"ASSOCIATION OF INTERLEUKIN 6 IMMUNOHISTOCHEMISTRY EXPRESSION WITH PLASMA ELISA INTERLEUKIN 6 LEVELS IN INVASIVE DUCTAL CARCINOMA BREAST"

BY

DR. ANKITA GIRDHAR, MBBS

DISSERTATION SUBMITTED TO
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH
TAMAKA, KOLAR, KARNATAKA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR IN MEDICINE
IN
PATHOLOGY

UNDER THE GUIDANCE OF
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DEPARTMENT OF PATHOLOGY
SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

JUNE 2023

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AT R.L. JALAPPA HOSPITAL AND RESEARCH CENTRE, KOLAR

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Abstract

Background: The most prevalent kind of cancer in women affecting one in eight of them is breast carcinoma. (BC). Due to the gradual shift in Indian women's lifestyles, breast cancer has lately overtaken cervical cancer as the most frequent carcinoma in Indian women. IL6 is a cytokine of the pro-inflammatory type. IL 6 is produced by fibroblasts and macrophage cells. The IL 6/STAT 3 pathway is inhibited by anti-IL 6 agents or anti-IL 6 monoclonal antibodies, which were developed in response to the function of IL 6 pathways in targeted therapy for breast malignancy

Aims and objectives:
1 To determine the proportion and intensity of expression of IL-6 in tissue sections of Invasive Ductal carcinoma breast.
2 To estimate plasma Interleukin 6 levels by ELISA method in Invasive Ductal Breast carcinoma
3 To evaluate the association between immunohistochemistry Interleukin 6 expressions versus plasma (ELISA) Interleukin 6 levels.

Materials and methods:
It was a Laboratory observational cross-sectional type of research done on Primary breast carcinoma specimens. The study duration was 18 months (January 2021 to June 2022). The study included all the fresh cases of Invasive Ductal Carcinoma breast (IDC) which was diagnosed by Fine Needle Aspiration Cytology (FNAC) and confirmed by Trucut biopsy and mastectomy specimens. Immunohistochemistry was performed on formalin fixative tissue sections with embedding by paraffin. Retrieval of antigen was done by using microwave in Initial Tris-EDTA at pH= 9 after which the endogenous peroxidase activity was destroyed with 3% hydrogen peroxide. The immunohistochemistry expression analysis was done using IHC scoring system. Plasma of newly diagnosed malignancy breast patients was taken for measuring IL6 levels by ELISA method.

Results:

Among 50 cases of IDC, the mean IHC expression of IL6 observed was 201.6±88.4. The mean ELISA IL6 expression observed was 46.13±9.98. The ELISA IL6 showed a statistically significant association with perimenopausal phase (p=0.011. Her2 new positive expressed (p=0.014), Ki-67 proliferation index (p=0.04), and Her2 enriched molecular category (p=0.04). IHC IL6 did not show any association with clinicopathological parameters. A negligible positive correlation means as IHC IL6 showed increased expression, ELISA IL6 also showed increase in values (p=0.217) as per Pearson coefficient

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ACKNOWLEDGEMENT

I begin by expressing my immense gratitude to the Almighty Lord for his blessings.

My continued reverence and acknowledgment to the beloved teacher and guide **Dr. Kalyani R**, Professor and HOD of Department of Pathology, who handpicked this topic and graced study officially with her constant support and expert advice, her encouragement, wise constructive judgment the painstaking effort to weed out errors, and her affection during study leave permanently indebted to her. I dedicate a good part of the work to her.

Sincere thanks to **Dr. Krishna Prasad ,** Professor and Head of Department of surgery**,** to consent to be the co-guide and give timely help throughout the PG career.

I take this opportunity to express my humble and sincere gratitude and indebtedness to the teacher **Dr. Kalyani R**, Professor and Head of Department, for her expert advice, constant support, encouragement, and timely help in every aspect.

I would like to express my gratitude to **Dr. Harendra Kumar M.L,** Professor, for his constant guidance, support, and encouragement.

I express my deep, immense gratitude and humble thanks to **Dr. T.N. Suresh,** Professor, for his support, advice, and encouragement.

I express my deep, immense gratitude and humble thanks to **Dr. Subhashish Das**, Professor, for his advice and encouragement throughout the study.

I express my deep, immense gratitude and humble thanks to **Dr. Hemalatha A**, Professor, for her advice and encouragement throughout the study.

I express my sincere thanks to **Dr. Supreetha M S**, **Dr. Shilpa M D**, **Dr.Sindhu C**, **Dr. Haritha** Assistant Professors, for their constant guidance and encouragement in preparing this dissertation.

I want to convey my sincere thanks to **Dr. Manjula K**, Professor, **Dr. Swaroop Raj B.V,** and **Dr. Sushan Shweta Jayekar**, Associate Professors, for their kind help, constant support, and expert advice in preparing this dissertation.

My loving family members **Dr. Ashok, Smt. Rajni and Dr Ankur** who will always be the most significant source of strength and inspiration for their unconditional support in every aspect of life.

I express my sincere thanks to my senior residents, **Dr. Nikhil, Dr. Sowjanya, Dr.Soumya, Dr. Princy** for their support and love in every aspect of life. I express my immense gratitude and special thanks to seniors **Dr. Gaurav and Dr. Priyanka** for their support.

I express my sincere thanks to my batchmates and friends, **Dr. Satadruti Chakraborty, Dr. Snigdha, Dr.Amrutha, Dr.Jahnavi Reddy, Dr. Ayswaria, Dr.Nagaraju and Dr. K.Sudarshan.**

I enjoyed working with my juniors **Dr. Haneena, Dr. Zubiya, Dr. Divya, Dr. Sahiti, Dr. Queen, Dr. Ambika, Dr. Priyanka, Dr. Deepika, Dr. Bhadra, Dr. Deepa, Dr. Kamala, Dr. Sushma, Dr.Manju, Dr. Nikitha, Dr. Pratibha** and **Dr. Sharju** and thank them for their kind cooperation.

I am thankful to technical staff and all non-teaching staff for their invaluable help, without whom this study would not have been possible.

Thank you, everyone.

Date : Signature of the Candidate

Place: KOLAR **Dr. Ankita Girdhar**

LIST OF ABBREVIATIONS

IHC – Immunohistochemistry

IL 6- Interleukin 6

ELISA – Enzyme linked immunosorbent assay

FNAC - Fine Needle Aspiration Cytology

ER – Estrogen Receptor

PR – Progesterone Receptor

Her 2 – Human epidermal growth factor receptor 2

IDC – Infiltrating Ductal Carcinoma

TDLU – Terminal duct lobular unit

WHO – World Health Organisation

AJCC – American Joint Committee on Cancer

H&E – Hematoxylin and eosin

NPI – Nottingham Prognostic index

TBS **–** Tris buffer Solution

ASCO – American Society of Clinical Oncology

CAP – College of American Pathologists

BRCA1 – Breast Cancer gene 1

BC - Breast carcinoma

SBR – Scarff Bloom Richardson grading

NOS – Not other specified

CAFs - Cancer associated fibroblasts

SDF-1 - Stromal cell-derived factor

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ABSTRACT:

Background

Breast carcinoma (BC) is one of the commonest malignancy in women affecting 1 in 8 women. IL6 is a cytokine of the pro-inflammatory type. The source of IL 6 is macrophage cells and fibroblasts. The role of IL 6 pathways in targeted therapy in breast cancer results in the development of anti-IL 6 agents or anti IL 6 monoclonal antibodies, which act as an inhibitor of the IL 6/STAT 3 pathway.

Aims and objective:

1. To determine the proportion and intensity of immunohistochemistry expression of IL 6 in tissue sections in Invasive Ductal carcinoma breast.
2. To estimate plasma Interleukin 6 levels by ELISA method.
3. To evaluate the association between immunohistochemistry Interleukin 6 expressions versus plasma (ELISA) Interleukin 6 levels.

Materials and methods

Laboratory observational cross-sectional study done on Primary breast carcinoma specimens. Duration of study was 18 months (January 2021 to June 2022). The study included all the fresh cases of Invasive Ductal Carcinoma Breast (IDC) which were diagnosed by Fine Needle Aspiration cytology (FNAC) and confirmed by Trucut biopsy and mastectomy specimens. Immunohistochemistry (IHC) was performed as per the Standard operating procedures (SOP's) of the department. IHC expression was evaluated utilizing H-score. Plasma of same diagnosed malignancy breast patients was taken for measuring IL6 levels by ELISA method.

Results:

Among 50 cases of IDC, the mean IHC expression of IL6 observed was 201.6±88.4. The mean ELISA IL6 expression observed was 68.13±89.98. The ELISA IL6 showed a statistically significant association with premenopausal phase (p=0.01), Her2 neu positive expression (p=0.04)), low Ki67 proliferation index (p=0.04), and Her2 enriched molecular category (p=0.04). IHC IL6 did not show any association with clinicopathological parameters. A negligible positive correlation means as IHC IL6 showed increased expression, ELISA IL6 also showed increase in values (p=0.217) as per Pearson coefficient.

Conclusion:

This study showed that with increasing IHC expression ELISA levels also increased. The ELISA IL6 levels showed a significant association with premenopausal phase**,** Her 2 neu positive expression**,** low Ki67 proliferative index and Her 2 enriched molecular category**.** This shows the potential of ELISA IL6 being a marker of good prognostic parameters.

Key words : Breast malignancy, IL6, Immunohistochemistry, ELISA

INTRODUCTION

INTRODUCTION:

Breast carcinoma is the most common malignancy in the female population, accounting for 11.7% of all cancer cases. It is the leading cause of death in women. This disease has become a severe threat worldwide due to the increasing incidence in developing and developed countries.

The incidence of breast cancer worldwide in 2020 was 22,61,419 (11.7%), and the number of deaths was 6,84,996 (6.9%). Among females, the incidence rate of BC is one of the leading cause of cancer death worldwide.[1] In India, recently breast carcinoma has overtaken carcinoma of the cervix to become the most common carcinoma among Indian women due to the gradual change in the lifestyle of Indian women.[1] In India, as per the GLOBOCAN data 2020, BC accounted for 13.5% (178361) of all cancer cases and 10.6% (90408) of all deaths with a cumulative risk of 2.81.[1] Approximately one in four women was newly detected and died due to BC in India.[2] The prevalence of Breast Cancer in Kolar reported is 6.41%.[3] In India, the age-adjusted incidence of BC among females is 25.8 per 1, 00,000 and the death rate is 12.7 per 1,00,000. The proportion of BC in Bangalore is 34.4%.[2] Breast cancer has a high risk of recurrence and metastasis.[4]

The College of American Pathologists (CAP) have identified many prognostic and predictive factors to guide the clinical management of affected women. The prognosis and treatment of patients depend on a few established parameters like tumor size, histological grade, histological subtype, lymph node status, overexpression of HER2/neu, estrogen receptor (ER), and progesterone receptor (PR), IL 6 status.[5]

IL6 is a cytokine of the pro-inflammatory type. The source of IL 6 is macrophage cells and fibroblasts. Interleukin 6, an acute phase protein, affects B cells and the induction of Th17 cells. Many types of cells release it in the tumor microenvironment, such as cancerous cells, and it affects tumorigenesis.[6,7]

For the evolution of Breast cancer, the stromal cells of the breast cancer microenvironment, which consist of fibroblasts, immune cells, endothelial cells, and adipocytes with altered phenotype and function, play a significant role and are the main objective for future treatment. High expression of IL 6 was produced by multidrug-resistant breast cancer cells, and it had been observed in exogenous adipocyte derived IL 6, which produces multiple chemotherapeutic drug resistance.[8]

The role of IL 6 pathways in targeted therapy in breast cancer resulted in the development of anti-IL 6 agents or anti IL 6 monoclonal antibody, which acted as an inhibitor of the IL 6/STAT 3 pathway.[8] For overall survival in Invasive BC, preoperative plasma IL 6 expression levels may be an independent prognostic factor.[9] In Breast Cancer, raised IL 6 levels were found in serum and the tumor site.[10]

AIMS AND OBJECTIVE

AIMS AND OBJECTIVE

Aim:

To derive the significance between Interleukin 6 immunohistochemistry levels and plasma Interleukin 6 levels in Invasive Ductal Carcinoma Breast.

Objectives:

1. To determine the proportion and intensity of immunohistochemistry expression of IL 6 in tissue sections in Invasive Ductal carcinoma breast.
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REVIEW OF LITERATURE

REVIEW OF LITERATURE :

The breast is a modified sweat gland in both females and males but is rudimentary in males. In females, it is well developed after puberty and is a vital accessory organ of the female reproductive system.[11,12]

Embryology and Development of Breast:

The breast develops from the ectodermal thickening that extends from the axilla to the groin called the milk line, mammary line, mammary ridge, or the line of Schultz. During 4th week of gestation, the milk line appears, but most of its extent, except the pectoral region, disappears. During the 7th week of pregnancy, the milk line or mammary line extends from the base of the forelimb to the region of the hindlimb. Most mammary lines disappear after their formation, and minor portions remain in the thoracic region and penetrate the underlying mesenchyme. Mammary pit is formed with the remaining part of the mammary ridge. From these pits, around 15-20 secondary buds will grow, and subsequently, these buds undergo divisions and subdivisions, forming lobes of the mammary gland. The glands and stroma are derived from the ectoderm and the mesoderm respectively. [11,13,14] During puberty, estrogen induces growth, and progesterone with prolactin aids in secondary alveoli development.

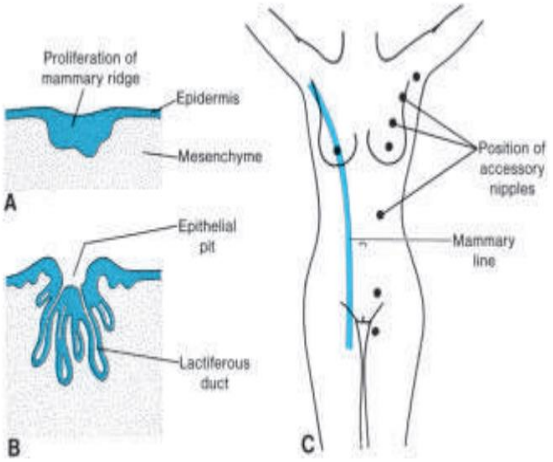


Fig 1 - A and B. Sections through the developing mammary gland at the third and eighth months, respectively. C. Positions of accessory nipples (blue line, mammary line).[13]

Gross Anatomy of Breast:

Breast is situated in the pectoral region. It extends vertically from the second to seventh rib and horizontally between the mid axillary line and lateral border of the sternum. It lies in the superficial fascia, and a small extension upwards and laterally pierces the deep fascia in the axilla is called the axillary tail of Spence.[15,16] The retro mammary space contains loose areolar tissue that separates the mammary gland from the pectoralis fascia that covers the pectoralis major muscle. Due to this loose areolar tissue, the breast can freely move over the pectoralis major muscle. Parts of the pectoralis minor, serratus anterior, and external oblique muscle lie deep in the breast.[16, 17]

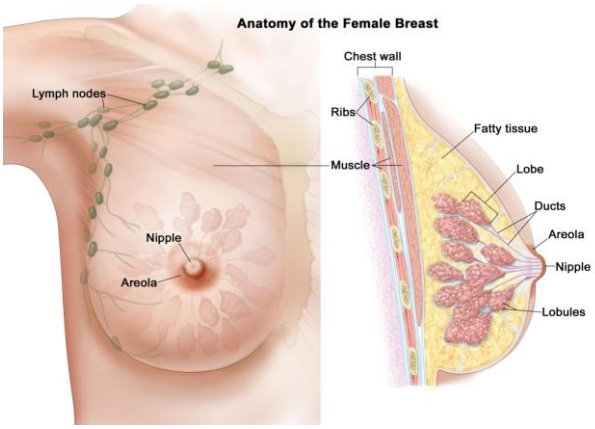


Fig 2: Anatomy of Female Breast[17]

Skin covers the entire mammary gland with a circular pigmented area called an areola. Areola is present around the base of a conical projection called nipple. Nipple is pigmented and present at the level of the fourth intercostal space. Skin of the nipple and areola are devoid of hair.[16,1718,19]

The breast parenchyma contains glandular tissue of 15-20 lobes of a cluster of alveoli that secretes milk and drains the lactiferous duct. These lactiferous ducts pierce the nipple and open on it. The dilatated terminal end of each duct is called the lactiferous sinus. The main duct branches repeatedly give rise to multiple terminal duct-lobular unit (TDLU) consisting of terminal ducts leading to a lobule with numerous acini.

The supporting framework of the glandular component is formed by fibro fatty stroma. The interlobular stromal tissue is more vascular and collagenous around each duct, and it separates the lobules with moderately dense collagenous tissue. The fatty stroma forms the main bulk of the mammary gland, while the attachment of skin and gland to the pectoralis fascia is by a fibrous part called the suspensory ligaments (of Cooper). [16,17,18,19]

Blood Supply Breast:

Mammary gland is a highly vascular organ. Breast is supplied by the branch of an internal thoracic artery, a lateral branch of a posterior intercostal artery, and the thoracoacromial, lateral and superior thoracic branches of the axillary artery. The posterior surface is relatively avascular, and its venous drainage follows the arteries. Near the base of the nipple, the veins form superficial and deep sets of an anastomotic venous circle. The superficial venous set drains into the superficial veins of the lower neck and to the internal thoracic vein, while the deep veins drain into the internal thoracic, posterior intercostal, and axillary veins.[16,17]

Lymphatic Drainage Breast:

The parenchyma of the breast along with the nipple and areolar skin is drained by the deep lymphatic channels while rest of the skin over the breast is drained by the superficial lymphatics.

The lymph from the breast drains into,

1. The axillary nodes chiefly anterior group which accounts for about 75% lymphatic drainage. They are divided into the anterior, posterior, lateral, central and apical groups.
2. The internal mammary nodes present along the internal thoracic vessels drains about 20%.
3. The supraclavicular, cephalic and posterior intercostal nodes which drains around 5% of lymph.
4. Lymph also drains into the subdiaphragmatic and sub peritoneal lymph plexus.
5. Cancer cells obstructing the superficial lymphatic vessels leads to edema of the skin causes orange peel like skin called as Peau d' orange appearance. It plays an important role in staging the carcinoma of breast.[16,17]

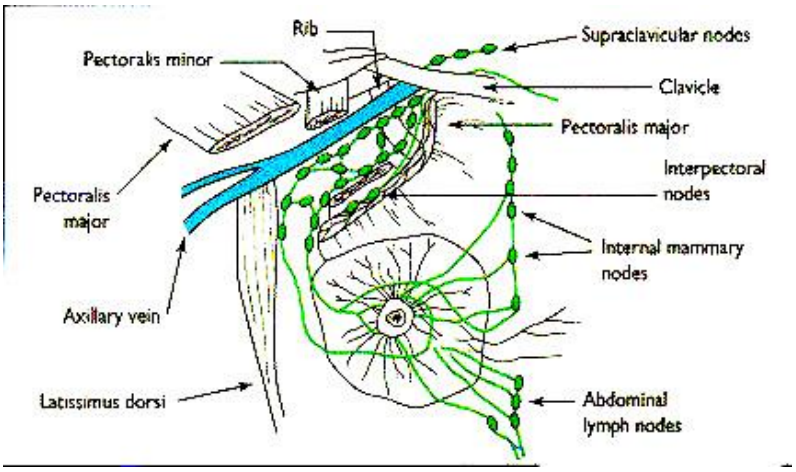


Fig 3: Lymphatic Drainage of Breast[16]

Nerve Supply of Breast:

Lateral and anterior cutaneous branches of 2nd to 6th intercostal nerves innervate the breast.[16]

Normal Histology of Breast:

The ducts and the acini are bilayered structures. The basal myoepithelial cells lie between the luminal epithelium and the basement membrane of the alveoli and surrounding the ducts. The luminal layer consists of tall columnar cells in larger ducts and cuboidal epithelial cells in smaller ducts and acini. They have a secretory function. The milk secreted in the alveoli passes into and along the lactiferous ducts due to the contraction of these cells. Around the epithelial lining of the ducts, a discontinuous layer of stellate myoepithelial cells having pale cytoplasm are seen. Near the nipple, the terminal parts of the lactiferous ducts are lined by keratinizing stratified squamous epithelium.[20]

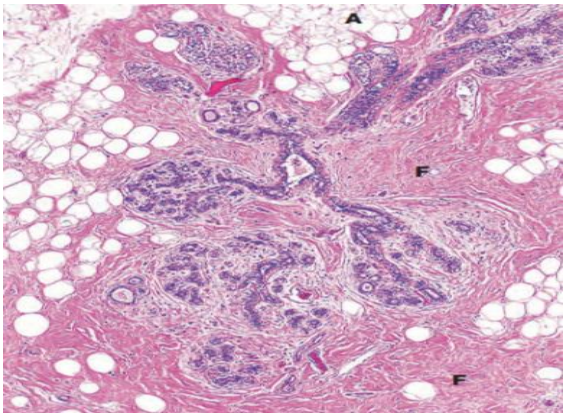


Fig 4: Normal female breast tissue adult shows terminal duct–lobular units (TDLU) at low magnification. The extensive branching duct system is surrounded by relatively dense fibrous interlobular tissue **F** and adipose tissue **A.**[20]

RISK FACTORS:

The incidence of breast carcinoma increases with the following risk factors:

1. Positive family history
2. Female sex
3. Early menarche
4. Germline mutation of tumor suppressor genes
5. Age – peak incidence at 70 – 80 years
6. Hormonal therapy
7. Nulliparous women or women who had their first child birth after 35years
8. Benign breast disease that revealed atypical hyperplasia or proliferative changes in previous biopsies

9. Obesity
10. Radiation exposure
11. Carcinoma of the contralateral breast or endometrium
12. Reduced duration of breast feeding
13. Environmental toxins (organochlorine pesticides)[21]

ETIOPATHOGENESIS:

Carcinoma breast are a clonal proliferation of cells with multiple genetic abnormalities influenced by inherited susceptibility genes and exposure hormones. Around 12% of carcinoma breast are familial, which occurs due to susceptibility genes inheritance. Some of the known susceptibility genes are– BRCA1, BRCA2, TP53, and CHEK 2 (tumor suppressor genes). The remaining breast cancer cases are due to significant risk factors, which are related to hormone exposure as well as environmental factors like radiation.[4,22,23]

- **Age:** The risk of developing breast cancer increases with age. By using the Surveillance, Epidemiology, and End Results (SEER) database, the probability of a woman in the United states developing breast cancer is a lifetime risk of 1 in 8; 1 in 202 from birth to age 39 years of age, 1 in 26 from 40-59 years, and 1 in 28 from 60-69 years.
- **Personal history:** A history of breast cancer is also a significant risk factor for developing a second ipsilateral or contralateral breast cancer. The most common cancer among breast cancer survivors is metachronous contralateral breast cancer.
- **Breast pathology**: Proliferative breast disease is associated with an increased risk of breast cancer. Proliferative breast lesions without atypia, including usual ductal hyperplasia, intraductal papillomas, sclerosing adenosis, and fibroadenomas, confer only a small increased risk of breast cancer development, approximately 1.5-2 times that of the general population.
- **Family history**: A woman's risk of breast cancer is increased if she has a family history of the disease.
- **Genetic predisposition**: Approximately 20%-25% of breast cancer patients have a positive family history, but only 5%-10% of breast cancer cases demonstrate an autosomal dominant inheritance.
- **Early menarche:** Early age at menarche is a risk factor among pre-and postmenopausal women for developing breast cancer. Delay in menarche by two years is associated with a corresponding risk reduction of 10%.

- **Parity and age at first full-term pregnancy:** Nulliparous women are at an increased risk for developing breast cancer compared to parous women.
- **Breast feeding:** Evidence suggests that breast feeding has a protective effect against the development of breast cancer.
- **Age at menopause:** Later onset of menopause has also been associated with increased breast cancer risk.
- **Hormone replacement therapy (HRT):** Evidence suggests a relationship between the use of hormone replacement therapy (HRT) and breast cancer risk. Breast cancers related to HRT use are usually hormone receptor positive. When compared with patients who do not use HRT, breast cancer risk is higher in HRT users.

- **Obesity:** Obesity, specifically in postmenopausal women, has also been shown to increase a woman's risk of breast cancer.
- **Radiation:** Radiation exposure from various sources, including medical treatment and nuclear explosion increases the risk of breast cancer.
- Radiation to the chest wall for childhood cancer treatment increases breast cancer risk linearly with chest radiation dose.[22,23]

MOLECULAR MECHANISM OF CARCINOGENESIS:

The hypothesis for breast cancer is that; resident breast tissue stem cells are the cell of origin for all breast cancers. First, these cells have to acquire a driver mutation. Then any one of the three major genetic pathways of carcinogenesis follows, these are:

1. The dominant pathway is linked with losses of chromosome 16q, gain of chromosome 1q, or activating mutations in PIK3CA. This pathway results in ER positive and HER2 negative cancers.
2. The pathway associated with HER2 gene amplification gives rise to HER2 positive cancers.
3. HER2 negative, ER negative cancers develop through a pathway independent of HER2 gene amplification and ER mediated changes in gene expression.[23,24,25]

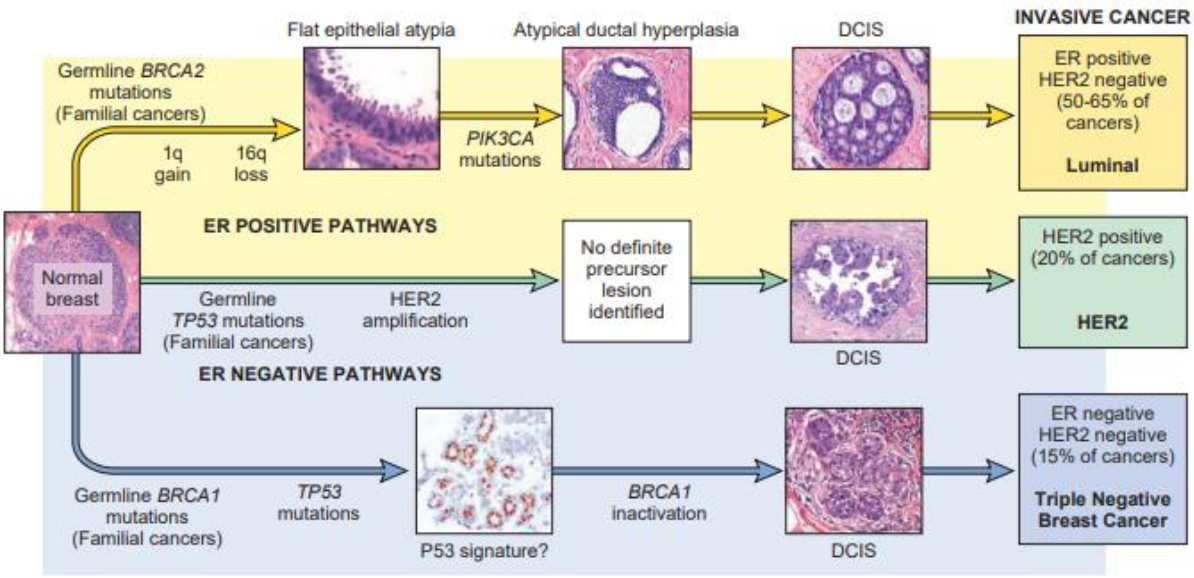


Fig 5: Major pathways of breast cancer development [23]

The box with the question mark indicates that no precursor lesions have been identified—perhaps because lesions progress quickly to carcinoma.

Molecular Regulation of Carcinogenesis[23,24,25]

- Chemical carcinogenesis: metabolism of chemicals by xenobiotic metabolizing enzymes:
- Electrophilic intermediates from numerous chemicals can form covalent bonds with nucleophilic regions on nucleotides within DNA, particularly proto-oncogenes or tumor suppressors. In the absence of accurate DNA repair of adducts, these chemical modifications can lead to changes in the encoded genes. This, in turn, can begin transforming a normal cell into a cancerous cell.
- Regulation of xenobiotic metabolizing enzymes by transcription factors:
- Glucocorticoid receptor and the estrogen receptor (ER) ligands regulate cellular function by regulating the expression of genes. Structural similarities between xenobiotics and natural chemicals can cause toxicity/cancer by interfering with normal homeostasis.
- Molecular and cellular signaling that cause carcinogenesis:
- As the tumor continues to grow, changes in cellular metabolism occur, which can include increased utilization of different substrates for energy to “feed” the tumor and

deprive surrounding normal cells of critical nutrients. New blood vessels can form (angiogenesis) because of a tumor's anoxic condition that provides more oxygen to be delivered to the tumor. This is mediated by growth factor/growth factor receptors (eg, vascular endothelial growth factor [VEGF]).[24,25]

CLINICAL FEATURES:

With the invention of routine screening techniques, more number of asymptomatic cases are being detected in recent years. However, many studies have established the difference in the frequency distribution of benign and malignant diseases between age groups. Benign lesions are more common among young female whereas, carcinomas are the common cause of symptoms in older women.[23]

Many patients present with complaints of lump in breast, with or without pain. Nipple abnormalities like retraction, discharge, eczema or distortion are other signs which are less common. Triple assessment by clinical examination, radiography (mammography / ultrasound) and tissue sampling (using core needle biopsy or fine needle aspiration cytology) is the mainstay for diagnosing of breast abnormalities. During clinical examination, assessment of the axillary lymph node is very important.[21]

LOCALISATION:

Most frequent origin of carcinoma breast is the epithelium of the terminal ductal-lobular unit (TDLU). Among 40-50% of cases the tumour arises in the upper outer quadrant of the breast. The frequency of breast cancer involvement of the other quadrants in descending order are central, upper inner, lower outer and the lower inner quadrant. Various morphological phenotypes in breast cancer, each having specific clinical characteristics or prognostic significance is there.[23,26,27,28]

TABLE 1 - WHO HISTOLOGICAL CLASSIFICATION OF BREAST TUMOURS:(2019)[26,27]

EPITHELIAL TUMOURS	
INVASIVE BREAST CARCINOMA	Infiltrating ductal carcinoma not otherwise specified (NOS) Oncocytic carcinoma Lipid rich carcinoma Glycogen rich Carcinoma Sebaceous carcinoma Lobular carcinoma NOS Tubular carcinoma Cribriform carcinoma Mucinous adenocarcinoma Mucinous cystadenocarcinoma NOS Invasive micropapillary carcinoma of breast Metaplastic carcinoma NOS
RARE AND SALIVARY GLAND TUMORS	Secretory carcinoma Acinic cell carcinoma Mucoepidermoid carcinoma Polymorphous adenocarcinoma Adenoid cystic carcinoma Classic adenoid cystic carcinoma Solid basaloid adenoid cystic carcinoma Adenoid cystic carcinoma with high grade transformation Tall cell carcinoma with reversed polarity
NEUROENDOCRINE NEOPLASMS	Neuroendocrine tumour, NOS Neuroendocrine tumour, grade 1 Neuroendocrine tumour, grade 2 Neuroendocrine carcinoma NOS Neuroendocrine carcinoma, small cell Neuroendocrine carcinoma, large cell
EPITHELIAL–MYOEPITHELIAL	Pleomorphic adenoma

TUMOURS	Adenomyoepithelioma Adenomyoepithelioma with carcinoma Epithelial-myoepithelial carcinoma
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)	Ductal carcinoma, non infiltrating, NOS DCIS of low nuclear grade DCIS of intermediate nuclear grade DCIS of high nuclear grade
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
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 Intraductal papillary adenocarcinoma with invasion
ADENOMAS	Tubular adenoma Lactating adenoma Duct adenoma NOS
MESENCHYMAL TUMOURS	
FIBROBLASTIC AND MYOFIBROBLASTIC TUMORS	Nodular fasciitis Myofibroblastoma

	Desmoid-type fibromatosis Inflammatory myofibroblastic tumour
PERIPHERAL NERVE SHEATH TUMORS	Schwannoma NOS Neurofibroma NOS Granular cell tumour NOS Granular cell tumour, malignant
SMOOTH MUSCLE TUMORS	Leiomyoma NOS Cutaneous leiomyoma Leiomyoma of the nipple and areola Leiomyosarcoma
ADIPOCYTIC TUMORS	Lipoma NOS Angiolipoma NOS Liposarcoma NOS
OTHER MESENCHYMAL TUMORS AND TUMOR – LIKE CONDITIONS	Pseudoangiomatous stromal hyperplasia
FIBROEPITHELIAL TUMOURS	Fibroadenoma NOS Phyllodes tumour NOS Periductal stromal tumour Phyllodes tumour, benign Phyllodes tumour, borderline Phyllodes tumour, malignant Hamartoma
TUMOURS OF THE NIPPLE	Nipple adenoma Syringoma NOS Paget disease of the nipple
MALIGNANT LYMPHOMA	Diffuse large B-cell lymphoma NOS Burkitt lymphoma NOS / Acute leukemia, Burkitt type Endemic Burkitt lymphoma Sporadic Burkitt lymphoma Immunodeficiency associated Burkitt lymphoma Breast implant associated anaplastic large cell

	lymphoma Mucosa associated lymphoid tissue lymphoma Follicular lymphoma NOS
METASTATIC TUMOURS	
TUMOURS OF THE MALE BREAST	Gynaecomastia Carcinoma Invasive carcinoma In situ carcinoma

INVASIVE DUCTAL CARCINOMA, NOT OTHERWISE SPECIFIED (NOS)

Invasive ductal carcinoma, not otherwise specified (ductal NOS) forms the most common group of invasive breast carcinoma. This heterogeneous group of tumours fails to exhibit distinct characteristics to be classified under a specific histological type. This group comprises around 40-75% of invasive carcinoma of breast and hence the most common type.[26,27]

These tumours do not have a specific gross findings. The size of tumour ranges from 1cm to 10cms. They usually have moderately or ill-defined edges, lack sharp border. Typically, ductal NOS carcinomas are firm to hard to palpate and may have a 'gritty' feel to cut with a knife. The cut surface appears grey-white with yellow streaks.

