally be performed before needle biopsies to prevent changes, such as hematomas, that could compromise diagnostic accuracy. Comparing current mammograms with previous images is also recommended. Suspicious mammographic findings include masses, architectural distortions, and microcalcifications, the latter being particularly important in detecting ductal carcinoma in situ (DCIS).[61,62]

2. Ultrasound

Ultrasound is frequently used to further evaluate clinically or mammographically suspicious breast masses, as well as to stage regional lymph nodes. It is the preferred imaging modality for women under 35 due to the density of their breast tissue, which limits the utility of mammography. In older women, ultrasound is used as an adjunct to mammography and for guiding biopsies, which have a lower false-negative rate when ultrasound-guided.[63,64]

3. MRI

The use of MRI in breast cancer diagnosis has increased over the past two decades due to its high sensitivity. However, MRI is less specific than other imaging methods and can lead to unnecessary biopsies, overtreatment, and delays in diagnosis. MRI should be reserved for specific indications and not used routinely. Additionally, MRI findings alone should not be used to guide surgical decisions, such as switching from breast-conserving surgery to mastectomy, without biopsy confirmation.[65]

2.4.3 Tissue sampling and Testing

1. Fine-Needle Aspiration (FNA)

Although the use of FNA for breast cancer diagnosis has declined, it remains valuable in certain clinical situations when performed by experienced hands. FNA can also evaluate hormonal receptor status and Her-2 status, although this is less reliable than with core biopsy.[60,66]

Advantages of FNA include its quick execution, minimal patient preparation, rapid results, and low cost.[67]

However, its limitations include less diagnostic material, lower sensitivity and specificity, and an inability to provide full histological information, such as tumor grade and invasiveness.[66]

Ultrasound-guided FNA is also frequently used to stage axillary lymph nodes, with abnormal epithelial cells indicating malignancy.[68,69]

2. Core biopsy

Core biopsy is the gold standard for percutaneous biopsy techniques, offering higher sensitivity and specificity than FNA. It allows for full histopathological diagnosis, including the tumor's biological features. However, core biopsy is more time-consuming, painful, and requires local anesthesia. It also necessitates a small incision, and patients on antithrombotic therapy may need to pause treatment before the procedure. Despite these drawbacks, core biopsy is the preferred method for non- palpable tumors.[60]

3. Punch biopsy

Punch biopsy is used to obtain samples from skin tumors to distinguish between benign and malignant lesions, such as Paget's disease or skin recurrences of breast cancer.[70]

2.5 Breast cancer Classifications and Assessment

2.5.1 Histological classification of breast cancer

Breast cancer can be broadly classified based on its developmental stage into two key categories: in situ and invasive cancers. In situ cancer remains confined to the epithelial compartment and is typically treatable through excision. In contrast, invasive cancer breaches the epithelial basement membrane and invades the surrounding connective tissue.[71]

In-situ breast cancer includes

1. Ductal Carcinoma In-Situ (DCIS)

DCIS originates within the lactiferous ducts and remains confined without infiltrating the surrounding breast tissue, as the basement membrane is intact. This form is considered non-invasive and is detected in approximately 20% of breast tumors via mammography. DCIS is categorized into subtypes, including papillary, micropapillary, cribriform, solid, and comedo. High-grade DCIS often serves as a precursor to invasive breast cancer, with frequent recurrence in the affected breast.[72,73]

2. Lobular Carcinoma in Situ (LCIS)

LCIS is typically not detectable via mammography, as it does not present with mass formation or calcification. It is usually discovered incidentally during pathological exams and is considered a marker for increased breast cancer risk rather than a direct precursor. LCIS is histologically characterized by uniform, small polygonal or cuboidal cells with a high nuclear-to-cytoplasmic ratio. It is important to differentiate LCIS from atypical lobular hyperplasia.[74,75]

3. Paget's disease

Paget's disease involves malignant epithelial cells (Paget cells) within the nipple's epidermis, extending to the areola and adjacent skin. It is usually associated with intraductal tumors and is considered non-invasive or minimally invasive.

However, if significant invasion occurs (>1 mm), it is classified as invasive cancer. The prognosis depends on the presence of underlying breast cancer.[71,76]

Invasive breast cancers

1. Invasive Ductal Carcinoma (IDC)

IDC is the most common form of invasive breast cancer, accounting for 70-80% of cases, and is also referred to as invasive carcinoma of no special type (NST).[77]

IDC is further classified based on its histological structure into subtypes like tubule-forming, solid, and scirrhous. Tumor cells are typically pleomorphic, with prominent nucleoli and frequent mitoses. Necrosis and calcifications are present in more than half of IDC cases.[78,79]

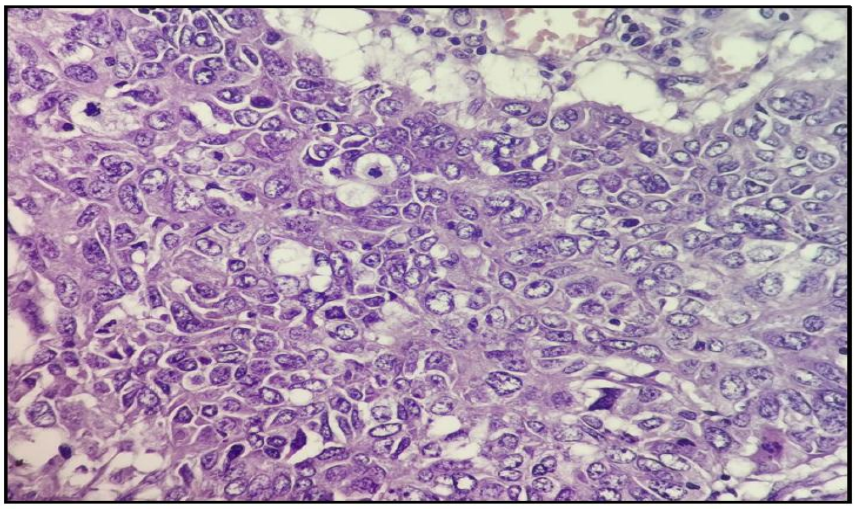

Figure 2.3: Invasive ductal carcinoma, Showing a solid growth Pattern and Marked nuclear pleomorphism.[77]

2. Invasive lobular carcinoma

Representing 5-15% of all invasive breast cancers, ILC commonly affects older women. It is characterized by small, loosely cohesive cancer cells that infiltrate in single-file patterns, known as the classic type. Tubule formation is rare, and ILC cells often exhibit intracytoplasmic lumina filled with mucin. ILC may also display pleomorphic features, with cytological atypia and infrequent mitoses.[80,81]

3. Tubular carcinoma

Tubular carcinoma, comprising about 2% of all breast cancers, is defined by well-differentiated tubular structures. It typically affects women aged 50-60. Cancer cells show mild atypia, forming irregular but clearly defined tubules. Non-typical cases are classified as IDC, tubule-forming type.[71,82]

4. Invasive cribriform carcinoma

This subtype, resembling cribriform DCIS, is characterized by invasive cancer forming cribriform-like structures. It is associated with a favorable prognosis and accounts for 1-3.5% of breast cancer cases, commonly affecting patients around 50 years old.[83,84]

5. Mucinous carcinoma

Mucinous carcinoma is defined as breast neoplasms with mucinous component that comprises >90% of the tumor, characterized by the production of large amounts of mucus by cancer cells, which accumulate in the stromal tissue. This subtype constitutes approximately 2% of all breast cancer cases and is characterized by cancer cell nests floating in extracellular mucus.[71,82]

6. Carcinoma with medullary features

This type accounts for about 5% of breast cancers and is associated with better clinical outcomes and a lower likelihood of axillary lymph node involvement. It typically affects patients around 50 years old and is often linked to BRCA1 gene mutations. Histologically, medullary carcinoma forms well-circumscribed tumors with marked lymphocytic infiltration and pronounced nuclear atypia.[85]

7. Apocrine carcinoma

Comprising 1-4% of breast cancers, apocrine carcinoma is characterized by apocrine metaplasia with eosinophilic cytoplasm and intracytoplasmic granules. PAS-positive staining is common, and bizarre tumor cells with multilobulated nuclei may also be observed.[82,86]

8. Metaplastic carcinoma

Metaplastic carcinoma presents either as monophasic (Single metaplastic component) or biphasic (Two or more components). It can include metaplastic histologies like squamous or spindle cells, often combined with an adenocarcinoma component, most commonly IDC. Subtypes of metaplastic carcinoma include fibromatosis-

like carcinoma, low-grade and high grade adenosquamous carcinoma, high grade spindle cell carcinoma, and high number of morphologies within mixed metaplastic carcinomas.[87]

World Heath Organization classification (5th edition 2019) (table 1.1).

Table 2.1: World Heath Organization classification latest update.[88]

Benign epithelial Proliferations and Precursors • Usual ductal hyperplasia • Columnar cell lesions, including flat epithelial atypia • Atypical ductal hyperplasia
Adenosis and Benign sclerosing lesions • Sclerosing adenosis • Apocrine adenoma • Microglandular adenosis • Radial scar / complex sclerosing lesion
Adenomas • Tubular adenoma, NOS • Lactating adenoma • Duct adenoma, NOS
Invasive breast carcinoma • Infiltrating duct carcinoma NOS • Oncocytic carcinoma • Lipid-rich carcinoma • Glycogen-rich carcinoma • Sebaceous carcinoma • Lobular carcinoma NOS • Tubular carcinoma • Cribriform carcinoma NOS • Mucinous adenocarcinoma • Mucinous cystadenocarcinoma NOS • Invasive micropapillary carcinoma of breast • Apocrine adenocarcinoma • Metaplastic carcinoma NOS
Epithelial-myoepithelial tumours • Pleomorphic adenoma • Adenomyoepithelioma NOS • Adenomyoepithelioma with carcinoma • Epithelial-myoepithelial carcinoma
Papillary neoplasms • Intraductal papilloma
• Ductal carcinoma in situ, papillary • Encapsulated papillary carcinoma • Encapsulated papillary carcinoma with invasion • Solid papillary carcinoma in situ • Solid papillary carcinoma with invasion • lntraductal papillary adenocarcinoma with invasion
Rare and Salivary gland-type tumors • Acinar cell carcinoma • Adenoid cystic carcinoma ○ Classic adenoid cystic carcinoma ○ Solid-basaloid adenoid cystic carcinoma ○ Adenoid cystic carcinoma with high-grade transformation • Secretory carcinoma • Mucoepidermoid carcinoma • Polymorphous adenocarcinoma • Tall cell carcinoma with reversed polarity
Non-invasive lobular neoplasia

• Atypical lobular hyperplasia • Lobular carcinoma 'in situ NOS ○ Classic lobular carcinoma in situ ○ Florid lobular carcinoma .in situ ○ Lobular carcinoma in situ, pleomorphic
Ductal carcinoma in situ (DCIS) • lntraductal carcinoma, non-Invasive, NOS ○ DCIS of low nuclear grade ○ DCIS of intermediate nuclear grade ○ DCIS of high nuclear grade
Neuroendocrine neoplasms • Neuroendocrine tumor NOS • Neuroendocrine tumor, grade 1 • Neuroendocrine tumor, grade 2 • Neuroendocrine carcinoma NOS • Neuroendocrine carcinoma, small cell • Neuroendocrine carcinoma, large cell

2.5.2 Histological grading system

The Elston-Ellis modification of the Scarff-Bloom-Richardson grading system, commonly known as the Nottingham grading system, is the most widely used method for histological grading of breast cancer. This system assesses tumors based on three key morphological features.[89,90]

(a) Tubule formation

Tubule or gland formation is evaluated based on the percentage of tubular or glandular structures with well-defined lumina. Only structures with clear central lumina surrounded by polarized cells are counted (90):

i. Score 1: More than 75% of the tumor forms tubules or gland
ii. Score 2: Tubule/gland formation is observed in 10–75% of the tumor.
iii. Score 3: Less than 10% of the tumor consists of tubular or glandular structures.

(b) Nuclear pleomorphism

This assessment evaluates the variability in the size and shape of tumor cell nuclei, typically focusing on the least differentiated part of the tumor. Tumor cell nuclei are compared with those of normal epithelial cells.

- ❖ Score 1: Nuclei closely resemble normal epithelial cells with minimal pleomorphism; nucleoli and chromatin patterns are inconspicuous.
- ❖ Score 2: Nuclei are 1.5–2 times larger than normal cells, with moderate pleomorphism and still inconspicuous nucleoli.
- ❖ Score 3: Nuclei are over twice the size of normal cells, with significant pleomorphism, vesicular chromatin, and prominent nucleoli.[90]

(c) Mitotic count

Mitotic activity is measured in the most proliferative area of the tumor, typically at the periphery. The score is based on the number of clearly identified mitotic figures in a specified area or high-power field (HPF), with cutoffs determined by the field size.[90]

- ❖ Score 1: Low mitotic count.
- ❖ Score 2: Moderate mitotic count.
- ❖ Score 3: High mitotic count

Final grading[90]

- ❖ Grade 1: Combined score of 3–5.
- ❖ Grade 2: Combined score of 6–7.
- ❖ Grade 3: Combined score of 8–9

2.5.3 Clinical staging (TNM)

Breast cancer progression is categorized into different stages, from in situ cancer to invasive cancer, including spread to regional lymph nodes and distant organs. The TNM system is the most commonly used staging method, based on the evaluation of tumor size (T), lymph node involvement (N), and the presence of distant metastasis (M).[91,92]

- ❖ Primary tumor (or T)
- ❖ Lymph nodal status (or N)
- ❖ Systemic metastasis (or M).

Table 2.2: TNM staging according to latest guidelines update according to The 8th Edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual (93).

