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2	Dr Jhanavi Reddy, Dr Kalyani R	L-152137/2024	Association of Immunohistichemistry Leptin expression with plasma ELISA leptin levels in invasive ductal carcinoma of breast	2024	3-158
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(57) Abstract:
The present invention relates to a method for enhancing the antioxidant and anti-inflammatory properties of curcumin, a bioactive compound derived from Curcuma longa. The method involves formulating curcumin into bioavailable delivery systems, such as nanoemulsions, liposomes, solid lipid nanoparticles (SLNs), and bioadhesive systems, to improve its solubility, stability, and absorption. Additionally, chemical modifications of curcumin are made to produce derivatives with increased therapeutic efficacy. The formulation is evaluated through various biochemical, molecular, and in vivo assays to assess its effects on oxidative stress and inflammation. The invention also explores the synergistic potential of combining curcumin with other bioactive compounds and its application in treating diseases associated with chronic inflammation and oxidative damage, such as arthritis, cardiovascular disease, neurodegenerative disorders, cancer, and diabetes. Personalized medicine approaches are also incorporated, enabling tailored treatment regimens to optimize curcumin's therapeutic potential for individual patients.

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ASSOCIATION OF IMMUNOHISTOCHEMISTRY LEPTIN EXPRESSION WITH PLASMA ELISA LEPTIN LEVELS IN INVASIVE DUCTAL CARCINOMA BREAST

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ASSOCIATION OF IMMUNOHISTOCHEMISTRY LEPTIN EXPRESSION WITH PLASMA ELISA LEPTIN LEVELS IN INVASIVE DUCTAL CARCINOMA BREAST

BY
DR. YEDUGURI JAHNAVI REDDY, MBBS

DISSERTATION SUBMITTED TO
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TAMAKA, KOLAR, KARNATAKA
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UNDER THE GUIDANCE OF
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PRIOR PERMISSION TO START OF STUDY

The Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the study entitled **"Association of Immunohistochemistry Leptin expression with plasma Elisa Leptin levels in invasive ductal carcinoma breast"** being investigated by **Dr. Yeduguri Jahnavi Reddy,** Dr. Kalyani Raju & Dr. P N Sreeramulu[1] in the Departments of Pathology & Surgery[1] at Sri Devaraj Urs Medical College, Tamaka, Kolar. **Permission is granted by the Ethics Committee to start the study.**

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Tamaka, Kolar.

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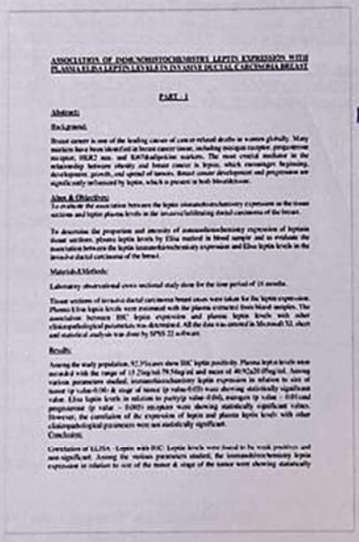

ASSOCIATION OF IMMUNOHISTOCHEMISTRY LEPTIN EXPRESSION WITH PLASMA LEVELS OF LEPTIN AMONG WOMEN IN INVASIVE DUCTAL CARCINOMA BREAST

PART - I

Abstract:

Background:

Breast cancer is one of the leading causes of cancer-related deaths in women globally. Many markers have been identified as breast cancer tumor, including estrogen receptor, progesterone receptor, HER2 neu and Ki67(Leptin) markers. The most crucial mediator in the relationship between obesity and breast cancer is leptin, which encourages beginning, development, growth, and spread of tumors. Breast cancer development and progression are significantly influenced by leptin, which is present in both bloodstream.

Aims & Objectives:

To evaluate the association between the leptin immunohistochemistry expression in the tissue sections and leptin plasma levels in the invasive/infiltrating ductal carcinoma of the breast.

To determine the proportion and intensity of immunohistochemistry expression of leptin in tissue sections, plasma leptin levels by Elisa method in blood sample and to evaluate the association between the leptin immunohistochemistry expression and Elisa leptin levels in the invasive ductal carcinoma of the breast.

Materials & Methods:

Laboratory observational cross sectional study done for the time period of 24 months.