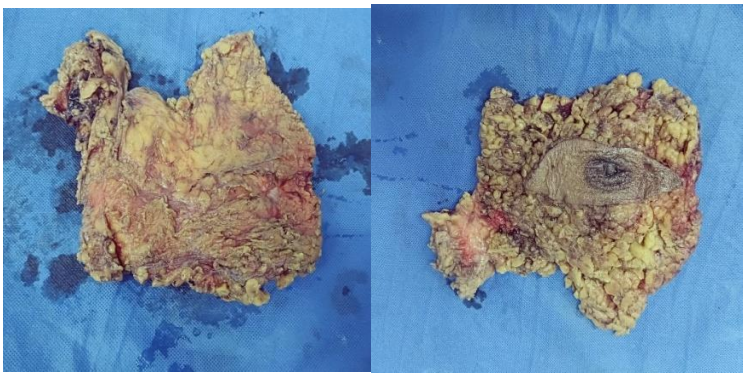


Figure 6: Gross image of mastectomy specimen with axillary clearance B/3399/22

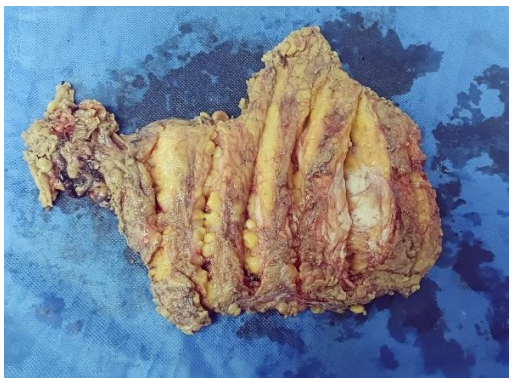


Figure 7: Cut surface showing grey white homogenous solid area B/3399/22

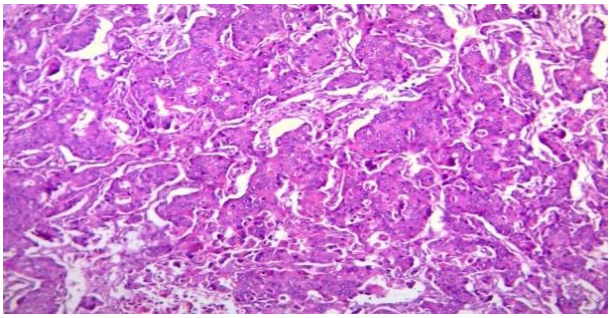


Figure 8: Microphotograph (400X): showing tumor cells are arranged nests and in sheets, individual cells are round to oval with pleomorphic vesicular nuclei having prominent nucleoli. Periphery showing chronic lymphocytic infiltration.

The tumour cells may be arranged in clusters, cords or trabeculae. Some of the tumours are composed mainly of tumour cells in solids or syncytial infiltrative growth pattern with little stroma in-between. In few cases, glandular differentiation may be seen in the form of tubular structures with a central lumen among the tumour cell groups.

The tumour cells have a variable presentation. They usually have abundant eosinophilic cytoplasm, uniform, round, or highly pleomorphic nuclei, with prominent multiple nucleoli. Mitotic activity varies from low to extensive. Among 80% of cases, foci of associated precursor lesions like ductal carcinoma in situ (DCIS) have been recorded. Such associated precursor lesions were of a higher grade comedo type, with a mixture of other patterns.

Invasive carcinoma of breast that are ≤1mm in size are known as microinvasive carcinoma.[26,27]

ONCOCYTIC CARCINOMA:

These tumours have clinical features resembling to that of invasive ductal carcinoma NOS type. The tumour should comprise >50% of cancer cells showing strong positivity for mitochondrial immunohistochemical staining to be classified under this category.

The tumour is composed predominantly of solid sheets, nests and islands with pushing borders. Rarely, papillary, glandular and plexiform pattern are also seen. The tumour cells display abundant granular eosinophilic cytoplasm with distinct cell borders and round centrally placed nuclei with prominent nucleoli. The characteristic appearance of the cytoplasm is due to abundant number of mitochondria.[27,28]

LIPID-RICH CARCINOMA:

Another variant of invasive breast carcinoma composed of tumour cells with concentrated cytoplasmic neutral lipids in the form of intracytoplasmic vacuoles. These vacuoles can be stained with lipid stains like Sudan III or Oil Red O. The tumour cells have irregular nuclei displaying moderate to severe atypia with one or more nucleoli.

In most cases, these tumours are of higher histological grade. Prominent mitotic activity is a common feature. Most of these tumours are negative for ER and PR factor, and have a higher tendency for HER2 positivity and high rate of proliferation. [27,28]

GLYCOGEN-RICH CARCINOMA:

Glycogen-rich clear cell carcinoma (GRCC), a variant of invasive breast carcinoma, contain polygonal to round cells with sharp well defined cell borders displaying clear or finely granular cytoplasmic vacuoles and round to oval nuclei and clumped chromatin with prominent nucleoli. The cytoplasmic vacuoles contain, PAS positive, diastase sensitive glycogen.[28]This variant of breast carcinoma is found to be more likely associated with triple negative, a higher grade, advanced stage cancer.

SEBACEOUS CARCINOMA:

It is a rare variant of invasive breast carcinoma showing prominent sebaceous differentiation. This variant originates from the mammary gland parenchyma.

The tumour cells range from small monomorphic cells to pleomorphic large cells with dominant clear to vacuolated cytoplasm and eccentrically placed nuclei. The cytoplasmic vacuoles stain positive for Oil Red O. Another feature of tumour is composed of ovoid to

spindled cells with non- vacuolated cytoplasm, located towards the periphery of the lobules. Many mitotic figures can be seen. The tumour cells are positive for adipophilin[27,28]

INVASIVE LOBULAR CARCINOMA:

Invasive lobular carcinoma forms 5-15% of invasive breast tumours.

Grossly, invasive lobular carcinoma are difficult to define due to the diffuse growth pattern of cancer. The borders are irregular and poorly defined.[27,29]

Invasive lobular carcinoma is formed by proliferation of discohesive, individually dispersed small cells, infiltrating to a fibrous connective tissue. A typical pattern where tumour cells are arranged in single linear cords infiltrating into stroma with thin rim of cytoplasm, rarely with intracytoplasmic lumen that harbours a mucoid material. The nuclei of cancer cells are round or notched ovoid. These tumours show many mitotic figures. About 90% of cases are associated with lobular carcinoma in situ (LCIS).

TUBULAR CARCINOMA:

Pure tubular carcinoma forms <2% of invasive breast carcinoma.

On Gross examination, tubular carcinoma is smaller in size ranging from 0.2 to 2cms in diameter. The pure tubular type has a prominent stellate configuration with radiating arms with central yellow flecks, due to stromal elastosis.

Tubular carcinoma is composed of open tubules lined by a single layer of epithelial cells with a clear lumen. A part of these tubules appear angulated while others are generally rounded or oval. The malignant epithelial cells are regular and small in size with mild nuclear pleomorphism. Myoepithelial cells are not present. Another important feature is cellular desmoplastic stroma around the tubules. Calcification may be present.[27,29]

CRIBRIFORM CARCINOMA:

Cribriform carcinoma accounts for 0.8 to 3.5% of breast carcinoma. It has an excellent prognosis. The tumour is composed of cancer cells arranged as invasive islands with angulation, which have well defined spaces, formed by arches of cells forming a sieve-like or cribriform pattern. Apical snouts are a common finding in this variety. Tumour cells are small with mild to moderate nuclear pleomorphism with reactive fibroblastic stroma. Mitotic figures are rare..[27,29]

MUCINOUS ADENOCARCINOMA:

Pure mucinous carcinoma forms about 2% of all forms of carcinoma breast. It has a favourable prognosis. On examination, these are soft in consistency and have a typical glistening gelatinous cut surface. Size of tumour is highly variable ranging from <1cm to >20cms (but average is 2.8cms)

The tumour is composed of proliferation of round cells with eosinophilic cytoplasm. Tumour cells are arranged in clusters as well as dispersed singly in pools of mucin, with intervening delicate fibrous septa containing capillary sized vessels. Mitosis, atypia and micro calcifications are not frequent features. The pools of mucin are positive for mucicarmine.[27,29]

MUCINOUS CYSTADENOCARCINOMA:

Mucinous cystadenocarcinoma is a rare subtype of invasive breast carcinoma. On gross examination, tumour is well-circumscribed, solid and cystic, with the cysts containing gelatinous material.

This form of carcinoma is characterized by cystic structures lined by tall columnar cells with stratification, papillae formation and tufting. The neoplastic epithelium has abundant intracytoplasmic mucin and cystic spaces lack myoepithelial lining.[27,29]

INVASIVE PAPILLARY CARCINOMA:

Invasive papillary carcinoma is a rare type of invasive adenocarcinomas characterized predominantly of papillary morphology (>90%) in the invasive component. The papillae is formed by malignant epithelial cells related intimately to fine fibro vascular core. Myoepithelial cells are not present at the periphery and along the papillary stalks. [27,29]

INVASIVE MICROPAPILLARY CARCINOMA:

Pure invasive micropapillary carcinoma of breast is very rare form, and forms 0.9-2% of breast cancers. These tumours are characterized by morula like aggregates of cuboidal to columnar tumour cells which lacks fibrovascular core, surrounded by empty stromal spaces. The neoplastic cell clusters are characterized by a reverse polarity i.e., "inside-out" pattern.

They are associated with more frequent peritumoral lymphovascular invasion and axillary nodal involvement. Hence these subtypes of carcinomas have worse prognosis.[27,29]

CARCINOMA WITH APOCRINE DIFFERENTIATION:

Apocrine carcinoma is another rare subtype that is common among older women. On examination presents with a firm, poorly circumscribed mass. The tumour cells are characterized with large abundant granular eosinophilic or vacuolated cytoplasm, round to oval enlarged nuclei exhibiting moderate to marked atypia with prominent nucleoli. The cells are arranged in solid sheets with moderate to high mitotic rate. [27,29]

METAPLASTIC CARCINOMA:

Metaplastic carcinoma is a group of neoplasms formed by differentiation of neoplastic epithelium into squamous cells and/or mesenchymal-looking elements (spindle, osseous, chondroid, rhabdoid). Constitutes for only about 0.2-1% of all breast cancers, these tumours have age distribution and clinical features similar to that of ductal carcinoma NOS. This carcinoma consists of a heterogenous group of tumours. Hence a descriptive classification system was adopted. [27,29]

Descriptive classification of metaplastic carcinoma[27,29]: -

1) Low-grade adenosquamous carcinoma
2) Fibromatosis-like metaplastic carcinoma
3) Spindle cell carcinoma
4) Squamous cell carcinoma
5) Mixed metaplastic carcinoma
6) Metaplastic carcinoma with mesenchymal differentiation

The AJCC 8th edition has also given staging by pTNM (tumour, nodal status and metastasis) classification, which is the most widely employed system.[29]

pTNM CLASSIFICATION OF TUMORS OF THE BREAST: [27,29]

TABLE 2 - pT – Primary tumour

pTx	Tumor cannot be assessed
pT0	No evidence of primary tumour
pTis	Ductal carcinoma in situ, Paget's disease, encapsulated papillary carcinoma and solid papillary carcinoma
pTis (DCIS)	Ductal carcinoma in situ without invasive carcinoma
pTis(Paget's)	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 peaud'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)

TABLE 3 - Lymph nodes (pN)

pNx	Lymph nodes cannot be assessed
pN0	No regional lymph node metastasis histologically
pN0(i-)	No regional lymph node metastasis by histology or immunohistochemistry
pN0(+)	Isolated tumour cells (cluster ≤ 0.2 mm and < 200 cells)
pN0(mol+)	RT-PCR positive but negative by light microscopy
pN1mi	Micrometastasis (tumour 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

TABLE 4 - Distant metastasis (M)

M0	No distant metastasis
pM1	Distant metastasis histologically proven > 0.2 mm

TABLE 5 - HISTOPATHOLOGICAL STAGING :[29,30,31,32]

Stage	Tumor	Lymph node	Metastasis
Stage 0	**Tis**	**N0**	**M0**
Stage IA	**T1**	**N0**	**M0**
Stage IB	**T0, T1**	**N1mi**	**M0**
Stage IIA	**T0, T1**	**N1**	**M0**
	T2	**N0**	**M0**
Stage IIB	**T2**	**N0**	**M0**
	T3	**N0**	**M0**
Stage IIIA	**T0,T1,T2**	**N2**	**M0**
	T3	**N1, N2**	**M0**
Stage IIIB	**T4**	**N0, N1, N2**	**M0**
Stage IIIC	**Any T**	**N3**	**M0**
Stage IV	**Any T**	**Any N**	**M1**

GRADING OF INVASIVE CARCINOMA OF BREAST:

The histologic appearance of a tumour can be matched with its degree of malignancy.[33] The assessment of the degree of differentiation using morphology of the tumour cells provides a useful prognostic information in carcinoma breast.[33,34]

Many studies have shown a significant association between histological grade and survival rate in patients with invasive breast cancer. It is a powerful prognostic factor and hence an important component in a pathology report of breast carcinoma. One of the most commonly used systems is the **Nottingham histologic score system**, otherwise called the **Elston-Ellis modification of Scarff-Bloom-Richardson grading system.**[29,35]

METHOD OF GRADING:

The grading of invasive carcinomas of breast done based on an evaluation of tubule or gland formation, mitotic count ad nuclear pleomorphism. A numerical scoring system ensures that each factor is assessed individually. Slide fields comprising representative tumour cell burden should be assessed.[29,36,37]

Tubules and glandular acini are the structures exhibiting clear central lumina. Cut-off points of 75% and 10% of tumour area composed of glandular differentiation are used to allocate the score.

Assessment of nuclear pleomorphism depends on the nuclear regularity in size and shape compared to adjacent normal epithelium. Additional features useful in allocating scores for pleomorphism are increased irregularity of nuclear margins and number of nucleoli.

Mitosis evaluation done based on defined mitotic figures counted. Number of mitosis per 10 high power fields counted for assigning a score. Peripheral leading edge of the tumour selected for assessment of mitotic count.

The grade is assigned based on score by adding 3 values [29]

TABLE 6 – MODIFIED SCARFF-BLOOM-RICHARDSON HISTOLOGICAL GRADING

FEATURES	SCORE
TUBULE / GLAND FORMATION	
>75% of tumour	1
10-75% of tumour	2
<10% of tumour	3

NUCLEAR PLEOMORPHISM	
Small, regular and uniform cells	1
Moderate increase and variability in size	2
Marked variation	3
MITOTIC COUNT / 10 high power fields	
0-5	1
6-10	2
>11	3

Grade 1 (well differentiated) - 3-5 points

Grade 2 (moderately differentiated) -6-7 points

Grade 3 (poorly differentiated) - 8-9 points

EVOLUTION OF PROGNOSTIC INDEX IN BREAST CARCINOMA:

Von Hansemann is the one behind many of the histological grading systems used for grading cancer. He explained that, the greater the degree of nuclear atypia, the greater the risk of metastasis occurring.[34]

Greenough developed a grading system for carcinoma breast, based on the cytological and histological features, which divides the cancers into 3 histological grades. This system includes the proportion of tubule formation, variations in size of both cells and nuclei, the secretory activity of tumour cells, nuclear hyperchromatism and mitotic count

Patey and Scarff simplified Greenough's method by taking into account only 3 features including tubule formation, nuclear hyperchromatism and variation in size and shape of nucleus.[35]

This system of grading was further modified by Bloom and Richardson in 1957. They introduced a numerical scoring system to the existing method, such that it combines the nuclear pleomorphism with a measurement of differentiation as well as an assessment of mitotic activity.[34]

In 1957, Emad Rakha et al conducted a large study of 2219 cases. It was observed in the study that the histologic grade (by Nottingham modification by Bloom Richardson histological grading system) provides a very good prediction of prognosis in patients with invasive breast carcinoma and hence should be included in the breast carcinoma staging systems.[34]

Many prognostic factors for breast carcinoma have been described in the past, but only a very few retain their independent significance when multivariate analysis is done. Since prognosis is multifactorially determined, the best discrimination is achieved by integrating independently significant factors. Nottingham prognostic index is a method of grading done by integration of various prognostic factors, which is being used widely.

NOTTINGHAM PROGNOSTIC INDEX (NPI):

Prognosis assessment of breast carcinoma is essential as it aids the clinician to assess risk and tailor treatment plans for each patient.

First described by Galea in 1982, Nottingham prognostic index (NPI) is the only index available with both intra and inter-centre prospective validation.[29]

NPI takes into account the features namely tumour size, histological grade of the tumour and no. of lymph nodes with metastasis. In a study NPI was used to predict the survival of 80%, 42% and 13% in the 3 groups of breast cancer patients according to the NPI scores. Therefore, it is considered as a surrogate marker of aggressiveness in breast cancer.[33,34,35] NPI is a numerical value derived by adding the values of tumour size (maximum dimension in centimetres) multiplied by coefficient of 0.2, histological grade (according to Nottingham modification of Bloom Richardson grading system) and lymph node stage (expressed as a numerical score between 1 and 3).[36,29]

NPI= LN (1-3) + Grade (1-3) + [maximum diameter (cms) x 0.2]

Where, LN is a numerical score assigned to the number of nodes positive for malignancy as given as follows.[29]

1. 0 positive nodes
2. 1-3 positive nodes
3. ≥4 positive nodes

The previous system employed a 3-tiered classification, which was dividing the cases into good, moderate and poor prognostic groups based on cut-off points between the values ≤3.4, 3.4 to 5.4 and >5.4.

This system has been modified by a lot of researchers over the years, to a four to six-tiered classes, with slight variability in interpretation.[36]

PROGNOSTIC PARAMETERS IN INVASIVE BREAST CARCINOMA:

Prognostic factors are helpful for the selection of appropriate treatment in case of breast cancers but not specific predictors of response to a therapy. Patients with extremely good prognosis after surgical removal, may not need adjuvant chemotherapy or radiotherapy, as they themselves cause significant morbidity to the patient.[37,38] Likewise, patient with poor prognosis may have increased survival with an aggressive adjuvant approach. As invasive breast cancers follow markedly variable course, identifying prognostic factors plays a major role.[39,40] For the same reason, prognostic and predictive factors in breast carcinoma has become a important topic of studies in recent times.[41,42]

The prognosis of invasive breast carcinoma is associated with many clinical and pathological parameters as follows;

1) **Age of patient** - relative survival reduces after 50 years of age.[42,43]
2) **BRCA1 status –** breast cancer patient carrying BRCA1 mutation carry worst overall outcome in the absence of adjuvant therapy.[44,45]
3) **Pregnancy –** breast carcinomas that manifest during pregnancy or lactation are usually aggressive tumours with a poor prognosis.[46]
4) **Early diagnosis –** asymptomatic early detection breast cancer patients have a higher relative survival rate.

5) **Presence or absence of invasiveness –** about 50% of invasive breast carcinoma patients presents with local or distant metastasis at the time of diagnosis, so a poor prognosis.[44]
6) **Size -** the diameter of the tumour is correlated with the incidence of nodal metastasis and also survival rate.[45]
7) **Histological type –** some subtypes of invasive carcinoma breast including tubular carcinoma, invasive cribriform carcinoma, mucinous carcinoma, invasive lobular carcinoma, medullary carcinoma have been observed to have a more favorable prognosis.
8) Histological grade
9) **Tumour necrosis –** extensive tumour necrosis is found to be linked with lymph node metastasis and decreased survival rate.
10) **Tumour - Infiltrating lymphocytes (TILs)** - shows the immune response of the patient to tumour cells. Higher number of TILs have been found to be associated with a good prognosis.
11) **Lymph node metastasis –** more the number of nodes with tumour metastasis is associated with poor prognosis.[46]
12) **Radiation** [42]

STROMAL REACTION AND TUMOUR MICROENVIRONMENT:

The stromal component in IDC-NOS varies between highly cellular fibroblastic proliferation, marked hyalinization or foci of periductal or perivenous elastosis, scant element of connective tissue. Some lDC-NOS show a fibrotic focus, which is an area of exaggerated reactive tumour stroma formation > 1 mm within the tumour, with or without coagulative necrosis and these cases have been found with a more- aggressive behaviour.

TILs (Tumour Infiltrating Lymphocytes) are mononucleated lymphoid cells infiltrating the tumour and also its stroma. In recent years it has been identified as an important prognostic marker, with high numbers of TILs associated with better outcome and better response to neoadjuvant therapy in triple-negative and HER2- positive breast carcinomas. Recent studies also suggest that TILs may also affect cancer invasion and metastasis. TILs are found to be affected by the subtype of breast cancer. Higher TILs density is associated with Hormone-receptor negative (HER2 enriched and triple negative) subtypes of breast carcinoma. The biological association between TILs and primary breast carcinoma also differ

between ER-positive and ER-negative cases. For example, High TIL expression was associated with poor prognosis in ER-positive patients, whereas that in ER-negative patients was a good prognostic marker. TILs have a strong prognostic value in improving estimates of distant recurrence-free survival, disease-free survival, and overall survival in early-stage TNBCs treated with standard adjuvant/neoadjuvant chemotherapy.[26,27,47]

Quantification of TILs - done on H&E-stained tissue sections from most representative tumour block. TILs should be scored in the stroma between the areas of carcinoma, and all mononuclear cells (lymphocytes and plasma cells) should be included. Stromal TILs should be scored as a percentage of the stromal areas alone. Carcinomatous area should not be considered under the area studied. Peritumoral follicular aggregates and tertiary lymphoid structures with germinal centres should not be included in the stromal TIL assessment.[26,27,48]

IMMUNOHISTOCHEMISTRY (IHC) :

Immunohistochemistry is a technique, which depends on antigen antibody recognition. It is used for localizing specific antigen in cells or tissues using a light microscope. Even though the history of IHC dates back to 1940s, only since the early 1990s, this method is widely used in surgical pathology.[49]

The enzymatic label (horseradish peroxidase) which was developed by Avrameas, Nakane and colleagues made it possible to visualise the labelled antibody, in the presence of a chromogenic substrate system, using light microscope.[49] In recent times IHC has been adapted for diagnostic purposes, identification and demonstration of prognostic and predictive markers.

BIOLOGIC MARKERS IN BREAST:

ESTROGEN RECEPTOR (ER):

This is a nuclear marker demonstrated both in ductal and lobular epithelium, with a higher proportion observed in lobules than in ducts. Among premenopausal women, there is usually an inverse relationship between the expression of estrogen receptor and markers of cell proliferation. The ER positive cells do not express the marker of proliferation (anti Ki-67), whereas the Ki-67 positive cells are usually ER negative. The proportion of cells that express ER gradually increases with age, but remains relatively stable after menopause.[48]

There are two forms of ER (ER α and ER β) observed to be expressed in normal breast tissue. The expression of ER α levels varies with the phase of menstrual cycle, whereas ER β

does not show any such variations. Myoepithelial cells do not show immunoreactivity for ER α, but expresses ER β. Higher levels of ER β was observed to be protective against neoplastic progression in breast.

PROGESTERONE RECEPTOR (PR):

The progesterone receptor is also known as NR3C3 (nuclear receptor subfamily 3, group C, member 3). It is a nuclear protein which gets activated by the steroid hormone progesterone. This nuclear marker is expressed in both lobular and ductal epithelial cells. The expression of PR does not show any variations with the menstrual cycle.[48,49]

PROTOCOL FOR REPORTING ER AND PR IHC:

ASCO and CAP have issued recommendations for reporting the results of immunohistochemically assays for ER and PR. Various studies suggest that cases with higher hormone receptor levels associated with good chance of response to hormonal therapy. Expression of receptors by as low as 1% of tumour cells has also been associated with clinical response. After consideration of these results, the guidelines recommend classifying all cases with at least 1% positive cells as receptor positive. [50,51,52]

SCORING SYSTEM FOR ER AND PR EVALUATION:

There are two popular scoring systems, the Allred and H scores, used for quantification of the immunoreactivity of tumour cells for estrogen and progesterone receptor.

The Allred score is used for quantifying ER and PR expression by tumour cells. It is a combination of the percentage of positive cells and the intensity of staining in the majority of the carcinoma. The two scores are added to obtain a final score. (ALLRED SCORE)

TABLE 7 – ALLRED SCORING: PROPORTION SCORE

Proportion Score (PS)	% Positive Cells
0	0
1	<1%
2	1% - 10%
3	11% - 33%
4	34% - 66%
5	>67%

TABLE 8 – ALLRED SCORING: INTENSITY SCORE

Intensity score (IS)	Intensity of positivity
0	None
1	Weak
2	Intermediate
3	Strong

The 2 scores are added for a total score.[27,29]

INTERPRETATION:

Negative - 0, 2

Positive - 3, 4, 5, 6, 7, 8

OTHERS:

Normal epithelial cells of breast consistently express anti-apoptotic protein Bcl-2. Variable expression of lactalbumin, gross cystic disease fluid protein-15, casein and CD117 along with cytokeratins 7, 8, 18 and 19 are also observed in the epithelial cells. Myoepithelial cells strongly express S-100 along with variable expression of cytokeratins 5, 6, 14 and 17.

HER-2/neu:

HER2/neu is a proto-oncogene product also called as c-er B-2. The amplification and overexpression of this protein in breast carcinoma is associated with poor survival outcome.[51,52]

Its major significance is that it is a known predictor of response to targeted therapy against this transmembrane protein.[44,52,53] The association of HER2/neu with breast carcinoma was first observed by Vijver et al in 1988.[54]

Azizun-nisa et al in a study with 150 cases, conducted in 2008 concluded that 24.7% of breast cancer cases have HER2/neu overexpression and the overexpression is associated with increase in tumour size and grade.[55]

ASCO and CAP have issued recommendations for reporting the results of HER2 testing. It recommends that intratumoral heterogeneity of HER2 gene amplification be reported when present.[56]

TABLE 9- REPORTING RESULTS OF HER2 NEU TESTING BY IHC:[29]

IHC RESULTS	CRITERIA
Negative (Score 0)	No immunoreactivity or immunoreactivity in ≤10% of tumour cells.
Negative (Score 1)	Faint weak immunoreactivity in >10% of tumour cells but only a portion of the membrane is positive.
Equivocal (Score 2)	Weak to moderate complete membrane immunoreactivity in >10% of tumour cells or circumferential intense membrane staining in ≤30% of cells.
Positive (Score 3)	More than 30% of the tumour cells must show circumferential intense and uniform membrane staining. A homogeneous (chicken wire) pattern should be present.

PROLIFERATION INDEX:

Antigen Ki-67 is a cellular marker, which codes for proliferation encoded by MKI-67 gene. This protein can be detected by monoclonal antibody Ki-67 (MIB1). It is a nuclear protein that is essential for cellular proliferation and associated with ribosomal RNA transcription.

Ki-67 antigen is present during all the active phases of cell cycle including G1, S, G2 and mitosis, but is absent in resting cells (G0). With this feature Ki-67 is recognized as an excellent marker to assess the growth fraction of a given cell population. The percentage of tumour cells that are positive for KI-67 often correlates with the clinical course of the carcinoma breast.[57,58]

RECEPTOR STATUS:

IHC has been used for the evaluation of receptor status of breast carcinoma, which assess the presence of estrogen receptor, progesterone receptor and Her2 receptor. Assessment is very essential for selection of appropriate usage of targeted therapy. Targeted therapy has become one of the most effective adjuvant treatments for breast carcinoma, which has added to better outcome.

Receptor status evaluation done to categorize breast carcinoma into several molecular subtypes. The following are the clinicopathological definitions of invasive breast

carcinoma subtypes as adopted by the thirteenth St. Gallen international breast cancer conference held in 2013.[59]

MOLECULAR CLASSIFICATION OF BREAST CARCINOMA[27]

1. **Luminal A-like**

- ER – positive
- PR – positive
- Her2 – negative
- Ki 67 (proliferation index) - low (<15%)
- Expression of luminal (low-molecular-weight) cytokeratins, and high expression of hormone receptors and associated genes.
- ~60% of invasive breast cancers.