Staged part	Stage	Description
Primary Tumor (pT)	pTX	Cannot be assessed
	pT0	No evidence of primary tumor
	pTis	Ductal carcinoma in situ, Paget disease, encapsulated papillary carcinoma, and solid papillary carcinoma
	pTis (DCIS)	Ductal carcinoma in situ without invasive carcinoma
	pTis (Paget)	Paget disease without invasive carcinoma

	pT1mi	Tumor ≤ 1 mm
	pT1a	Tumor > 1 mm but ≤ 5 mm
	pT1b	Tumor > 5 mm but ≤ 10 mm
	pT1c	Tumor > 10 mm but ≤ 20 mm
	pT2	Tumor > 20 mm but ≤ 50 mm
	pT3	Tumor > 50 mm
	pT4a	Extension to chest wall (not including pectoralis muscle)
	pT4b	Edema (including peau d'orange), ulceration of skin, or ipsilateral satellite skin nodules
	pT4c	Both T4a and T4b
	pT4d	Inflammatory carcinoma (involves > 1/3 of the breast skin, primarily a clinical diagnosis)
Regional Lymph Nodes (pN)	pNX	Cannot be assessed
	pN0	No regional lymph node metastasis histologically
	pN0(i-)	No regional lymph node metastasis by histology or immunohistochemistry
	pN0(i+)	Isolated tumor cells (cluster ≤ 0.2 mm and < 200 cells)
	pN0(mol+)	RT-PCR positive but negative by light microscopy
	pN1mi	Micrometastasis (tumor deposit > 0.2 mm and ≤ 2.0 mm or ≤ 0.2 mm and > 200 cells)
	pN1a	Metastasis in 1 - 3 axillary lymph nodes with at least 1 tumor deposit > 2.0 mm
	pN1b	Metastasis in internal mammary sentinel lymph node with tumor deposit > 2.0 mm
	pN1c	pN1a and pN1b
	pN2a	Metastasis in 4 - 9 axillary lymph nodes with at least 1 tumor deposit > 2.0 mm
	pN2b	Metastasis in clinically detected internal mammary nodes with pathologically negative axillary nodes
	pN3a	Metastasis in ≥ 10 axillary lymph nodes with at least 1 tumor deposit > 2.0 mm or metastasis to infraclavicular lymph node
	pN3b	Positive internal mammary node by imaging with pN1a or pN1b
	pN3c	Metastasis in ipsilateral supraclavicular lymph node
Distant Metastasis (pM)	pM1	Distant metastasis histologically proven > 0.2 mm

2.5.4 Immunohistochemistry (IHC)

Is a widely used technique to analyze intracellular proteins and cell surface markers in various tissues, including the breast. It employs antibodies to detect specific proteins, aiding in the identification of tumor subtypes, tissue origin, and differentiation between primary and metastatic cancers. IHC also provides prognostic information and helps predict the response to therapies. Advances in antibody development and antigen retrieval techniques have increased the diagnostic, prognostic, and therapeutic value of IHC in breast cancer pathology.[94,95]

IHC plays a crucial role in solving diagnostic challenges such as distinguishing between benign and malignant tumors, differentiating in situ from invasive cancers, identifying microinvasion, and confirming the breast as the primary site in cases of metastatic cancer. Even in the absence of advanced molecular testing, IHC remains helpful in identifying histologic subtypes and molecular phenotypes. Normal mammary gland tissue contains luminal, basal, and myoepithelial cells, each expressing distinct proteins such as cytokeratins, epithelial membrane antigen, estrogen, and progesterone receptors, as well as markers like smooth muscle actin and p63 in myoepithelial cells. These markers are essential for accurately diagnosing and characterizing breast tumors.[95,96]

Hormone receptors

A key breakthrough in breast cancer management was the discovery that tumors expressing hormone receptors (estrogen and progesterone) are more likely to respond to hormone therapies. Estrogen receptor (ER) status is a strong predictor of response to treatment, while progesterone receptor (PR) status is a less reliable indicator.[97,98]

Tumors are classified as hormone receptor-positive if 1–10% of the invasive cancer cells stain for these receptors, with the staining intensity (weak, moderate, or strong) also considered. Proper tissue fixation, typically within 1 hour of removal, is crucial for reliable results. Fixation time should range from 6 to 72 hours.[97,99]

Hormone receptor evaluation can also be performed using techniques like in situ hybridization or PCR on paraffin-embedded tissues. ER-positive tumors account for about 80% of breast cancers and are generally well-

differentiated, whereas ER- negative tumors often show higher histologic grades. Certain tumors, such as medullary and metaplastic carcinomas, are typically ER-negative, while others, like mucinous, tubular, and lobular carcinomas, are more often ER-positive. ER status is usually stable during disease progression, although rare cases of ER-negative tumors becoming ER-positive have been observed, particularly after tamoxifen therapy.[100,101]

HER-2/Neu expression

HER-2/neu is one of the first oncogenes studied in invasive breast cancer, and it is identified in 10–20% of breast cancer patients. HER-2 status is a marker for sensitivity to Herceptin (trastuzumab) and resistance to tamoxifen. Common methods for detecting HER-2 include.[102]:

- Immunohistochemistry (IHC)
- Fluorescence in situ hybridization (FISH)
- Chromogenic in situ hybridization (CISH)

- **Including silver in situ hybridization (SISH)**

These methods are the gold standard for determining HER-2 status, and several have been approved by the U.S. FDA since 1998.[103]

IHC is often used as a screening test, with FISH used to confirm equivocal results, as FISH is more time-consuming and costly than IHC.[95] IHC assesses the overexpression of HER-2 protein on the cell surface, while FISH evaluates HER-2 gene amplification. In most HER-2-positive cancers, overexpression correlates with gene amplification.[94]

The American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines recommend the following scoring for HER-2 by IHC:

- 0 and 1+: Negative

0: No staining or faint/incomplete membrane staining in ≤10% of tumor cells.

1+: Incomplete faint staining in >10% of tumor cells.

- 2+: Weakly positive

Weak to moderate complete membrane staining in >10% of tumor cells.

- 3+: Positive

Intense circumferential membrane staining in >10% of tumor cells.

Further evaluation is unnecessary for tumors that are definitively positive (3+) or negative (0/1+). Equivocal (2+) cases should be tested for gene amplification using FISH.[103]

2.5.5 Molecular classification of breast cancer

A landmark 2000 study published in Nature highlighted the molecular subtypes of breast cancer based on gene expression patterns of hormone receptors. This molecular classification has been shown to have high prognostic and predictive value in invasive ductal carcinoma (IDC) and is now recommended for all invasive breast cancers.[104] The molecular classification groups breast cancers into four major subtypes (Table 1.2):

Table 2.3: Overview of different molecular subtypes.[67]

Molecula r subtype	Gene profile	IHC phenotype		Histolofic subtype	Survival
Luminal A	High expression of luminal epithelial genes and ER related genes	ER+, PR≥20%, HER2−, Ki67low		Tubular Carcinoma, low-grade IDC-NST, classic ILC	Good
Luminal B	Higher level of proliferation and HER2-related genes than luminal A	HER2 (−)	ER+ PR- HER2- high levels of Ki- 67	IDC-NST, pleomorphic ILC	Intermediate
		HER2	ER+		
		(+)	HER2+		
			any level of PR		
			any level of Ki-67		
HER	High expression of	ER−,		Mostly	Poor
2/neu	HER2-related	PR−,		pleomorphic ILC	
	genes; low	HER2+			
	expression of ER				
	related genes				
TNBC	High expression of	ER−,		High-grade IDC-	Poor
	basal epithelial and	PR−,		NST, metaplastic	
	proliferation	HER2−		carcinoma,	
	genes; low			Medullary	
	expression of			carcinoma,	
	HER2-related and			adenoid cystic	
	ER related genes			carcinoma	

1. Luminal A subtype

Luminal A tumors are ER and/or PR positive, HER2 negative, and have low Ki67 expression (<20%). They are typically low grade, slow-growing, and associated with a good prognosis. These cancers respond well to hormone therapy (e.g., tamoxifen, aromatase inhibitors) and have less need for chemotherapy.[105,106]

2. Luminal B subtype

Luminal B tumors are higher grade and have a worse prognosis than Luminal

A. They are ER positive but may be PR negative, with high Ki67 expression (>20%). These tumors benefit from both hormone therapy and chemotherapy.[107,108]

This subtype is divided into[109]

a) Luminal B (HER2-): ER+, PR-, HER2-, Ki67 high.
b) Luminal B (HER2+): ER+, HER2+, any level of PR and Ki67

3. HER2/neu subtype

This subtype accounts for 10–15% of breast cancers and is characterized by high HER2 expression and absence of ER and PR. These tumors are fast-growing but have seen improved outcomes with HER2-targeted therapies.[103,110]

4. Triple Negative Breast Cancer (TNBC)

It is identified by surrogate immunohistochemistry with negative reactions to ER, PR, and HER2. TNBCs are responsible for 30% of breast cancer deaths, although they account for only 10% to 20% of breast cancers.[111,112] Most high- grade TNBCs are of NST histology, with a small proportion being metaplastic carcinomas. Because of the lack of targetable biomarkers, there are no optimal therapeutic strategies yet. Even with a relative sensitivity to neoadjuvant chemotherapy (NACT), and up to 39% of patients end up with residual cancer have significantly worse prognoses (triple-negative paradox).[113,114]

Efforts have been made to further classify TNBC in the past years. Lehmann et al refined classification categorized TNBC into 4 sustainable subtypes: BL1, BL2, MES, and LAR, and stated that each had distinctive clinicopathologic characters. Two basal-like types (BL1 and BL2), with a basal pattern of gene expression, but showing differences in the immune response; BL1 is the largest group, comprising about 35% of the cases. This group demonstrates the best responses to NACT, and therefore has the best overall survival and disease recurrence–free survival. Comparably, BL2 has the lowest response to NACT, with a worse disease recurrence–free survival. The MES subtype is characterized by a lack of lymphocytic infiltrates and low lymph node metastasis but high lung metastasis. Lobular carcinoma exclusively falls into LAR, which, not surprisingly, has a lower histologic grade, with frequent lymph node and bone metastases. One of the luminal androgen receptor type (LAR), which presents differential expression of genes involved in androgen metabolism.[115–117]

In spite of its straightforward definition, managing this subtype has posed significant challenges clinically, owing to its morphological, molecular, and clinical diversity, compounded by the absence of targeted therapies.[118]

2.6 Histologic subtypes of TNBC

The majority of triple-negative breast cancers (TNBCs) are high-grade invasive ductal carcinomas (IDCs), also known as invasive carcinomas of no special type. These tumors are typically characterized by pushing borders, significant nuclear pleomorphism, a high mitotic index, and the presence of necrosis and lymphocytic infiltration. However, rare histologic subtypes also exhibit the TNBC phenotype or are enriched for it. These include carcinomas with apocrine differentiation, carcinomas with medullary features, and metaplastic breast carcinomas (MBCs). Despite the distinct histologic characteristics, these subtypes share similar genomic landscapes with conventional TNBCs, though they may present with clinically relevant differences.[119] (Figure 2.6).

2.6.1 High grade triple negative breast cancer

1. High grade Invasive Ductal Carcinoma (IDC)

Feature of Triple negative IDC listed above.

2. Carcinoma with medullary features

In the 5th edition of the WHO classification, medullary carcinoma was reclassified as a pattern within invasive ductal carcinoma of no special type, due to overlapping features with basal-like tumors and BRCA1-mutated breast cancers.[120,121] These tumors exhibit high-grade features, a dense lymphocytic infiltrate, and are often well-circumscribed. They are associated with a favorable prognosis and are responsive to chemotherapy.[122,123] Medullary carcinomas are overrepresented in the immunomodulatory subgroup of TNBCs, with potential for treatment de-escalation studies.[124,125]

3. Metaplastic breast carcinoma

Metaplastic breast carcinomas are a diverse group of invasive tumors characterized by differentiation toward squamous or mesenchymal components. The incidence of MBC varies between 0.2% and 1.0% of breast cancers.[126,127] MBCs are often classified as claudin-low or basal-like subtypes. Spindle cell- predominant tumors typically fall into the claudin-low subtype, while chondroid tumors align with the mesenchymal subtype. These tumors frequently harbor PIK3CA alterations, DNA repair deficiencies, and stem-like features that make them susceptible to immune therapy. [128–132]

4. Carcinoma with apocrine differentiation

It is characterized by large cells displaying abundant eosinophilic granular cytoplasm and enlarged nuclei with prominent nucleoli, closely resembling apocrine sweat glands. Notably, these tumors frequently exhibit

androgen receptor positivity and demonstrate HER2 amplification in 30–60% of cases. They tend to align with the luminal androgen receptor and immune signature molecular subtypes of triple- negative breast cancer (TNBC).[124] However, a recent series observed that they often lack expression of programmed death-ligand 1 (PD-L1).[133] While most cases are sporadic, these tumors can also occur in individuals with germline PTEN pathogenic variants.[134]

5. Triple-negative invasive lobular carcinoma

There is growing awareness of invasive triple-negative lobular carcinoma (ILC) as a rare presentation of E-cadherin-negative breast cancer. These tumors typically display apocrine morphology, characterized by eosinophilic cytoplasm and prominent nucleoli and account for 0.9–2.3% of invasive lobular breast cancers.[135,136] Triple-negative ILC tends to affect older individuals compared to other groups.[137] While reports on triple-negative ILC are limited in size, typically involving dozens of cases, emerging evidence suggests that patients with this subtype experience poorer outcomes compared to those with triple-negative invasive ductal carcinoma or hormone receptor-positive ILC.[135] Despite lower levels of Ki67 in triple-negative ILC compared to triple-negative invasive ductal carcinoma, outcomes appear to be worse for the former.[137]

2.6.2 Low grade triple negative breast cancer

Low-grade triple-negative disease involves a spectrum of rare lesions, spanning from neoplasms with uncertain metastatic possibility to well-defined special types of invasive carcinoma.[138]

Low-grade TNBC is divided into

1) Low-grade carcinomas, which involves certain subtypes of metaplastic carcinoma (MC), fibromatosis-like carcinoma and low grade Adeno-squamous carcinomas, exhibit typical triple-negative breast cancer (TNBC) genomic profiles but display a low-grade morphology and generally have a favorable prognosis.[139]

2) Carcinomas resembling those found in salivary glands, termed salivary-like carcinomas, exhibit characteristic genetic alterations, a lack of frequent TP53 mutations, and low levels of genetic instability. Other rare histologic special types of low-grade TNBC include low-grade variants of metaplastic breast carcinomas (MBCs) and solid papillary carcinoma with reverse polarity (SPCRP).[139]

1. Acinic cell carcinoma

Acinic cell carcinoma is exceptionally rare, typically presenting as a palpable lump with an average size of 3.5 cm. It exhibits variable patterns, including solid, microglandular, nested, and clear-cell formations, composed of cells with granular cytoplasm and large serous differentiation reminiscent of acinic cells.[140] Its histochemical and immunohistochemical profile is distinctive: PAS-positive, PAS- diastase-positive, epithelial membrane antigen (EMA)-positive, lysozyme-positive, A1AT-positive, GCDFP-15-positive, and S100-positive.[141] It has been suggested to originate from microglandular adenosis, where acinar serous differentiation is also observed, although many, if not all, of these carcinomas are believed to arise from microglandular adenosis.[142] These tumors exhibit mixed prognoses; when pure and displaying a low-grade morphology, the prognosis tends to be favorable, even in the presence of local or distant recurrences or metastases.[143] However, they are often associated with a high-grade component, leading to more frequent recurrences.[141]

2. Salivary Gland–Like Tumors of the Breast

a. Adenoid cyctic carcinoma

Adenoid cystic carcinomas represent a salivary gland-type tumor with low malignant potential, characterized by a composition of both myoepithelial and epithelial cells. The classic adenoid cystic carcinoma typically displays a cribriform pattern and a basophilic matrix. These tumors often harbor MYB-NFIB fusions, although MYBL1 rearrangements or MYB amplification may also occur.[144] While classic adenoid cystic carcinoma is the most commonly diagnosed form, two less common and more aggressive subtypes have been identified: solid basaloid and high-grade transformational adenoid cystic carcinomas. Typically, these tumors manifest as a palpable mass in older patients.[145] In spite of their triple-negative phenotype, classic adenoid cystic carcinomas generally have an excellent prognosis, and surgical intervention is usually curative. It's worth noting that tumors with similar histology and molecular phenotype arising in the salivary gland exhibit markedly different clinical behaviors. Numerous retrospective series have indicated limited to no benefit from chemotherapy in classic adenoid cystic carcinomas.[146] On the other hand, the less common subtypes, solid basaloid and high-grade transformational adenoid cystic carcinomas, tend to have a more aggressive clinical course. While data is limited due to small sample sizes, the potential benefit from chemotherapy in these subtypes cannot be ruled out.[145]

b. Secretory carcinoma

Secretory breast carcinoma presents with characteristic features uncommon in breast oncology. These tumors often display abundant intracellular and extracellular secretions, resembling thyroid follicles. A key characteristic is the presence of the ETV6-NTRK fusion gene,[147,148] encoding a tyrosine kinase that activates RAS- MAPK and PI3K pathways, driving oncogenesis. Treatment with TRK inhibitors has shown profound and enduring responses, with reports of dramatic responses to larotrectinib in advanced pediatric secretory breast carcinoma.[149] Typically, these tumors manifest as slow-growing, painless, and mobile masses. Despite being triple- negative, they carry an excellent prognosis and

can usually be managed with local therapies alone. While rare, distant metastases may occur, warranting consideration of TRK inhibitors in such cases.[150]

c. Adenomyoepithelioma (AMEs)