Tissue sections of invasive ductal carcinoma breast cases were taken for the leptin expression. Plasma Elisa leptin levels were estimated with the plasma extracted from blood samples. The association between IHC leptin expression and plasma leptin levels with other clinicopathological parameters was determined. All the data was entered in Microsoft XL sheet and statistical analysis was done by SPSS 22 software.

Results:

Among the study population, 97.3% cases show IHC leptin positivity. Plasma leptin levels were recorded with the range of 15.2 ng/ml-79.94ng/ml and mean of 46.9(±20.05)ng/ml. Among various parameters studied, immunohistochemistry leptin expression in relation to size of tumor (p value-0.04) & stage of tumor (p value-0.03) were showing statistically significant value. Elisa leptin levels in relation to parity(p value -0.04), estrogen (p value - 0.01) and progesterone (p value - 0.02) receptors were showing statistically significant values. However, the correlation of the expression of leptin and plasma leptin levels with other clinicopathological parameters were not statistically significant.

Conclusion:

Correlation of ELISA - Leptin with IHC Leptin levels were found to be weak positives and non-significant. Among the various parameters studied, the immunohistochemistry leptin expression or relation to size of the tumor & stage of the tumor were showing statistically

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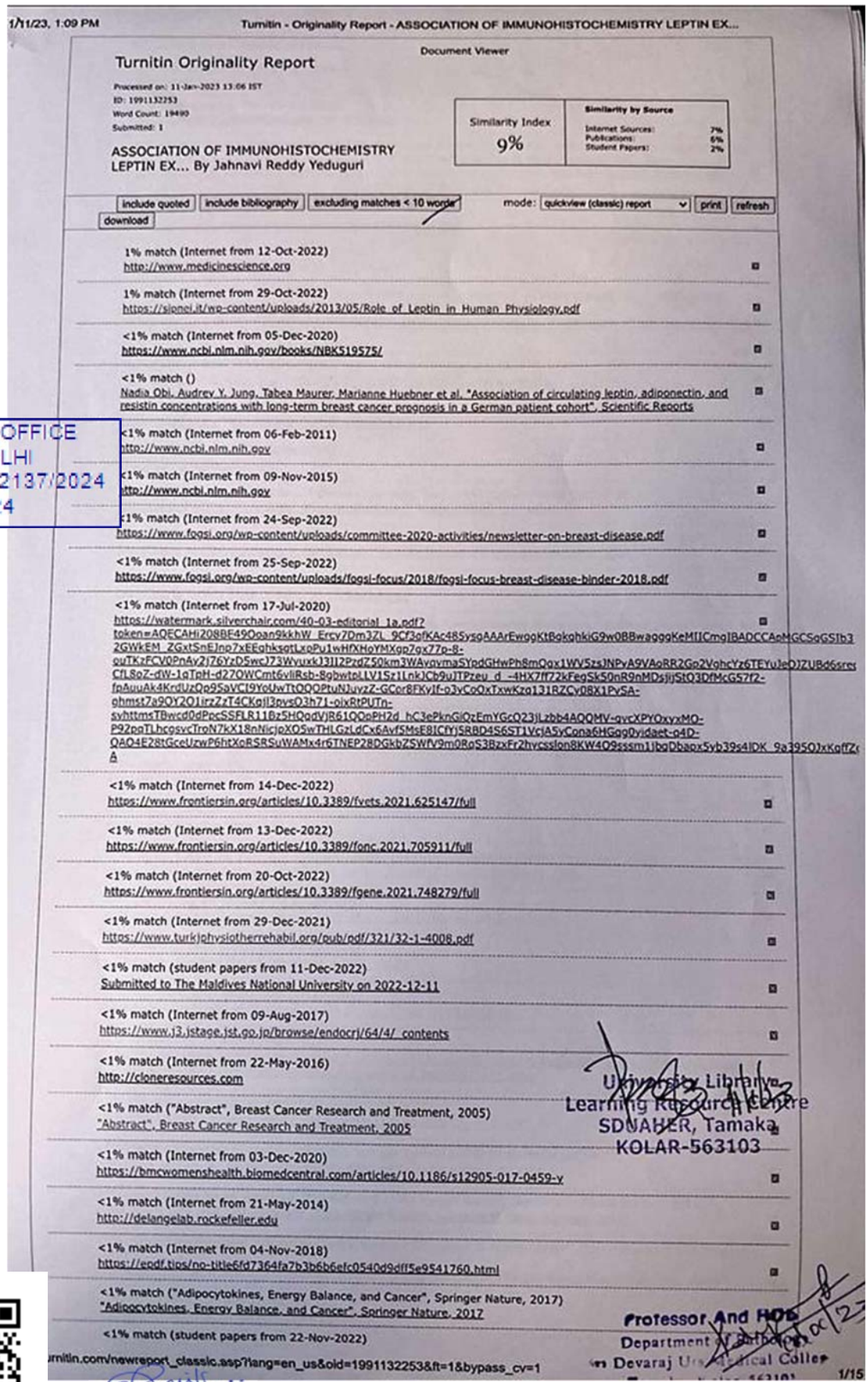