2. **Luminal B-like (HER2-negative)**

- ER – positive
- Her2 – negative
- Expression of luminal (low-molecular-weight) cytokeratins and moderate to weak expression of progesterone receptor and associated genes.
- At least one of the following
    - Ki 67 (proliferation index) – high (>15%)
    - PR – low or negative
- ~10% of invasive breast cancers

3. **Luminal B-like (HER2-positive)**

- ER – positive
- Her2 – amplified or overexpressed
- Ki 67 (proliferation index) -any
- PR – any

4. **HER2 ENRICHED**

- Her2 – amplified or overexpressed
- ER – absent
- PR – absent

- ~15% of invasive breast cancers
- High proliferation rate TP53 mutation common
- May be high grade and node positive

5. Basal like or Triple negative breast carcinoma

- ER – absent
- PR – absent
- Her2-negative High expression of basal epithelial genes, basal cytokeratins
- ~15% of invasive breast cancers
- High proliferation rate TP53mutation common
- BRCA1dysfunction (germline,sporadic)

These molecular classes play a important role in clinical evaluation because each subtype have different prognosis and different response to specific therapies.[59]

- ER positive / PR positive cancers have a better prognosis compared to ER positive/ PR negative tumours which in turn have a better prognosis than ER negative / PR negative cancers.[58]
- Her2/neu receptor considered as both a prognostic and predictive marker in breast carcinoma, is observed to be overexpressed in about 10-20% of invasive breast carcinomas.
- Amplification of Her2 receptor is associated with a poor prognosis. [60,61,62]

CYTOKINES:

Cytokines are large group of proteins, glycoproteins or peptides that are secreted by specific cells of immune system. The term cytokine has been used to include variety of factors including Interleukins, Colony Stimulating Factors, Interferons, Growth factors. Cytokines are principally synthesized by leukocytes and they, primarily acted on (other) leukocytes, and thus could be called interleukins (ILs).[63,64]

Cytokines regulate the proliferative, differentiation and maturation events though they are in anomolar-to-picomolar concentrations, as they have high affinity for their receptors. Cytokines are synthesized locally and also immediately secreted when stimulated.[63]

Cytokines show properties of pleiotropism and redundancy. Many cytokines are pleiotrophic, means having multiple activation on different target cells and or overlapping

cell regulatory actions.[64] Cytokines interact in a network firstly by inducing each other, secondly, trans modulating cell surface receptors and thirdly, by a synergistic, additive or antagonistic interaction on cell function.[65] Cytokines are either pro inflammatory or anti-inflammatory in nature. In periodontal diseases, the balance between the pro-inflammatory and anti-inflammatory cytokines is altered leading to the excessive production of pro inflammatory cytokines in comparison to antiinflammatory cytokines. A number of studies have shown that a variety of inflammatory mediators, such as interleukin (IL)-1β, IL-6, IL-8, prostaglandins, tumour necrosis factor-α (TNF-α) and matrix metalloproteinases (MMPs) are involved in pathogenesis of periodontal diseases.[66]

Functions of cytokines:

Functions of cytokines are divided into four broad categories, depending on
biologic actions of a particular cytokine

1. Mediators of natural immunity, which are triggered by infectious agents from mononuclear phagocytes.
2. Regulators of lymphocyte growth, activation, and differentiation, which are elicited in response to specific antigen recognition by T lymphocytes.
3. Regulators of immune-mediated inflammation, which activate non-specific inflammatory cells elicited response to specific antigen recognition by T lymphocytes.
4. Stimulators of immature leukocyte growth and differentiation, which are produced by both stimulated lymphocytes as well as other cells.[66]

The Cytokine Family, IL6:

Interleukins are large group of cytokines (IL-1 to IL-17) produced mainly by T-cells, some are also produced by mononuclear phagocytes and tissue cells. They perform a variety of functions, Main role directing other cells to divide and differentiate. Each interleukin acts on a specific group of cells which has the correct receptors for that interleukin. The cytokines IL-6, IL-1β, and TNF-α are found to be increased in most inflammatory states and also remain the main stay of therapeutic intervention. Although mostly regarded as a pro-inflammatory cytokine, IL-6 also has many regenerative or anti-inflammatory activities.[67]

IL-6 was first detected in T-Lymphocyte supernatant cultures. It induced maturation of B cells into immunoglobulin producing plasma cell and it was shown by in situ immuno-

histochemical staining and hybridisation that IL-6 was produced by various cells including fibroblasts, endothelial cells, T-Cells,49 B-Cells, monocytes, macrophages, keratinocytes.[68,69]

The Interleukin-6 (IL6) along with the group of similar interleukins structural features and signalling machinery cytokines are referred to as the IL6 or IL6-like family. As the main feature of IL6 is the transmembrane signalling receptor glycoprotein 130, (this signal transducer is also known as CD130) or IL6 signal transducer.[70]Along with interleukin-27, interleukin-37 and interleukin-35 have been described by different authors as belonging to either the IL6 or IL12 cytokine families.[71,72]

IL6-like family receptors:

IL6-like family receptors present a modular structure with conserved motifs. Both signalling and non-signalling receptors include a single immunoglobin-like domain (IGD) and a cytokine-homology region (CHR), made up of cytokine-binding domains (CBD). Signalling receptors also has a membrane-proximal element including several copies of a fibronectin type III-like (FNIII) domain. The ectodomain of the shared signal transducer gp130/IL6ST contains of 6 domains, with the 3 membrane-distal ones (D1-D3) being essential for binding to the cytokine (and the non-signalling receptor, where this is required). Other signalling receptors, such as LIFR and OSMR, present larger ectodomains consisting of variations of this modular structures. [73]

Physiological role of IL6:

IL6 in the human body is termed as acute phase proteins, haematopoiesis, antigen specific immune responses, inflammation and cellular metabolism. In normal adipose tissue, plasma IL 6 is mainly secreted by none of adipocyte members. There are two types of IL6 receptors – transmembrane IL 6 receptor (expressed on cell surface) and soluble IL 6 receptor (circulation). IL6 also identified with viral infections, Juvenile rheumatoid arthritis, rheumatoid arthritis and in systemic sclerosis. [62,63]

Adipokines are molecules which are secreted by adipocytes (endocrine function). Cancer associated adipocytes (adipocytes next to invasive cancer breast) have a role in breast cancer progression and metastasis and secrete adipokines and Cytokine IL 6 is also expressed by adipocytes during inflammation. IL 6 has relationship with development of stem cell phenotype, cachexia, angiogenesis and resistance to therapy in breast cancer. IL 6 has classical pathway where JAK/STAT 3 signalling pathway is abolished by upregulation of

suppressor of cytokine signalling 3 gene via homologous or heterologous feedback regulation.[74,75,76]

Mechanism of Action:

Cytokines form oligomeric protein complexes, which binds with high affinity to the transmembrane receptors. These binding results gp130/IL6ST homo- or heterodimers which trigger intracellular signaling.[73,74,75,76]

Dimerisation of gp130/IL6ST, results in the signal transduction and activation of 3 major downstream pathways can take place. It involves

1. The Ras-Raf mitogen-activated protein kinase (MAPK/MERK/ERK) signalling cascade,
2. The Janus-activated kinase—signal transducer and activator of transcription (JAK/STAT) pathway,
3. The phosphoinositol-3 kinase—protein kinase B/Akt (PI3K/AKT) pathway. [77]
4. The IL6 and related cytokines has a wide range of functions due to the pleiotropic effects of these signalling pathways along with their own complexes.

Cancer promoting effects of cytokines:

Cytokines possess tumour-promoting effects due to

- **Intrinsic processes:** Such as cell proliferation, differentiation, invasion , survival and metastasis,
- **Extrinsic processes:** Such as modulation of inflammation and angiogenesis which affect the tumour microenvironment.[78]
- IL6 also contributes to disease progression and also in development of treatment resistance.[79]
- Breast cancer results in generation of anti-IL 6 agents or anti IL 6 monoclonal antibody which works as inhibitor of IL 6/STAT 3 pathway.[8] For overall survival in Invasive breast cancer, preoperative plasma IL 6 expression levels works as a independent prognostic factor. IL 6 is identified as poor independent prognostic marker in breast cancer. In breast cancer, raised IL 6 levels were found along in serum as well as in tumour site.
- IL 6 secreted from non-stem cells play major role in conversion of non-cancer stem cells to cancer stem cells through activation of JAK1-STAT 3 –OCT 4 signal

transduction pathway. Cachexia is generalized inflammatory state where IL 6 plays a role.

- IL 6 induced development of cachexia leads to altered metabolism which is co-cultured with cancer cells.
- IL 6 is also associated with atrophy and increased catabolism of muscle protein. Depletion of adipose tissue stores, loss of skeletal mass in several organs and weight loss are peculiar features of cachexia.
- Therapeutic resistance is linked with increased expression of IL 6.High expression of IL6 has led to multidrug resistant cancer cells as well as poor therapeutic gain, tumor relapse and aggressive tumor growth.
- IL 6 mediated STAT 3 activation produces high expression of multidrug resistance genes MDR1 and C/EBPBETA and C/EBPDELTA (CCAAT enhancer binding protein family of transcription factors).
- Recombinant IL 6 had radio protective effect according to some studies but not conclusive heighten the role of adipocyte derived IL6 in adipocyte induced therapeutic resistance in breast cancer cells.[76,77]

Role of Circulating IL6 Level as a Biomarker:

- Higher levels were also observed in patients with widely dispersed metastatic BC compared to single metastatic disease, in recurrent compared with non-recurrent disease and in progressive compared to stable disease.
- Elevated serum levels were also linked with worse prognosis and survival, as well as reduced response to chemo- or endocrine therapy.
- Multivariate analysis has confirmed that IL6 is an independent poor prognostic indicator in metastatic BC.
- A meta-analysis also found that high IL6 expression is associated with poor overall survival. In short, despite varied reports on IL6's role from in vitro studies, clinical evidence firmly supports that IL6 is involved in and a biomarker of BC development and progression.
- Despite the evidence on its prognostic and potentially predictive value of serum IL6 levels, prospective studies are required before their assessment can be applied in BC detection and monitoring or to guide treatment selection. Studies have reported

changes in IL6 levels during treatment with taxane-based chemotherapy, but not with anthracycline-based chemotherapy or endocrine therapy. [76]

In a study done by **Ahmad N et al** (2018) association of IL 6 with good prognosis among early stage of invasive BC for immunohistochemistry is well established. Median H score for IL 6 expression were 80 out of 1190 and 349(29.3%) case were showing high IL 6 expression but there was no significant relation between IL 6 expression and either stage, LN involvement or vascular invasion which was analyzed on basis of immunohistochemistry. But IL 6 expression was significantly associated with patients above 40 years, lower tumor size, lower tumor grade, lower Nottingham prognostic index, positive estrogen receptor status and positive progesterone receptor status.[79]

In a study done by **Shimura T. et al** reported that IL 6 >= 10.0 pg. /ml had bad overall survival and recurrence free survival compared those patients with IL 6 <10.0 pg. /ml. There were no statistical significant differences in pathological T factor, TNM stage, pathological N factor and administration of neoadjuvant or adjuvant chemotherapy, progesterone receptors, Estrogen receptors or HER 2 expression levels, lymphatic invasion or microscopic vascular invasion associated with IL 6.[9]

Goswami et al study showed a significant correlation of IL-6 levels with lymph node involvement, mitotic index, tumor grade and adipose tissue invasion. The study also showed that there is progressive increase in IL-6 levels as the stage of disease progresses.[80] It is B cell stimulatory factor and therefore is responsible for effector B cell differentiation of antibody producing cells, which are synthesized by vascular endothelial cells and mononuclear phagocytes.

MATERIALS AND METHODS

MATERIALS AND METHODS:

Study Design: Laboratory observational cross sectional study.

Place of Study: Department of Pathology, SDUMC, Tamaka, Kolar.

Source of data:

Primary breast carcinoma specimens will be collected from the Department of Surgery and Department of Pathology from Sri Devraj Urs Medical College, Tamaka, Kolar.

Duration of Study:

18 months (January 2021 to June 2022)

Sample Size:

Sample size of 50 estimated by based on expression of IL 6 expression in Breast carcinoma based on serum IL 6levels IL 6 >10 pg. /ml had poor overall survival compared to IL 6 < 10 pg. /ml as reported in the study with 95% confidence interval and an absolute error of 12%, the sample size will be 50, Expected proportion = 75.[79] Formula used:

$$n = \frac{Z^2_{1-\alpha/2} p (1-p)}{d^2}$$

P: Expected proportion

D: Absolute Precision

- 1-α/2 : Desired Confidence level

Inclusion Criteria:

All the fresh cases of Invasive Ductal Carcinoma Breast diagnosed by Fine Needle Aspiration Cytology and confirmed by Trucut biopsy and mastectomy specimens are included in the study.

Exclusion Criteria:

All the cases post chemotherapy, post Radiotherapy carcinoma breast, metastatic deposits in breast, recurrence of breast carcinoma, any other malignancy, Chronic inflammatory disorders are excluded from the study.

Methods:

Ethical clearance was obtained from Institutional ethical committee before conducting the study.

Informed consent was obtained from the study participants before starting the study.

All freshly diagnosed Primary Invasive Ductal Carcinoma Breast cases by FNAC/ trucut biopsy were included. Case details were collected from the case files or by interaction with patient which include – age, clinical presentation, physical examination findings including relevant laboratory and radiological investigations. In local physical examination, the site of the lesion, the size of the tumor, involvement of surrounding structures, number of palpable Lymph nodes and in addition side and quadrant of breast, Involvement of Nipple and Areola including skin changes, BMI of the patient was noted and the patient would be classified as being normal/overweight/obese /severe obesity/morbid obesity/super obesity according to Asian BMI criteria.

The breast tissue either Trucut or Mastectomy specimen was fixed in 10% Neutral Buffered Formalin overnight and then grossed as per the SOP of the lab and representative bits were given from the tumour proper, resected margins including skin, nipple and areola. The tissue bits were processed as per the protocol of the lab. Tissue sections were stained with H and E stains. The tissue sections were screened and analyzed for histomorphological features including histopathological type and grade of tumour. The clinical stage of the tumour was noted.ER, PR, Her 2 neu, Ki 67 status were taken from department records. Tissue sections were subjected to Immunohistochemistry for Interleukin 6 and procedure was as per the manufacturer's protocol.

6 ml of blood sample was taken in K2 EDTA vacutainer from the patient following Histopathological Confirmation of Diagnosis by FNAC or TRUCUT Biopsy before the patient undergoes Mastectomy since IL 6 levels might get altered after the removal of tumor and centrifuged at 1500 rpm for 10 min, the plasma was separated, the separated plasma was subjected to ELISA IL6 estimation.

Tissue IHC IL6 Protocol :

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue section. The section was deparaffinized with standard xylene then rehydrated using graded alcohol and then rinsed by water. Antigen retrieval was done by using microwave in Tris-EDTA buffer at pH= 9 and then treated with 3% hydrogen peroxide to reduce endogenous peroxidase activity. The tissue section was the incubated with at 4^0 C and finally subjected to primary antibody(Gene Tex) anti IL-6. Primary antibody category number GTX109204, Rabbit polyclonal IgG type antibody with reactivity on human and mouse.The sections then incubated with secondary antibody (DBS -Diagnostic bio system). 3, 3-diaminobenzidinetetrahydrochloride (DAB solution) then used as chromogens and then after counterstaining with hematoxylin, sections were dehydrated, mounted and examined under microscope.

The immunohistochemistry scoring was done as follows:

Based on H score, intensity grading {H score were calculated by multiplying percentage area scoring positive by respective intensity using formula:

(% of cells stained weak x 1) + (% of cells stained moderate x 2) + (% of cells stained strong x 3) (range 0- 300).[79]

Adopted from Bhaumik A, Das S, Roy S, Chowdhury B Immunohistochemically diagnosis of Breast cancer cases with prognostic markers ER, PR and Her-2/1 Neu.2015.[81]

Plasma ELISA IL-6 procedure :

Diaclone Human IL6 ELISA kit – Insert version 12.

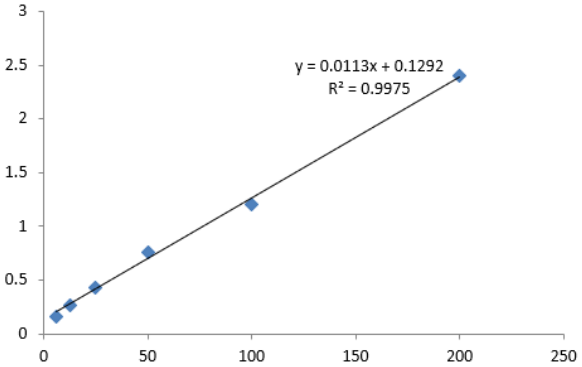


Fig 9 – Normal standard curve ELISA IL6

Preparation of standard curve was done. 100ul of each Sample, Control and zero (appropriate Standard Diluent) in duplicate to appropriate number of wells was added. Then 50ul of diluted Biotinylated Anti-IL-6 to all wells was added. All the wells were covered with plastic plate cover and incubated at room temperature (18 to 25°C) for 1 hour.

Then after 1 hour cover was removed and the plate was washed as follows:

a) The liquid was aspirated from each well

b) 0.3 ml of 1x Wash Buffer into each well was dispensed

c) The contents of each well were aspirated

d) Step b and c were repeated another two times

100ul of diluted Streptavidin-HRP solution into all wells was added. All the wells were covered with plastic plate cover and incubated at room temperature (18 to 25°C) for 1 hour. Then after 1 hour cover was removed and the plate was washed with steps as mentioned above.

100ul of ready-to-use TMB Substrate into all wells was added and incubated in the dark for 12-15 minutes" at room temperature. The plate was wrapped in aluminium foil. 100ul of H_2SO_4 was added.

The immunohistochemistry interleukin 6 expression was correlated with plasma ELISA interleukin 6 results.

STASTICAL ANALYSIS

The findings were entered in MS excel sheet and statistical analysis were done. The Continuous data like Interleukin 6 levels were analyzed by Mean and standard deviation with confidence intervals and categorical data presented by frequency and percentage.

To compare ELISA with Immunohistochemistry findings, sensitivity and specificity analysis were used.

Chi – square test used for testing significance of difference in proportions between two methods.

P value <0.05 was considered as statistically significant.

All the data entered in Microsoft XL sheet and statistical analysis was done by using IBM SPSS software version– 22.

RESULTS

RESULTS:

TABLE 10 : BASIC CHARACTERISTICS OF STUDY POPULATION

BASIC CHARACTERISTICS		FREQUENCY	PERCENTAGE (%)
AGE CATEGORY	35 - 45	6	12.0
	46 - 55	18	36.0
	56 - 65	20	40.0
	66 – 75	6	12.0
MENOPAUSAL STATUS	PREMENOPAUSAL	13	26.0
	POSTMENOPAUSAL	37	74.0
PARITY	MULTIPARA	47	96
	PRIMIPARA	3	4
BMI	UNDERWEIGHT	14	28.0
	NORMAL	32	64.0
	OVERWEIGHT	4	8.0
TUMOR INFILTRATING LYMPHOCYTES	ABSENT	33	66
	PRESENT	17	34
LYMPHOVASCULAR INVASION	ABSENT	49	98
	PRESENT	1	2
pT (TUMOR SIZE)	T1	13	26
	T2	33	66
	T3	3	6
	T4	1	2
METASTATIC LYMPH NODES	ABSENT	48	96.0
	PRESENT	2	4.0
STAGING	I	12	24
	II	36	72
	III	2	4
NOTTINGHAM GRADING	GRADE 1	10	20
	GRADE 2	23	46
	GRADE 3	17	34
NOTTINGHAM PROGNOSTIC INDEX (NPI)	MODERATE	11	22
	GOOD	39	78
ER	NEGATIVE	29	58
	POSITIVE	21	42
PR	NEGATIVE	31	62
	POSITIVE	19	38
HER2 NEU	NEGATIVE	35	70
	POSITIVE	15	30

Ki67	<14%	21	42
	>14%	29	58
MOLECULAR	LUMINAL A	8	16
	LUMINAL B	13	26
	HER-2 ENRICHED	9	18
	TRIPLE NEGATIVE	20	40

TABLE 11: MEAN IL-6 IHC AND IL-6 ELISA

IL-6	**IHC**		**ELISA**	
	MEAN	**SD**	**MEAN**	**SD**
MEAN+SD	201.60	78.04	68.13	37.70

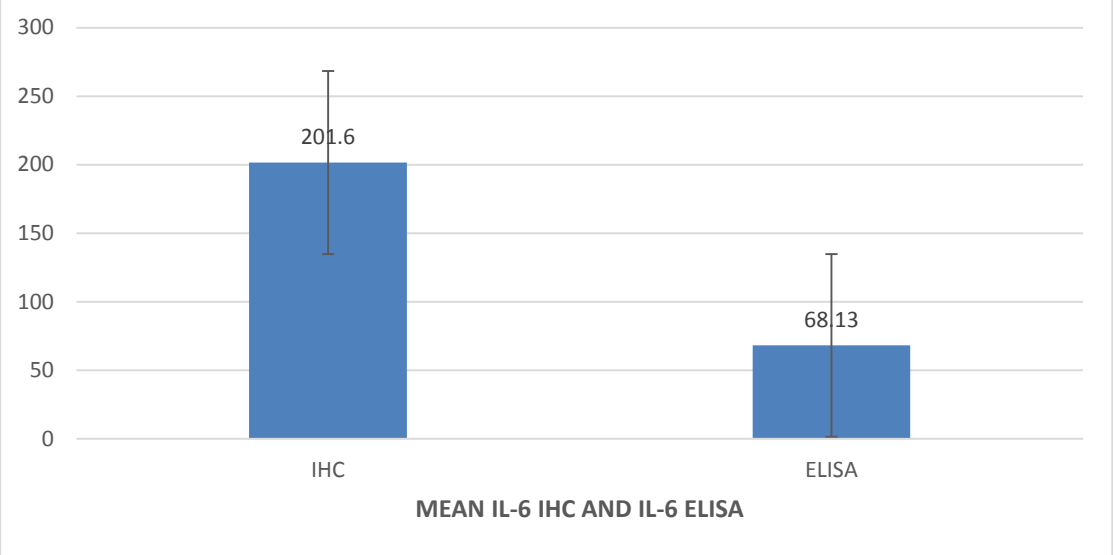


CHART 1 : BAR DIAGRAM SHOWING ASSOCIATION OF MEAN IL-6 IHC AND IL-6 ELISA

The Mean values of IL6 by IHC method 201.60 ± 78.04 and by ELISA IL 6 was 68.13 ± 37.70

TABLE 12: CORRELATION BETWEEN ELISA-IL6 AND IHC-IL6

ELISA- IL6	IHC IL-6
Pearson Correlation	0.178
Sig. (2-tailed)	0.217

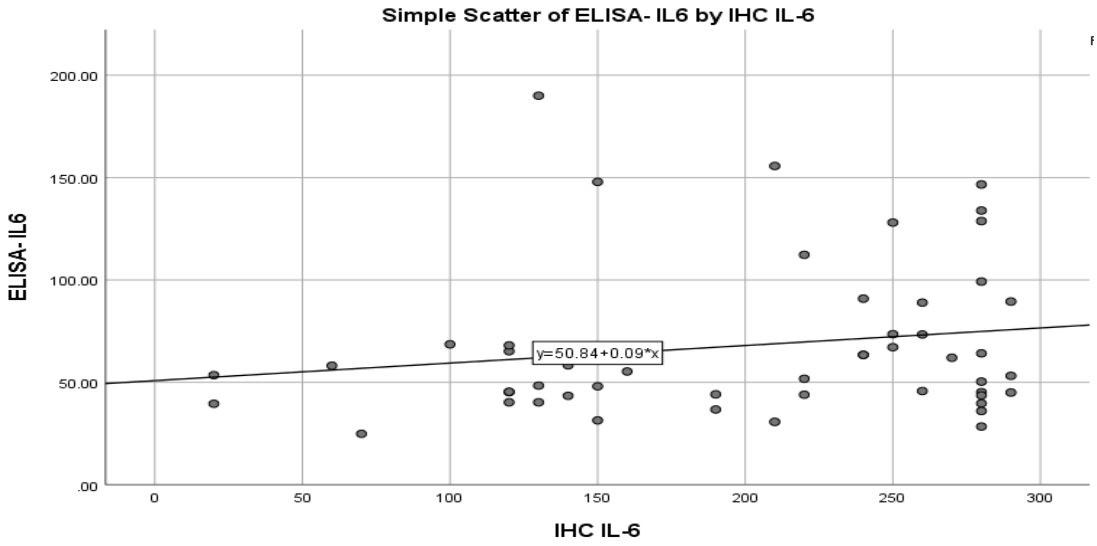


CHART 2 – Scatter diagram showing correlation between IHC IL6 and ELISA IL6

Negligible positive correlation was found between IHC IL-6 and ELISA IL-6 i.e., when the IHC IL-6 values increase, the ELISA IL-6 values also increase. This positive correlation was not found to be statistically significant (P=0.217) i.e., only 79% of the studies done elsewhere would get similar results.

TABLE 13: ASSOCIATION OF AGE DISTRIBUTION WITH IHC IL-6 AND ELISA IL6

AGE GROUP	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
35-45	6	12	196.29	109.96	62.04	19.50
46-55	18	36	209.44	64.12	70.50	40.29
56-65	20	40	198.01	80.56	76.38	41.76
66-75	6	12	195.00	93.50	39.60	9.12
Total	50	100	201.60	50.97	68.13	37.70
p value			0.964		0.203	

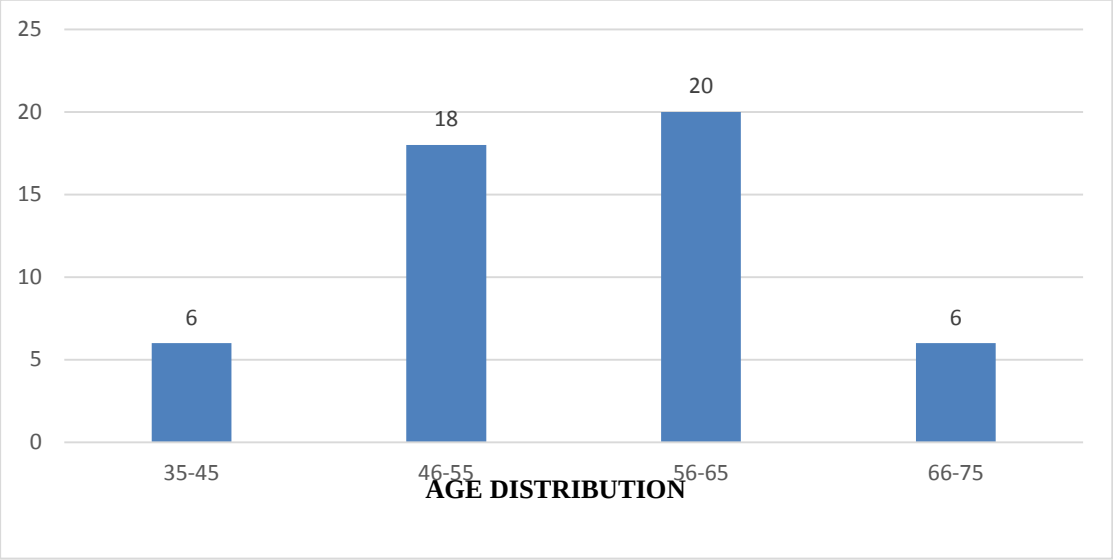


CHART 3: BAR DIAGRAM SHOWING THE AGE DISTRIBUTION OF SUBJECTS IN THE STUDY

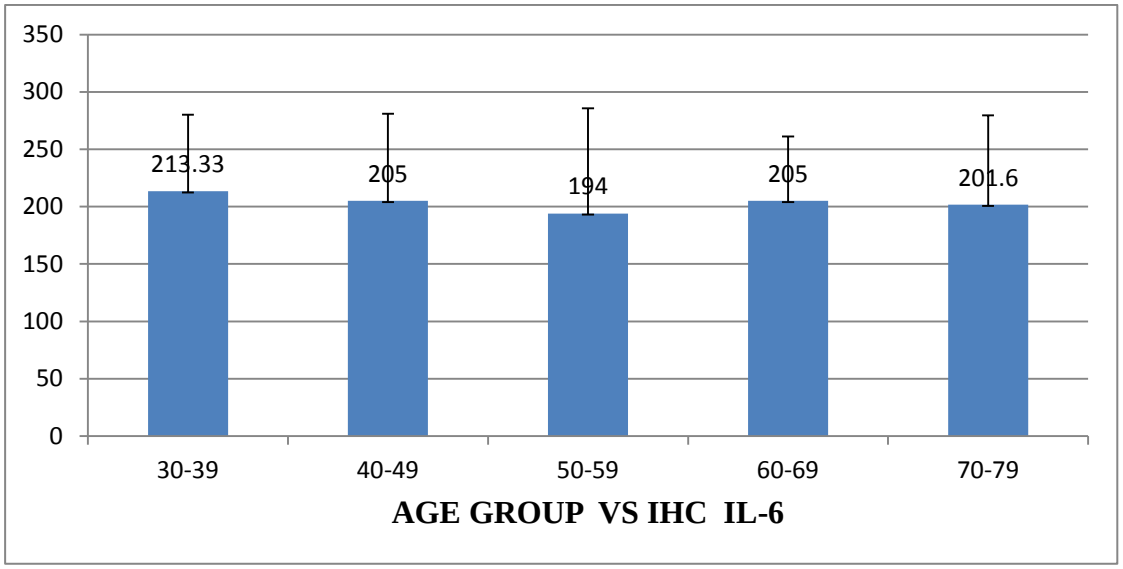


CHART 4 : BAR DIAGRAM SHOWING THE ASSOCIATION OF AGE DISTRIBUTION WITH IHC IL-6

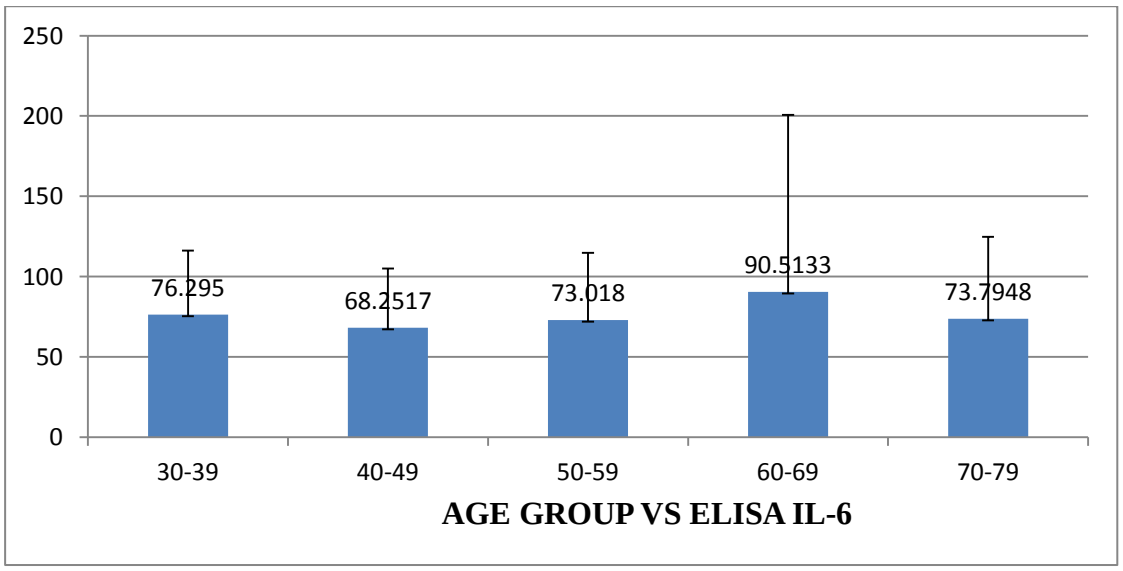


CHART 5 : BAR DIAGRAM SHOWING THE ASSOCIATION OF AGE DISTRIBUTION WITH ELISA IL-6

In current study, 20(40%) of the study population were in 56 to 65 years age group and mean IHC IL-6 were more prevalent in the 46 to 55 years age group. Mean ELISA IL-6 was highest among 56 to 65 years age group. But it was discovered that there was no significant correlation between the study groups.