It is an extremely uncommon tumor, typically appearing in women over 60, often presenting as nipple discharge when it affects large ducts in the retroareolar region. This neoplasm forms glandular structures comprising both epithelial and myoepithelial cells, with the latter being more predominant, set within a stroma less prominent than that of pleomorphic adenoma. Various patterns may be discerned, including lobed, papillary, tubular, and mixed, sharing resemblances with epithelial-myoepithelial carcinomas of salivary glands. Immunophenotypically, the tumor can exhibit triple-negative characteristics in around 40% of cases, with the epithelial component typically showing positivity for CK AE1/AE3, EMA, and carcinoembryonic antigen (CEA), while the myoepithelial component stains positive for p63, S100, and smooth muscle actin (SMA). Genetically, it displays a mutation profile akin to epithelial-myoepithelial carcinomas of salivary glands, with recurrentsomatic mutations in the HRAS and PI3K-AKT pathways, present in over 50% of cases, often with genetic heterogeneity. Although most adenomyoepitheliomas (AMEs) follow a benign clinical course, progression to carcinomas of varying grades, often exhibiting metaplastic and myoepithelial differentiation, and metastatic disease in AMEs lacking recognizable histologically defined carcinoma have been reported.[151]

d. Polymorphic adenocarcinoma

This neoplasm is exceedingly uncommon, characterized by an invasive breast carcinoma displaying a central solid pattern, encircled by a consistent and uniform band of cells. Immunophenotypically, it typically exhibits a triple-negative profile (nearly 100%), along with positivity for Bcl2, E-cadherin, PGFA, and CK7, as well as p-cadherin. Notably, it lacks expression of androgen receptors (AR), EMA, and CD117. Genetically, it resembles its salivary gland counterpart, with a high frequency of somatic activating mutations in PRKD1.[152]

e. Mucoepidermoid carcinoma

This tumor is relatively rare and typically presents as a solid or cystic mass in young women under the age of 40. Histologically, it is characterized by a proliferation of basaloid, epidermic, and mucinous cells, with a notable absence of true keratinization.[120] In cases of low-grade tumors, cystic areas may be observed. Immunophenotypically, it is almost always triple negative, with positivity for p63 (in basaloid and epidermic cells), CK7 (in mucinous cells), EGFR, EMA, CEA, p-cadherin, and PAS staining. It falls under the basal-like subtype. Genetically, these tumors are marked by the t(11;19) translocation and the presence of the CRTC1-MAML2 fusion protein.[152]

2.7 The Role of SOX10 in Triple-Negative Breast Cancer

SOX10 (SRY-Box Transcription Factor 10) is a transcription factor primarily known for its role in the development and maintenance of neural crest-derived cells, such as melanocytes (due to its ability to promote melanin synthesis and melanocyte proliferation) and Schwann cells. Recent research has identified SOX10 as a significant player in certain cancers, particularly those derived from neural crest lineages, like melanoma.[153] However, its emerging role in breast cancer, especially in the aggressive triple-negative breast cancer (TNBC) subtype, has gained attention due to its association with tumor progression, metastasis, and poor clinical outcomes.[154–156]

2.7.1 SOX10 Expression in TNBC

SOX10 is frequently overexpressed in TNBC. Several studies have demonstrated that SOX10 is predominantly expressed in basal-like breast cancers, which overlap significantly with TNBC. SOX10 has been linked to the maintenance of basal cell identity in these tumors and is associated with more aggressive tumor phenotypes.[157] Elevated levels of SOX10 in TNBC are correlated with higher rates of proliferation, invasion, and metastasis, making it a potential marker for poor prognosis in this subset of breast cancer patients.[8]

2.7.2 SOX10 and Cancer Stem Cells in TNBC

SOX10 is strongly associated with the cancer stem cell (CSC) phenotype in TNBC. Cancer stem cells are a subpopulation of tumor cells with the ability to self-renew, initiate tumors, and contribute to metastasis. SOX10 is believed to regulate the stemness properties of CSCs in TNBC, promoting their survival and enhancingtheir ability to drive tumorigenesis and metastasis.[154] These SOX10-expressing cancer stem cells are more likely to be resistant to conventional chemotherapies, which poses significant challenges for treatment.[158]

In TNBC, SOX10 helps maintain the stem-like characteristics of cancer cells by promoting the expression of other stemness-associated genes, such as NANOG and OCT4. This regulation supports the long-term propagation of CSCs, which can repopulate the tumor after chemotherapy, contributing to relapse and metastasis.[159]

2.7.3 SOX10 in Epithelial-Mesenchymal Transition (EMT) and Metastasis

Epithelial-mesenchymal transition (EMT) is a biological process in which epithelial cells lose their adhesion properties and acquire mesenchymal, invasive traits. EMT plays a critical role in the metastatic spread of TNBC, and SOX10 has been identified as a key regulator of this process in TNBC cells. SOX10 promotes EMT by upregulating EMT-associated transcription factors, which facilitate the loss of epithelial characteristics and the gain of mesenchymal traits.[160]

The activation of EMT by SOX10 allows TNBC cells to become more motile, invasive, and capable of infiltrating surrounding tissues and distant organs. This is particularly relevant in TNBC, which is known for its high metastatic potential. Studies have shown that SOX10 expression is strongly correlated with distant metastasis in TNBC patients, particularly to the lungs, liver, and brain. In addition, SOX10-driven EMT may also contribute to the formation of circulating tumor cells (CTCs), which are critical for the spread of TNBC to distant sites.[160,161] A meta- analysis revealed a significant correlation between SOX10 overexpression and both the TNM stage and grade of breast cancer. The findings demonstrated a strong association between SOX10 overexpression with tumor grade and stage of metastasis in different populations.[162]

2.7.4 SOX10 and Chemoresistance in TNBC

One of the major challenges in treating TNBC is the development of chemoresistance, which limits the efficacy of standard chemotherapy. SOX10 has been implicated in promoting chemoresistance in TNBC through various mechanisms, including the activation of DNA repair pathways, the upregulation of anti-apoptotic proteins, and the maintenance of CSC populations.[155]

SOX10 has been shown to increase the expression of genes involved in drug efflux, such as ATP-binding cassette (ABC) transporters, which pump chemotherapy drugs out of cancer cells, reducing their intracellular concentrations and thus their cytotoxic effects. Furthermore, SOX10-expressing cells are often more resistant to apoptosis due to the upregulation of survival pathways such as the PI3K/AKT and NF-κB signaling pathways.[163]

2.7.5 SOX10 as a Prognostic Marker in TNBC

Due to its significant role in promoting tumorigenesis, metastasis, and chemoresistance, SOX10 has been suggested as a potential prognostic marker for TNBC. High levels of SOX10 expression are associated with poor clinical outcomes, including shorter overall survival and increased likelihood of metastasis. SOX10 significantly promotes the clinical progression of breast cancer, leading to poor prognosis of breast cancer patients, especially in TNBC.[8,156]

2.7.6 Therapeutic Targeting of SOX10 in TNBC

Given its critical role in TNBC progression and chemoresistance, SOX10 represents a promising therapeutic target. Inhibiting SOX10 activity or blocking its downstream signaling pathways could reduce tumor growth, metastasis, and resistance to therapy in TNBC patients.[8]

3. MATERIALS AND METHODS

3.1 Study Design and Study population

A cross sectional study which included 50 cases of triple negative breast carcinoma, extended from 1st March 2024 to 1st February 2025 in Al-Yarmouk teaching hospital and private laboratory in Baghdad.

3.2 Criteria for patients selection

3.2.1 Inclusion criteria

- Triple Negative Breast Cancer (TNBC) diagnosed using immunohistochemistry;
- Negative ER;
- Negative PR;
- Negative HER-2/neu.
- Not on neoadjuvent therapy;
- Wide local excision with axillary clearance;
- Lumpectomy with axillary clearance;
- Mastectomy with axillary clearance;
- Tru-cut biopsy with known pathological TNM.

3.2.2 Exclusion criteria

- Patients on neoadjuvent therapy;
- If the primary tumor showed ER, PR and/or Her2/neu positive;
- Tru-cut biopsy with unknown pathological TNM.

3.3 Immunohistochemistry

3.3.1 Principle of staining

PolyExcel two step detection systems is non-biotin, micro polymer based on an HRP labeled polymer, which was conjugated with secondary antibodies. For IHC staining, rabbit primary antibody was added to tissue which bound to tissue specific antigens in the specimen then any excess antibody was removed by washing, then secondary antibody or poly excel HRP labeled polymer added and reacted with primary antibody. Again, any excess secondary antibody was removed by washing; the end brown color which formed as 3-3 diaminobenzidine HCL (DAB) which in turn oxidized by denoting electrons to activate HRP/H2O2 reaction.

3.3.2 Materials

The Instruments and Apparatus used in the current investigation are listed below

Apparatus	Company/ Origin
Automated upright Microscope System with LED illumination for life Sciences Leica DM 4000 B LED	Leica-microsystems
Centrifuge	Hettich Universal(Germany)
Cold Plate for Modular Tissue Embedding System Leica EG1150 C	Leica-biosystems
Electrical oven	Memmert (Germany)
Heated Paraffin Embedding Module Leica EG1150 H	Leica-biosystems
Mettler H54 A.R. Micobalance	Karl Kolp (Germany)

Microplate reader	Biotec EL x 800(Germany)
Microplate washer EL x 50	Biotec EL x50 (Germany)
Multistainer Leica ST 5020	Leica biosystems
Orbital Shaker	GFL (Germany)
pH meter	Inolab (Germany)
Semi-automated Rotary Microtome	Leica-biosystems
Leica RM2245	
Semi-encolsed Benchtop Tissue Processor Leica TP 1020	Leica-biosystems
Thermostatic waterbath	Germany (Tiawan)
Vortex Mixer	Cleaver (Germany)

List of Antibodies, Dyes and Chemicals used in the study

Antibody	**Company / Origin**
SOX10 (Clone: EP268) Rabbit Monoclonal Antibody	PathnSitu (USA)

Dyes and Chemicals

Dyes and Chemicals	**Company / Origin**
Secondary detection system	PathnSitu (USA)
3-3 diaminobenzidine HCL (DAB)	PathnSitu (USA)
Phosphate buffer saline (PBS)	(DAKOR, Demark)
Hydrochloric acid solution 34-37% (250ml)	Fluka (India)
Formaldehyde 37-41% (2.5 L)	Sigma Chemicals LTD (Germany)
Acetic Acid Glacial (2.5 L)	Scharlau (Spain)
Citric acid powder	Thomas Baker (India)
Ethanol absolute 99.9% (2.5 L)	Scharlau (Spain)
Xylene mixture of isomers (2.5 L)	Scharlau (Spain)
Paraffin pellets (2.5 Kg)	Scharlau (Spain)

1. The primary antibody

The antibody is intended for use to qualitatively identify the SOX10 antigen by light microscopy in formalin fixed, paraffin embedded (FFPE) tissue sections using immunohistochemical (IHC) detection methodology. Interpretation of any positive or negative staining must be complemented with the evaluation of proper known controls (Positive and Negative) and must be made within the context of the patient's clinical history and other diagnostic tests.

Routinely processed, FFPE tissues were used as they were suitable for use with this primary antibody, when used PathnSitu's Poly Excel HRP/DAB detection system. The tissue fixative used was 10% neutral buffered formalin as recommended. Thickness of the sections were 2-5µm. Slides were stained once the sections are made as antigenicity of the cut sections may diminish over a period of time. As recommended, a known stained positive and negative controls were simultaneously prepared with unknown specimens.

2. Secondary detection system

Polyexcel HRP/DAB detection system two step (code PEH002) and DAB chromogen was used. The materials used were Peroxidae block, HRB and DAB chromogen.

3. Primary antibody diluents 1% bovine serum albumin (BSA) and 0.05% sodium azide (NaN3).

4. Antigen retrieval solution: Tris-EDTA buffer (Cat#PS009) was used as antigen retrieval solution.

5. Heat retrieval method: Sections were retrieved under high pressure for 25 minutes using pressure cooker, sections were allowed solution to cool at the room temperature, the tissue sections/slides were later transferred to the distilled water prior to the primary antibody application.

6. Phosphate buffer solution (PBS)

7. Ethanol alcohol absolute 99.9%

8. Xylene

9. D. W.

10. Aqueous mounting media

3.3.3 Preparation of tissue Section and Reagents for IHC

1. Paraffin embedded sections were cut into 3 µm thickness to obtain optimum resolution after staining.
2. Then the sections were placed into floatation water path preheated to 45℃ for decompression of the section.
3. The section was placed on a positively charge slide as a flat and wrinkled free as possible to optimize stain contact with tissue, with painted portion faced up.
4. Allowing the tissue to dry at room temperature.

5. Absolute ethanol was diluted with D.W. to prepare concentrations of 95, 70 and 50% of alcohol.
6. Phosphate buffer saline (PBS) tablets were dissolved in DW, for each tablet dissolved in 100 ml of D.W.
7. Sodium citrate buffer (10 ml sodium citrate, 0.05 % Tween pH 6)- Tri – Sodium citrate (dehydrated) (2.94g)- DW (1000ml)- All were mixed well to dissolve, and then the pH was adjusted to 6 by adding 1N HCL, 0.5 ml of Tween 20was added and mixed well- Finally, the mixture was stored at room temperature for 3 months and at 4℃ for longer storage.

3.3.4 Immunohistochemical procedure

1. Slide backing

The slides were placed in a semi-vertical position in hot air oven preheated to 65℃.

2. Deparaffinization

The tissue section was dewaxed in xylene as following

- Xylene for 3 min then
- Fresh xylene for 3 min then
- Xylene 1:1 absolute ethanol for 3 min.

3. Rehydration

The slide was submerged through descending concentrations of alcohol jars that contained approximately 250 ml of each of the following

- Absolute ethanol for 3 min then
- Fresh absolute ethanol for 3 min then
- 95% ethanol for 3 min then
- 70% ethanol for 3 min then
- 50% ethanol for 3 min.

The slides were kept in tap water until ready to perform antigen retrieval where at no time from this level onward the slide should not dry. Drying out causes nonspecific antibody binding and therefore high background staining, these notes were recommended by manufacture.

4. Heat induced epitope

Slides were placed in a container and covered with 10 mmol sodium citrate buffer, at pH 6. Then heated to 95℃ for 20 min.