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Date :

Signature of the Candidate

Place: KOLAR

Dr. Yeduguri Jahnavi Reddy

LIST OF ABBREVIATIONS

BC – Breast cancer

IHC – Immunohistochemistry

ER – Estrogen Receptor

PR – Progesterone Receptor

Her 2 – Human epidermal growth factor receptor 2

DALY - Disability-adjusted life years

IDC – Infiltrating Ductal Carcinoma

CCL 2 – Chemokine ligand 2

CCL 5 – Chemokine ligand 5

IL 6 – Interleukin 6

IGF 1 – Insulin like growth factor 1

ELD – Extralobular ducts

TD – Terminal ducts

L – Lobules

WHO – World Health Organisation

DCIS – Ductal carcinoma in situ

AJCC – American Joint Committee on Cancer

H&E – Haematoxylin and Eosin

NPI – Nottingham Prognostic index

TBS – Tris buffer Solution

HR – Hormone Receptors

Ob – Leptin gene

ObR – Leptin Receptor

DPX - Dibutylpthalate Polystyrene Xylene

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ASSOCIATION OF IMMUNOHISTOCHEMISTRY LEPTIN EXPRESSION WITH PLASMA ELISA LEPTIN LEVELS IN INVASIVE DUCTAL CARCINOMA BREAST

ABSTRACT:

BACKGROUND:

Breast cancer is one of the leading causes of cancer-related deaths in women globally. Many markers have been identified in breast cancer tissue, including estrogen receptor, progesterone receptor, HER2 neu, Ki67 & adipokine markers. The most crucial mediator in the relationship between obesity and breast cancer is leptin, which encourages beginning, development, growth, and spread of tumors. Breast cancer development and progression are significantly influenced by leptin, which is present in both blood & tissue.

AIMS & OBJECTIVES:

To evaluate the association between the leptin immunohistochemistry expression in the tissue sections and leptin plasma levels in the invasive/infiltrating ductal carcinoma of the breast.

To determine the proportion and intensity of immunohistochemistry expression of leptin in tissue sections, plasma leptin levels by Elisa method in blood sample and to evaluate the association between the leptin immunohistochemistry expression and Elisa leptin levels in the invasive ductal carcinoma of the breast.

MATERIALS & METHODS:

Laboratory observational cross-sectional study done for the time period of 18 months.

Tissue sections of invasive ductal carcinoma breast cases were taken for IHC leptin expression. Plasma Elisa leptin levels were estimated with the plasma extracted from blood samples. The association between IHC leptin expression and plasma leptin levels with other clinicopathological parameters was determined. All the data was entered in Microsoft XL sheet and statistical analysis was done by SPSS 22 software.

RESULTS:

ıg the study population, 92.3%cases show IHC leptin positivity. Plasma leptin levels recorded with the range of 13.21ng/ml-79.54ng/ml and mean of 40.92±20.05ng/ml.

Among various parameters studied, immunohistochemistry leptin expression in relation to size of tumor (p value-0.04) & stage of tumor (p value-0.05) were showing statistically significant value. Elisa leptin levels in relation to parity (p value–0.04), estrogen (p value – 0.01) and progesterone (p value – 0.005) receptors were showing statistically significant values. However, the correlation of IHC expression of leptin and plasma leptin levels with other clinicopathological parameters were not statistically significant.

CONCLUSION:

Correlation of ELISA –Leptin with IHC- Leptin levels were found to be weak positives and non-significant. Among the various parameters studied, the immunohistochemistry leptin expression in relation to size of the tumor & stage of the tumor were showing statistically significant value. Elisa leptin levels in relation to parity, estrogen receptor and progesterone receptor were showing statistically significant values.

KEY WORDS:

Breast Cancer, Leptin, Immunohistochemistry, Elisa.