TABLE 14: ASSOCIATION OF MENOPAUSAL PHASE WITH IHC IL-6 AND ELISA IL6

MENOPAUSAL PHASE	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
Premenopausal	13	26.0	216.92	83.20	90.32	37.47
Postmenopausal	37	74.0	196.22	76.60	60.33	35.01
Total	50	100	201.60	78.04	68.13	37.70
p value			0.416		**0.012**	

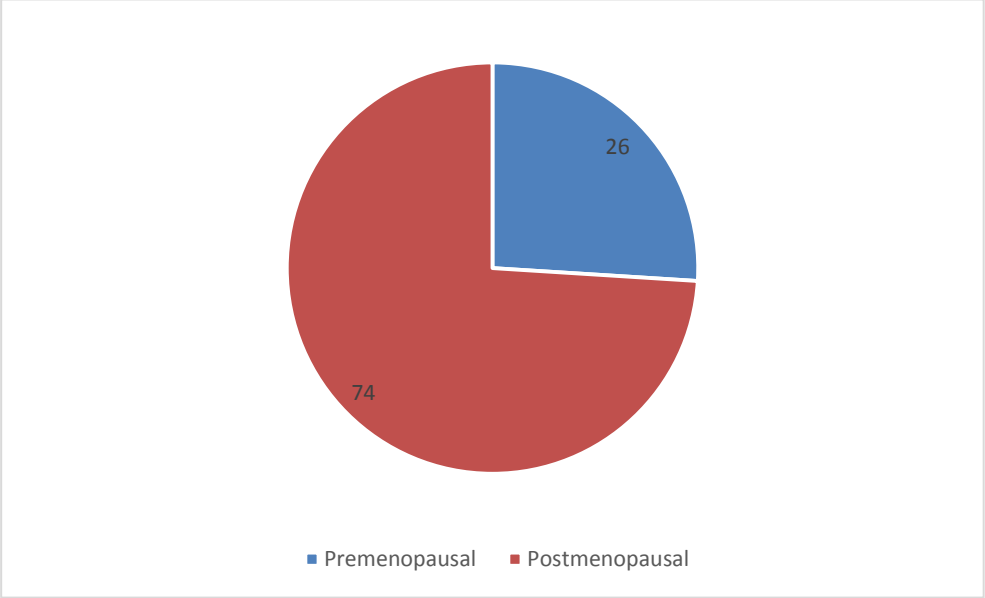


CHART 6 : PIE DIAGRAM SHOWING MENOPAUSAL PHASE OF SUBJECTS IN THE STUDY

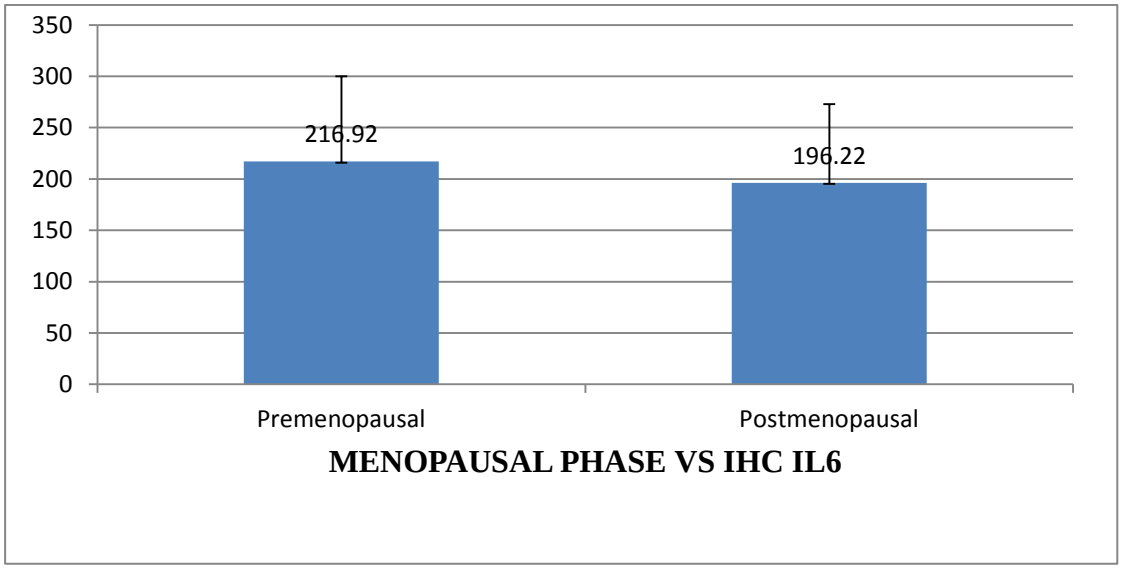


CHART 7 : BAR DIAGRAM SHOWING THE ASSOCIATION OF MENOPAUSAL PHASE WITH IHC IL-6

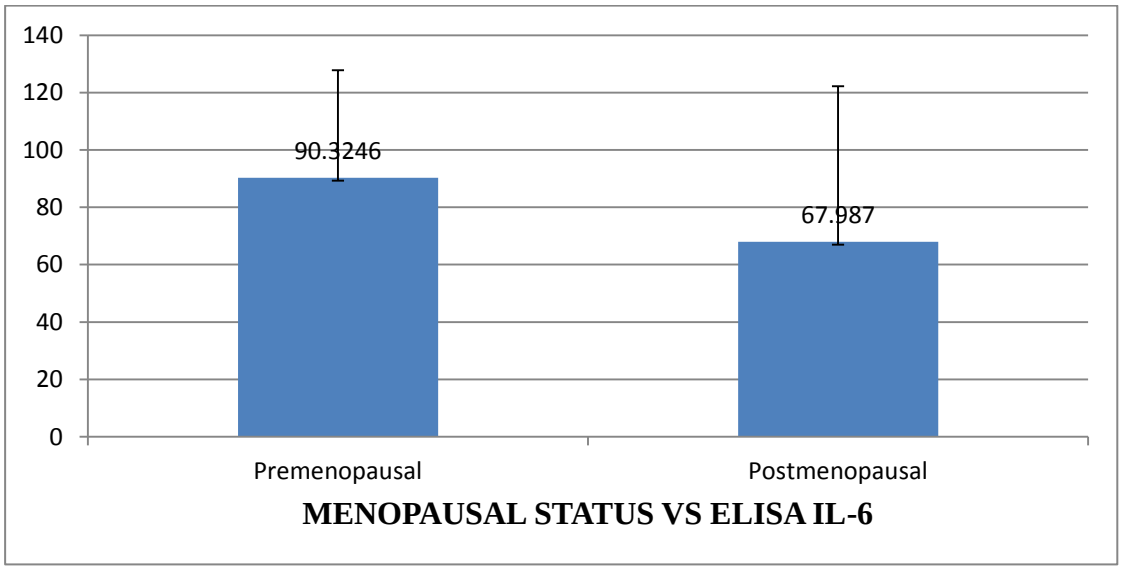


CHART 8 : BAR DIAGRAM SHOWING THE ASSOCIATION OF MENOPAUSAL PHASE WITH ELISA IL6

In present study, 37(74%) of the study population were in postmenopausal status but showing both mean IHC IL-6 and mean ELISA IL-6 predominance among premenopausal group. But there was no association between menopausal phase and IHC IL6 sample population. But menopausal phase with ELISA IL6 sample population was showing significance.

TABLE 15: ASSOCIATION OF PARITY WITH IHC IL-6 AND ELISA IL6

PARITY	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
Primipara	3	6	206.67	66.583	40.2500	6.75567
Multipara	47	94	201.28	79.335	69.9147	38.18512
Total	50	100	201.60	50.97	68.13	37.70
p value			0.904		0.189	

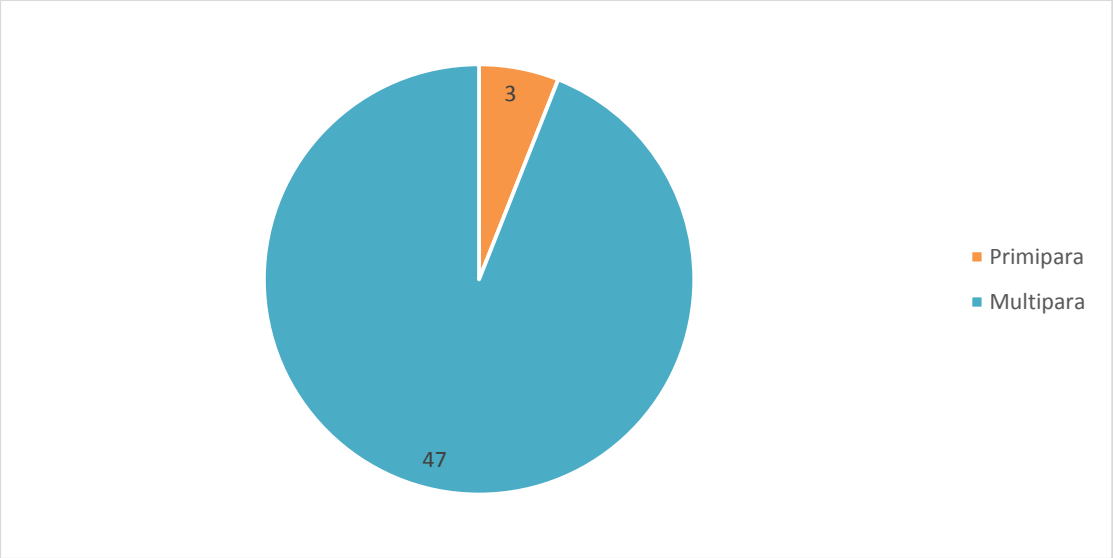


CHART 9: PIE DIAGRAM SHOWING DISTRIBUTION OF PARITY

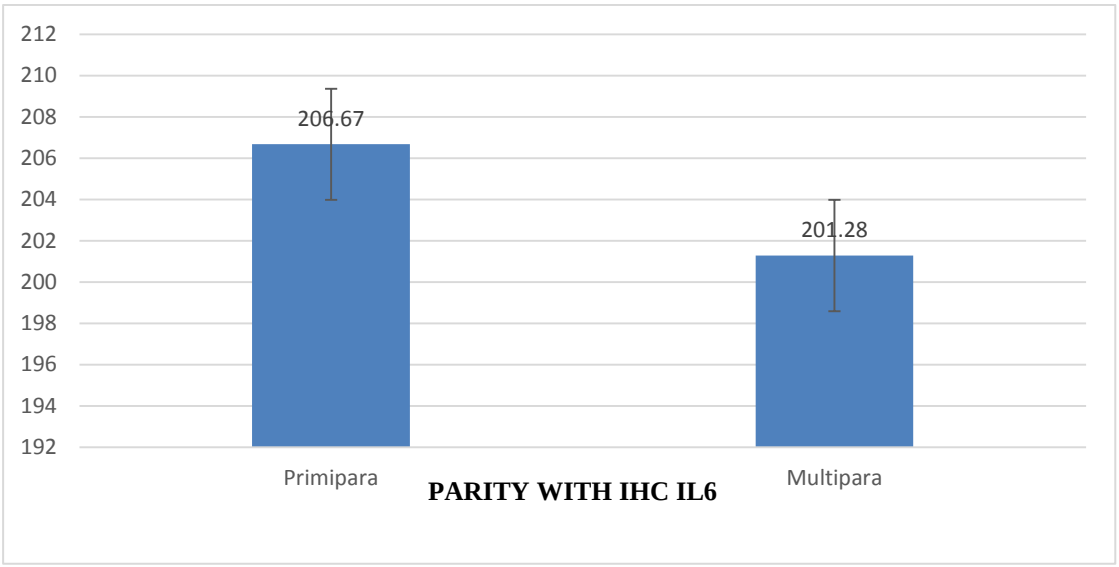


CHART 10: BAR DIAGRAM SHOWING THE ASSOCIATION OF PARITY WITH IHC IL-6

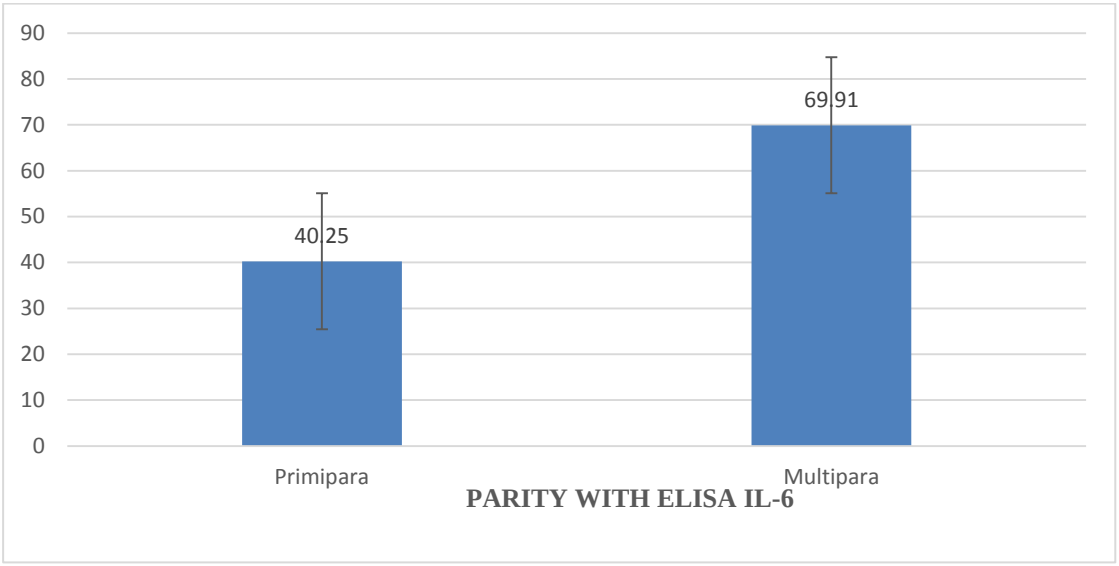


CHART 11: BAR DIAGRAM SHOWING ASSOCIATION OF PARITY WITH ELISA IL6

In the present study, 94% of participants were multipara with mean IHC IL-6 predominance among primipara. Mean values of ELISA IL6 was showing predominance among multipara. But it was not determined that there was a substantial statistical difference between the groups.

TABLE 16: ASSOCIATION OF BMI WITH IHC IL-6 AND ELISA IL6

BMI	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
Underweight (< 18.5)	14	28	208.57	71.88	68.52	34.97
Normal (18.5-24.9)	32	64	198.44	81.56	68.18	39.23
Overweight (25-29.9)	4	8	202.50	89.58	66.35	44.78
TOTAL	50	100	201.60	78.04	68.13	37.70
p VALUE			0.924		0.995	

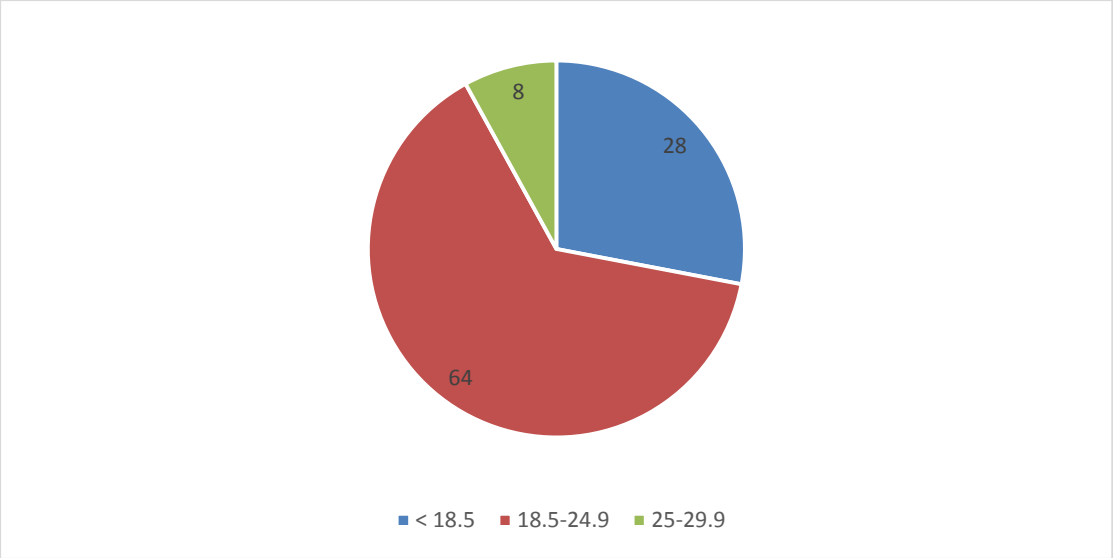


CHART 12: PIE DIAGRAM SHOWING DISTRIBUTION OF BMI

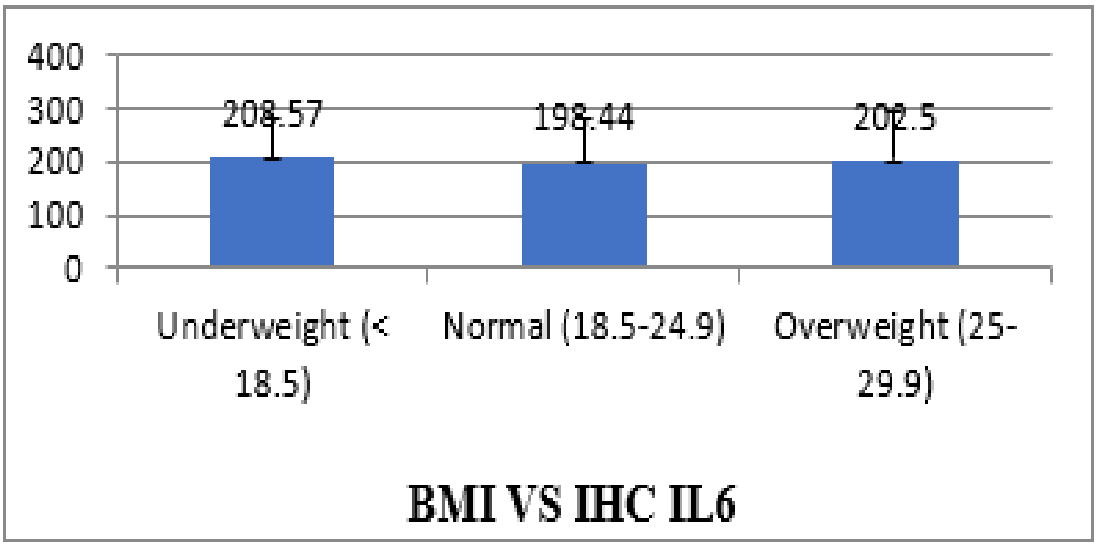


CHART 13 : BAR DIAGRAM SHOWING ASSOCIATION OF DISTRIBUTION OF BMI WITH IHC IL-6

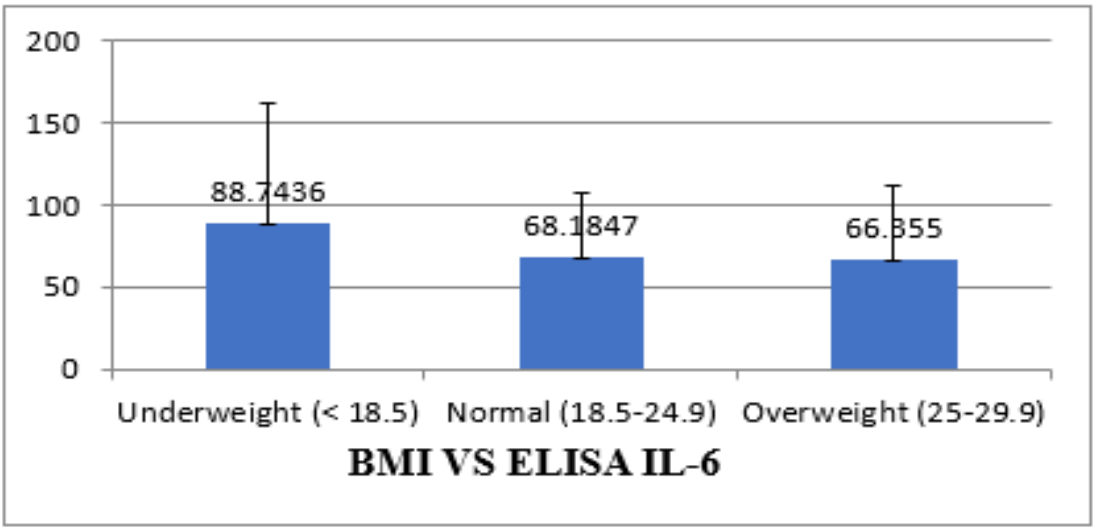


CHART 14 : BAR DIAGRAM SHOWING DISTRIBUTION OF BMI WITH ELISA IL6

In current study, 32 (64%) of the study population were in Normal BMI level with mean IHC IL-6 predominance among underweight BMI levels. Mean ELISA IL-6 was among underweight BMI level 14(28%). But the difference between the groups was not found to be showing any association.

TABLE 17: DISTRIBUTION OF TUMOR INFILTRATING LYMPHOCYTES WITH IHC IL-6 AND ELISA IL6

TUMOR INFILTRATING LYMPHOCYTES	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
Absent	33	66.0	190.91	79.93	64.93	33.09
Present	17	34.0	222.35	71.98	74.35	45.82
Total	50	100	201.60	78.04	68.13	37.70
p VALUE			0.180		0.408	

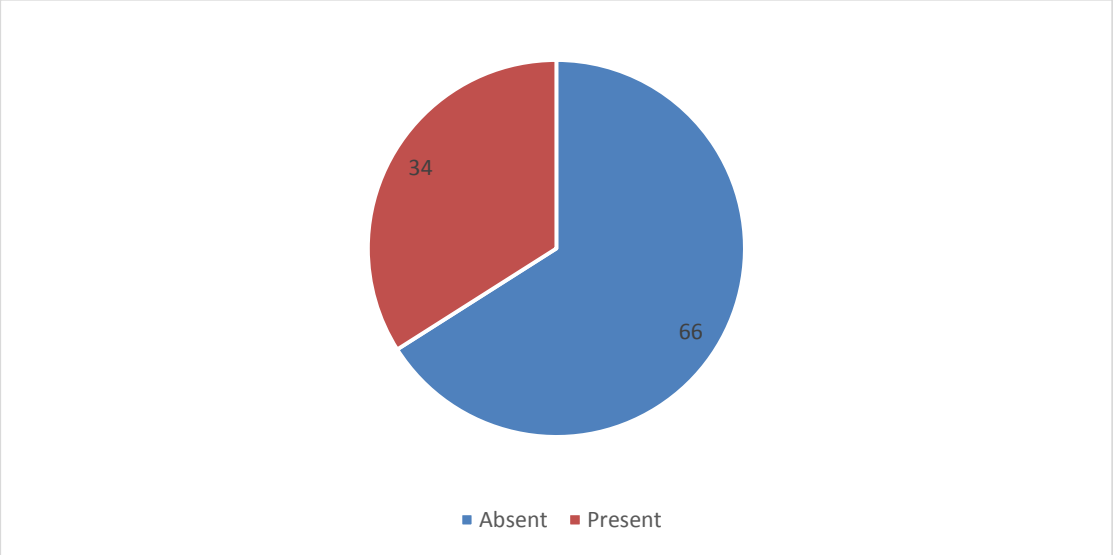


CHART 15: PIE DIAGRAM SHOWING DISTRIBUTION OF TUMOR INFILTRATING LYMPHOCYTES

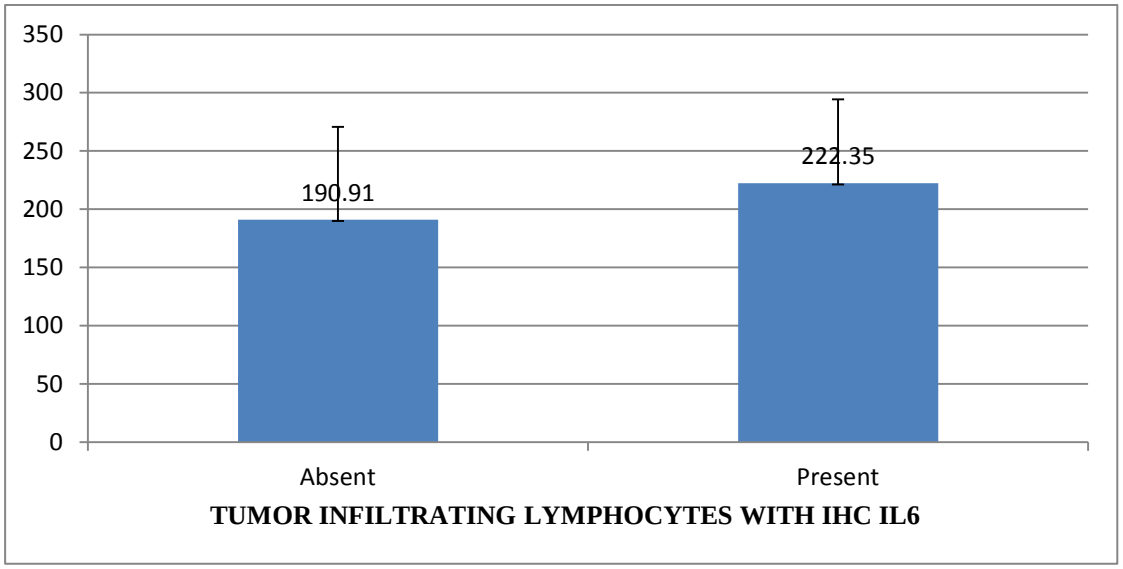


CHART 16: BAR DIAGRAM SHOWING DISTRIBUTION OF TUMOR INFILTRATING LYMPHOCYTES WITH IHC IL-6

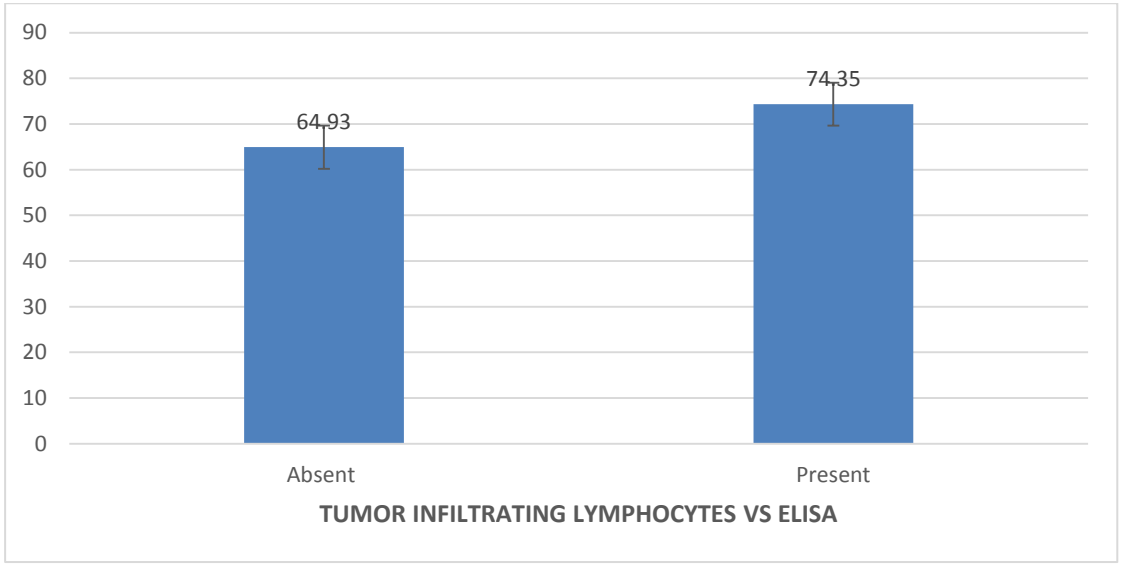


CHART 17 : BAR DIAGRAM SHOWING DISTRIBUTION OF TUMOR INFILTRATING LYMPHOCYTES WITH ELISA IL6

In this study, 33(66%) of the study population were not having Tumor Infiltrating Lymphocytes but mean IHC IL 6 and mean ELISA IL-6 predominance among population with positive Tumor Infiltrating Lymphocytes 17(34%). But the difference between the groups was not found to be showing any association.

TABLE 18: DISTRIBUTION OF LYMPHOVASCULAR INVASION WITH IHC IL-6 AND ELISA IL6

LYMPHO-VASCULAR INVASION	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
Absent	49	98.0	204.29	76.48	69.01	37.56
Present	1	2.0	70.00	-	24.85	-
Total	50	100	167.87	76.67	68.13	37.70
p VALUE			0.089		0.337	

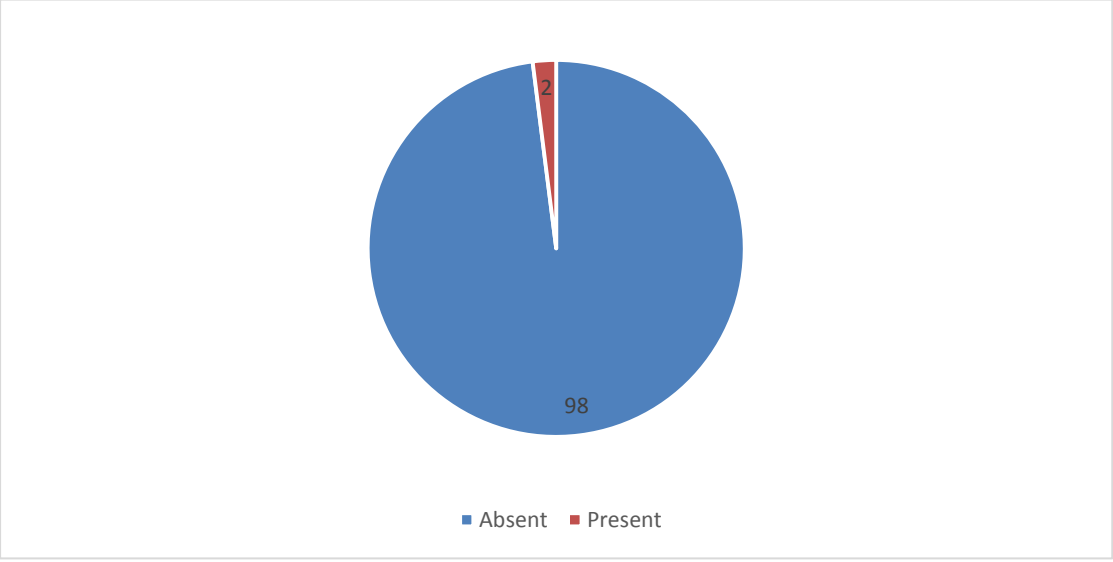


CHART 18: PIE DIAGRAM SHOWING DISTRIBUTION OF LYMPHOVASCULAR INVASION

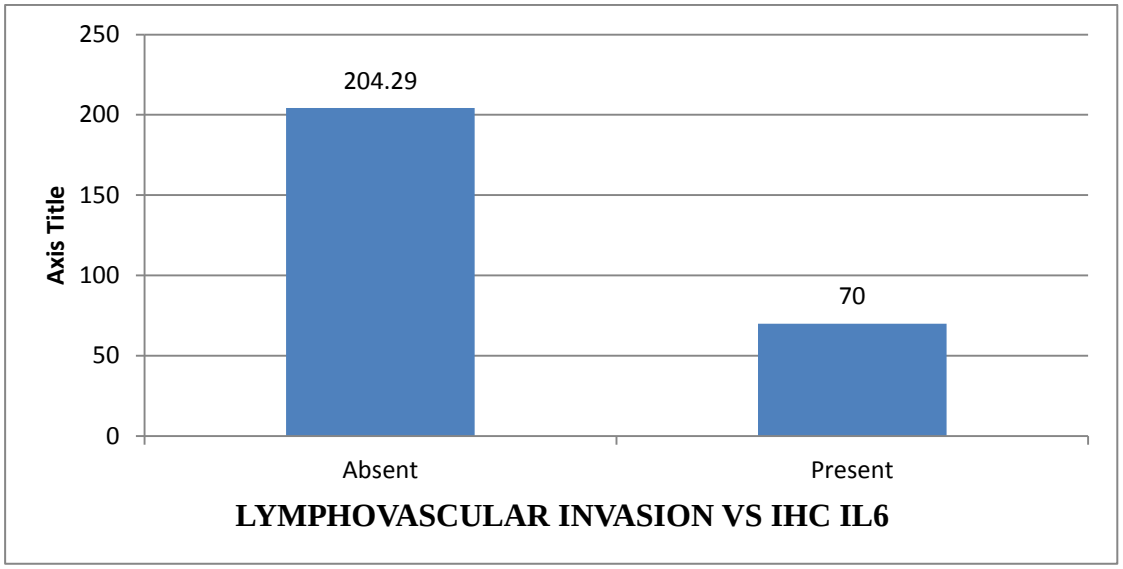


CHART 19: BAR DIAGRAM SHOWING DISTRIBUTION OF LYMPHOVASCULAR INVASION WITH IHC IL6

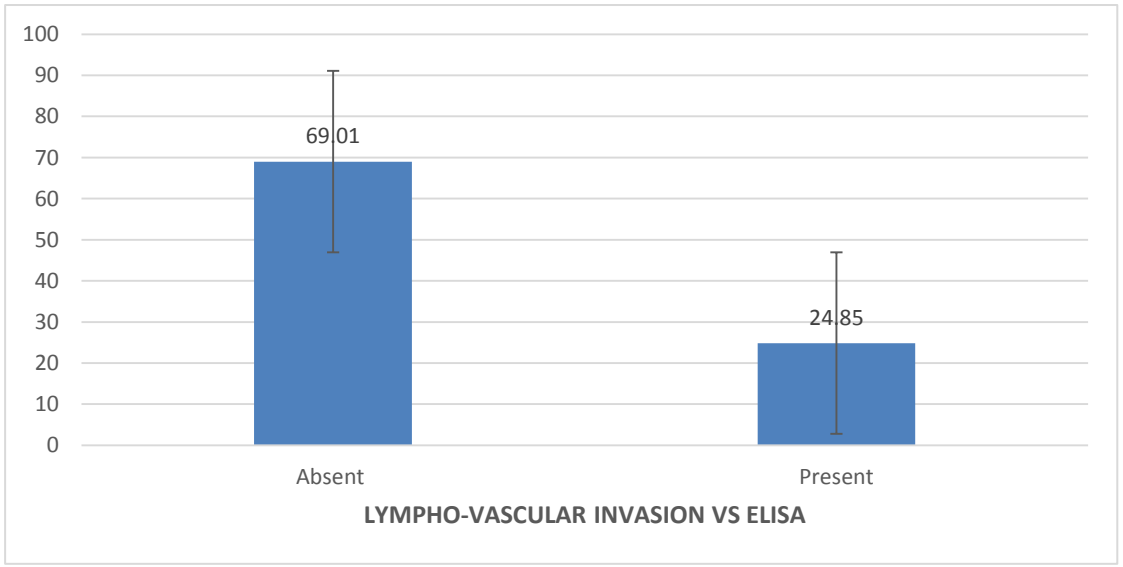


CHART 20 : BAR DIAGRAM SHOWING DISTRIBUTION OF LYMPHOVASCULAR INVASION WITH ELISA IL6

In current study, 49(98%) of the study population were not having Lymphovascular Invasion with mean IHC IL 6 predominance among them. Mean ELISA IL-6 was among study population having Lymphovascular Invasion 1(2%). However, there was no significant difference between the sample groups.