Fresh buffer was added and heated at 95℃ for 5 min. Slides were allowed to cool in the buffer for 20 min, wash in deionized H2O three times for 2 min each. Excess liquid from the slides was aspirated.

Cooling for 20 min was the suggested time for antigen retrieval by manufacture, less than 20 min may leave the antigen under retrieval leading to weak staining, however, more than 20 min may leave them over retrieved leading to non-specific background staining with increased chance of section dissociatiation from slide.

5. Tissue sectioning and circled with pap pen, hydrophobic barrier was made by pen to retain aqueous solution within determined area eliminating use of extra reagent.

6. Peroxidase block

Aliquot of 50 µl of peroxidase blocking reagent was placed onto sections and incubated for 10 min in humid chamber.

7. Washing

Slides were washed with phosphate buffer saline for 2-5 min with gentle agitation then drained and blotted.

8. Protein block

Protein was blocked with 10% of normal serum 1% BSA in TBS for 10 min at room temperature, then the slide was drained without rinsing and wiped around the section with tissue paper.

9. Primary Antibody & Negative control reagent

Aliquot of 50 µl of pre-diluted primary antibody was placed into the sections and incubated for 30 min at room temperature in PathnSitu polyExcel detection system. For 12 hr the slides were placed in fresh Phosphate buffer saline PBS 2x5 with simple agitation then drained and blotted.

10. Secondary antibody

A few drops of secondary antibodies were applied to cover the specimen and then incubated for 10 min at room temperature. Then the slides were rinsed with Phosphate buffer saline PBS for few min then drained and blotted.

11. Poly Ecel Poly HRP

Sufficient drops of PolyExcel PolyHRP were applied to cover the specimen and incubated for10 min at room temperature in a humid chamber.

12. Substrate chromogen reagent

The DAB-substrate solution was prepared freshly in each run and used within one hr aliquot of 50 drops of Stunn DAB substrate and one drop of chromogen in dark field. Then several drops of substrate-chromogen were applied to cover the specimen and incubated for 5 min at room temperature, then rinsed gently with running tap water for 4 min as recommended by the manufacture.

13. Hematoxylin counter stain

The slides then immersed in hematoxyline for 1 min then slides were rinsed slowly with running tap water then a D.W. for 3 min and then drained.

14. Mounting

One to two drops of mounting medium were applied onto the sections then quickly covered with cover slips and left to dry overnight.

15. Examination

The slides were examined under a light microscope for assessment of immunostaining.

3.3.5 Quality control

The recommended positive tissue control for SOX10 Receptor is melanoma. A positive internal control of myoepthilial cells (Figure 4.22) and negative tissue control (Example, LN or tonsil) was used with every staining procedure performed for monitoring the correct performance of processed tissue and test reagents.[164] A negative tissue controls provided an indication of nonspecific background staining. If the results were not expected in positive and negative controls, the test was considered invalid and the entire procedure was cross verified.

3.4 Microscopic study

A digital light microscope (Micros Austria) was used in the examination of slides, each field was obtained from the region of 5 zones of the slide (corners and the center) which were randomly selected, then the image captured in high definition (HD) using the same device built in camera that displays the image on the LCD screen.

3.5 Data collection

All cases were retrieved from the archives of the teaching labs of Histopathology Department in AL-Yarmouk teaching hospital in Baghdad and of private laboratories.

3.6 Immunohistochemical Evaluation of SOX10 Expression

The evaluation of SOX10 expression in triple-negative breast cancer (TNBC) was performed using two scoring systems: the semi-quantitative H-score method and the immunoreactivity score (IRS) both based on previously published literature. The H-score method combines the percentage of stained tumor cells and the intensity of staining into a single numerical score.[165] Similarly, the IRS incorporates both the staining intensity and the proportion of positive cells to provide a comprehensive assessment of SOX10 expression.[166] All evaluations were reviewed by a specialist to ensure accuracy and consistency in scoring.

H-Score calculation

The H-score was calculated using the formula:

H-score = (% of weak-staining cells×1) + (% of moderate-staining cells×2) + (% of strong-staining cells×3)

Where:

- Intensity score:
  - 0 = No staining
  - 1 = Weak staining
  - 2 = Moderate staining
  - 3 = Strong staining
- Percentage of stained cells: The proportion of tumor cells showing staining at each intensity level, expressed as a percentage of the total number of cells.

The resulting H-score ranged from 0 (no staining in tumor cells) to 300 (100% of cells stained with strong intensity).

- **Scoring procedure**

1. Assessment of intensity levels
- The intensity of SOX10 staining was evaluated in the nuclei of tumor cells and categorized as:
  - 0: No staining
  - 1: Weak staining
  - 2: Moderate staining
  - 3: Strong staining

2. Threshold for positivity
- An H-score of ≥1 was considered positive for SOX10 expression.
- Tumors with an H-score of 0 were classified as negative.

Immunoreactivity score for SOX10 Expression

The immunohistochemical evaluation of SOX10 expression in triple-negative breast cancer (TNBC) was conducted by assessing the nuclear immunoreactivity of tumor cells. A scoring system was applied that incorporated both the percentage of positively stained cells and the intensity of staining to calculate an immunoreactivity score.

1. Scoring system

2. Percentage of positive cells
- 0: <1% positive cells
- 1: 1%–10% positive cells
- 2: 11%–50% positive cells
- 3: 51%–100% positive cells

3. Staining intensity
- 0: Negative (no staining)
- 1: Weak staining
- 2: Moderate staining
- 3: Strong staining

4. Calculation of the immunoreactivity score: The immunoreactivity score was calculated as the product of the percentage of positive cells score and the staining intensity score.

Immunoreactivity Score =
(Percentage of Positive Cells Score) × (Staining Intensity Score)

5. Interpretation of Scores
- Negative: Score of 0 or 1
- Low Positive: Score of 2
- Intermediate Positive: Score of 3 or 4
- High Positive: Score of 6 or 9

6. Evaluation procedure

The nuclear staining of SOX10 in TNBC tumor cells was reviewed by pathologists to ensure consistency and accuracy. The proportion of cells with nuclear staining and intensity were assessed in the sample. Immunoreactivity scores were determined for each tumor

specimen based on the defined criteria.

3.7 Ethical consideration

All ethical considerations were approved by the regional ethical committee as presented in the appendix (Issue no.: Path 46 / Date: 22/4/2024).

3.8 Statistical analysis

Data analysis was conducted using SPSS version 27 (Statistical Package for the Social Sciences). Of the 50 TNBC cases, only the 47 cases with invasive ductal carcinoma of no special type (IDC-NST) were included in the statistical analysis. The three rare histologic subtypes (one metaplastic carcinoma and two medullary carcinomas) were excluded from inferential analysis to prevent compromising statistical power. However, these cases were retained in the cohort and analyzed descriptively to preserve the overall sample size for clinicopathological correlations.

Descriptive statistics were used to summarize the data, including measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of differences between quantitative variables was assessed using the ANOVA test for comparisons among more than two independent means, and the Mann-Whitney U test for non-normally distributed data in two independent groups. For comparisons of more than two independent groups with non-parametric data, the Kruskal-Wallis test was applied. Differences in categorical variables were tested using the Pearson Chi-square test (χ^2), with Yates' correction or Fisher's exact test when appropriate.

Additionally, correlation analysis was performed using Pearson's correlation coefficient for normally distributed continuous variables and Spearman's rank correlation for non-parametric data. Statistical significance was considered at a P- value ≤ 0.05.[167]

4. RESULTS

4.1 Age distribution of the patients

All the 50 patients were females and no males were invloved in the study.

The mean age of the participants was 52.38 ± 10.3 years, the age distribution of patients revealed that the majority were aged 50–59 years, with 17 (34.0%) patients. This was followed by age group 40–49 years and 60–69 years, each comprising 12 (24.0%) patients. Patients aged 30–39 years included 7 (14.0%) patients, while those aged 70-79 years were only 2 (4.0%) patients (Table 4.1).

Table 4.1: Age distribution for patients of the study.

	Groups	Frequency	Percent
Age	30-39 years old	7	14.0
	40-49 years old	12	24.0
	50-59 years old	17	34.0
	60-69 years old	12	24.0
	70-79 years old	2	4.0
	Total	50	100.0
	Mean±SD	52.38±10.3	

4.2 Histologic charactristics of the tumor

4.2.1 Microscopic subtype of the tumor

The majority of the patients were diagnosed with the invasive ductal carcinoma (IDC) NSF subtype, comprising 47 (94%) patients (Figure 4.11 to 4.14). A smaller proportion of patients had the IDC with medullary pattern, which accounted for 2 (4%) patients (Figure 4.15 and 4.16) and only one patient (2%) had Metaplastic carcinoma, NOS (Figure 4.1, 4.17 and 4.18).

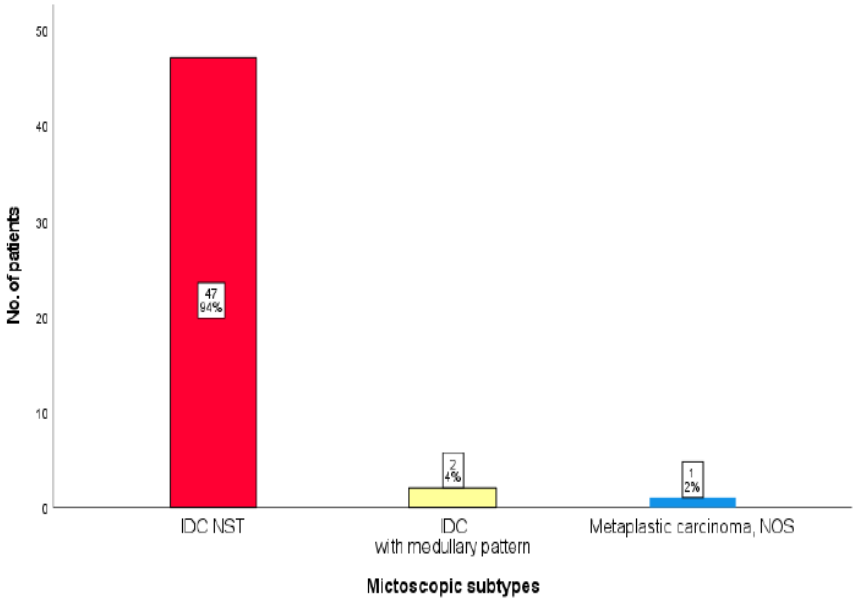

Figure 4.1: Microscopic subtypes for tumours of patients of the study.

4.2.2 Histologic grade of the tumor

The tumor grade distribution showed that Grade III tumors were the most common, observed in 30 (60%) patients (Figure 4.11 and 4.12). While, grade II tumors, was found in 20 (40%) patients (Figure 4.2, 4.13 and 4.14).

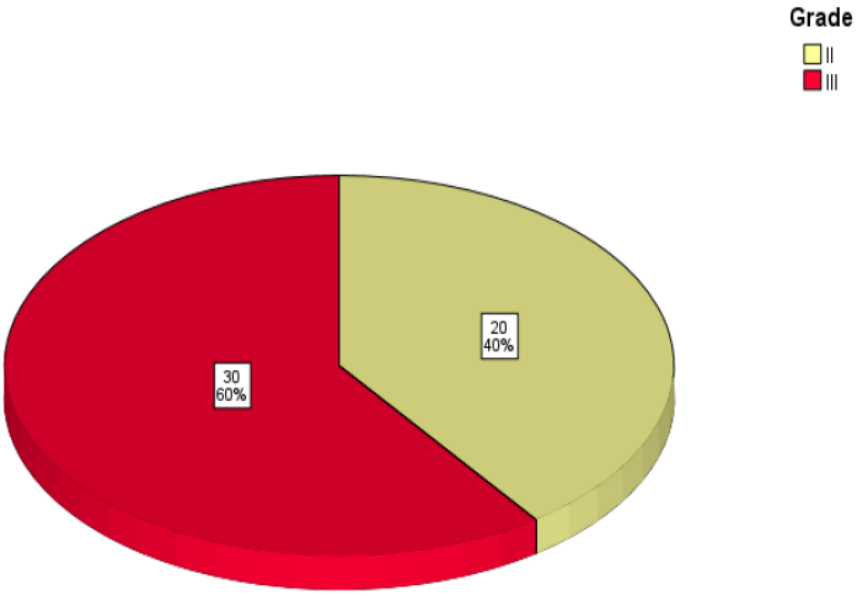

Figure 4.2: Histological grade of the tumors for patients of the study.

4.2.3 TNM Stage of the tumor

4.2.3.1 T-stage of the tumor

For the T stage of the patients. The majority of patients were classified as T3, accounting for 19 (38%) patients. This was followed by T2, observed in 14 (28%) patients, and T1, with 12 (24%) patients. While patients with stage T4 were 5 (10%) patients (Table 4.3).

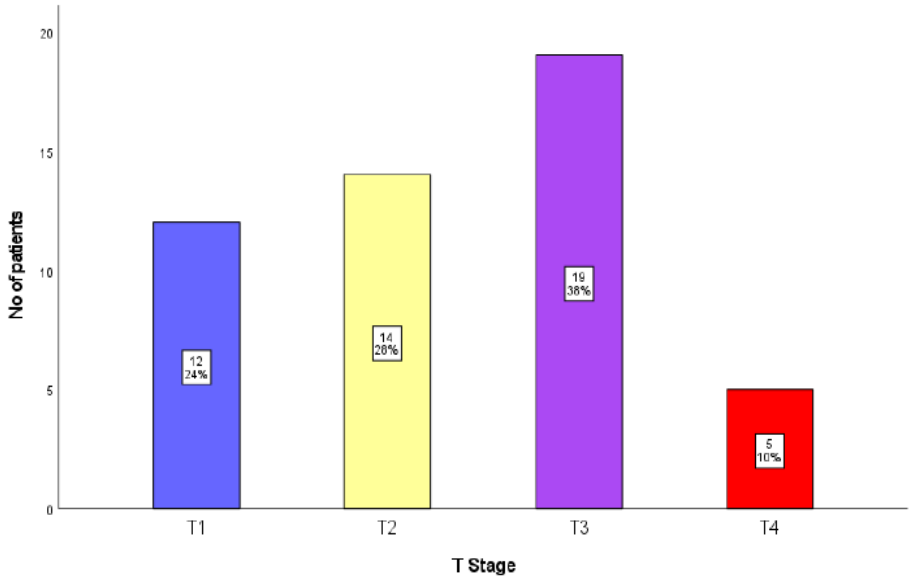

Figure 4.3: T-stage of the tumors for patients of the study.

4.2.3.2N stage

The of lymph node (N) staging showed an equal distribution of patients N1 and N2 stages represented, with 19 (38%) patients each. The remaining 12 (24%) patients were classified as N0 (Figure 4.4).