INTRODUCTION:

Breast cancer (BC) is one of the leading causes of cancer-related deaths in women globally. BC is the second most frequent type of cancer overall.[1] In 2018, there were 6,26,679 breast cancer deaths and 20,88,849 new cases of the disease worldwide (11.6%).[2] According to the American Cancer Society, breast neoplasms are the most common kind of cancer among women, accounting for over 1,700,000 newly diagnosed cases and 5,80,000 BC deaths in the US in 2015.[1] According to the Saudi Cancer Registry, BC has a comparable ranking among malignancies and neoplasms in Saudi Arabia, accounting for 25.8% of all recorded neoplasms in females in 2012.[1] In India, the incidence of BC in female population is 25.8 per 1,00,000 and the mortality rate is 12.7 per 1,00,000. Incidence of breast cancer rate in Bangalore is 34.4%.[3] Prevalence of the breast cancer in kolar district was reported as 6.4 % of total female cancers.[4]

According to the WHO, there would be 6,85,000 deaths and 2.3 million new cases of BC worldwide in 2020.[5] The most frequent malignancy in the globe as of the end of 2020 was breast cancer, which had been diagnosed in 7.8 million women in the five years prior.[5] Breast cancer is the type of cancer that causes the most disability-adjusted life years (DALY) loss in women worldwide.[6]

The lining cells (epithelium) of the glandular tissue's ducts (85%) or lobules (15%) are where breast cancer begins. The cancer is initially contained within the duct or lobule ("in situ"), where it often exhibits no symptoms and carries a minimal risk of disseminating (metastasis)[5]. Humans have been aware of breast cancer since the time of the Ancient Egyptians.[7]

In every nation in the globe, women can get breast cancer (BC) at any age after puberty, and the prevalence increases as people age. Little changed in breast cancer mortality from the 1930's to the 1970's. In countries with early detection systems combined with various forms of therapy to eradicate invasive sickness, survival rates started to increase in the 1980's.[8]

Many markers have been identified in breast cancer tissue, including the estrogen receptor (ER), the progesterone receptor (PR), Human epidermal growth factor receptor 2 (HER 2Neu), and Ki67. Some cancer-associated adipokines, such as leptin, adiponectin, Interleukin ·6), chemokine ligand 2 (CCL-2), chemokine ligand 5(CCL-5), and others, are being ›yed in the diagnostic methods, therapy, and further prognosis of breast cancer.[9]

Abdominal obesity contributes to the creation of an environment that favors cancer growth.[10] Obesity has been linked to cancer, namely breast, endometrial, ovarian, thyroid, and prostate cancer.[11]

The most crucial mediator in the relationship between obesity and breast cancer is leptin, which encourages the beginning, development, growth, and spread of tumors.[12]Through its interactions with other signaling molecules such estrogen receptor, growth factors, notch, and inflammatory factors, leptin increases the risk of breast cancer.[13] Breast cancer cells invade more readily when the epidermal growth factor receptor is transactivated by leptin and insulin like growth factor 1 (IGF-I) signaling.[14]

RESEARCH QUESTION:

Does plasma leptin level have any association with immuno-expression of leptin in tissue sections in cases of invasive/infiltrating ductal carcinoma of the breast?

AIMS AND OBJECTIVES

AIM:

To evaluate the association between the leptin immunohistochemistry expression in the tissue sections and leptin plasma levels in the invasive/infiltrating ductal carcinoma of the breast.

OBJECTIVES:

1) To determine the proportion and intensity of immunohistochemistry expression of leptin in tissue sections in the invasive/infiltrating ductal carcinoma of the breast.

2) Estimation of plasma leptin levels by Elisa method in the invasive/infiltrating ductal carcinoma of the breast.

3) To evaluate the association between the leptin immunohistochemistry expression and Elisa leptin levels the invasive/infiltrating ductal carcinoma of the breast.