TABLE 19: DISTRIBUTION OF TUMOR SIZE (pT) WITH IHC IL-6 AND ELISA IL6

pT	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
T1	13	26	207.57	71.88	73.79	64.79
T2	33	66	213.22	76.60	78.98	54.18
T3	3	6	201.65	66.53	69.34	34.65
T4	1	2	197.22	75.60	69.98	55.18
Total	50	100	203.65	66.63	70.34	34.65
p value			0.950		0.850	

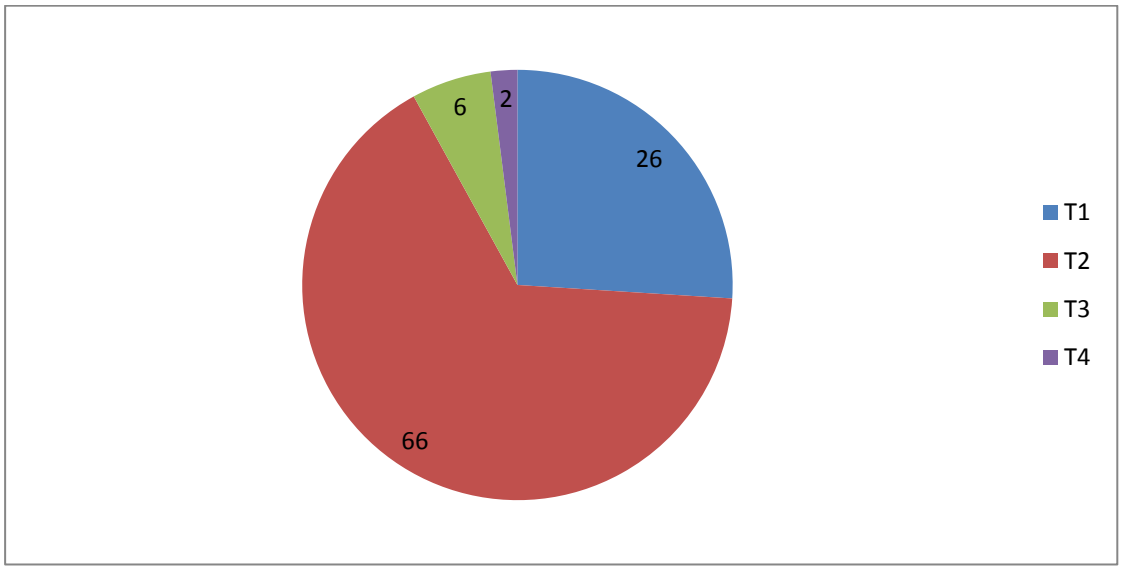


CHART 21: PIE DIAGRAM SHOWING TUMOR SIZE DISTRIBUTION

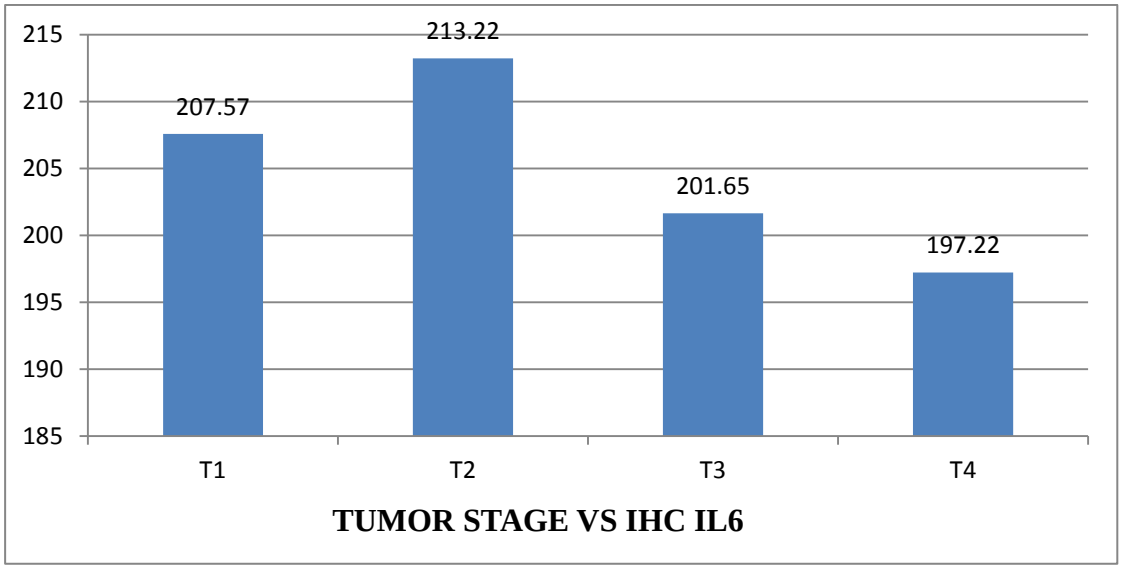


CHART 22 : BAR DIAGRAM SHOWING TUMOR SIZE (pT) DISTRIBUTION WITH IHC IL6

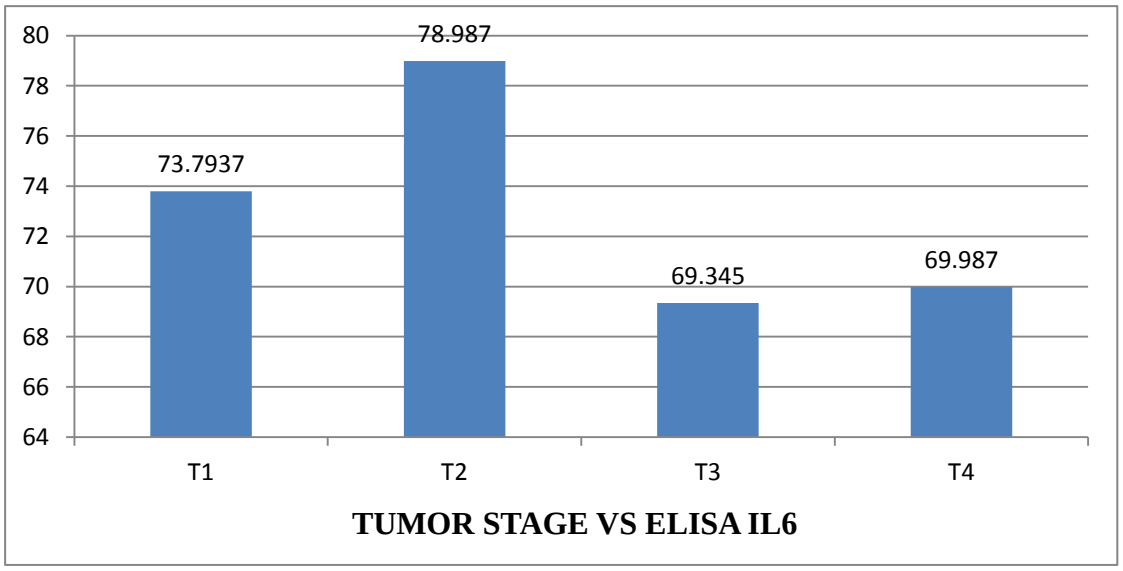


CHART 23 : BAR DIAGRAM SHOWING TUMOR SIZE (pT) DISTRIBUTION WITH ELISA IL6

In current study, 33(66%) of the sample population were falling under T2 staging. Mean IHC IL-6 were more prevalent in the T2 stage group. Mean ELISA IL-6 was highest among T2 stage group. However, it was not determined that the differences between the groups were substantial.

TABLE 20 : DISTRIBUTION OF METASTATIC LYMPH NODES (pN) WITH IHC IL-6 AND ELISA IL6

METASTATIC LYMPH NODES (pN)	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
Negative	48	96.0	202.08	78.33	75.07	51.64
Positive	2	4.0	190.00	98.99	42.99	3.87
Total	50	100	198.67	85.45	58.56	25.67
p VALUE			0.833		0.389	

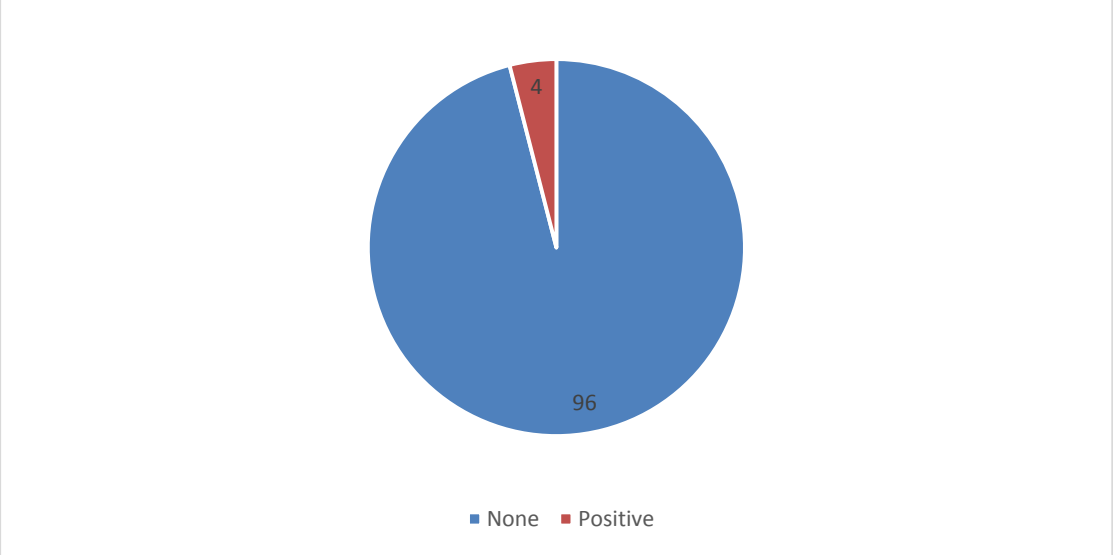


CHART 24 : PIE DIAGRAM SHOWING DISTRIBUTION OF METASTATIC LYMPH NODES (pN)

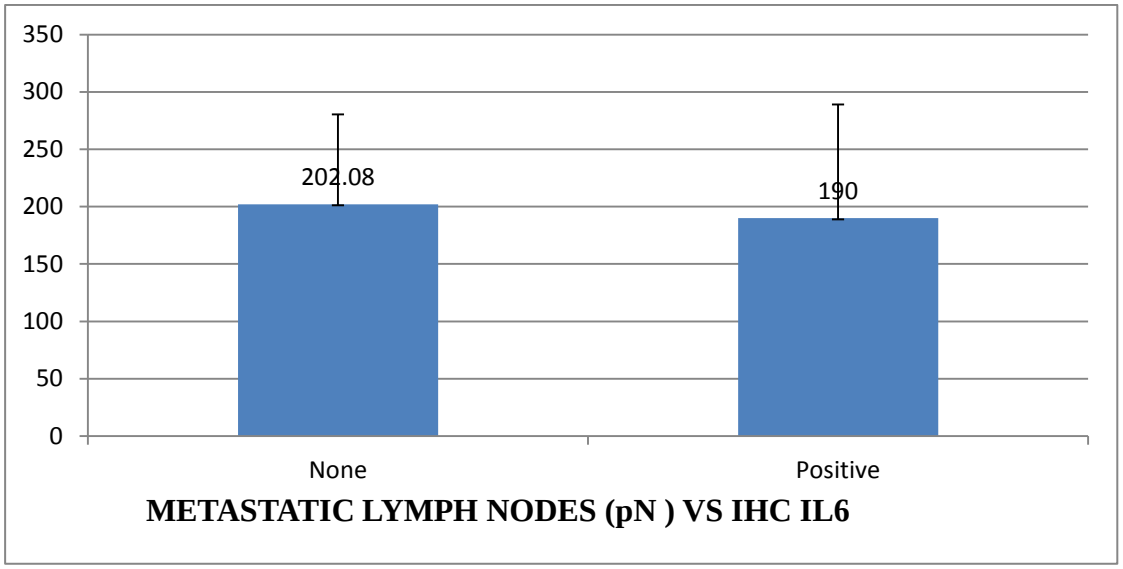


CHART 25 : BAR DIAGRAM SHOWING DISTRIBUTION OF METASTATIC LYMPH NODES (pN) WITH IHC IL-6

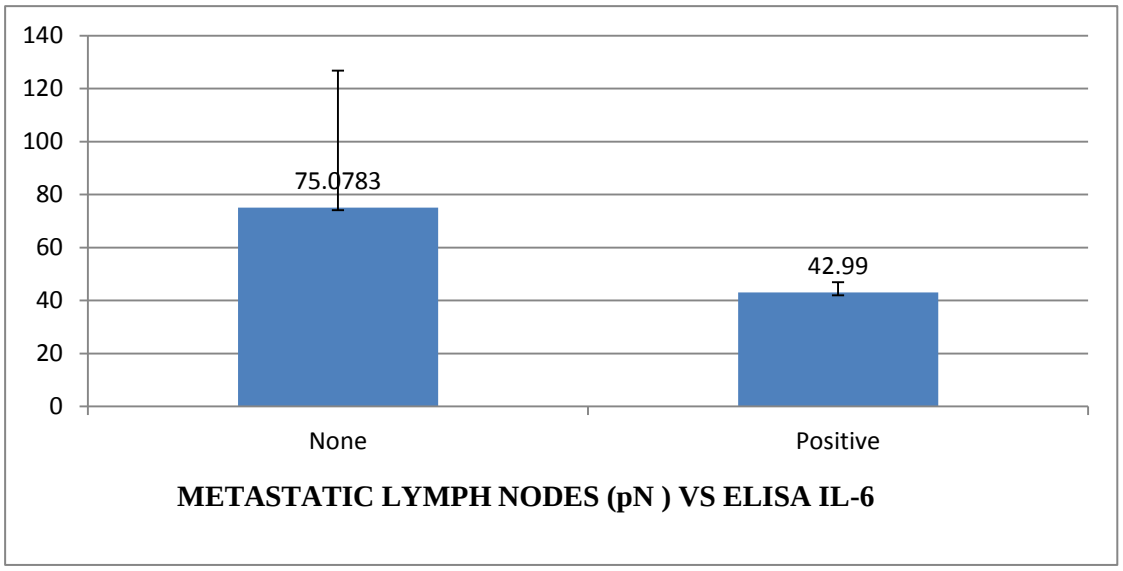


CHART 26 : BAR DIAGRAM SHOWING DISTRIBUTION OF METASTATIC LYMPH NODES (pN) WITH ELISA IL6

In this study, 48 (96%) of the study population were not having Metastatic lymph nodes with mean IHC IL-6 predominance among them. Mean ELISA IL6 was also showing predominance among non-metastatic study population. However, it was not determined that the differences between the groups were substantial.

TABLE 21: ASSOCIATION OF TUMOR STAGING WITH IHC IL-6 AND ELISA IL6

STAGING	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
I	12	24	169.17	78.21	93.09	40.71
II	36	72	211.39	78.27	67.12	36.73
III	2	4	220.00	-	43.96	-
Total	50	100	213.45	75.65	65.65	34.56
p VALUE			0.443		0.363	

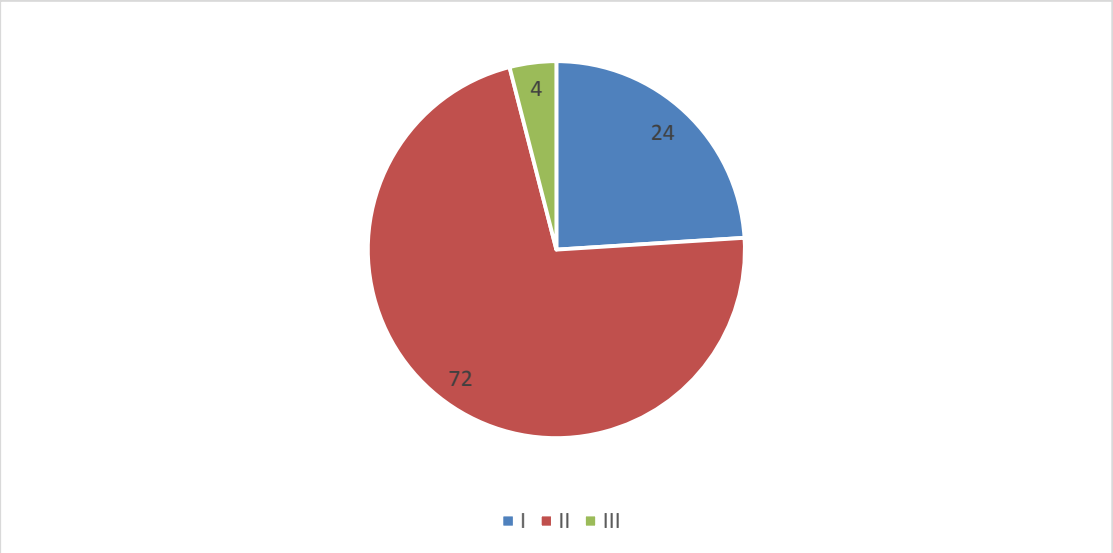


CHART 27 : PIE DIAGRAM SHOWING TUMOR STAGING DISTRIBUTION

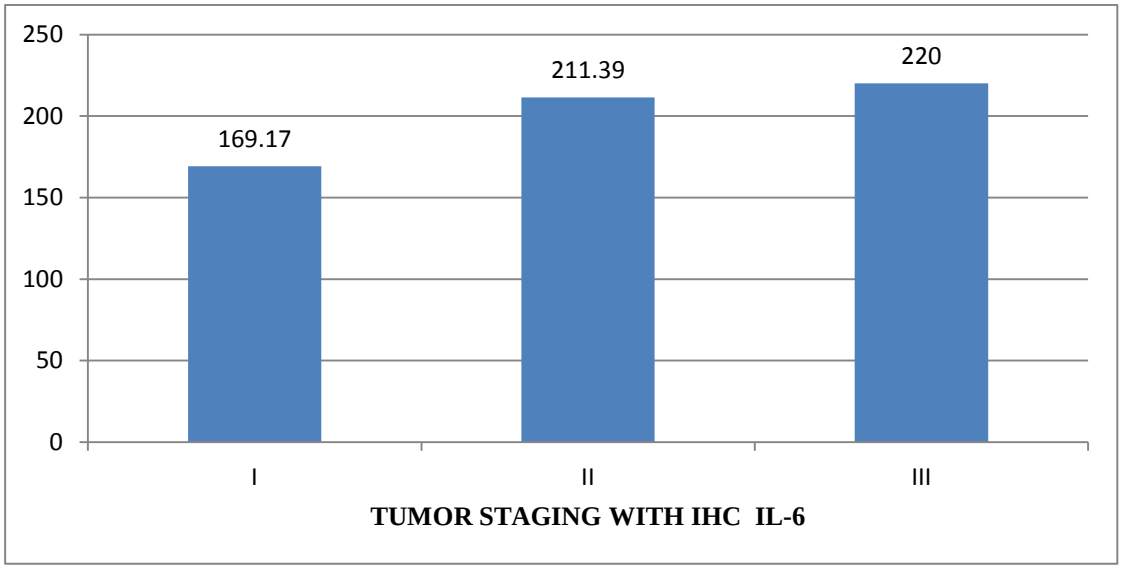


CHART 28 : BAR DIAGRAM SHOWING DISTRIBUTION OF TUMOR STAGING WITH IHC IL-6

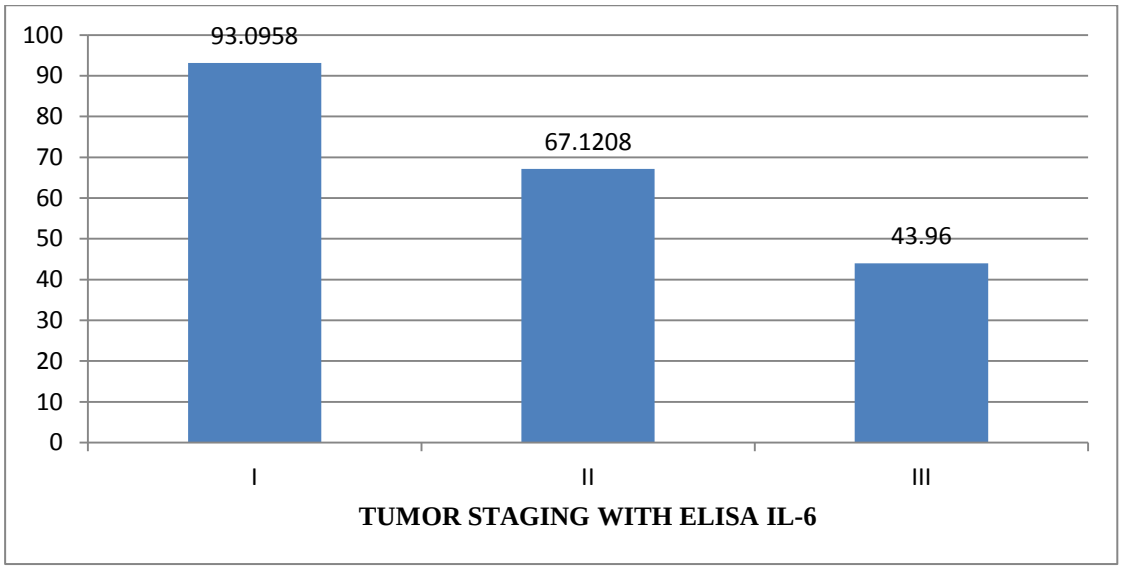


CHART 29 : BAR DIAGRAM SHOWING DISTRIBUTION OF TUMOR STAGING WITH ELISA IL-6

In this study, 36(72%) of the study population were having stage 2 with mean IHC IL 6 showing predominance among moderate stage 3 study population. Mean ELISA IL-6 was showing predominance among mild stage 1 study population. But it was determined that there was no significant difference between the sample groups.

TABLE 22 : DISTRIBUTION OF MODIFIED SBR GRADING WITH IHC IL-6 AND ELISA IL6

MODIFIED SBR GRADING	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
Grade 1	10	20	183.00	66.67	57.50	16.58
Grade 2	23	46	200.43	90.02	90.69	66.06
Grade 3	17	34	214.12	68.01	60.50	32.23
Total	50	100	211.65	66.53	68.34	34.65
p VALUE			0.613		0.093	

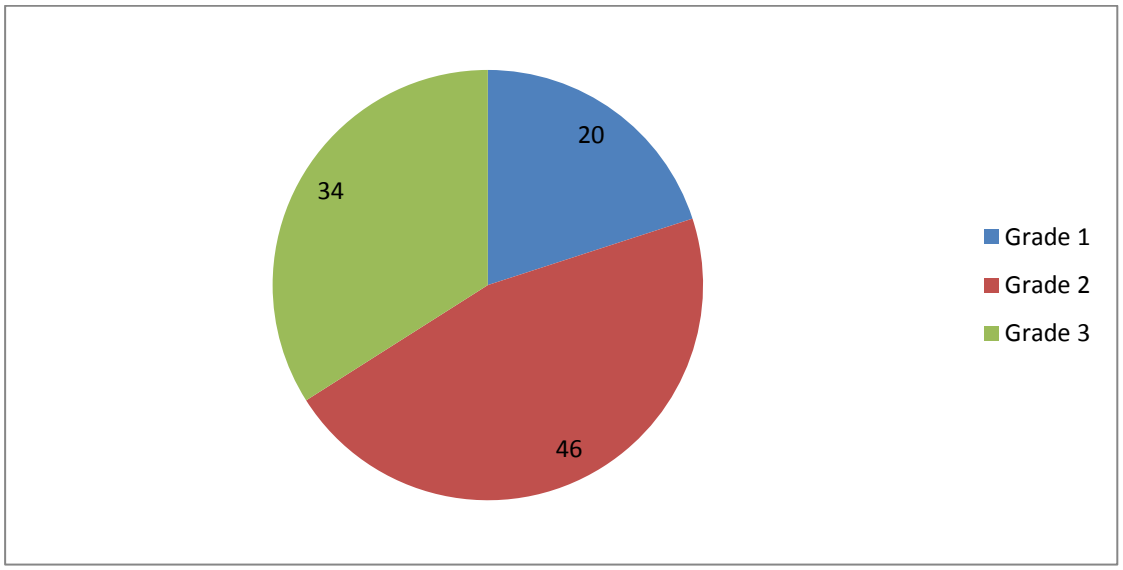


CHART 30 : PIE DIAGRAM SHOWING DISTRIBUTION OF MODIFIED SBR GRADING

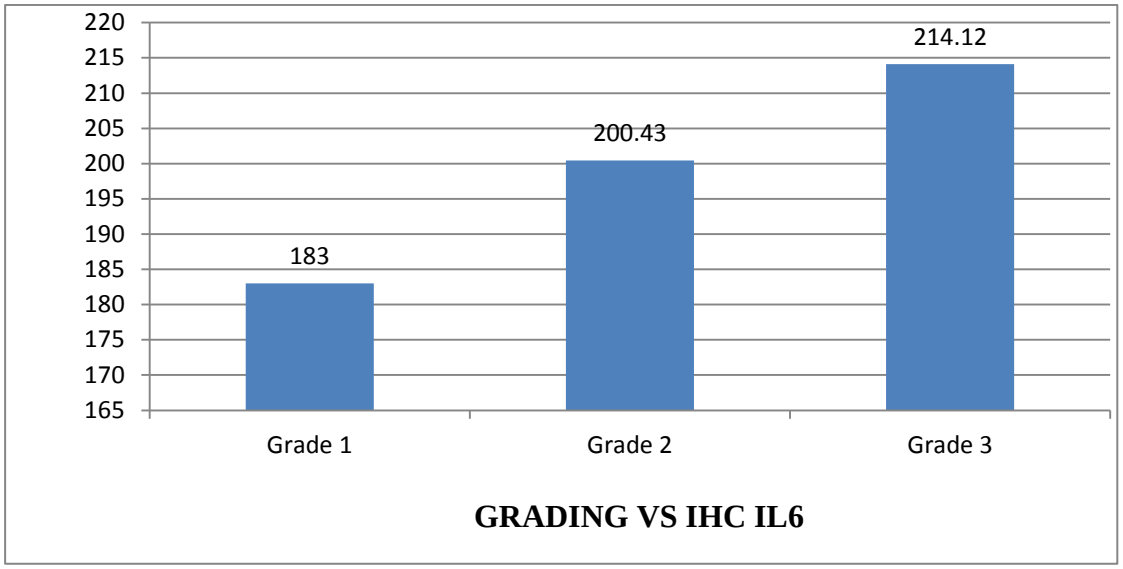


CHART 31: BAR DIAGRAM SHOWING DISTRIBUTION OF MODIFIED SBR GRADING WITH IHC IL-6

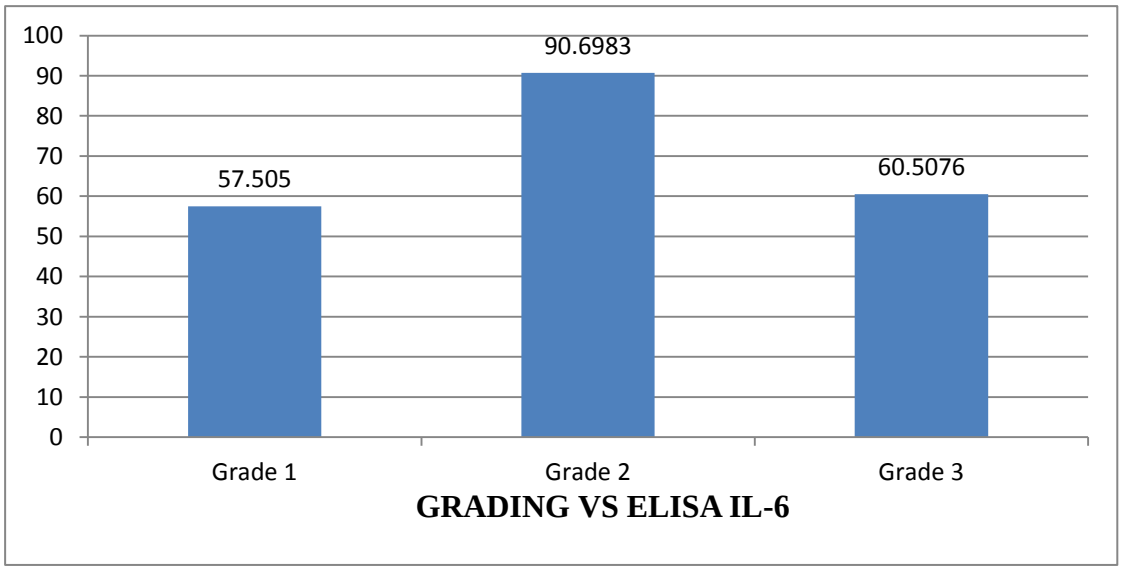


CHART 32: BAR DIAGRAM SHOWING DISTRIBUTION OF MODIFIED SBR GRADING WITH ELISA IL6

In current study, 23(46%) of the study population were Grade 2 with mean IHC IL 6 among Grade 3 study population showing predominance among them. Mean ELISA IL-6 was showing predominance among Grade 2 study population. But it was determined that there was no significant difference between the sample groups.