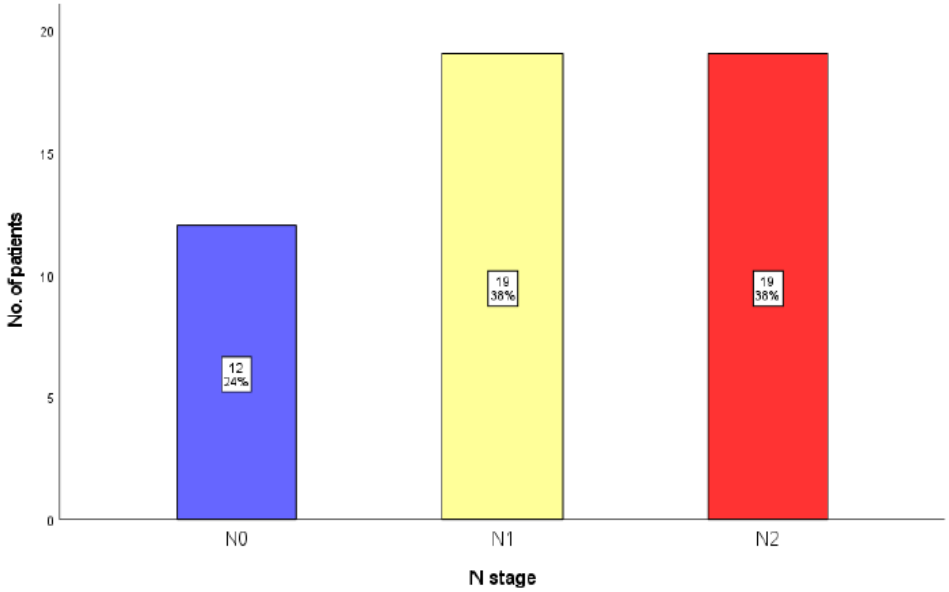

Figure 4.4: N-stage of the tumors for the patients of the study.

4.2.4 Tumor size and lymph node number

The mean tumor size was (4.33±2.62) cm. The mean number of lymph nodes (LN) involved was (2.56±2.45) (Table 4.2).

Table 4.2: Tumor size and lymph node number for patients of the study.

Tumor characteristics	Mean	Std. Deviation	Minimum	Maximum
Size	4.33	2.624	1.2	7
LN	2.56	2.459	0	9

4.2.5 Clinicopathological characteristics of different tumor subtypes

IDC with Grade III tumors observed in 57.4% of cases, while T3-T4 tumors accounted for 46.8%. Nodal involvement (N1-N2) was seen in 74.5% of IDC cases. IRS negativity was present in 40.4%, while high IRS

positivity was observed in 38.3%, and a positive H-score was found in 74.5%. IDC with medullary features showed that both patients had Grade III. Tumor size was T2-T3 for both patients, and both had nodal involvement. IRS and H-score were negative in both patients. Metaplastic carcinoma presented in one patients only who had Grade III, T4 tumor and nodal involvement (N2). Negative IRS and H-score were notable as shown in table 4.3 and figure 4.21.

Table 4.3: Characteristics of different tumor subtypes.

			Tumor subtypes			Total
			IDC	IDC/medullary features	IDC/metaplastic	
Grade	II	No.	20	0	0	20
		%	40.0%	0.0%	0.0%	40.0%
	III	No.	27	2	1	30
		%	54.0%	4.0%	2.0%	60.0%
T	T1	No.	12	0	0	12
		%	24.0%	0.0%	0.0%	24.0%
	T2	No.	13	1	0	14
		%	26.0%	2.0%	0.0%	28.0%
	T3	No.	18	1	0	19
		%	36.0%	2.0%	0.0%	38.0%
	T4	No.	4	0	1	5
		%	8.0%	0.0%	2.0%	10.0%
N	N0	No.	12	0	0	12
		%	24.0%	0.0%	0.0%	24.0%
	N1	No.	17	2	0	19
		%	34.0%	4.0%	0.0%	38.0%
	N2	No.	18	0	1	19
		%	36.0%	0.0%	2.0%	38.0%
IRS	Negative	No.	19	2	1	22
		%	38.0%	4.0%	2.0%	44.0%
	Low pos.	No.	7	0	0	7
		%	14.0%	0.0%	0.0%	14.0%
	Int. pos.	No.	3	0	0	3
		%	6.0%	0.0%	0.0%	6.0%
	High pos.	No.	18	0	0	18
		%	36.0%	0.0%	0.0%	36.0%
H-score	Negative	No.	12	1	0	13
		%	24.0%	2.0%	0.0%	26.0%
	Positive	No.	35	1	1	37
		%	70.0%	2.0%	2.0%	74.0%
Total		No.	47	2	1	50
		%	94.0%	4.0%	2.0%	100.0%

4.3 Immunoreactivity score (IRS) of SOX10

The immunoreactivity scores (IRS) showed that 19 (40.43%) of the patients had negative immunoreactivity score. Low positive immunoreactivity was observed in 7 (14.89%) patients, while the intermediate positive immunoreactivity was seen 3 (6.38%) patients. Patients with high positive immunoreactivity were 18 (38.3%) patients (Figure 4.5, 4.19, 4.20 and 4.21).

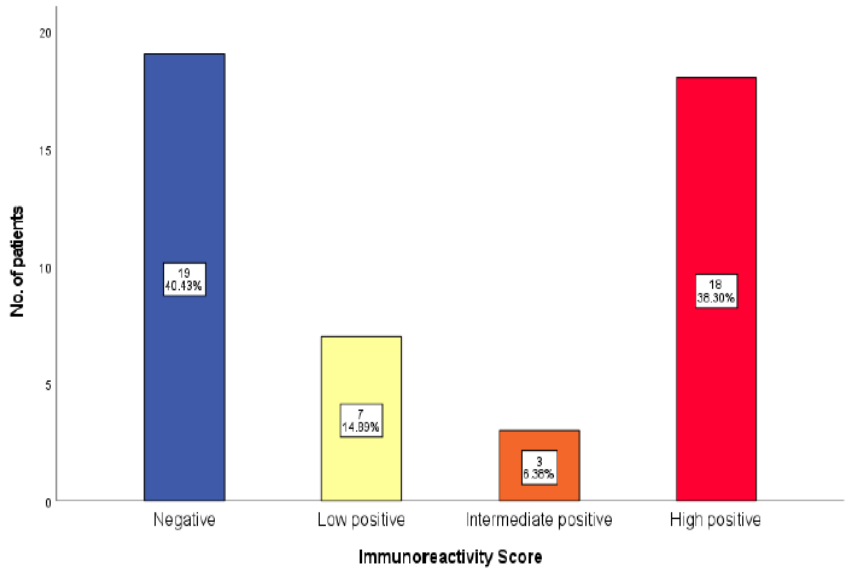

Figure 4.5: Immunoreactivity score (IRS) of SOX10 for patients of the study.

4.3.1 Immunoreactivity score and patients' age

The association of immunoreactivity score with age showed a statistically significant assocaition with p-value of 0.03 as shown in table 4.4 below.

Table 4.4: The association of immunoreactivity score of SOX10 with age for patients of the study.

			Immunoreactivity Score				Total	X^2	P-value
			Neg.	Low pos.	Int. pos.	High pos.			
Age grou ps	30-39 years old	No	1	1	2	3	7	18.1	0.037*
		%	5.3%	14.3%	66.7%	16.7%	14.9%		
	40-49 years old	No	5	1	1	4	11		
		%	26.3%	14.3%	33.3%	22.2%	23.4%		
	50-59 years old	No	10	4	0	2	16		
		%	52.6%	57.1%	0.0%	11.1%	34.0%		
	60-69 years old	No	2	1	0	8	11		
		%	10.5%	14.3%	0.0%	44.4%	23.4%		
	70-79 years old	No	1	0	0	1	2		
		%	5.3%	0.0%	0.0%	5.6%	4.3%		
Total		No	19	7	3	18	47		
		%	100%	100%	100%	100%	100%		

Statistically significant at p-value<0.05, X^2: Pearson chi-square value.

4.3.2 Immunoreactivity score and histologic features of the tumor

Tumor grade, T-stage and N-stage all showed a statistically significant assocaition with immunoreactivity score with p-value<0.05 as shown in table 4.5 below.

Table 4.5: The association of grade, T-stage and N stage with immunoreactivity score of SOX10 for patients of the study.

			Immunoreactivity Score				Total	X^2	P-value
			Neg.	Low pos.	Int. pos.	High pos.			
Grade	II	No	13	2	0	5	20	8.88	0.023*
		%	27.7%	4.3%	0.0%	10.6%	42.6%		
	III	No	6	5	3	13	27		
		%	12.8%	10.6%	6.4%	27.7%	57.4%		
T-stage	T1	No	11	0	0	1	12	35.1	0.001*|
		%	57.9%	0.0%	0.0%	5.6%	25.5%		
	T2	No	6	4	2	1	13		
		%	31.6%	57.1%	66.7%	5.6%	27.7%		

	T3	No	1	3	0	14	18		
		%	5.3%	42.9%	0.0%	77.8%	38.3%		
	T4	No	1	0	1	2	4		
		%	5.3%	0.0%	33.3%	11.1%	8.5%		
N-stage	N0	No	9	1	1	1	12	15.9	0.005*
		%	47.4%	14.3%	33.3%	5.6%	25.5%		
	N1	No	5	5	2	5	17		
		%	26.3%	71.4%	66.7%	27.8%	36.2%		
	N2	No	5	1	0	12	18		
		%	26.3%	14.3%	0.0%	66.7%	38.3%		
Total		No	19	7	3	18	47		
		%	100.0%	100.0%	100.0%	100.0%	100.0%		

*Statistically significant at p-value<0.05, X^2: Pearson chi-square value.

4.3.3 Immunoreactivity Score and Tumor size

One-way ANOVA test to assess the statistical association between tumor size and immunoreactivity score showed a statistically significant difference with p-value of 0.001. Post-hoc pair-wise analysis to assess the source of statistical signifcance showed that the only statistical significant associatiom between negative immunoreactivity score group and high positive immunoreactivity score with p- value of 0.001 as shown in table 4.6 below.

Table 4.6: The association of tumor size with immunoreactivity score of SOX10 for patients of the study.

		Mean	Std. Deviation	95% Confidence Interval for Mean		F-test	P-value
				Lower Bound	Upper Bound		
Size	Negative	2.63	2.14	1.6	3.67	7.89	0.001*
	Low positive	4.78	2.04	2.89	6.68		
	Intermediate positive	5.23	1.96	.35	10.11		
	High positive	5.53	1.47	4.8	6.26		
	Total	4.23	2.26	3.56	4.89		
Post hoc test: Tukey HSD							
Size	Negative	Low positive					0.062
		Intermediate positive					0.134
		High positive					0.001*
	Low positive	Intermediate positive					0.986
		High positive					0.810
	Intermediate positive	High positive					0.994

*Statistically significant at p-value<0.05.

4.3.4 Immunoreactivity Score and LN number

Meanwhile, there was no statistically significant association for LN number and immunoreactivity score of SOX10 with p-value of 0.06 as shown in table 4.7 below.

Table 4.7: The association of lymph node number with immunoreactivity score of SOX10 for patients of the study.

		Mean	Std. Deviation	95% Confidence Interval for Mean		F-test	P-value
				Lower Bound	Upper Bound		
LN	Negative	1.94	2.43	.77	3.12	2.62	0.063
	Low positive	2.28	1.25	1.12	3.44		
	Intermediate positive	2.00	1.73	-2.30	6.3		
	High positive	3.72	1.77	2.83	4.6		
	Total	2.68	2.12	2.05	3.3		

4.4 H-score for patients of the study

The mean H-score for the patients was (109.28±112.44). The scores ranged from 0 to 285 as shown in table 4.8 below.

Table 4.8: H-score distribution for patients of the study.

Mean	Std. Deviation	Std. Error of Mean	Minimum	Maximum
109.2766	112.43988	16.40104	0	285.00

Patients with negative SOX-10 expression when H-score was used with a cutoff point <1% was 12 (25.53%) while

35 (74.47%) had positive SOX-10 expression as shown in figure 4.6.

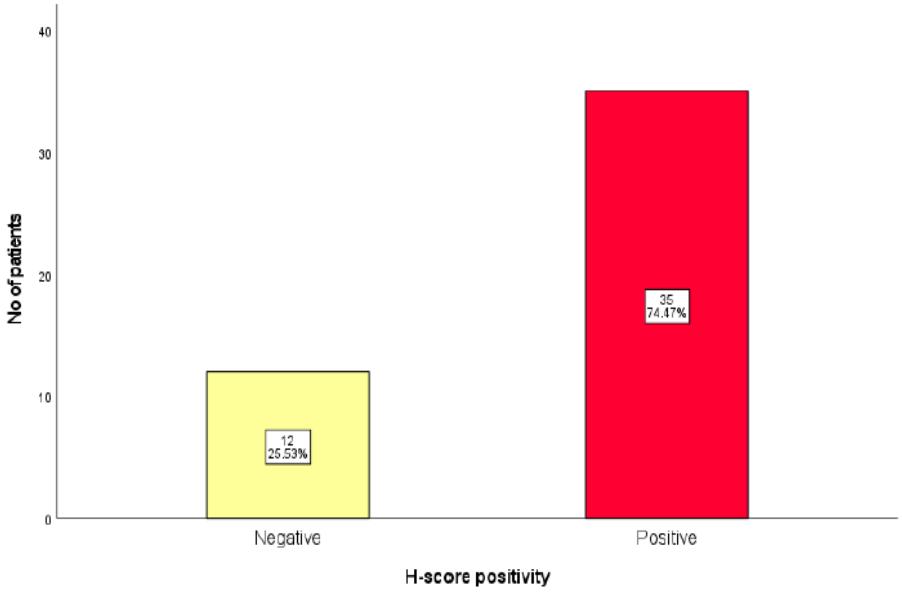

Figure 4.6: SOX-10 status using H-score for patients of the study.

4.4.1 H-score and clinical and pathological characteristics

The analysis of H-scores across different age groups revealed the overall Kruskal-Wallis's test showed a statistically significant difference in H-scores among the age groups (p = 0.041). Pairwise comparisons further highlighted significant associations. The H-score in the 50–59 years old group was significantly lower than that in the 30–39 years old group (p = 0.043) and the 60–69 years old group (p =0.003). No statistically significant differences were observed between other age group pairs (p > 0.05) (Table 4.9).

Table 4.9: The association of age with H-score for patients of the study.

	Mean	SD	95% Confidence Interval for Mean		Kruskal Wallis test	P-value
			Lower Bound	Upper Bound		
30-39 years old	117.14	76.74	46.16	188.12	9.94	0.041*
40-49 years old	108.09	118.11	28.73	187.44		
50-59 years old	50	89.44	2.33	97.66		
60-69 years old	187	112.16	111.64	262.35		
70-79 years old	135	190.91	-1580.33	1850.33		
Total	109.27	112.43	76.26	142.29		
Post-hoc analysis						
30-39 years old			40-49 years old			0.357
			50-59 years old			0.043*
			60-69 years old			0.615
			70-79 years old			0.657
40-49 years old			50-59 years old			0.228
			60-69 years old			0.107
			70-79 years old			0.908
50-59 years old			60-69 years old			0.003*
			70-79 years old			0.455
60-69 years old			70-79 years old			0.436

*Statistically significant at p-value<0.05.

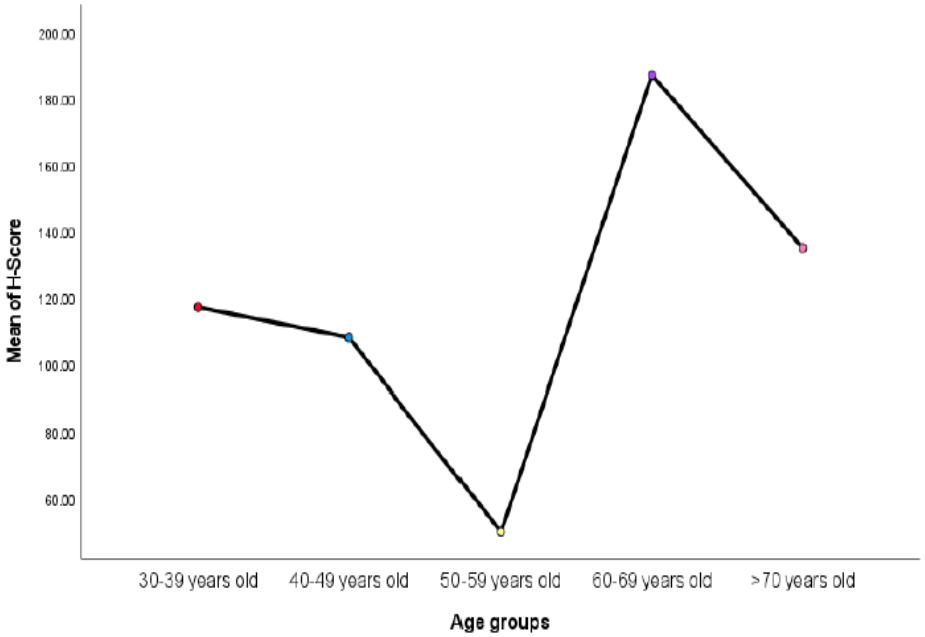

Figure 4.7: The association of age with H-score for patients of the study.

The analysis of H-scores across tumor grades revealed statistically significant differences (p = 0.018) (Table 4.10).

Table 4.10: The association of tumor grade with H-score for patients of the study.

	Mean	SD	95% Confidence Interval for Mean		Mann- Whitney U	P-value
			Lower Bound	Upper Bound		
II	75.7	113.76	22.4	128.9	378.5	0.018*
III	134.1	106.77	91.9	176.3		
Total	109.2	112.43	76.2	142.2		

*Statistically significant at p-value<0.05.

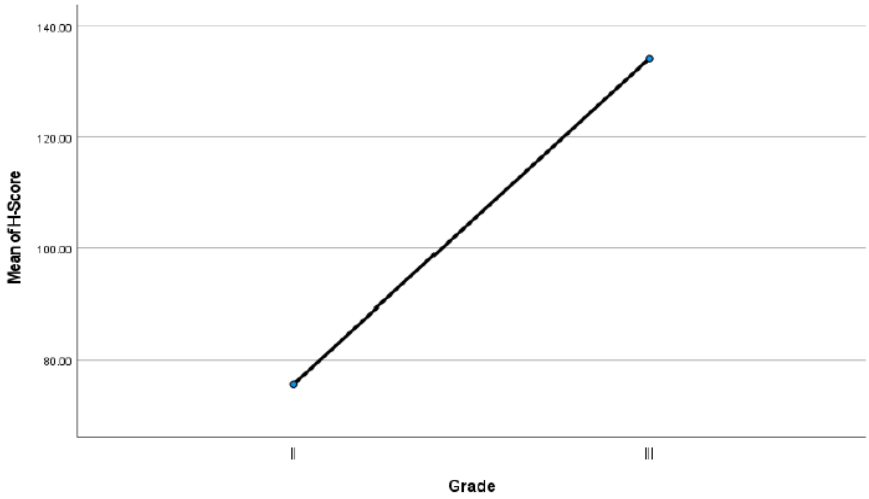

Figure 4.8: The association of tumor grade with H-score for patients of the study.

The association of H-scores across different T stages revealed statistically significant differences (p = 0.001). Pairwise comparisons showed significant differences between T1 and T3 tumors (p = 0.001) as well as between T2 and T3 tumors (p = 0.001). No significant differences were observed between all other pair- wise comparisons (p-value>0.05) (Table 4.11)

Table 4.11: The association of T-stage of the tumor with H-score for patients of the study.

	Mean	SD	95% Confidence Interval for Mean	Kruskal Wallis	P-value

			Lower Bound	Upper Bound	test	
T1	25.33	77.26	-23.75	74.42	22.68	0.001*
T2	56.3	73.89	11.65	100.96		
T3	193.05	92.00	147.3	238.80		
T4	156.25	129.44	-49.72	362.22		
Total	109.27	112.43	76.26	142.29		
T1			T2			0.810
			T3			0.001*
			T4			0.058
T2			T3			0.001*
			T4			0.200
T3			T4			0.870

*Statistically significant at p-value<0.05.

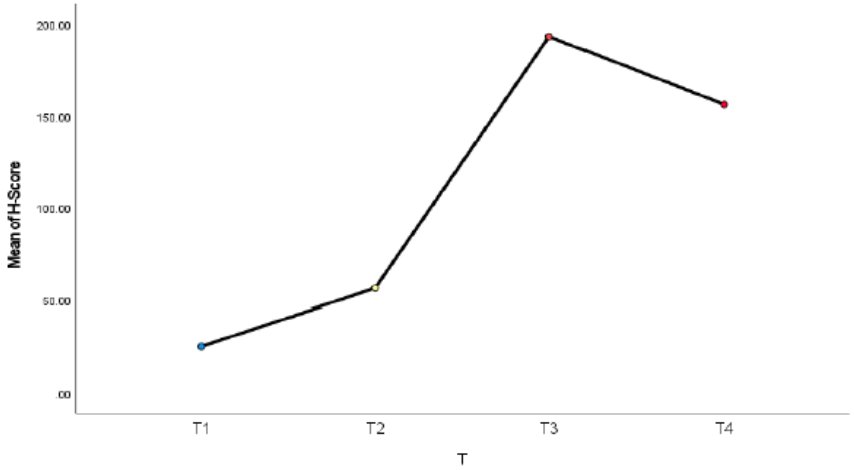

Figure 4.9: The association of T-stage of the tumor with H-score for patients of the study.

Analysis of H-scores across different N stages revealed statistically significant differences (p = 0.002). Pairwise comparisons showed significant differences between N0 and both N1 and N2 (p-value<0.05). However, the difference between N1 and N2 was not statistically significant (p = 0.098) (Table 4.12).

Table 4.12: The association of N-stage of the tumor with H-score for patients of the study.

	Mean	SD	95% Confidence Interval for Mean		Kruskal Wallis test	P-value
			Lower Bound	Upper Bound		
N0	38.75	80.62	-12.47	89.97	12.27	0.002*
N1	91.17	92.98	43.37	138.98		
N2	173.38	116.94	115.23	231.54		
Total	109.27	112.43	76.26	142.29		
N0			N1			0.048*
			N2			0.001*
N1			N2			0.098

*Statistically significant at p-value<0.05.

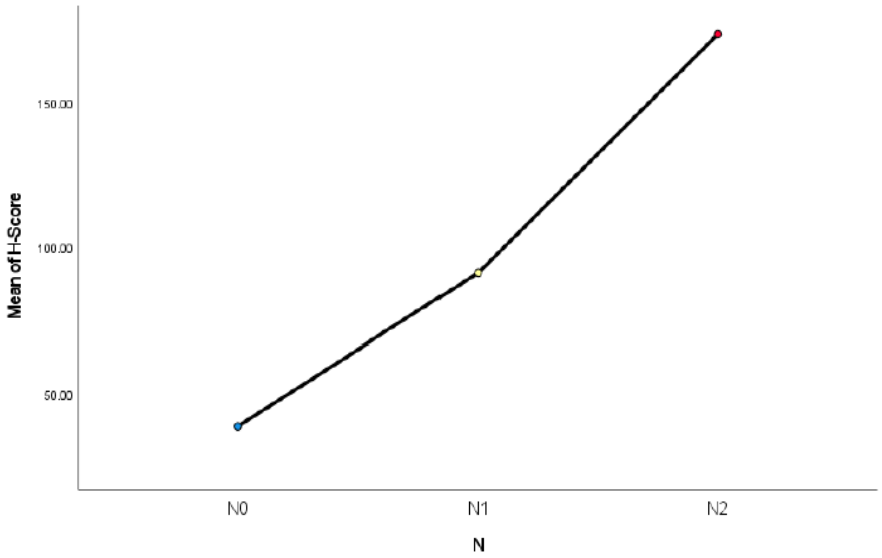

Figure 4.10: The association of N-stage of the tumor with H-score for patients of the study.

4.4.2 Correlation analysis for H-score with clinical and pathological factors

The Spearman's correlation analysis revealed statistically significant associations between the H-score and several clinical and pathological factors. A moderate positive correlation was observed between the H-score and T stage, N stage, number of lymph nodes involved, and tumor size with p-value of 0.001. These findings indicate that higher H-scores are associated with more advanced tumor characteristics.

However, the correlation between H-score and age was not statistically significant (p = 0.380) (Table 4.13).

Table 4.13: The correlation of age, T-stage, N-stage, LN number and tumor size with H-score for patients of the study.

		Age	T	N	LN	Size
H-Score	Spearman's Correlation	0.131	0.646	0.511	0.472	0.581
	P-value	0.380	0.001*	0.001*	0.001*	0.001*

*Statistically significant at p-value<0.05.

4.4.3 Histological assessment of studied specimens

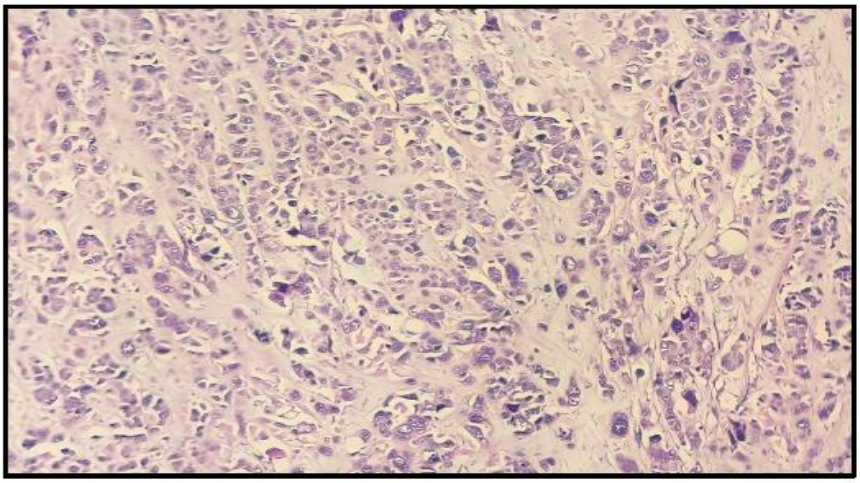

Figure 4.11: Invasive ductal carcinoma, no special type (IDC NST), Grade III, The tumor displays densely cellular, irregular infiltrating nests with marked stromal desmoplasia. High-grade cytological features include

nuclear pleomorphism and frequent mitotic figures, 10x (H&E stain).

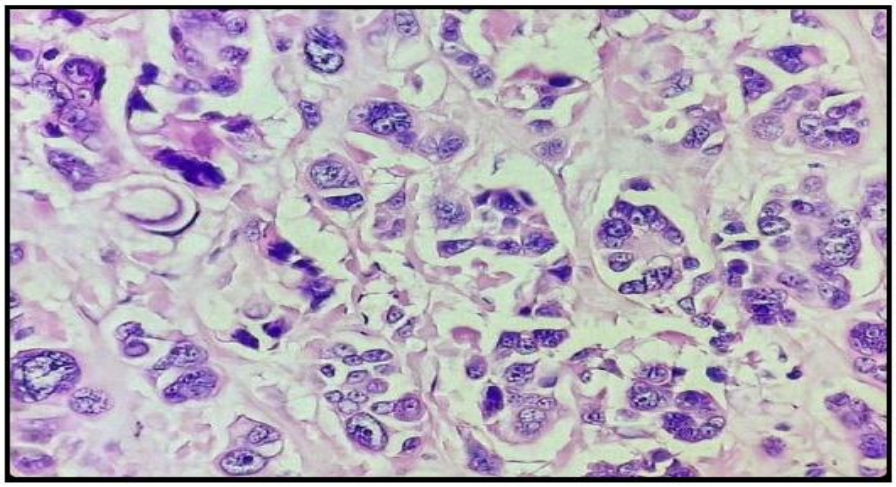

Figure 4.12: Invasive ductal carcinoma of no special type (NST), Grade III, showing markedly pleomorphic nuclei with coarse chromatin, prominent nucleoli, and frequent mitotic figures, 40x (H&E stain).

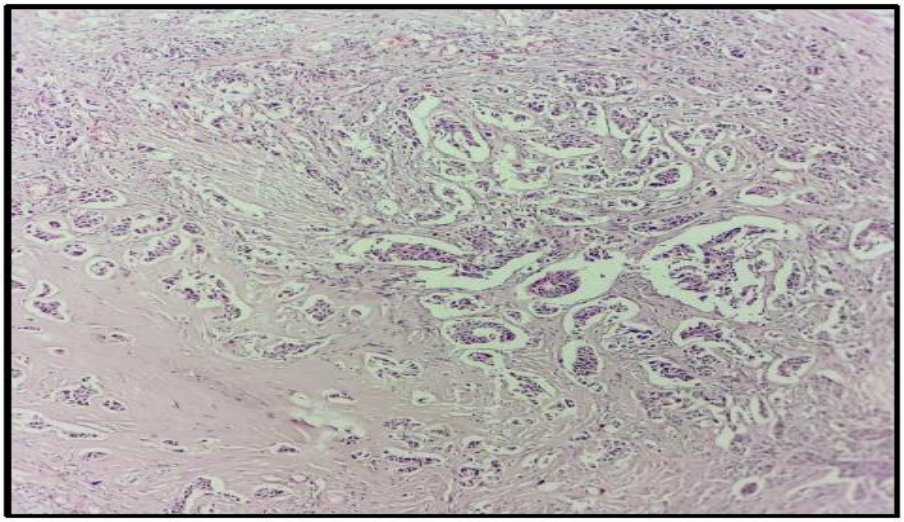

Figure 4.13: Invasive ductal carcinoma, no special type (IDC NST), Grade II. Demonstrates irregular tumor nests infiltrating the stroma, associated with desmoplastic reaction and intermediate-grade cytological features, 10x (H&E stain).

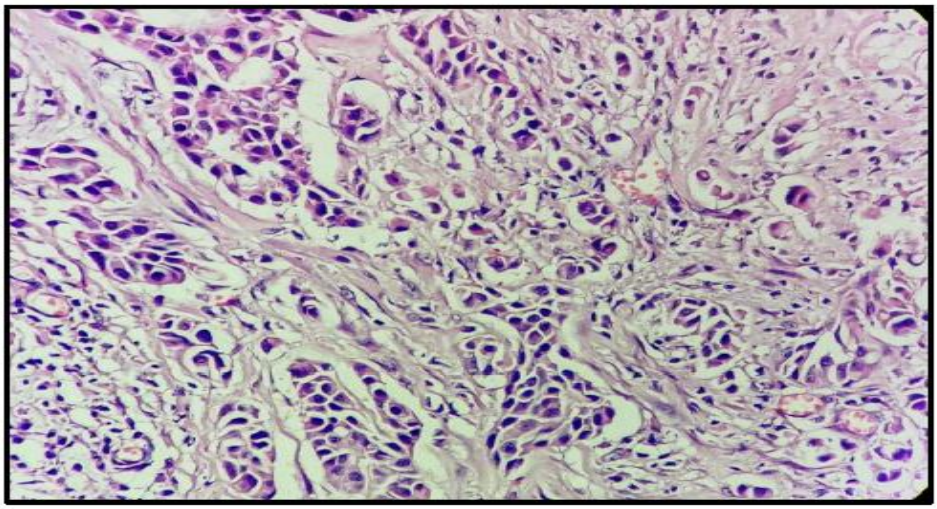

Figure 4.14: Invasive ductal carcinoma, no special type (IDC NST), Grade II, 40x (H&E stain).