TABLE 23: DISTRIBUTION OF NPI WITH IHC IL-6 AND ELISA IL6

NPI	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
Good (<3.4)	39	78	194.62	81.36	77.58	54.80
Moderate (3.4–5.4)	11	22	226.36	61.85	60.34	32.65
Total	50	100	223.54	78.78	72.34	43.45
p value			0.237		0.327	

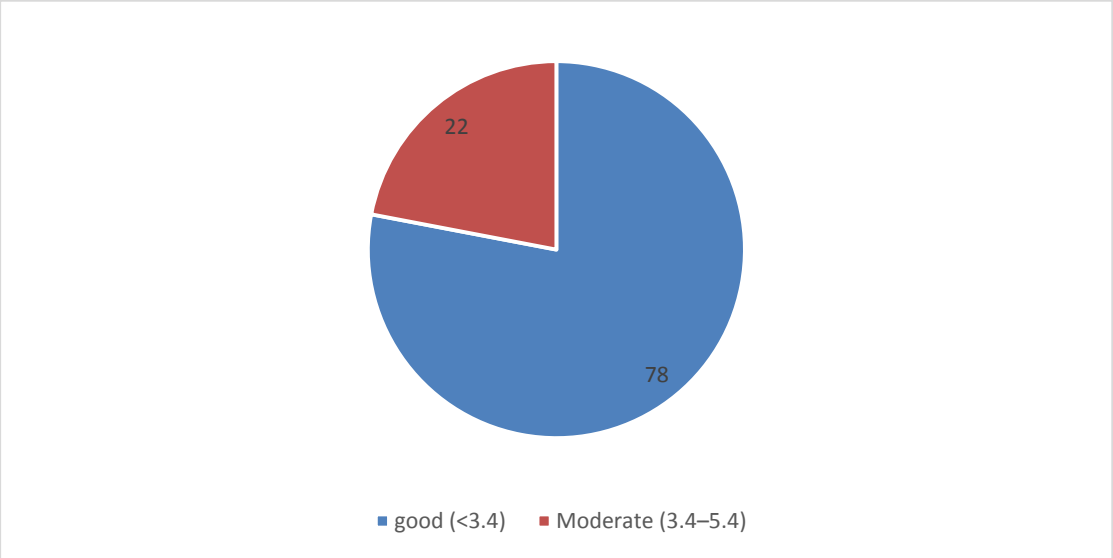


CHART 33 : PIE DIAGRAM SHOWING DISTRIBUTION OF NPI

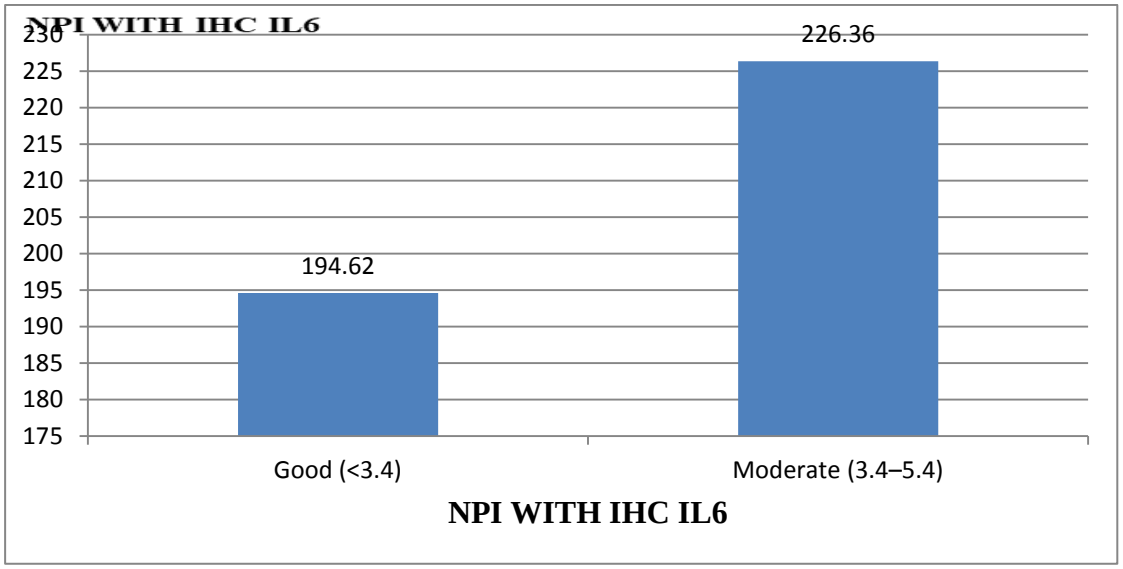


CHART 34 : BAR DIAGRAM SHOWING DISTRIBUTION OF NPI WITH IHC IL-6

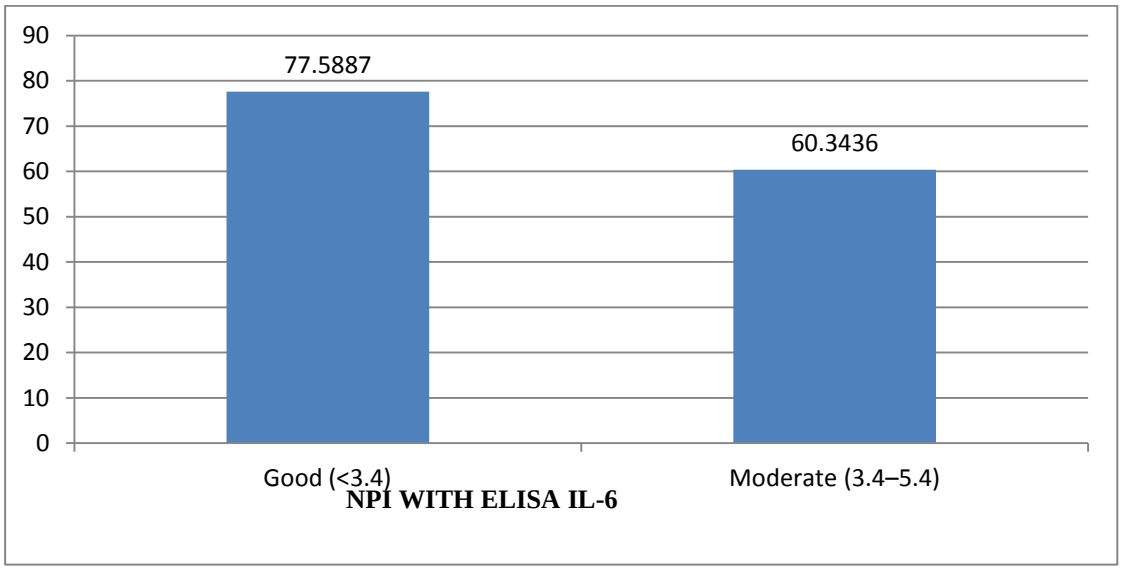


CHART 35 : BAR DIAGRAM SHOWING DISTRIBUTION OF NPI WITH ELISA IL6

In current study, 39 (78%) of the study population were having good NPI with mean IHC IL 6 and mean ELISA IL-6 predominance among them. But it was determined that there was no significant difference between the sample groups.

TABLE 24: DISTRIBUTION OF ER STATUS WITH IHC IL-6 AND ELISA IL6

ER	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
Positive	21	42	195.24	71.80	82.86	48.41
Negative	29	58	206.21	83.21	67.22	52.60
Total	50	100	201.76	77.87	77.86	49.76
	p value		0.629		0.289	

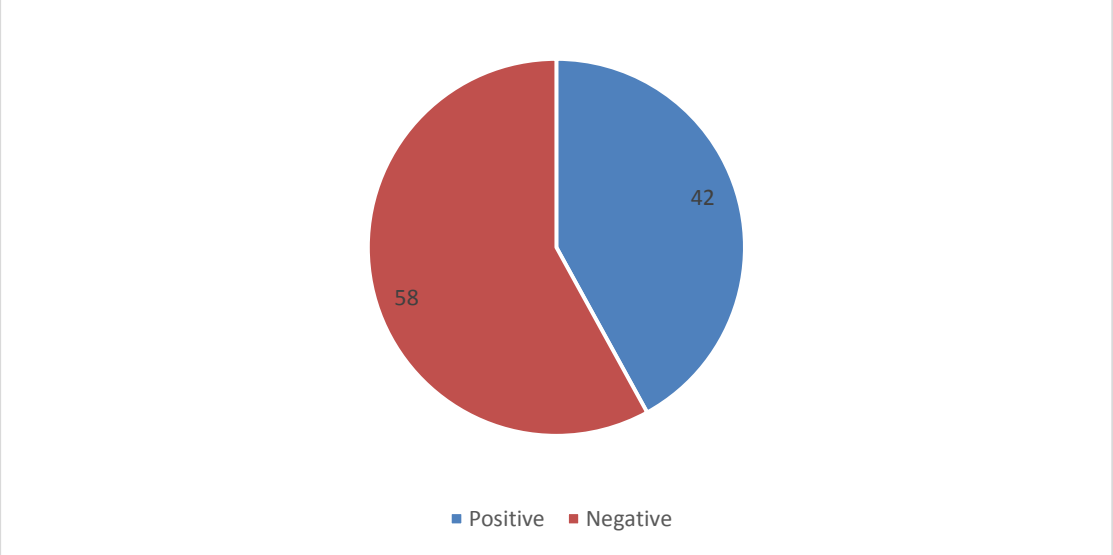


CHART 36 : PIE DIAGRAM SHOWING ER STATUS

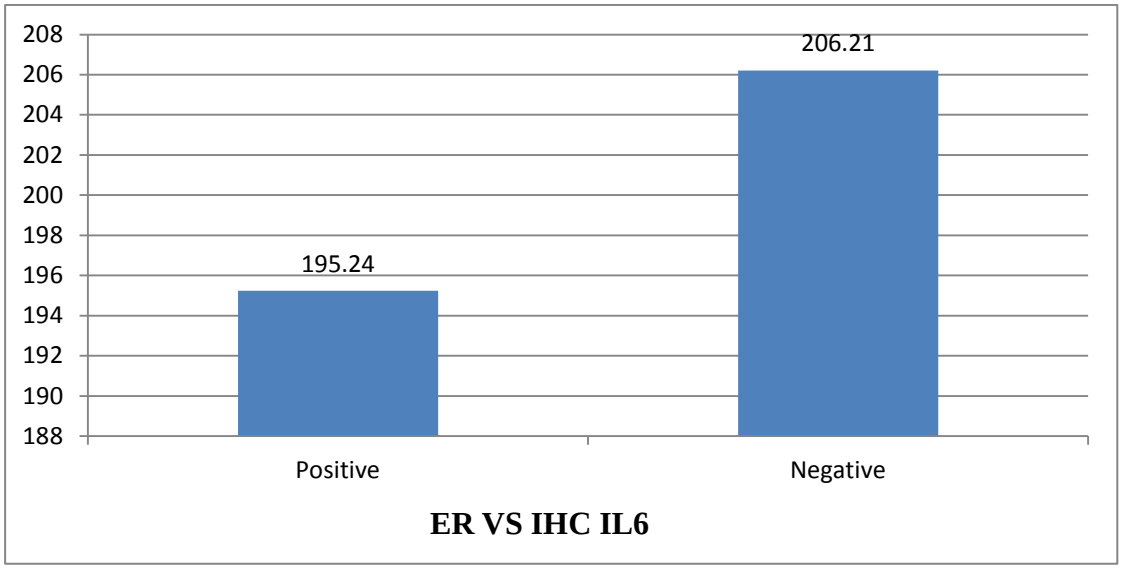


CHART 37 : BAR DIAGRAM SHOWING DISTRIBUTION OF ER STATUS WITH IHC IL-6

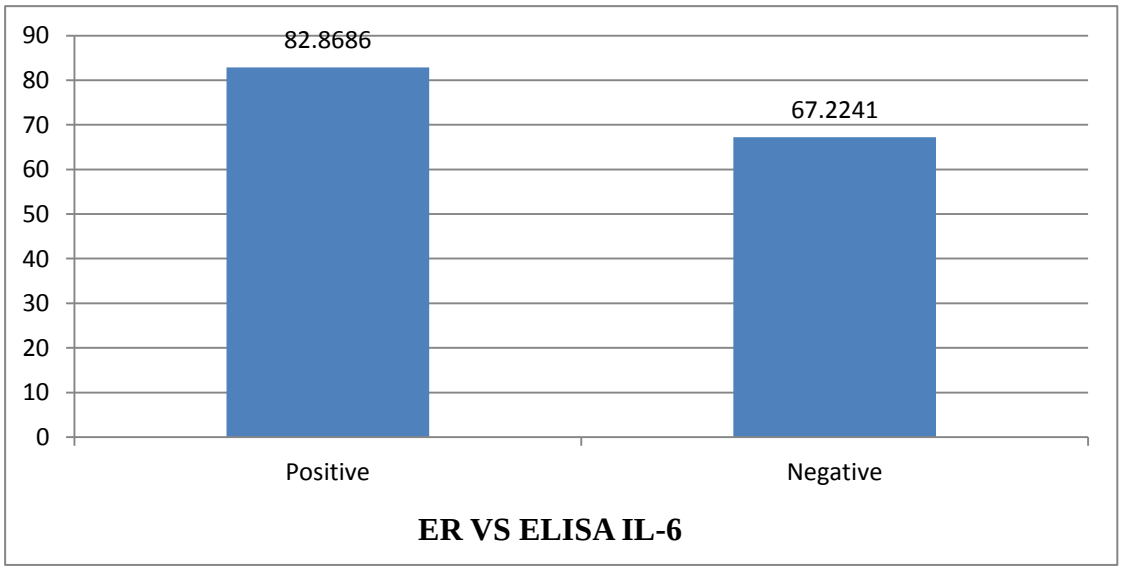


CHART 38 : BAR DIAGRAM SHOWING DISTRIBUTION OF ER STATUS WITH ELISA IL6

In present study, 29 (58%) of the study population were having negative ER expression with both mean IHC IL 6 and Mean ELISA IL-6 predominance among them. But it was determined that there was no significant difference between the groups.

TABLE 25: DISTRIBUTION OF PR STATUS WITH IHC IL-6 AND ELISA IL6

PR	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
Positive	19	38	199.47	74.34	80.89	48.20
Negative	31	62	202.90	81.41	69.44	32.90
Total	50	100	201.54	76.65	75.76	50.65
p value			0.882		0.446	

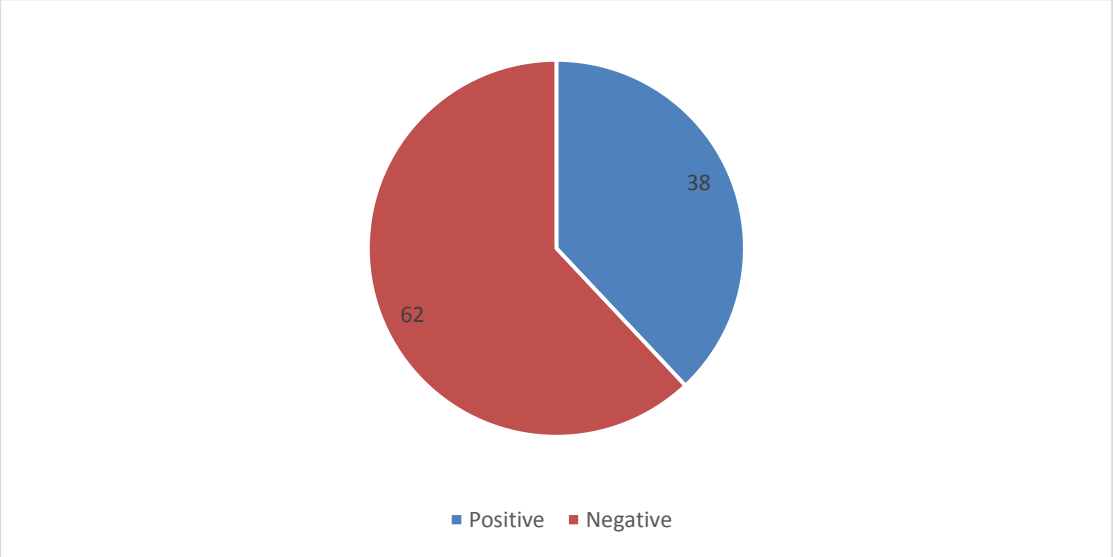


CHART 39 : PIE DIAGRAM SHOWING PR STATUS

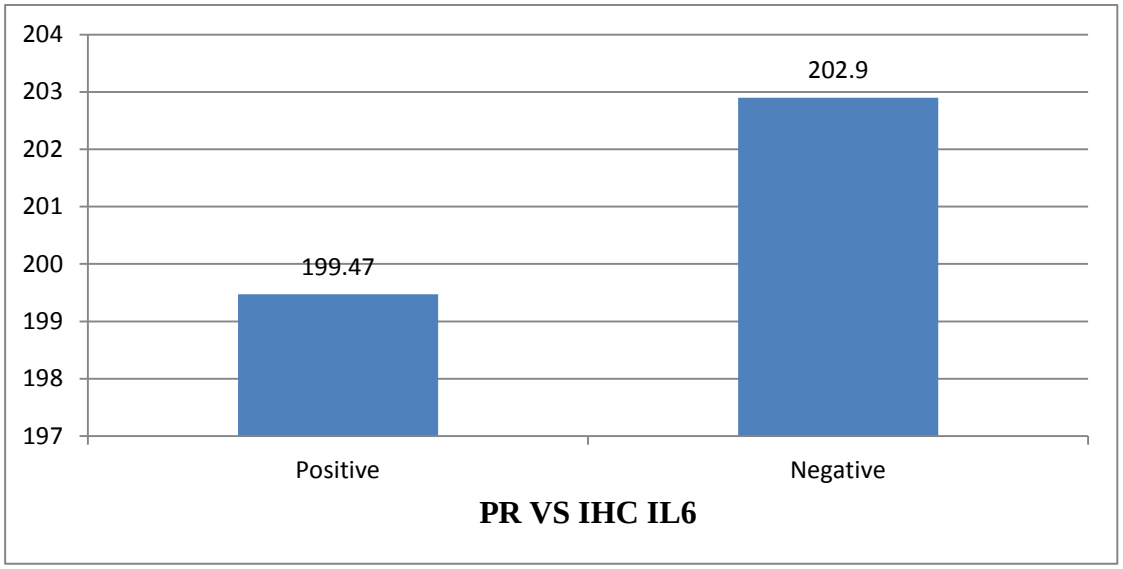
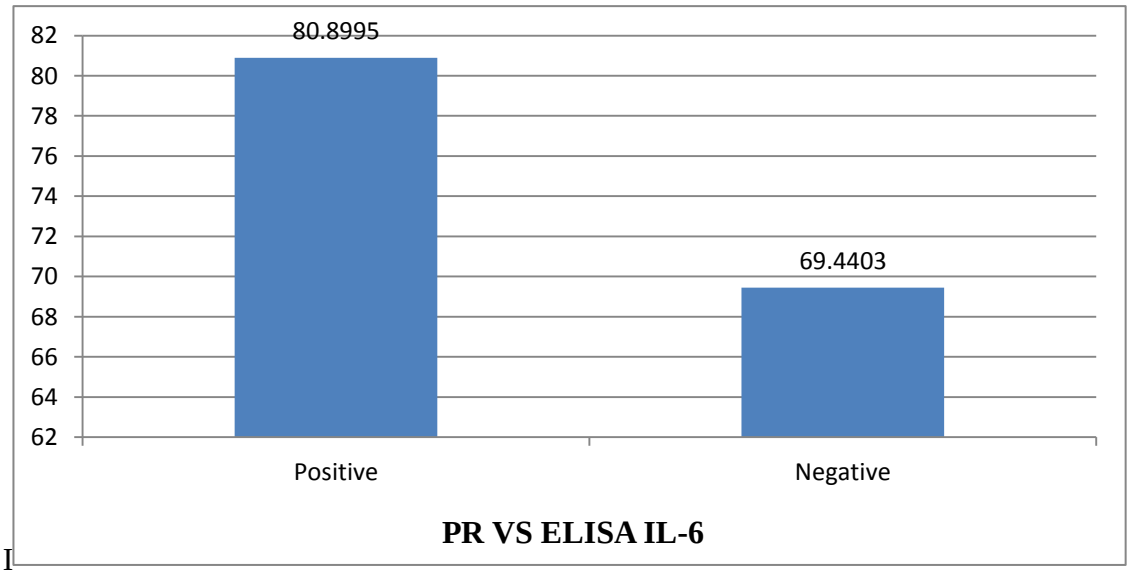


CHART 40 : BAR DIAGRAM SHOWING DISTRIBUTION OF PR STATUS WITH IHC IL-6



I

CHART 41 : BAR DIAGRAM SHOWING DISTRIBUTION OF PR STATUS WITH ELISA IL6

In present study, 31(62%) of the study population were having no PR expression with mean IHC IL 6 study population. Mean ELISA IL-6 showing predominance among PR positive expression study population. But the difference between the groups was not found to be showing any significance.

TABLE 26: DISTRIBUTION OF Her 2 Neu STATUS with IHC IL-6 AND ELISA IL6

Her 2 neu	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
Positive	15	30	212.00	66.35	96.14	71.81
Negative	35	70	197.14	83.05	64.21	36.15
Total	50	100	208.87	75.65	78.54	45.65
p VALUE			0.543		**0.041**	

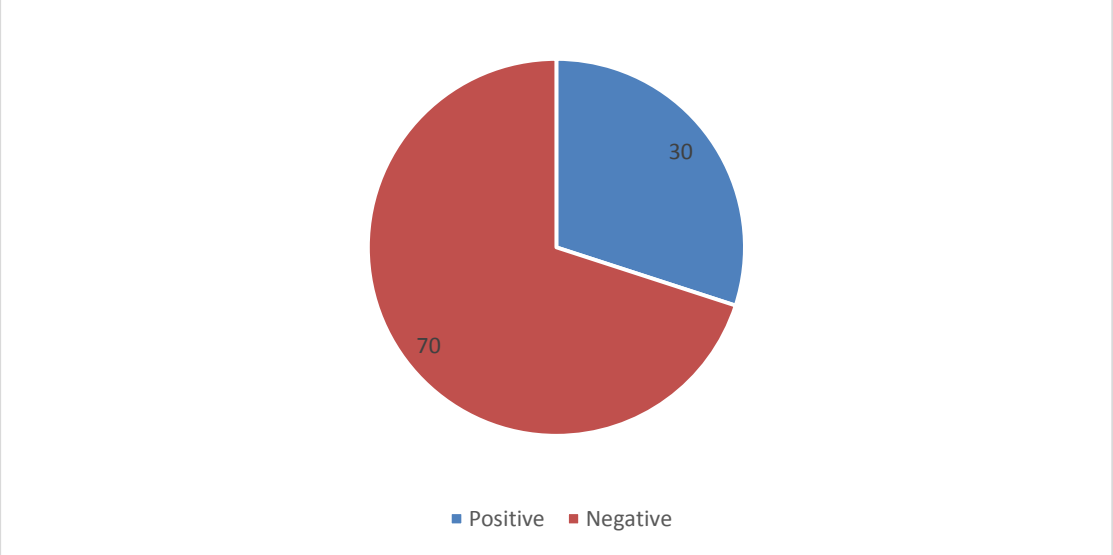


CHART 42 : PIE DIAGRAM SHOWING Her 2 Neu STATUS

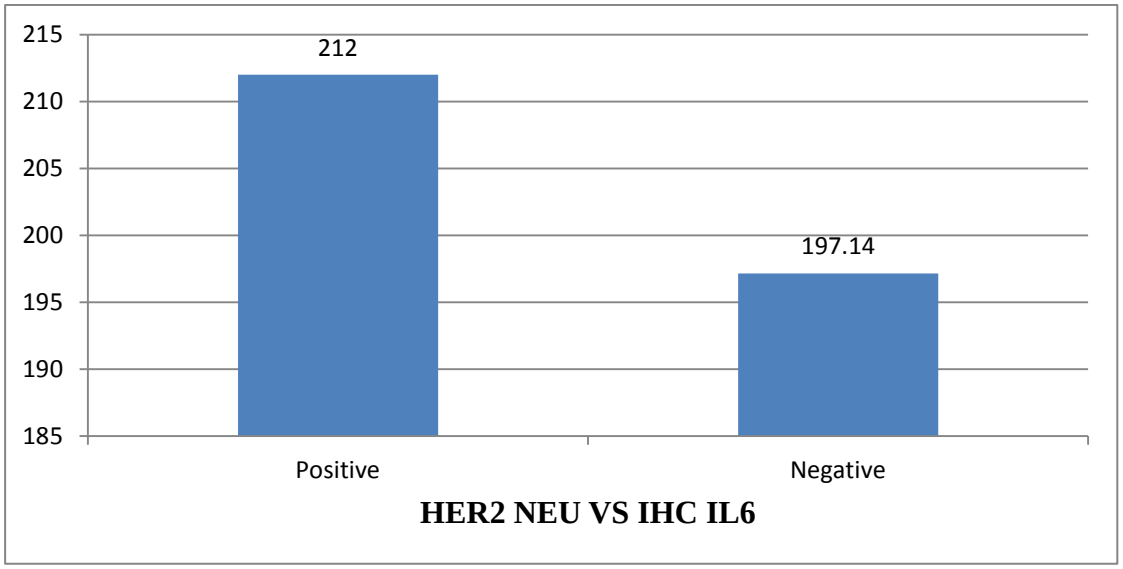


CHART 43 : BAR DIAGRAM SHOWING DISTRIBUTION OF Her 2 Neu STATUS with IHC IL-6

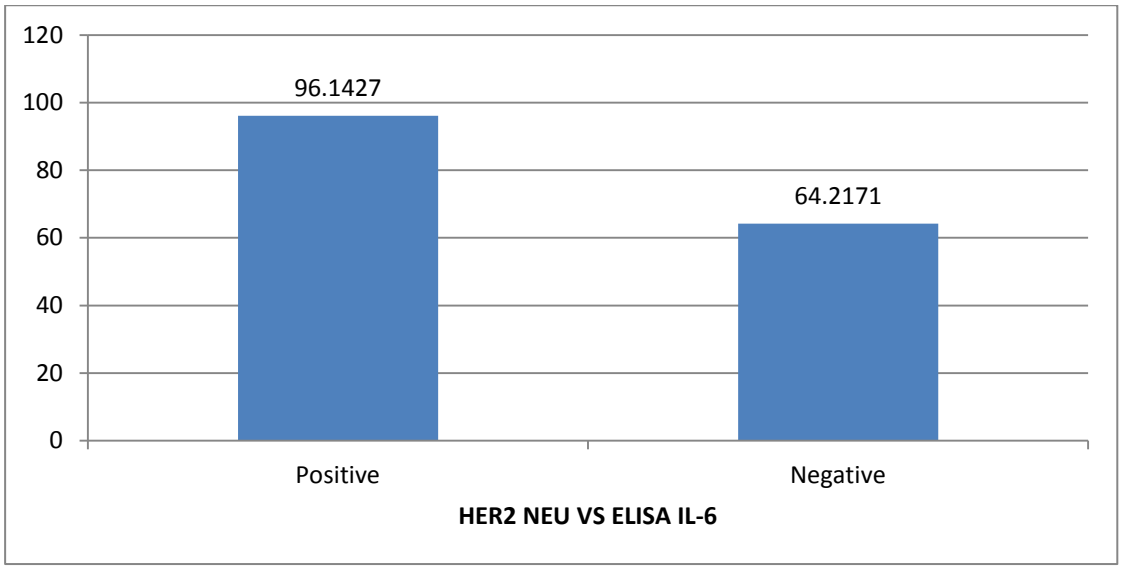


CHART 44 : BAR DIAGRAM SHOWING DISTRIBUTION OF Her 2 Neu STATUS with ELISA IL6

In present study, 35(70%) of the study population were found to be Her 2 neu negative expression. 30% of the study population were found to be Her 2 neu positive. The mean IHC IL 6 and ELISA IL6 of Her 2 neu positive study population were showing predominance among them. The association was found to be statistically significant between ELISA IL-6 and Her 2 neu status of the sample participants.

TABLE 27: DISTRIBUTION OF Ki67 with IHC IL-6 AND ELISA IL6

Ki67	FREQUENCY	PERCENTAGE	IHC		ELISA	
			MEAN	SD	MEAN	SD
<14%	21	42	186.19	77.42	86.56	65.19
>14%	29	58	212.76	77.91	64.54	36.06
Total	50	100	198.76	77.76	77.12	44.78
p VALUE			0.543		**0.041**	

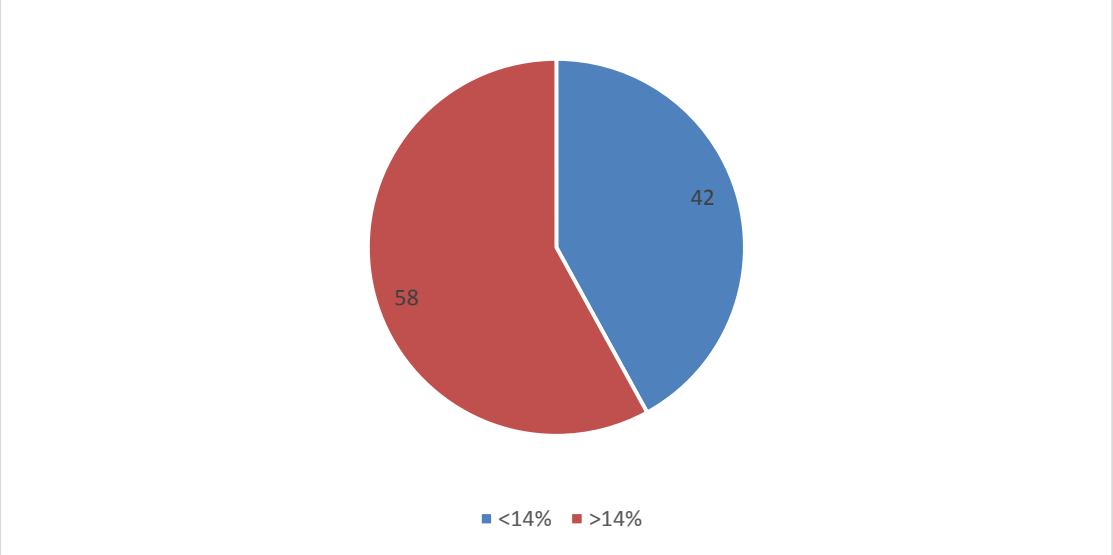


CHART 45 : PIE DIAGRAM SHOWING Ki67 DISTRIBUTION

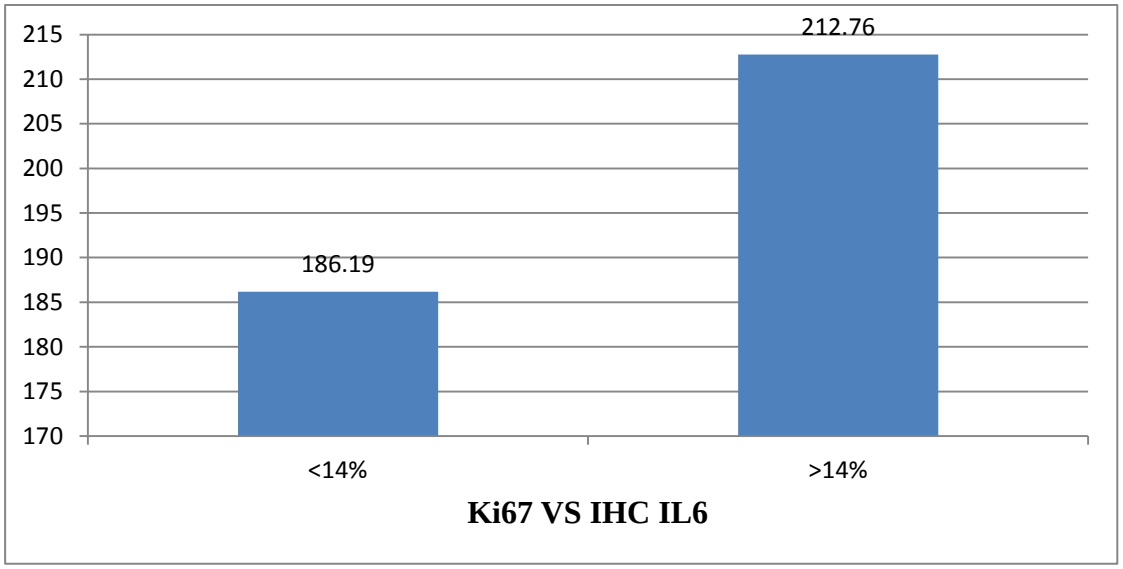


CHART 46 : BAR DIAGRAM SHOWING DISTRIBUTION OF Ki67 WITH IHC IL-6

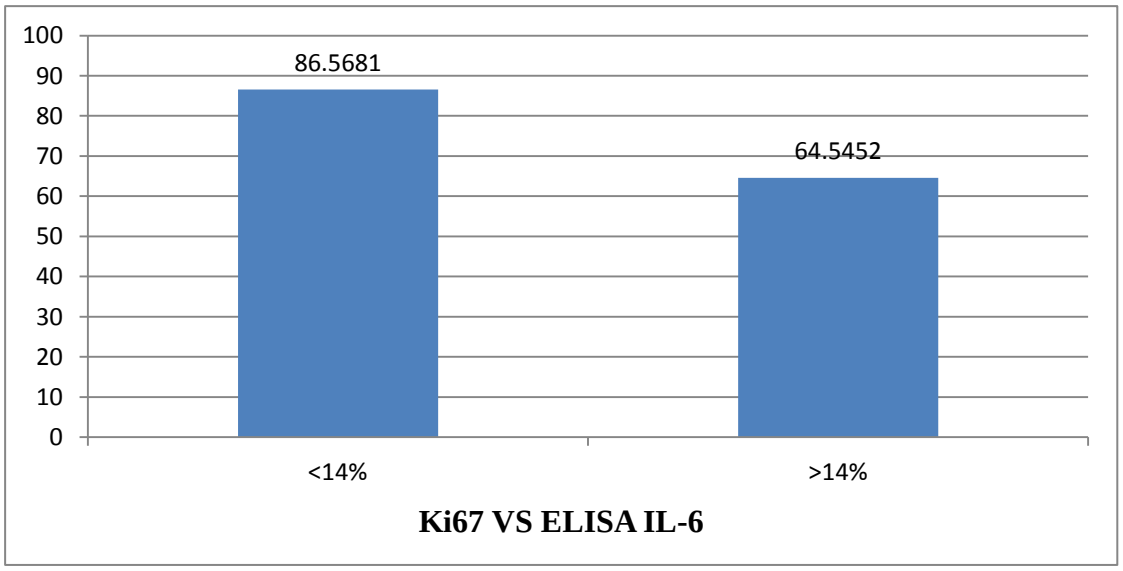


CHART 47 : BAR DIAGRAM SHOWING DISTRIBUTION OF Ki67 WITH ELISA IL6

In current study, 58% of the study population were found to have Ki67 value more than 14% and showing mean IHC IL 6 with predominance among them. The mean ELISA IL 6 of study population with Ki67 less than 14% were showing predominance among them. The association was found to be statistically significant between ELISA IL-6 and Ki67 of the study participants.