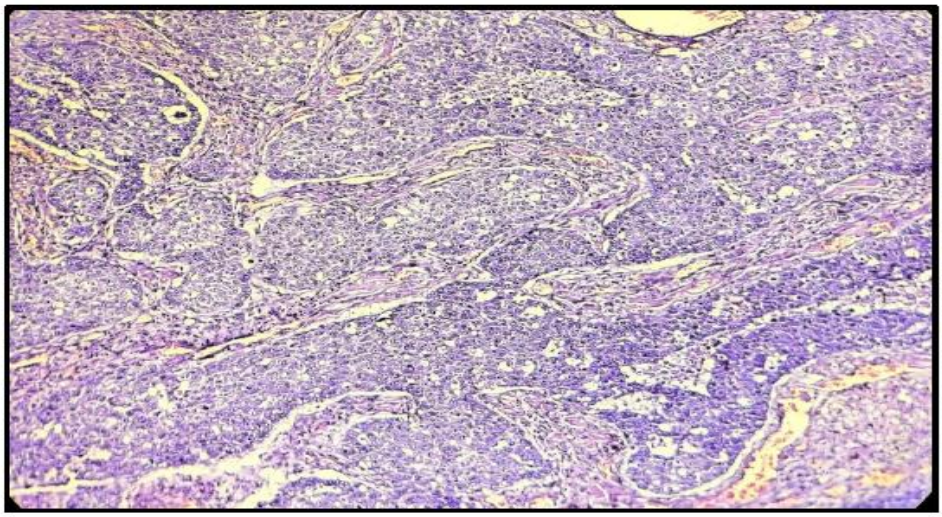

Figure 4.15: Invasive ductal carcinoma, no special type (IDC NST), with medullary pattern showing syncytial sheets of pleomorphic cells, and a prominent lymphoplasmacytic infiltrate surrounding the tumor nests, 10x (H&E stain).

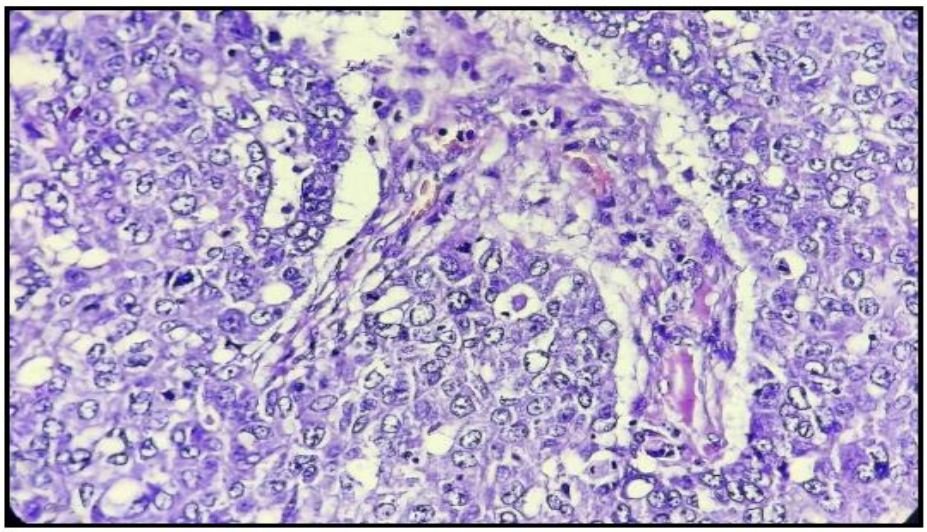

Figure 4.16: Invasive ductal carcinoma, no special type (IDC NST), with medullary pattern demonstrating large tumor cells with vesicular nuclei, prominent nucleoli, and frequent mitotic activity. A dense lymphocytic infiltrate is present at the tumor periphery, 40x (H&E stain).

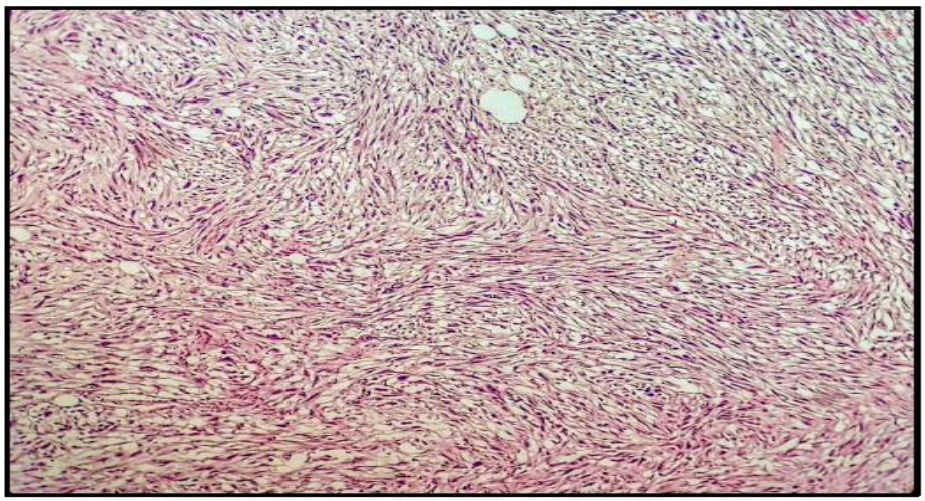

Figure 4.17: Metaplastic carcinoma, not otherwise specified (NOS), showing spindle cell morphology, 10x (H&E stain).

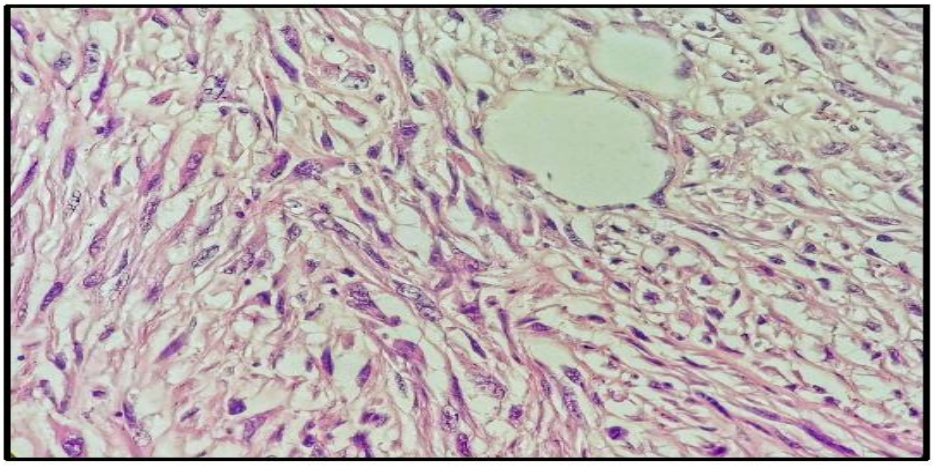

Figure 4.18: Metaplastic carcinoma, not otherwise specified (NOS), showing spindle cell morphology with marked nuclear atypia, 40x (H&E stain).

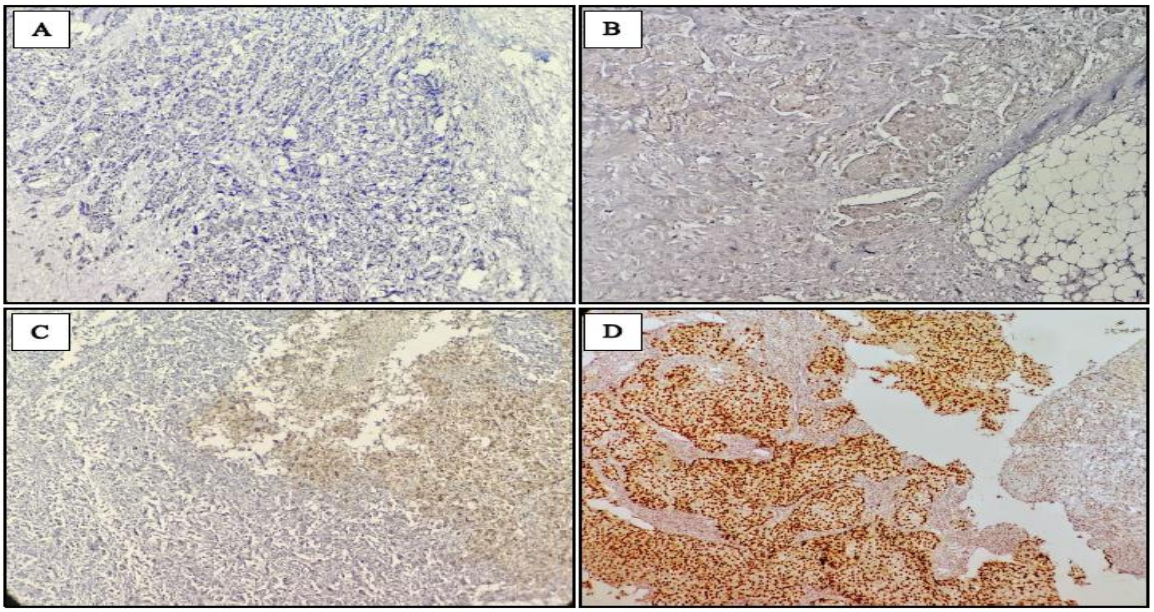

Figure 4.19: Immunohistochemical (IHC) analysis of SOX10 expression in triple- negative breast cancer (TNBC) tissues
(A) Negative SOX10 expression (0% positive tumor cells).
(B) Weak SOX10 expression (<10% tumor cells with faint staining).
(C) Moderate SOX10 expression (11-50% tumor cells with distinct nuclear staining).
(D) Strong SOX10 expression (>50% tumor cells with intense nuclear staining) 10x.

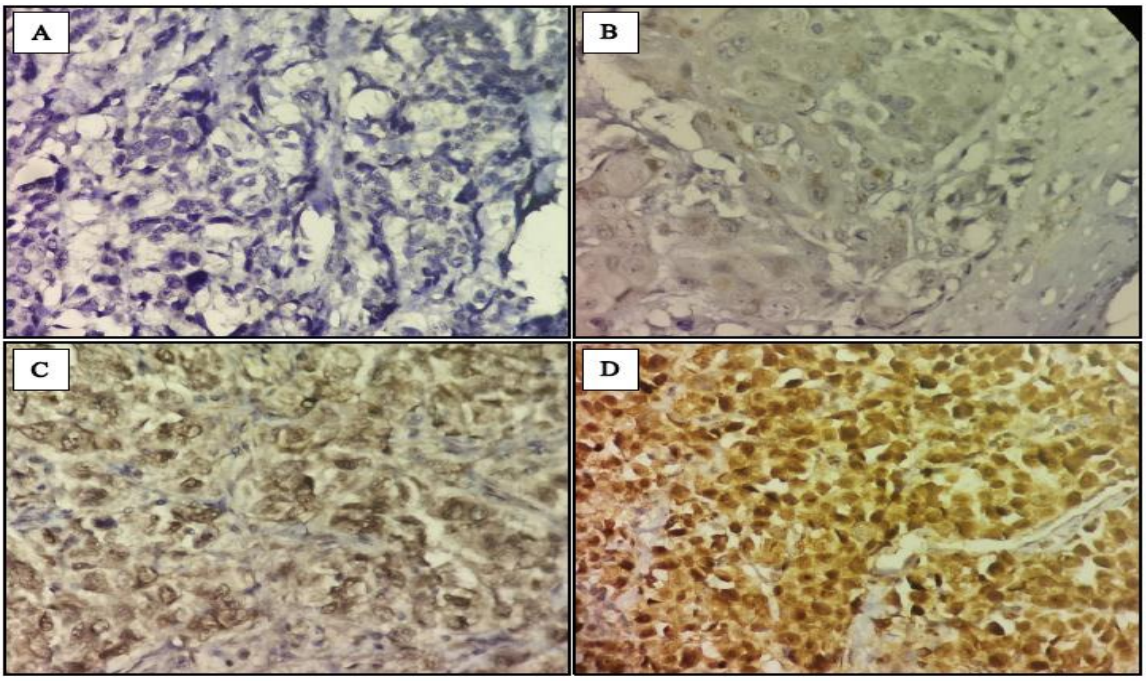

Figure 4.20: Immunohistochemical (IHC) analysis of SOX10 expression in triple- negative breast cancer (TNBC) tissues
(A) Negative SOX10 expression (0% positive tumor cells).
(B) Weak SOX10 expression (<10% tumor cells with faint staining).
(C) Moderate SOX10 expression (11-50% tumor cells with distinct nuclear staining).
(D) Strong SOX10 expression (>50% tumor cells with intense nuclear staining) 40x.

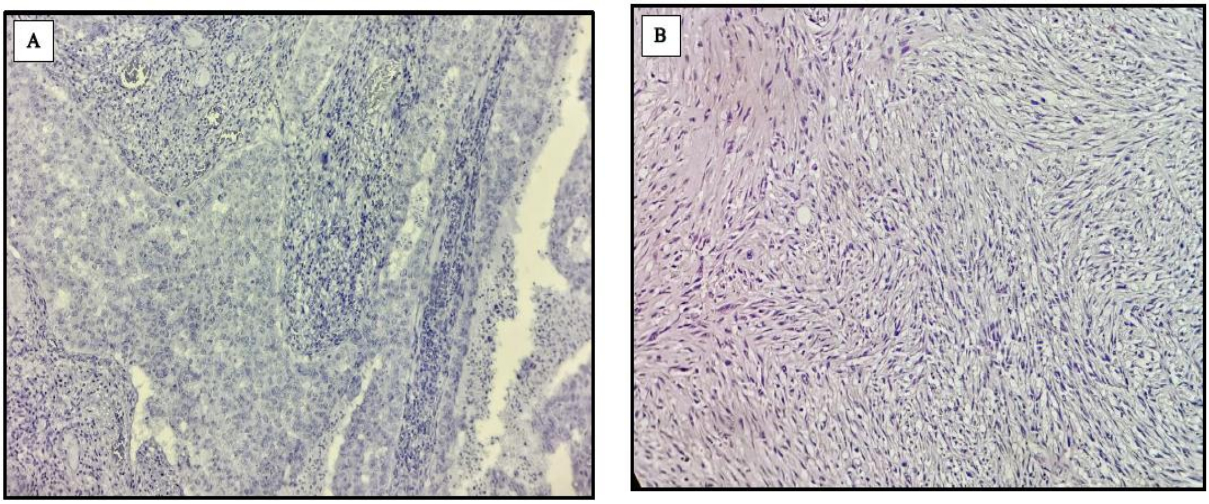

Figure 4.21: SOX10 immunohistochemical expression in triple-negative breast cancer (TNBC) subtypes.
(A) Invasive ductal carcinoma (IDC) with medullary pattern showing negative SOX10 nuclear staining.
(B) Metaplastic carcinoma demonstrating absence of SOX10 nuclear expression 10x.