TABLE 28 : DISTRIBUTION OF MOLECULAR EXPRESSION WITH IHC IL-6 AND ELISA IL6

Molecular	Frequency	Percentage	IHC IL6		ELISA IL6	
			MEAN	SD	MEAN	SD
Luminal A	8	16	205.00	76.15	96.11	52.77
Luminal B	13	26	189.23	71.46	74.72	45.76
HER 2-ENRICHED	9	18	230.00	65.57	178.765	77.76
Triple negative	20	40	195.50	89.47	54.38	18.07
Total	50	100	74.54	46.06	68.13	37.70
p value			0.660		0.051	

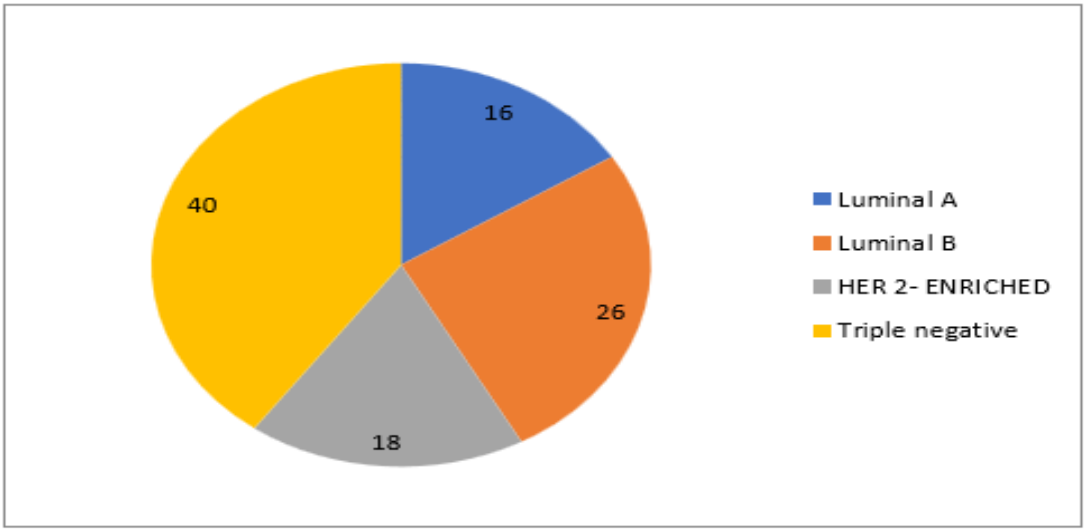


CHART 48 : BAR DIAGRAM SHOWING MOLECULAR EXPRESSION

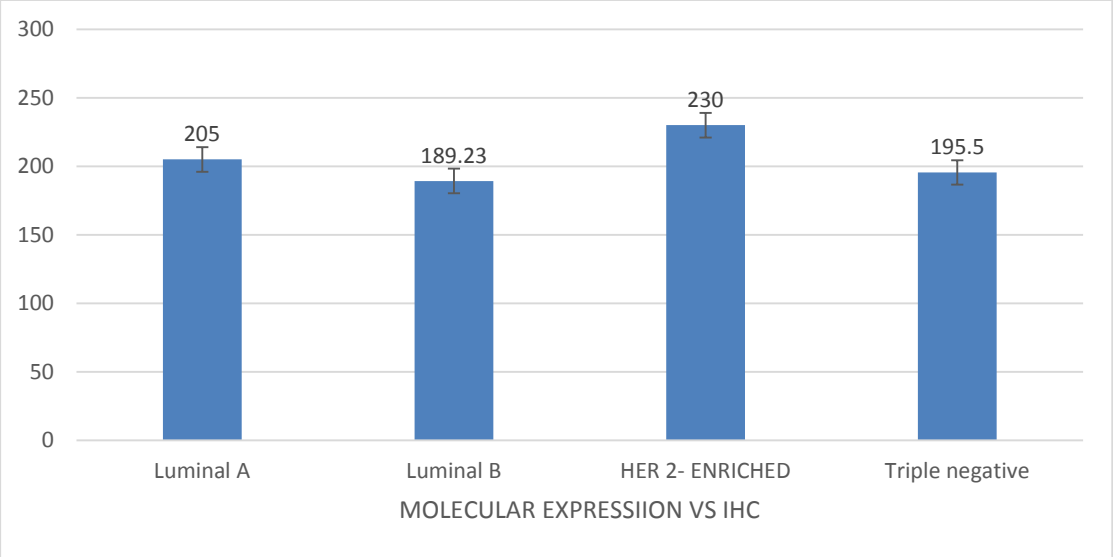


CHART 49 : BAR DIAGRAM SHOWING DISTRIBUTION OF MOLECULAR EXPRESSION AND IHC IL 6

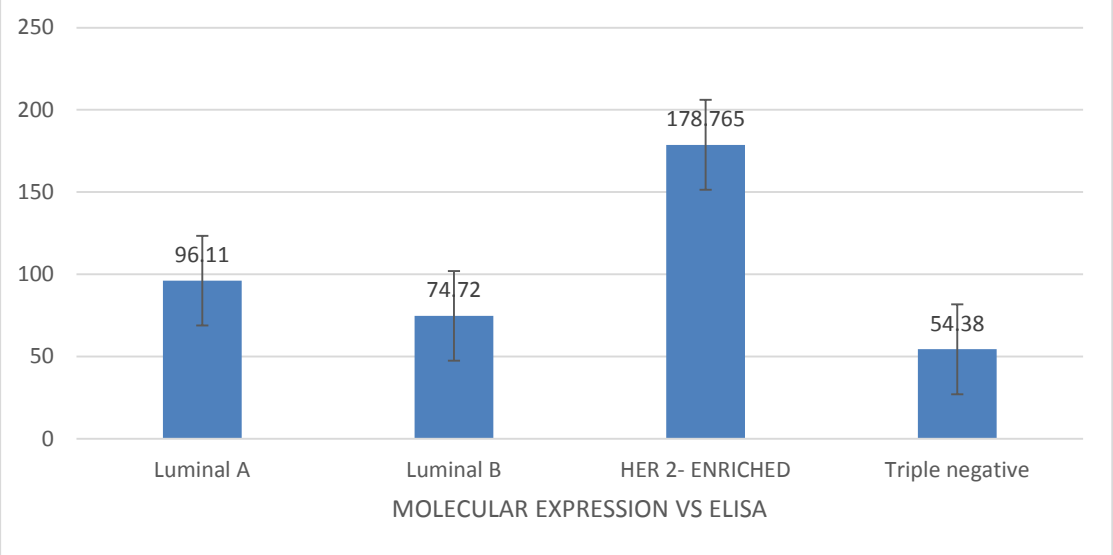


CHART 50 : BAR DIAGRAM SHOWING DISTRIBUTION OF MOLECULAR EXPRESSION AND ELISA IL6

In current study, 40% of the study participants were found to be triple negative on molecular expression. 26% of the study participants had Luminal B, 18% had Her 2-enriched and 16% had Luminal A on molecular expression. The IL-6 on IHC and ELISA were found to be more among HER 2-enriched molecular expression. The association was not found to be showing statistical association. IL-6.

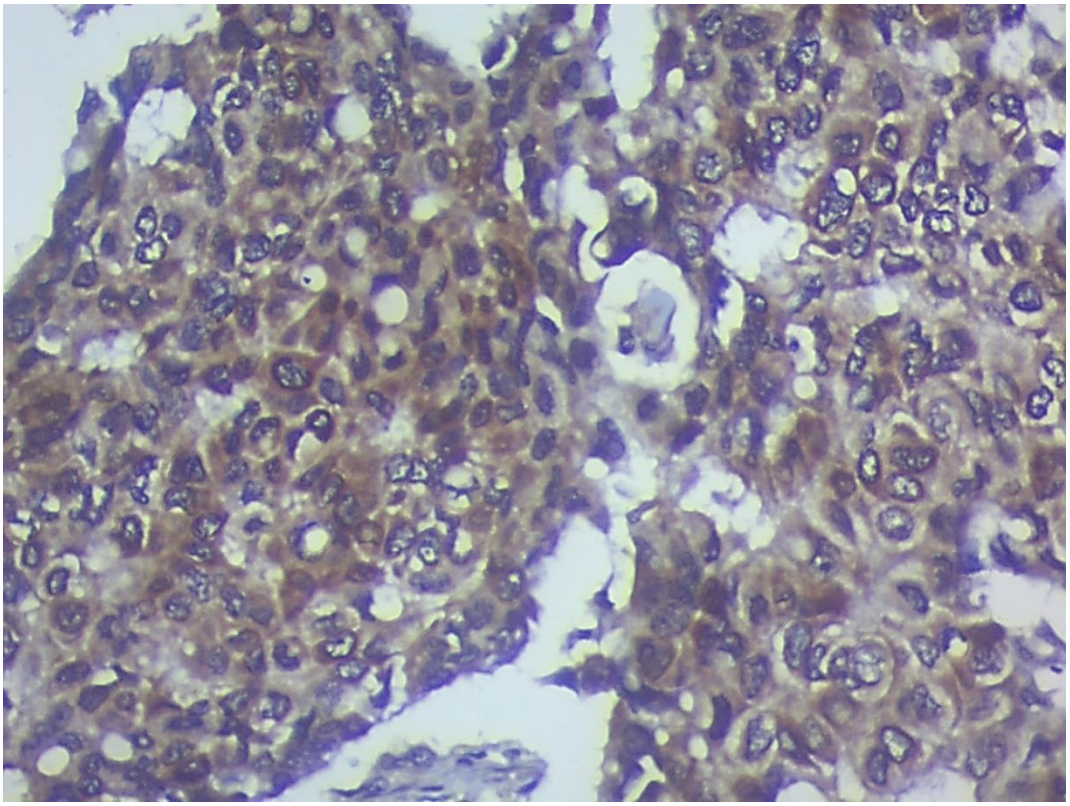


Figure 10 : Microphotograph showing low expression of IHC IL6 (IHC IL6, 100X)

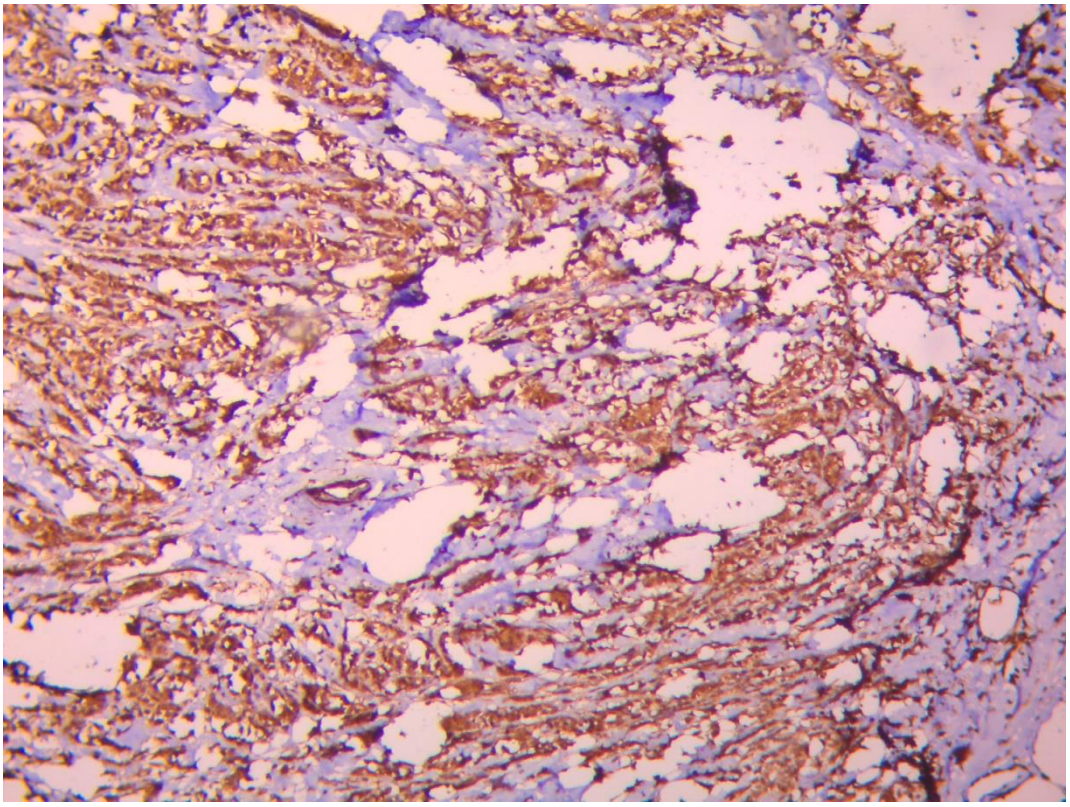


Figure 11 : Microphotograph showing strong expression of IHC IL6 (IHC IL6, 100X)

DISCUSSION

DISCUSSION:

A number of factors produced by Cancer associated fibroblasts (CAFs) had been shown to be involved in promoting malignant transformation in epithelial cells, these include TGFß and CXCL12, stromal cell-derived factor 1 (SDF-1). These factors contributed to a variety of responses that helped the tumour in different ways. [82] It has been established that persistent inflammation in the tumour microenvironment fosters tumour growth and develops resistance to radiation and chemotherapy. Breast cancer is one of many cancers where interleukin-6 (IL-6) cytokine overexpression in the tumour microenvironment has been seen. Tumor cells and fibroblasts associated with the tumour were the main sources of IL-6 release in the tumour microenvironment. The importance of IL-6 immunopathogenic function and its signalling in the development, metastasis, and therapy of tumours has been well established by a number of studies. [9]

Age:

In present study, majority 20 (40%) of the study population were in 56 to 65 years age group with an average age of 56.7 years. In a study done by Lee JS et al., average age of 49.8 years in breast malignancy was stated. [84] In a study done by Osuala KO et al., age of Carcinoma breast patients ranged from 30 to 81 years with an average age of 49.5 years. [82]

Table 29: Comparison of Present study with other studies with respect to mean age

Study	Year	Mean age
Present study	2022	56.7 ± 5.6
Lee JS et al[83]	2019	49.8±10.2
Osuala KO et al [82]	2015	49.5 ± 6.75

Menopausal status:

In present study, 13 (26%) of the study population were in premenopausal status and 37 (74%) postmenopausal status. In a study done by Surakasula A et al., 54% of the study population attained postmenopause, and risk of breast malignancy was high in late menopause but less risk Compared to premenopausal women of the same age group. Lack of awareness, poverty, and a lack of screening tools were the main causes of late stage presentation. [84]

Table 30 – Comparison of Present study with other studies with respect to postmenopausal phase

Study	Year	**Postmenopausal**
Current study	2022	74%
Surakasula A et al[84]	2014	54%

Premeopausal:

In present study 26% of samples were in premenopausal status and limited literature available on this to discuss. In a study done by Surakasula A et al.,[84] premenopausal women had more risk of Carcinoma breast as compared to postmenopausal of same age.

Table 31 – Comparison of Present study with other studies with respect to premenopausal phase

Study	**Year**	**Premenopausal**
Present study	2022	74%
Surakasula A et al[84]	2014	46%

Parity:

In the present study, only 3 (6%) of study population were primipara, and 47(94%) were multipara. In a study by Fortner RT et al., [85]13% of participants were primipara, and 77% were multipara. Parity was associated with a lower risk of ER positive breast cancer but not associated with ER negative disease. In a study by Lee JS et al, 80.9% of study population were multipara. High parity was associated with increased of breast malignancy.[83]

Table 32 : Comparison of Present study with other studies with respect to Primipara

Study	**Year**	**PRIMI PARA**
Present study	2022	6%
Fortner RT et al[85]	2019	13%

Table 33 : Comparison of Present study with other studies with respect to Multipara

Study	Year	MULTI PARA
Present study	2022	94%
Fortner RT et al. [85]	2019	77%
Lee JS et al	2019	80.9%

BMI:

In the present study, 32 (64%) of the study population were normal BMI level, 14 (28%) of the study population were underweight and 8% of participants were overweight.

In the study by Jee et al. [86], 53% of the study population were in normal BMI levels & found that a higher BMI could increase breast cancer risk in the Korean population. However, higher BMI could be a protective factor against breast cancer risk for premenopausal women.

Conversely, in the study by Palmer et al., 73% of the study population were in normal BMI levels, demonstrating that lower BMI was associated with breast cancer risk [87] For premenopausal women, a higher BMI could decrease breast cancer risk. However, higher BMI was associated with increased breast cancer risk in postmenopausal women. The exact mechanism behind the association between BMI and breast cancer risk was uncertain, but there were some potential hypotheses.[87]This dose- response metaanalysis of prospective cohort type study showed that every 5kg/m^2 increase in BMI will lead to breast malignancy risk to 2% among females.

Table 34 : Comparison of Present study with other studies with respect to BMI

Study	Year	Normal BMI
Present study	2022	64%
Jee et al. [86]	2008	53%
Palmer et al[87]	2007	73%

IHC IL6 and ELISA IL6 :

In a study by Osuala KO et al., the Mean IHC IL 6 Value was 75.76±34.65 IHC. About 65% of patient samples had IHC IL-6 expression that was positive.[82] Chronic inflammation in the tumour microenvironment had been demonstrated to promote tumour growth and to create resistance to radiation and chemotherapy. Interleukin-6 (IL-6) cytokine overexpression in the tumour microenvironment had been found in a variety of malignancies, including breast cancer. The primary sources of IL-6 release in the tumour microenvironment were tumour cells and tumor-associated fibroblasts. Numerous research had established the role of IL-6 immunopathogenic function and its signalling in the development, metastasis, and treatment of tumours. [9]

Age in relation to IHC IL6:

In present study, mean values of IL6 by IHC method was highest in 46 to 55 years of age i.e 209.51 ± 64.12. In a study done by Osuala KO et al., [82]age of Carcinoma breast patients ranged from 30 to 81 years with an average age of 49.5 years and 65% cases were showing IHC IL 6 expression. Although there was no correlation between age and IHC IL6 but high expression of IL6 marker was associated with poor outcome.

AGE with ELISA IL6:

In current study, mean ELISA IL-6 with 76.38 ± 41.76 was highest among 56 to 65 years age group. Limited data available in English literature on this to compare.

Menopausal phase with IHC IL6:

In current study, mean values of IL6 by IHC method was 216.92 ± 83.20 among premenopausal and 196.22 ± 76.60 among postmenopausal women respectively. Limited data available in English literature on this to compare.

Menopausal phase with ELISA IL6:

In current study, mean values of IL6 by ELISA method was 90.32 ± 37.47 among premenopausal and 60.33 ± 35.01 among postmenopausal women respectively. Limited data available in English literature on this to compare.

Parity and IHC IL6:

In the current study, only 3(6%) of participants were primipara. Mean values of IHC IL6 among primipara was 206.67± 65.58 and among multipara was 201±79.3 respectively. Limited data available in English literature on this to compare.

Parity and ELISA IL6:

In the present study 47(94%) of participants were multipara. Mean values of ELISA among primipara was 40.25 ± 6.75 and multipara was 69.9±38.18 respectively. Limited data available in English literature on this to compare.

BMI association with IHC IL6

In present study, 32 (64%) of the study population were in normal BMI levels (18.5-24.9) with mean IHC IL6 88.74±72.98,14(28%) were underweight (18.5) with mean IHC IL6 68.18±39.23,4(8%) were overweight with mean IHC IL-6 66.35 ± 44.78 respectively. Limited data available in English literature on this to compare.

BMI association with ELISA IL6

Mean ELISA IL-6 was among underweight BMI levels 14(28%). In a study done by Teixeira et al.,[88] ELISA IL 6 levels among normal group were 0.90 pg/ml compared to 0.64 pg/ml among the obese group. Limited data available in English literature on this to compare.

TIL association with IHC IL6

In current study, mean values of IHC IL 6 were 190.91±79.93 in absence of TIL(66%) and 222.35±71.98 in presence of TIL (34%) respectively. Limited data available in English literature on this to compare.

TIL association with ELISA IL6

In current study, mean values of ELISA IL 6 were 73.50±54.12 in absence of TIL(66%) and 74.35±45.82 in presence of TIL (34%) respectively. Limited data available in English literature on this to compare.

LVI association with IHC IL 6:

In current study, mean values of IHC IL 6 were204.79±76.48 in absence of LVI (98%) and 70 in presence of LVI (2%) respectively. Limited data available in English literature on this to compare.

LVI association with mean ELISA IL6

In present study, 49(98%) of the study population were not having lymphovascular Invasion(98%) with mean IHC IL 6 74.79±37.56 and 24.85 with presence of LVI (2%) respectively. Limited data available in English literature on this to compare.

pT association with IHC IL6:

In current study, mean values of IHC IL 6 were 207.57±71.88 among T1 stage, 213.22±76.60 among T2 stage, 201.65±66.53 among T3 stage and 197.22±75.60 respectively. Limited data available in English literature on this to compare.

pT association with ELISA IL6

In present study, mean ELISA IL-6 values were 73.79±64.79. In a study done by Fontanini G at al., no association was observed between IL-6 expression and tumour size or nodal status. [88] Limited English literature available on this aspect to compare.

pN (Metastatic lymph node) association with IHC IL6:

In present study, mean values of IHC IL 6 were 190.00±98.99 among those who had pN participants. Limited data available in English literature on this to compare.

pN (Metastatic lymph node) association with ELISA IL6:

In present study, mean values of IHC IL 6 were 42.99±3.87 among those who had pN participants. Limited data available in English literature on this to compare.

Tumor staging association with IHC IL6:

In present study, mean values of IHC IL 6 were 169.17±78.21 among stage I participants, 211.39±78.27 among stage II, 220 among stage III. Limited data available in English literature on this to compare.

Tumor staging association with ELISA IL6

In present study, 36(72%) of the study population were having stage 2 with mean IHC IL 6 predominance among them. Mean ELISA IL-6 values 67.12±36.73 was showing predominance among moderate stage 3 study population. In a study done by Osuala KO et al [82] showed that there is progressive increase in IL-6 levels as the stage of disease progresses. This increase was found to be statistically significant. Our results were in accordance with study done by Kozlowski et.al,[88] which assessed the concentration of IL-6 in blood serum of breast cancer patients to determine whether it correlated with the disease progression. On the contrary, 21% grade II or III ductal carcinomas were IL-6-negative. In a study done by Ravishankaran P et al. [89], with increasing degrees of tumour invasion, the median level of IL-6 increased proportionally with the stage of the cancer.

Table 35 : Comparison of current study with other studies with respect to tumor staging

Study	Year	Stage II Mean ELISA IL 6	Stage III Mean ELISA IL 6
Present study	2022	67.12±36.73	43.96
Ravishankaran P et al.[89]	2011	17.3±5.9	62.3±16.5

Grading association with IHC IL6:

In current study, mean values of IHC IL 6 were 57.50 ± 16.58 among grade I participants. Limited data available in English literature on this to compare.

Grading association with ELISA IL6 :

In current study, 46% study population were showing mean IHC IL6 values 200.43 ±90.02 among grade 2. But with increase grading , no association was seen.

In a study done by Fontanini G at al.,[90] a significant association was found between IL-6 expression and histological grading of the tumours; i.e. low-grade tumours (grade I) had higher IL-6 expression than high-grade tumours. Approximately one-fifth of investigated ductal carcinomas were classified as grade I. None of these well-differentiated tumours was IL-6-negative. Since high grade indicates less differentiated tumours, this finding suggested that reductions of IL-6 expression are associated with late stages of tumorigenesis.

ER positive expression and IHC IL6 :

In present study, mean values of IHC IL 6 were 82.86 ±48.41 among ER positive participants. Limited data available in English literature on this to compare.

ER positive expression and ELISA IL6 :

In present study, 42% of the study population were having positive ER expression in mean ELISA IL-6 predominance among them. But the difference between the groups was not found to be significant. Similar findings were also observed in a study done by Schillace RV et al.,[91] more expression of IL6 was observed among ER positive participants. IL-6 is found in ER positive tumors and was thought to synergize with estrogen to increase ER transcriptional activity.

ER negative expression and IHC IL6 :

In present study, mean values of IHC IL 6 were 206.21±83.21 among ER negative participants. Limited data available in English literature on this to compare.

ER negative expression and ELISA IL6 :

In present study, 29 (58%) of the study population were having negative ER expression with Mean ELISA IL-6 67.22 ±52.60 among them. But the difference between the groups was not found to be significant. In a study done by Fontanini G at al., 68% of the study population were having negative ER expression, among the 50 ER-negative tumours 36 (72%) were IL-6-positive. Similar findings were also observed in a study done by Schillace RV et al., less expression of ELISA IL6 was observed among ER negative participants.[91]

Table 36 – Comparison of current study with other studies with respect to ER negative expression

Study	Year	ER negative
Present study	2022	58%
Fontanini G[90]	1999	68%

PR negative expression and IHC IL6:

In current study, mean values of IHC IL 6 were 202.90±81.41 among PR negative participants. Limited data available in English literature on this to compare.

PR negative expression and ELISA IL6:

In present study, 31(62%) of the study population were having negative PR expression with Mean ELISA IL-6 69.44±32.90 showing predominance among them. In a study done by Schillace RV et al., more expression of IL6 was observed among PR negative participants.[91]

PR Positive expression and IHC IL6:

In present study, mean values of IHC IL 6 were 202.90±81.41 among PR positive participants. Limited data available in English literature on this to compare.

PR Positive expression and ELISA IL6:

In present study, 38% of the study population were having positive PR expression with Mean ELISA IL-6 showing lower among them. Similar findings were also observed in a study done by Schillace RV et al., reduced expression of ELISA IL6 was observed among PR positive study population.[91]

HER2 neu association with IHC IL6:

In present study, 70% study population with mean values of IHC IL 6 were 197.14±83.05 among HER2 NEU negative. Limited data available in English literature on this to compare.

HER2 neu association with ELISA IL6:

In present study, 35(70%) of the study population were having negative HER2 NEU expression with Mean ELISA IL-6 was showing predominance among Positive HER2 NEU expression study population 15(30%). Limited data available in English literature on this to compare.

Ki67 association with IHC IL6 :

In present study, mean values of IHC IL 6 were 186.19±77.42 among Ki67 <14%. Limited data available in English literature on this to compare.

Ki67 association with ELISA IL6 :

In present study, mean values of ELISA IL 6 were86.56±65.19 among Ki67 <14%. Limited literature available in English literature for comparison.

Molecular association with IHC IL 6:

In current study, mean values of IHC IL 6 were Luminal A (205.00±76.15), Luminal B (189.23±71.46), HER2-enriched (230±65.57) and Triple negative(195.50±89.47). Limited data available in English literature on this to compare.

Molecular association with ELISA IL 6:

In current study, mean values of ELISA IL 6 were Luminal A (96.11±52.77), Luminal B (74.72±45.76), HER2-enriched (178.76±77.76) and Triple negative (54.38±18.07).Limited data available in English literature on this to compare.

LIMITATIONS:

Small sample size and data was collected from single hospital, need to conduct multicentre study to assess status in a better way.

SUMMARY

SUMMARY:

1. In present study, majority, 40% of the total study population were in the range from 56 to 65 years age.
2. 74% of the total study population were in postmenopausal phase.
3. 94% of the total cases were multipara.
4. 64% of the total cases were having normal BMI.
5. 66% of the total cases were showing absence of tumor infiltrating lymphocytes.
6. 98% of the total cases were showing absence of lymphovascular invasion.
7. 66% of the total cases were showing pathological T2 staging.
8. 96% of the total cases were showing no metastatic lymph nodes.
9. 72% of the total cases were showing tumor stage II
10. 46% of the total cases were showing Modified SBR Grade 2.
11. 78% of the total cases were showing good NPI.
12. 58% of the total cases were showing ER negative expression.
13. 62% of the total cases were showing PR negative expression.
14. 70% of the total cases were showing Her 2 neu negative expression.
15. 58% of the total cases were showing high Ki67 proliferative index.
16. 40% of the total cases were in triple negative molecular category.
17. Mean IHC expression of IL6 observed was 201.6±88.4.
18. Mean IHC IL6 was showing predominance in age ranging from 46 to 55 years (36%) with value 209.44±64.12 with no statistical association.
19. Mean IHC IL6 was showing predominance in Premenopausal phase (26%) with value 216.92±83.20 with no statistical association.
20. Mean IHC IL6 was showing predominance in Primipara (6%) with value 206.67 ±66.58 with no statistical association.
21. Mean IHC IL6 was showing predominance in BMI with underweight (28%) with value 208.57c71.88 with no statistical association.
22. Mean IHC IL6 was showing predominance in presence of tumor infiltrating lymphocytes (34%) with 222.35±71.98 with no statistical association.
23. Mean IHC IL6 was showing predominance in absence of lymphovascular invasion (98%) with 204.29±76.48 with no statistical association.
24. Mean IHC IL6 was showing predominance in pathological T2 (66%) with 213.22±76.60 with no statistical association.

25. Mean IHC IL6 was showing predominance in group with no metastatic lymph nodes (96%) with 202.08±78.33 with no statistical association.
26. Mean IHC IL6 was showing predominance in stage II (72%) with 211.39±78.27 with no statistical association.
27. Mean IHC IL6 was showing predominance in Nottingham grade 3 (34%) with 214.12±68.01 with no statistical association.
28. Mean IHC IL6 was showing predominance in moderate NPI (22%) with 226.36±61.85 with no statistical association.
29. Mean IHC IL6 was showing predominance in ER negative expression (58%) with 206.21±83.21 with no statistical association.
30. Mean IHC IL6 was showing predominance in PR negative expression (62%) with 202.90±81.41 with no statistical association.
31. Mean IHC IL6 was showing predominance in Her 2 neu positive expression (30%) with 212±66.35 with no statistical association.
32. Mean IHC IL6 was showing predominance in Ki67 high proliferative index (58%) with 212.76±77.91 with no statistical association.
33. Mean IHC IL6 was showing predominance in Her 2 enriched molecular category (18%) with 230±65.57 with no statistical association.
34. The mean ELISA IL6 expression observed was 68.13±89.98
35. Mean ELISA IL6 was showing predominance in 56 to 65 years age group (40%) with 76.38±41.76 with no statistical association.
36. Mean ELISA IL6 was showing predominance in premenopausal phase (26%) with 90.32±37.4 **with statistical significance (p value = 0.01).**
37. Mean ELISA IL6 was showing predominance in multipara women (94%) with values 69.91±38.18 with no statistical association.
38. Mean ELISA IL6 was showing predominance in BMI underweight (<18.5)(28%) with 68.52±34.97 with no statistical association.
39. Mean ELISA IL6 was showing predominance in group with presence of tumor infiltrating lymphocytes (34%) with 74.35±45.82 with no statistical association.
40. Mean ELISA IL6 was showing predominance in group with absence of lymphovascular invasion (98%) with 69.01±37.56 with no statistical association.
41. Mean ELISA IL6 was showing predominance in pathological T2(66%) with 78.98±54.18 with no statistical association.

42. Mean ELISA IL6 was showing predominance in group with absence of metastatic lymph nodes (96%) with 75.07±51.64 with no statistical association.
43. Mean ELISA IL6 was showing predominance in tumor stage I (24%) with 93.09±40.71 with no statistical association.
44. Mean ELISA IL6 was showing predominance in Nottingham grade 2 (46%) with 90.69±66.06 with no statistical association.
45. Mean ELISA IL6 was showing predominance in good NPI (78%) with 77.58±54.80 with no statistical association.
46. Mean ELISA IL6 was showing predominance in ER positive expression (42%) with 82.86±48.41 with no statistical association.
47. Mean ELISA IL6 was showing predominance in PR positive expression (38%) with 80.89±48.20 with no statistical association.
48. Mean ELISA IL6 was showing predominance in Her 2 neu positive expression (30%) with 96.14±71.81 **with statistical association (p value = 0.04).**
49. Mean ELISA IL6 was showing predominance in low Ki67 proliferative index (42%) with 86.56±65.19 **with statistical association (p value =0.04).**
50. Mean ELISA IL6 was showing predominance in Her 2 enriched molecular category (18%) with 178.65±77.56 **with statistical association between ELISA IL-6 and Her 2 neu enriched category (p value = 0.04).**
51. Negligible positive correlation was found between IHC IL-6 and ELISA IL-6 i.e., when the IHC IL-6 values increase, the ELISA IL-6 values also increase. This positive correlation was not found to be statistically significant (p=0.217) i.e., only 79% of the studies done elsewhere would get similar results.

CONCLUSION

CONCLUSION:

In the present study, the mean IHC expression of IL6 observed was 201.6±88.4. The mean ELISA IL6 expression observed was 68.13±89.98.

In this study, we observed that with increasing IHC expression ELISA levels also increased.

The mean ELISA IL6 levels showed a significant association with premenopausal phase**,** Her 2 neu positive expression**,** low Ki67 proliferative index and Her 2 enriched molecular category**.** This shows the potential of ELISA IL6 being a marker of good prognostic parameters.

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BIBILIOGRAPHY

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ANNEXURE

INFORMED CONSENT FORM

STUDY TITLE: ASSOCIATION OF INTERLEUKIN 6 IMMUNOHISTOCHEMISTRY EXPRESSION WITH PLASMA ELISA INTERLEUKIN 6 LEVELS IN INVASIVE DUCTAL CARCINOMA BREAST

I, _____have read or have been read to me the patient information sheet and understand the purpose of the study, the procedure that will be used, the risk and benefits associated with my involvement in the study and the nature of information will be collected and disclosed during the study.

I have had my opportunity to ask my questions regarding various aspects of the study and my questions are answered to my satisfaction.

I, the undersigned, agree to participate in this study and authorize the collection and disclosure of my personal information for the dissertation.

Name and signature / thumb impression Date:

(Subject) Place:

Name and signature / thumb impression Date:

Place:

(Witness/Parent/ Guardian/ Husband)

PATIENT INFORMATION SHEET

STUDY TITLE: Association of interleukin 6 immunohistochemistry expression with plasma interleukin 6 levels in carcinoma breast.

PLACE OF STUDY: Department of Pathology, Sri Devaraj Urs Medical College, Kolar.

The main aim of the study is to check for the presence of Interleukin6 immunohistochemically expression in breast carcinoma and its correlation with plasma levels of interleukin 6.

You are requested to participate in a study conducted by the department of pathology as a part of dissertation. This study will be done on Invasive Ductal carcinoma specimens of the patients. The specimens will be collected from the Department of pathology, Sri Devaraj Urs Medical College, Kolar.

This study will be approved by the institutional ethics committee. The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate. You are requested to sign / provide thumb impression only if you voluntarily agree to participate in the study.

All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will not receive any monetary benefits to participate in this research.

This informed consent document is intended to give you a general background of study.

Please read the following information carefully and discuss with your family members. You can ask your queries related to study at any time during the study.

If you are willing to participate in the study you will be asked to sign an informed consent form by which you are acknowledging that you wish to participate in the study and entire procedure will be explained to you by the study doctor.
You are free to withdraw your consent to participate in the study any time without explanation and this will not change your future care.
For any clarification you are free to contact the investigator.

PRINCIPAL INVESTIGATOR:

Dr.Ankita Girdhar

Phone number: 9971509036

ಮಾಹಿತಿಯುತ ಸಮ್ಮತಿ ನಮೂನೆ

ಅಧ್ಯಯನ ಶೀರ್ಷಿಕೆ-

ಅಸೋಸಿಯೇಷನ್ ಆಫ್ ಇಂಟರ್ಲ್ಯೂಕಿನ್ 6 ಇಮ್ಯುನೊಹಿಸ್ಟೊಕೆಮಿಸ್ಟ್ರಿ ಎಕ್ಸ್‌ಪ್ರೆಶನ್ ಜೊತೆಗೆ ಪ್ಲಾಸ್ಮಾ ELISA ಇಂಟರ್‌ಲ್ಯೂಕಿನ್ 6 ಮಟ್ಟಗಳು ಆಕ್ರಮಣಕಾರಿ ಡಕ್ಟಲ್ ಕಾರ್ಸಿನೋಮ ಬ್ರೀಸ್‌ನಲ್ಲಿ.

ನಾನು, _____ ರೋಗಿಯನ್ನು ಓದಿದ್ದೇನೆ ಅಥವಾ ನನಗೆ ಓದಿದ್ದೇನೆ ಮತ್ತು ಅಧ್ಯಯನದ ಉದ್ದೇಶ, ಬಳಸಲಾಗುವ ಕಾರ್ಯವಿಧಾನ, ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಮತ್ತು ಮಾಹಿತಿಯ ಸ್ವರೂಪವನ್ನು ಅಧ್ಯಯನದಲ್ಲಿ ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ ಮತ್ತು ಬಹಿರಂಗಪಡಿಸಲಾಗುತ್ತದೆ.

ಅಧ್ಯಯನದ ವಿವಿಧ ಆಯಾಮಗಳ ಬಗ್ಗೆ ನನ್ನ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ನನಗೆ ಅವಕಾಶ ದೊರೆತಿತ್ತು ಮತ್ತು ನನ್ನ ಪ್ರಶ್ನೆಗಳಿಗೆ ನನ್ನ ತೃಪ್ತಿಗೆ ಉತ್ತರಿಸಲಾಗಿದೆ.

ನಾನು, ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಮತ್ತು ನನ್ನ ವೈಯಕ್ತಿಕ ಮಾಹಿತಿಯನ್ನು ಸಂಗ್ರಹಿಸುವುದು ಮತ್ತು ಬಹಿರಂಗಪಡಿಸುವಿಕೆಯನ್ನು ಈ ಲೇಖನಕ್ಕಾಗಿ ಅಧಿಕೃತಗೊಳಿಸಲು ನಾನು ಒಪ್ಪುತ್ತೇನೆ.

ಹೆಸರು ಮತ್ತು ಸಹಿ / ಹೆಬ್ಬೆಟ್ಟು ಗುರುತು ದಿನಾಂಕ:

(subject) ಸ್ಥಳ:

ಹೆಸರು ಮತ್ತು ಸಹಿ / ಹೆಬ್ಬೆಟ್ಟು ಗುರುತು ದಿನಾಂಕ:

ಸ್ಥಳ:

(ಸಾಕ್ಷಿ/ಪೋಷಕ/ ಪಾಲಕ/ ಪತಿ)

ರೋಗಿ ಮಾಹಿತಿ ಹಾಳೆ

ಅಧ್ಯಯನ ಶೀರ್ಷಿಕೆ: ಅಸೋಸಿಯೇಷನ್ ಆಫ್ ಇಂಟರ್ಲ್ಯೂಕಿನ್ 6 ಇಮ್ಯುನೊಹಿಸ್ಟೊಕೆಮಿಸ್ಟ್ರಿ ಎಕ್ಸ್‌ಪ್ರೆಶನ್ ಜೊತೆಗೆ ಪ್ಲಾಸ್ಮಾ ELISA ಇಂಟರ್‌ಲ್ಯೂಕಿನ್ 6 ಮಟ್ಟಗಳು ಆಕ್ರಮಣಕಾರಿ ಡಕ್ಟಲ್ ಕಾರ್ಸಿನೋಮ ಬ್ರೀಸ್ತನಲ್ಲಿ.

ಎಸ್ ಎಸ್ ಎಲ್ ಸಿ: ಕೋಲಾರ: ಶ್ರೀ ದೇವರಾಜ ಅರಸು ವೈದ್ಯಕೀಯ ಕಾಲೇಜು (ಎಸ್ ಎಸ್ ಎಲ್ ಸಿ) .

ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗವು ನಡೆಸಿದ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮ್ಮನ್ನು ವಿನಂತಿಸಲಾಗಿದೆ ಮತ್ತು ಡಿಸೆರ್ಟೇಶನ್ ನ ಭಾಗ. ಈ ಅಧ್ಯಯನವು ರೋಗಿಗಳ ಕಾರ್ಸಿನೋಮಾ ಮಾದರಿಗಳ ಮೇಲೆ ನಡೆಯುತ್ತದೆ. ಈ ಮೂಲಕ ಕಾಂಗ್ರೆಸ್ ಗೆ ಅನ್ನುರೋಗಶಾಸ್ತ್ರದಡಿಭಾಗದಿಂದ ಸಂಗ್ರಹಿಸಲಾಗುವುದು,Sri ದೇವರಾಜ್ ಉರುಸ್ ವೈದ್ಯಕೀಯ ಮತ್ತು ಕೋಲಾರ .ಈ ಅಧ್ಯಯನವನ್ನುಸಾಂಸ್ಥಿಕ ನೀತಿ ಶಾಸ್ತ್ರ ಸಮಿತಿ ಯು ಅಂಗೀಕರಿಸುತ್ತದೆ. ಮಾಹಿತಿ ಸಂಗ್ರಹಿತವನ್ನು ಕೇವಲ ಲೇಖನ ಮತ್ತು ಪ್ರಕಟಣೆಗಾಗಿ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ. ಯಾವುದೇ ಕಡ್ಡಾಯವಿಲ್ಲ ಭಾಗವಹಿಸಲು ಒಪ್ಪುತ್ತಾರೆ. ನೀವು ಹೆಬ್ಬೆಟ್ಟಿನ ಗುರುತನ್ನು ಮಾತ್ರ ನೀಡಿದರೆ ಮಾತ್ರ ಸಹಿ ಮಾಡಲು / ಒದಗಿಸಲು ನಿಮ್ಮನ್ನು ವಿನಂತಿಸಲಾಗಿದೆ

ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸ್ವಇಚ್ಛೆಯಿಂದ ಒಪ್ಪಿಕೊಳ್ಳಿ. ನಿಮ್ಮಿಂದ ಸಂಗ್ರಹಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿಡಲಾಗುವುದು ಮತ್ತು ಇದನ್ನು ಬಹಿರಂಗಪಡಿಸುವುದಿಲ್ಲ . ಅಥವಾ ಹೊರಗಿನವನಾದರೂ. ನಿಮ್ಮ ಗುರುತನ್ನು ಬಹಿರಂಗಪಡಿಸುವುದಿಲ್ಲ. ನೀವು ಯಾವುದೇ ಹಣಕಾಸಿಯನ್ನು ಪಡೆಯುವುದಿಲ್ಲ

ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಪ್ರಯೋಜನಗಳು.

ಈ ಮಾಹಿತಿಯುತ ಸಮ್ಮತಿ ದಸ್ತಾವೇಜು ನಿಮಗೆ ಅಧ್ಯಯನದ ಸಾಮಾನ್ಯ ಹಿನ್ನೆಲೆಯನ್ನು ನೀಡುವ ಉದ್ದೇಶವನ್ನು ಹೊಂದಿದೆ. ದಯವಿಟ್ಟು ಈ ಕೆಳಗಿನ ಮಾಹಿತಿಯನ್ನು ಎಚ್ಚರಿಕೆಯಿಂದ ಓದಿ ಮತ್ತು ನಿಮ್ಮ ಕುಟುಂಬ ಸದಸ್ಯರೊಂದಿಗೆ ಚರ್ಚಿಸಿ. ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂಧಿಸಿದ ನಿಮ್ಮ ಪ್ರಶ್ನೆಗಳನ್ನು ನೀವು ಕೇಳಬಹುದು. ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನೀವು ಸಿದ್ಧರಿದ್ದರೆ, ನಿಮಗೆ ಮಾಹಿತಿಯುತ ಸಹಿ ಮಾಡಲು ಕೇಳಲಾಗುತ್ತದೆ. ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಬಯಸುವುದನ್ನು ನೀವು ಒಪ್ಪಿಕೊಳ್ಳುವ ಸಮ್ಮತಿ ನಮೂನೆ ಮತ್ತು ಸಂಪೂರ್ಣ ಕಾರ್ಯವಿಧಾನವನ್ನು ಅಧ್ಯಯನ ವೈದ್ಯರು ನಿಮಗೆ ವಿವರಿಸುತ್ತಾರೆ. ನೀವು ಸ್ವತಂತ್ರರುಯಾವುದೇ ಸಮಯದಲ್ಲಿ ವಿವರಣೆ ಇಲ್ಲದೆ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮ್ಮ ಸಮ್ಮತಿಯನ್ನು ಹಿಂತೆಗೆದುಕೊಳ್ಳಿರಿ ಮತ್ತು ಇದು ಎಂಬ ಚಿಂತೆ ಯಲ್ಲಿದ್ದೀರಿ. ಯಾವುದೇ ಸ್ಪಷ್ಟನೆಗಾಗಿ ನೀವು ತನಿಖಾಧಿಕಾರಿಯನ್ನು ಸಂಪರ್ಕಿಸಲು ಸ್ವತಂತ್ರರು.

ಮುಖ್ಯ ತನಿಖಾಧಿಕಾರಿ : ಡಾII. ಅಂಕಿತಾ ಗಿರಿಧರ್

PATIENT PROFORMA

Name:	
Age:	Hospital Number:

Anonymised Sample No:

Chief complaint:

History of presenting illness:

Past history:

Personal history:

Menopausal state:

Premenopausal/Postmenopausal

BMI:

Normal/overweight/obese/severe obesity/morbid obesity/super obesity

Local examination:

Biopsy Number:

Gross:

Tumor size:

Microscopy:

Tumor grade:

Metastatic lymph node:

Lymphovascular invasion:

Tumor Infiltrating lymphocytes :

NPI:

Histopathological diagnosis:

Grading:

Immunohistochemical scoring:

Estrogen Receptor :

Progesterone Receptor:

Her 2 neu:

Ki 67:

KEYS TO MASTER CHART

P – Parity

L- living

BMI – Basal metabolic index

T – T staging according to 8th TNM Staging of breast carcinoma

N – N staging according to 8th TNM Staging of breast carcinoma

M – M staging according to 8th TNM Staging of breast carcinoma

ER – Estrogen Receptor protein

PR -Progesterone Receptor protein

Her2 neu – Human Epidermal Growth Factor Receptor 2 neu protein

NPI – Nottingham Prognostic Index

LA- Luminal A

LB- Luminal B

HER2 -E – Her2 neu enriched

TN – Triple negative

Pos – Positive

Neg – Negative

S.NO	AGE	HOSPITAL NUMBER	BIOPSY NO	DURATION OF LESION	MENOPAUSAL STATUS	FAMILY HISTORY	PARITY	BMI	TUMOR SIZE	TUMOR INFILTRATING LYMPHOCYTES	LYMPHOVASCULAR INVASION	METASTATIC LYMPH NODES	DISTANT METASTASIS	GRADING	STAGING	NPI	CLINICAL /RADIOLOGICAL STAGING	Pathological TNM staging	ER	PR	HER2 NEU	Ki67	MOLECULAR	IHC IL-6	ELISA- IL6
1	35Y/F	897214	B-33-21	12 months	Premenopausal	NIL	P2L2	18	50 X 40 mm	Present	Not seen	None	None	Grade 1	II	3	USG - BIRADS IV lesion , Clinical - T2N1M0	T2N0Mx	Neg	Neg	Neg	<14%	TN	260	88.92
2	40Y/F	901379	B-43-21	2 months	Premenopausal	NIL	P2L2	17	40 X 30 mm	Absent	Not seen	None	None	Grade 1	II	2.8	Clinical - T4bN2M1	T2N0Mx	Neg	Neg	Pos	>14%	HER2-E	270	62.02
3	57Y/F	903629	B-44-21	20 Days	Postmenopausal	NIL	P3L3	19	40 X 30 mm	Absent	Not seen	None	None	Grade 2	II	3.8	Clinical - T3N0M0	T2N0Mx	Neg	Neg	Neg	>14%	TN	290	89.45
4	58Y/F	892053	B-61-21	12 Months	Postmenopausal	NIL	P3L3	20	25 X 20 mm	Present	Not seen	None	None	Grade 1	II	2.6	Clinical - T2N0M0	T1N0Mx	Neg	Neg	Pos	<14%	HER2-E	240	63.43
5	57 Y/F	907808	B-65-21	4 Months	Postmenopausal	NIL	P2L2	21	40 X 35 mm	Absent	Not seen	None	None	Grade 3	II	4.8	Clinical - T2N0M0	T2N0Mx	Neg	Neg	Neg	>14%	TN	190	44.14
6	50Y/F	915709	B-150-21	4 Months	Postmenopausal	NIL	P1L1	22	20 X 20 mm	Present	Not seen	None	None	Grade 1	I	2.4	Mammography - BIRADS II,Clinical - T2N0M0	T1N0Mx	Neg	Neg	Neg	<14%	TN	190	36.71
7	57 Y/F	920033	B-198-21	12 Months	Postmenopausal	NIL	P2L2	20	25 X 20 mm	Absent	Not seen	None	None	Grade 1	I	2.9	Mammography - BIRADS IVA, Clinical - T3N0M0	T1N0Mx	Neg	Neg	Neg	>14%	TN	120	45.2
8	52 Y/F	923327	B-256-21	6 Months	Postmenopausal	NIL	P2L2	21	50 X 30 mm	Absent	Not seen	Positive	None	Grade 1	II	3	Mammography - BIRADS IVB,Clinical - T2N0Mx	T2N1Mx	Neg	Neg	Neg	>14%	TN	120	40.25
9	65 Y/F	930986	B-410-21	18 Months	Postmenopausal	NIL	P3L3	20	60 X 50 mm	Absent	Not seen	None	None	Grade 2	II	3.8	Clinical - T4bN2M1	T3N0Mx	Pos	Pos	Neg	>14%	LB	240	63.43
10	55Y/F	926208	B-451-21	2 Months	Premenopausal	NIL	P2L2	19	50 X 50 mm	Present	Not seen	None	None	Grade 2	II	3.4	Clinical - T4bN0M0	T2N0Mx	Neg	Neg	Pos	>14%	HER2-E	280	50.34
11	60 Y/F	915766	B-582-21	8 Months	Postmenopausal	NIL	P4L4	20	40 X 40 mm	Absent	Not seen	None	None	Grade 2	II	3.6	Clinical - T2N0M0	T2N0Mx	Pos	Neg	Pos	>14%	LB	160	55.29
12	71 Y/F	935455	B-632-21	6 Months	Postmenopausal	NIL	P5L5	18	30 X 20 mm	Present	Not seen	None	None	Grade 2	II	3.2	Clinical - T2N0M0	T2N0Mx	Neg	Neg	Neg	>14%	TN	280	45.2
13	65/F	946403	B-718-21	3 Months	Postmenopausal	NIL	P2L2	26	50 X 40 mm	Absent	Not seen	None	None	Grade 2	II	3.4	Clinical - T3N0Mx	T2N0Mx	Pos	Pos	Neg	>14%	LB	130	40.25
14	62/F	39318	B-818-21	6 Months	Postmenopausal	NIL	P2L2	18	50 X 40 mm	Present	Not seen	None	None	Grade 2	II	3.1	Clinical - T3N0Mx	T2N0Mx	Neg	Neg	Neg	<14%	TN	60	58.12
15	55/F	39217	B-856-21	12 Months	Postmenopausal	NIL	P3L3	20	20 X 20 mm	Absent	Not seen	None	None	Grade 1	I	2.8	Clinical - T2N0M0	T1N0Mx	Pos	Pos	Neg	>14%	LB	130	48.39
16	48/F	50456	B-887-21	10 Months	Premenopausal	NIL	P2L2	22	50 X 40 mm	Absent	Not seen	None	None	Grade 3	II	3.3	USG - BIRADS IV lesion , Clinical - T2N1M0	T2N0Mx	Neg	Neg	Neg	<14%	TN	260	73.35
17	65/F	50229	B-893-21	8 Months	Postmenopausal	NIL	P4L4	20	40 X 30 mm	Present	Not seen	None	None	Grade 2	II	3	Clinical - T2N0M0	T2N0Mx	Pos	Pos	Neg	<14%	LA	130	189.98
18	52/F	54427	B-910-21	18 Months	Postmenopausal	NIL	P2L2	18	82 X 72 mm	Absent	Not seen	None	None	Grade 3	III	3.8	Clinical - T4bN2M1	T4NoMx	Neg	Neg	Neg	<14%	TN	220	43.96
19	68/F	50119	B-993-21	18 Months	Postmenopausal	NIL	P3L3	19	25 X 20 mm	Absent	Not seen	None	None	Grade 2	I	2.9	Clinical - T2N1M0	T1N0Mx	Neg	Neg	Pos	>14%	HER2-E	290	45.03
20	45/F	56011	B-1113-21	12 Months	Postmenopausal	NIL	P2L2	18	40 X 35 mm	Present	Not seen	None	None	Grade 3	II	3.1	Clinical - T3N0M0	T2N0Mx	Neg	Neg	Neg	>14%	TN	280	39.72
21	72/F	54653	B-1171-21	4 Months	Postmenopausal	NIL	P2L2	17	20 X 20 mm	Absent	Not seen	None	None	Grade 2	I	2.8	Clinical - T2N0M0	T1N0Mx	Neg	Neg	Pos	<14%	HER2-E	150	31.44
22	56/F	879268	B-1218-21	4 Months	Postmenopausal	NIL	P3L3	19	25 X 20 mm	Absent	Not seen	None	None	Grade 2	I	2.9	Mammography - BIRADS II,Clinical - T2N0M0	T1N0Mx	Neg	Neg	Neg	<14%	TN	20	53.52
23	69/F	885577	B-1281-21	12 Months	Postmenopausal	NIL	P3L3	20	50 X 30 mm	Absent	Not seen	Positive	None	Grade 3	II	3.2	Clinical - T4N1M0	T2N1Mx	Neg	Neg	Neg	<14%	TN	260	45.73
24	53/F	883719	B-1302-21	6 Months	Postmenopausal	NIL	P2L2	21	60 X 50 mm	Present	Not seen	None	None	Grade 3	II	3.4	Clinical - T3N0Mx	T3N0Mx	Neg	Neg	Neg	>14%	TN	280	43.61
25	55/F	888033	B-1408-21	18 Months	Postmenopausal	NIL	P1L1	22	50 X 50 mm	Absent	Not seen	None	None	Grade 3	II	3.1	Clinical - T3N0Mx	T2N0Mx	Neg	Neg	Neg	>14%	TN	280	36
26	61/F	888835	B-1540-21	2 Months	Postmenopausal	NIL	P2L2	20	40 X 40 mm	Present	Not seen	None	None	Grade 3	II	3.2	Clinical - T2N0M0	T2N0Mx	Neg	Neg	Pos	>14%	HER2-E	280	99.19
27	65/F	886183	B-1673-21	8 Months	Premenopausal	NIL	P2L2	21	30 X 20 mm	Absent	Not seen	None	None	Grade 2	II	3	Mammography - BIRADS II,Clinical - T2N0M0	T2N0Mx	Neg	Neg	Neg	>14%	TN	240	90.87
28	53/F	61507	B-1697-21	24 Months	Postmenopausal	NIL	P2L2	21	50 X 40 mm	Present	Not seen	None	None	Grade 3	II	3.1	Clinical - T4bN0M0	T2N0Mx	Neg	Neg	Pos	>14%	HER2-E	210	30.69
29	67/F	58769	B-1705-21	24 Months	Postmenopausal	NIL	P2L2	20	50 X 40 mm	Absent	Seen	None	None	Grade 3	II	3.2	Clinical - T3N0M0	T2N0Mx	Pos	Pos	Neg	>14%	LB	70	24.85
30	49/F	87734	B-1858-21	12 Months	Premenopausal	NIL	P5L5	25	20 X 20 mm	Absent	Not seen	None	None	Grade 2	I	2.8	Clinical - T2N0M0	T1N0Mx	Pos	Pos	Neg	<14%	LA	280	128.74
31	58/F	62864	B-1923-21	36 Months	Postmenopausal	NIL	P4L4	20	50 X 40 mm	Present	Not seen	None	None	Grade 3	II	3.1	Clinical - T3bN0Mx	T2N0Mx	Pos	Pos	Neg	<14%	LA	290	53.17
32	52/F	62541	B-2059-21	18 Months	Postmenopausal	NIL	P3L3	21	40 X 30 mm	Absent	Not seen	None	None	Grade 3	II	3.2	Clinical - T3N0M0	T2N0Mx	Pos	Pos	Pos	<14%	LB	120	65.2
33	58/F	55208	B-2155-21	8 months	Postmenopausal	NIL	P4L4	20	40 X 30 mm	Absent	Not seen	None	None	Grade 3	s	2.9	Clinical - T3N1M0	T2NoMx	Pos	Pos	Pos	<14%	LB	220	112.28
34	65/F	57197	B-2192-21	12 months	Postmenopausal	NIL	P3L3	21	35 X 30 mm	Absent	Not seen	None	None	Grade 3	II	3	Mammography - BIRADS II,Clinical - T2N0M0	T2N0Mx	Pos	Pos	Neg	<14%	LA	140	43.43
35	64/F	63619	B-2278-21	10 months	Postmenopausal	NIL	P2L2	18	40 X 30 mm	Absent	Not seen	None	None	Grade 2	II	3.1	Clinical - T2N1M0	T2N0Mx	Pos	Pos	Neg	>14%	LB	220	51.75
36	47/F	63366	B-41-22	6 months	Premenopausal	NIL	P2L2	18	30 X 30 mm	Absent	Not seen	None	None	Grade 2	I	2.8	Clinical - T2N1M0	T1N0Mx	Neg	Neg	Pos	>14%	HER2-E	250	128.04
37	40/F	66662	B-102-22	8 months	Premenopausal	NIL	P2L2	19	40 X 30 mm	Absent	Not seen	None	None	Grade 2	II	3.2	Clinical - T2N0M0	T2N0Mx	Neg	Neg	Neg	>14%	TN	20	39.54
38	48/F	67214	B-165-22	9 months	Postmenopausal	NIL	P2L2	18	40 X 40 mm	Absent	Not seen	None	None	Grade 3	II	3.1	Clinical - T3N1M0	T2N0Mx	Neg	Neg	Neg	>14%	TN	250	67.15
39	41/F	65320	B-194-22	6 months	Premenopausal	NIL	P2L2	18	35 X 25 mm	Absent	Not seen	None	None	Grade 1	II	2.5	Clinical - T2N0M0	T2N0Mx	Pos	Pos	Neg	<14%	LA	250	73.52
40	57/F	63084	B-306-22	4 months	Postmenopausal	NIL	P2L2	19	40 X 30 mm	Absent	Not seen	None	None	Grade 2	II	2.8	Clinical - T4bN2M0	T2N0Mx	Pos	Pos	Neg	>14%	LB	280	133.88
41	40Y/F	901379	B-319-22	2 months	Premenopausal	NIL	P2L2	17	40 X 30 mm	Absent	Not seen	None	None	Grade 1	II	2.8	Clinical - T4bN2M1	T2N0Mx	Neg	Neg	Pos	>14%	HER2-E	100	68.57
42	50Y/F	915709	B-321-22	4 Months	Postmenopausal	NIL	P1L1	22	20 X 20 mm	Present	Not seen	None	None	Grade 1	I	2.4	Mammography - BIRADS II,Clinical - T2N0M0	T1N0Mx	Neg	Neg	Neg	<14%	TN	150	48.04
43	55Y/F	926208	B-343-22	2 Months	Premenopausal	NIL	P2L2	19	50 X 50 mm	Present	Not seen	None	None	Grade 2	II	3.4	Clinical - T4bN0M0	T2N0Mx	Pos	Pos	Pos	>14%	LB	280	147
44	65/F	946403	B-493-22	3 Months	Postmenopausal	NIL	P2L2	26	50 X 40 mm	Absent	Not seen	None	None	Grade 2	II	3.4	Clinical - T3N0Mx	T2N0Mx	Pos	Pos	Neg	>14%	LB	280	28.39
45	65/F	50229	B-690-22	8 Months	Postmenopausal	NIL	P4L4	20	40 X 30 mm	Present	Not seen	None	None	Grade 2	II	3	Clinical - T2N0M0	T2N0Mx	Pos	Pos	Neg	<14%	LA	280	64.14
46	72/F	54653	B-876-22	4 Months	Postmenopausal	NIL	P2L2	17	20 X 20 mm	Absent	Not seen	None	None	Grade 2	I	2.8	Clinical - T2N0M0	T1N0Mx	Pos	Pos	Pos	<14%	LB	120	45.38
47	53/F	883719	B-993-22	6 Months	Postmenopausal	NIL	P2L2	21	60 X 50 mm	Present	Not seen	None	None	Grade 3	II	3.4	Clinical - T3N0Mx	T3N0Mx	Neg	Neg	Neg	>14%	TN	140	58.3
48	49/F	87734	B-1097-22	12 Months	Premenopausal	NIL	P5L5	25	20 X 20 mm	Absent	Not seen	None	None	Grade 2	I	2.8	Clinical - T2N0M0	T1N0Mx	Pos	Pos	Neg	<14%	LA	120	68.04
49	58/F	62864	B-1114-22	36 Months	Postmenopausal	NIL	P4L4	20	50 X 40 mm	Present	Not seen	None	None	Grade 3	II	3.1	Clinical - T3bN0Mx	T2N0Mx	Pos	Neg	Neg	<14%	LA	150	147.86
50	47/F	63366	B-1167-22	6 months	Premenopausal	NIL	P2L2	18	30 X 30 mm	Absent	Not seen	None	None	Grade 2	I	2.8	Clinical - T2N1M0	T1N0Mx	Pos	Pos	Pos	>14%	LB	210	155.65