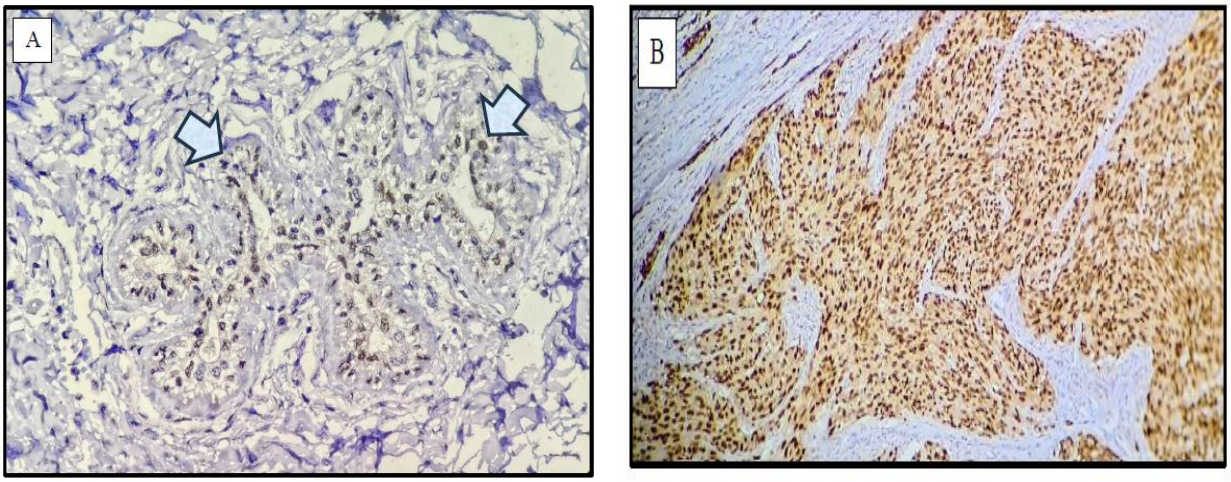

Figure 4.22: SOX10 immunohistochemical stain (10x magnification) a. demonstrating nuclear positivity in myoepithelial cells (arrow), serving as an internal control. The retained myoepithelial layer around benign ducts confirms proper assay performance and validates the technical adequacy of the staining procedure, b. demonstrates strong nuclear staining in melanoma serving as positive external control, this validates the specificity of the SOX10 antibody used in the study.

5. DISCUSSION

SOX10 has been studied in a variety of tumors, revealing its dual roles as an oncogene and a tumor suppressor depending on the tumor type.[168] In hepatocellular carcinoma, SOX10 overexpression drives oncogenic activity through the Wnt/double-stranded protein/TCF4 pathway, while in digestive system cancers, it acts as a tumor suppressor by inhibiting the Wnt/β-catenin signaling cascade.[169,170] These findings illustrate the context-dependent functions of SOX10 in tumorigenesis, sparking interest in its potential role in breast cancer. SOX10 has emerged as a critical regulator in the biology of triple-negative breast cancer (TNBC). TNBC is an aggressive breast cancer subtype with limited therapeutic options. The use of SOX10 as a diagnostic marker in TNBC was inspired by its observed overexpression in basal-like breast cancer (BLBC), a molecular subtype closely related to TNBC.[171] Immunohistochemical studies consistently demonstrated a marked elevation in SOX10 expression in BLBC, leading researchers to investigate its relevance in TNBC. Functionally, SOX10 is implicated in critical pathways that contribute to the aggressive nature of TNBC suggesting its role as a prognostic factor.[172] In this study, despite having three patients with rare histologic subtypes (metaplastic and medullary carcinomas), their small number precluded subgroup analysis for SOX10 expression. These tumors are known to exhibit distinct molecular profiles; however, the limited sample size rendered statistical comparisons with IDC-NST unreliable.

In reviewing the literature on SOX10 expression in triple-negative breast cancer (TNBC), it is evident that different studies have employed varying methodologies and cutoff points for assessing SOX10 expression. These include the use of the H- score and the immunoreactivity score (IRS), with differing thresholds such as 1% or 10% of stained cells for determining positivity, contribute to discrepancies in reported positivity rates, complicating comparisons between studies. In addition, the College of American Pathologists (CAP) has not established specific guidelines for the assessment of SOX10 expression in breast cancer. CAP provides general principles for the analytic validation of immunohistochemical assays but does not offer standardized protocols for SOX10 evaluation.[173]

In the present study, the evaluation of SOX10 expression using the immunoreactivity scoring system revealed that 59.57% of patients were categorized as having positive SOX10 expression. This finding is consistent with previous research. For example, Yoon E. et al. reported a 54.1% positivity rate using the IRS, while Lin X et al. in 2023 in China, employing a similar 10% cutoff point, observed positive SOX10 expression in 60.4% of cases (166,174). Similarly, Tariq et al. (2024, Pakistan) and Laurent E. et al. (2019, France) reported positivity rates of 58.3% and 62.3%, respectively, using a 10% cutoff for IRS.[175,176] These results collectively highlight a trend where the 10% cutoff, yields positivity rates around 60%, suggesting its reliability in identifying SOX10 expression in TNBC.

When using the H-score in this study, 74.4% of patients were classified as positive with a 1% cutoff point. This result aligns closely with findings from other studies using similar methodologies. For instance, Zhang DM et al. (2022, China) reported a positivity rate of 75.3%, while Qazi et al. (2020, USA) and Yoon et al. (2022, Canada) observed rates of 74% and 79.5%, respectively.[166,175,177] Studies by Lin X et al. (2021, China, 65.6%), Ali S et al. (2022, Pakistan, 82.6%), further highlight the consistency of results when employing a 1% cutoff for the H- score.[178,179] Interestingly, an Iraqi study conducted in Karbala in 2024 showed a relatively lower positivity rate of 43%

using the 1% cutoff, possibly reflecting differences in sample characteristics and size since only 30 patients were involved in that study.[180]

The comparison of these findings with previous studies demonstrates the benefits of both scoring systems in identifying SOX10 expression. For instance, the H-score tends to identify a higher proportion of positive cases due to its sensitivity in capturing even minimal expression levels, while the immunoreactivity score may apply stricter criteria by requiring both higher staining intensity and greater coverage. The variability in positivity rates between studies, on the other hand, emphasises the influence of methodological differences, such as scoring systems, cutoff thresholds, and sample heterogeneity.

High positive immunoreactivity for SOX10 was observed in 38.3% of the patients of the study. Comparable findings have been reported in the literature. For instance, Peevey J et al. in 2015 demonstrated high immunoreactivity scores (IRS) in 50% of TNBC cases, which is slightly higher than the prevalence observed in this study.[181] The difference in proportions could be attributed to variations in sample size, cohort characteristics, or methodology. These results suggest that a substantial proportion of TNBC tumors exhibit high SOX10 expression, reflecting the nuclear immunoreactivity of the protein. The high percentage of tumors with strong SOX10 immunoreactivity aligns with the notion that SOX10 is frequently overexpressed in basal-like and TNBC subtypes, as documented in prior studies. For instance, Cimino-Mathews et al. (2013) reported that SOX10 expression was prevalent in basal-like breast cancers, particularly TNBC, supporting its role as a biomarker for this aggressive subtype.[182]

Assessment of age showed a statistically significant association with SOX10 expression in triple-negative breast cancer (TNBC) patients for both the immunoreactivity score and H-score analysis for overall age group differences. Pair- wise analysis showed that patients in the 50–59 age group showed a significantly lower H-score compared to the 30–39 and 60–69 age groups. In contrast, several studies have reported no significant association between SOX10 expression and age in TNBC. For instance, Salman et al. (2024) in Karbala found no statistically significant difference in SOX10 expression across age groups.[180] Similarly, Jin L. et al. (2020) and Tariq M. et al. (2024) reported no significant association between age and SOX10 levels.[175,183] The lower expression in patients between 50-59 years old could be because this age group often represents a transitional period for women entering or being in postmenopause, where hormonal changes, particularly a significant drop in estrogen and progesterone levels, may influence tumor biology.[184] The difference between the studies could be the influence of the sample size or inherent variability within the age groups. While statistically significant, the finding might reflect a context-specific phenomenon meaning because of sample size and natural sample differences between age groups and only specific to this sample rather than a universal pattern that could be generalized to everyone, this is supported by the lack of statistically significant correlation between H-score and age which suggests that the relationship may not be linear and could be based on specific age brackets.

The relationship between SOX10 expression and tumor grade in triple-negative breast cancer (TNBC) highlights the potential role of SOX10 as a marker of tumor aggressiveness and differentiation. In this study, a statistically significant association was observed between tumor grade and SOX10 expression, as measured by both the immunoreactivity score (IRS) and H-score. Tumors of lower grade exhibited lower IRS, whereas higher grades were associated with increased IRS, indicating an upregulation of SOX10 expression in more aggressive tumors. This is further supported by statistically significant association observed between the H-score and tumor grade. This means that tumors with greater cellular atypia, mitotic activity, and loss of differentiation, showed stronger and more widespread SOX10 expression. These findings align with previous studies, further validating the association between SOX10 expression and tumor grade in TNBC. Salman et al. (2024, Karbala) similarly reported a statistically significant increase in H-scores with higher tumor grades, reflecting the progressive upregulation of SOX10 in more poorly differentiated tumors.[180] Lie JL et al. also demonstrated significant associations, showing that higher tumor grades were associated with increased SOX10 expression levels.[183]

The analysis of SOX10 expression in relation to tumor stage (T-stage) and nodal stage (N-stage) also revealed statistically significant associations in this study, with both the immunoreactivity score (IRS) and H-score showed a statistically significant association with these parameters. Specifically, a moderate positive correlation was observed between the H-score and both T-stage and N-stage, suggesting that higher SOX10 expression is associated with more advanced tumor and nodal stages. The H-score showed a moderate positive linear correlation with T-stage, N-stage, tumor size, and the number of lymph nodes involved. These findings suggest that higher SOX10 expression is associated with larger tumors and greater nodal involvement, emphasising its potential role in tumor aggressiveness and metastatic capability. these findings are consistent with the findings of Salman et al. (2024, Karbala), who also reported significant associations between SOX10 expression and both T-stage and N-stage.[180] Similarly, Jin L et al. (2020) found a positive association between SOX10 expression and these clinical parameters.[183] These studies collectively reinforce the association of SOX10 with tumor progression and nodal metastasis in TNBC.

The correlation with tumor size and lymph node involvement observed in this study provides additional

depth, emphasizing the progressive increase in SOX10 expression with advancing disease when H-score was used. The linear correlation across multiple parameters (T-stage, tumor size, N-stage, and lymph node involvement) suggests that SOX10 could serve as a comprehensive marker of tumor burden.

The significant positive correlation between tumor grade and SOX10 expression in this study suggests that SOX10 may contribute to the dedifferentiation of cancer cells, a hallmark of higher-grade tumors.[183] This finding is consistent with the established role of SOX10 in maintaining stemness and promoting epithelial-mesenchymal transition (EMT). EMT is a critical biological process through which cancer cells lose their epithelial characteristics, gain mesenchymal features, and acquire enhanced migratory and invasive capabilities. Studies have shown that SOX10 overexpression facilitates EMT by reducing epithelial markers like E-cadherin and increasing mesenchymal markers such as Vimentin and N-cadherin, thereby promoting tumor cell motility and metastasis.[185] Experimental evidence from other studies further supports SOX10's role in promoting tumor proliferation. Colony proliferation assays and flow cytometry have demonstrated that interference with SOX10 expression reduces the proliferative capacity of TNBC cells while inducing apoptosis. This dual effect underscores SOX10's intrinsic functions as a regulator of cell survival, differentiation, and proliferation.[186] In this study, the correlation between SOX10 expression and higher tumor grade may partly reflect its role in enabling cancer cells to bypass apoptotic mechanisms, thereby contributing to tumor progression. the links between SOX10 expression, immune modulation, and apoptosis resistance suggest that targeting SOX10 could offer therapeutic benefits.

The growing body of evidence on SOX10 as a therapeutic target opens new avenues for potential treatment strategies in triple-negative breast cancer (TNBC). Knockdown of SOX10 expression in TNBC cell lines has been shown to inhibit cell proliferation, migration, and invasion, suggesting that SOX10 contributes to the aggressive behavior of these cancer cells. In vivo experiments further support this, demonstrating that suppression of SOX10 leads to reduced tumor growth and metastasis. Mechanistically, SOX10 regulates genes involved in cell survival, migration, and stemness, making it an attractive target for therapy. Current research is exploring various therapeutic approaches, including small-molecule inhibitors, RNA interference-based therapies, and immunotherapies aimed at targeting SOX10. Additionally, combination therapies that integrate SOX10 inhibition with conventional treatments are under investigation to enhance anti-tumor efficacy. While these findings are promising, further research is necessary to fully elucidate the therapeutic potential of SOX10-targeted strategies and to evaluate their safety and effectiveness in clinical settings.[169,187]

6. CONCLUSION AND RECOMMENDATIONS

Conclusions

1. SOX10 expression is highly prevalent in TNBC, with both immunoreactivity score (IRS) and H-score confirming its widespread expression. Variability in SOX10 detection methods across studies, particularly regarding scoring systems and cutoff thresholds, affects reported positivity rates.
2. The H-score is more sensitive as it reflects a wider range of staining intensities and percentages. In contrast, the IRS provides a simpler, categorical approach.
3. SOX10 is a potential biomarker for TNBC prognosis, given its association with tumor differentiation, invasiveness, and metastatic potential.
4. A strong correlation was found between SOX10 expression and tumor size, grade, T-stage, and N-stage, supporting its involvement in tumor aggressiveness and metastasis.
5. Age-related differences in SOX10 expression were observed, but findings were inconsistent across studies, suggesting a potential influence of sample demographics or hormonal factors.
6. Expand study of SOX10 as a marker for breast cancer in patients with other subtypes than TNBC.

Recommendations

1. Standardization of SOX10 Detection Methods

- Future studies should adopt a uniform scoring system (such as a standardized H-score or immunoreactivity score) with consistent cutoff values to improve comparability between studies.

2. Exploration of SOX10 as a Therapeutic Target

- Preclinical and clinical studies should further evaluate SOX10-targeted therapies, including small-molecule inhibitors, RNA-based therapies, and immunotherapies to determine their safety and efficacy.

- Combination therapies that integrate SOX10 inhibition with existing TNBC treatments (chemotherapy, targeted therapy, or immunotherapy) should be explored for potential synergistic effects.

3. Integration of SOX10 into Clinical Practice

- If validated in further research, SOX10 testing should be incorporated into routine TNBC diagnostic and prognostic evaluations to help guide personalized treatment strategies.

4. Larger, Multicenter, Longitudinal Studies

- Future research should include larger patient cohorts from multiple centers to improve the generalizability and reliability of findings related to SOX10 expression in TNBC.

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