REVIEW OF LITERATURE:

EMBRYOLOGY & DEVELOPMENT:

EMBRYOLOGY:

At sixth week of pregnancy, mastogenesis, or the development of the breasts, begins. At the seventh week, milk line which forms a distinct linear elevation, emerges. The beginnings of breast, which will ultimately develop into the mature breast, are formed from the thicker white line towards the end of the eighth week. The number of basal cells increases during development. Nipple areolar complex will be formed at approximately 30 weeks of gestation as a result of papillary bag which will be blocked. At around 38-40 weeks, nipple develops.[15]

PRENATAL BIOPSY:

The establishment of primary/initial mammary bud and the formation of a primitive mammary gland are 2 basic stages/steps of prenatal breast development[15].Early embryogenesis is essentially hormone independent,[16] but second trimester development depends on hormones and regulatory factors.[17]

Notably, there are no gender differences in human breast growth during pregnancy. The progressive, unique phases of intrauterine breast development are described here, and they show notable variances at comparable stages and have a loose correlation with gestational age.[17]

FIRST TRIMESTER:

Progenitor cells unique to the mammary tissue can be detected as early as four to six weeks of gestation.[18] Around day 35 of pregnancy, the thoracic epidermis begins to grow paired areas of epithelial cells. The two ridges between the fetal axilla and inguinal area are known as the mammary crests or milk lines and are the result of these unique sites of growth.[18]

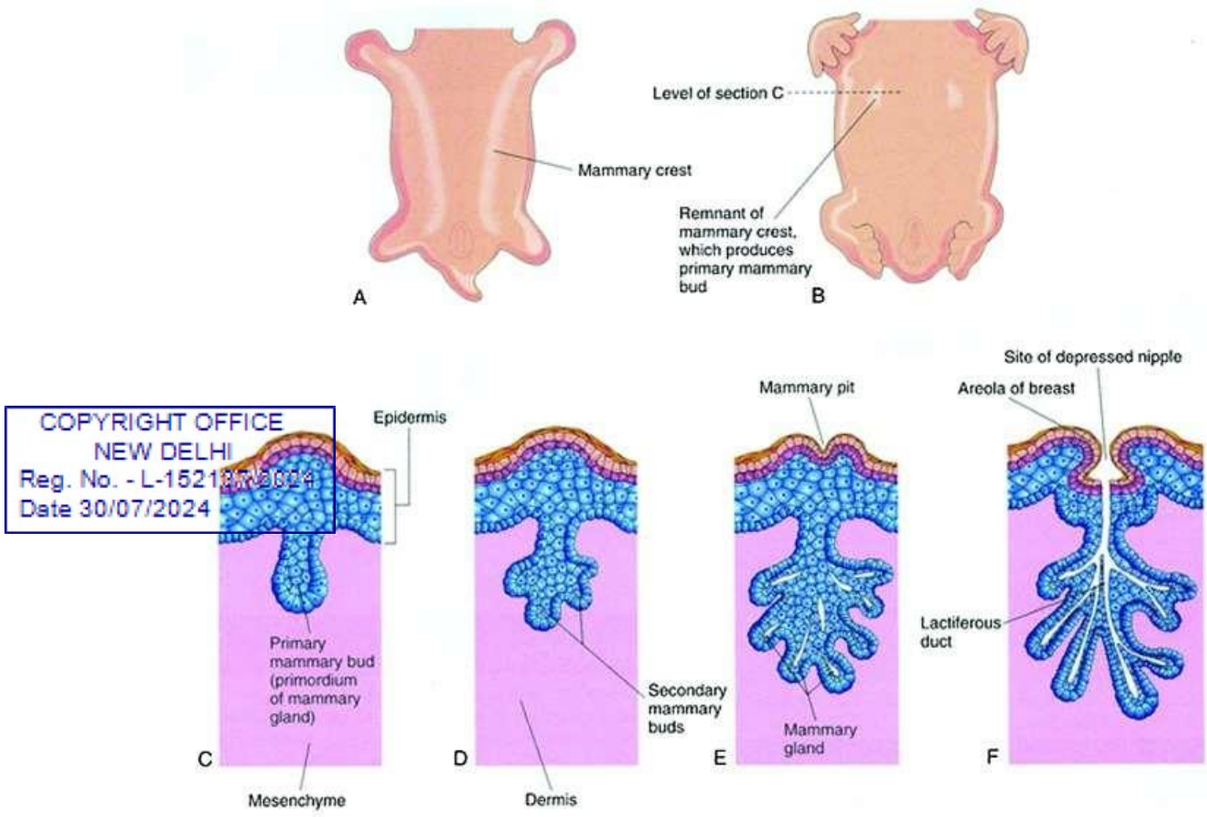


Figure 1: Embryology of normal breast. [19]

Under the inductive impact of regulatory substances released by the mesenchyme, the primary/initial mammary bud will start to form downwards and into the underlying mesenchyme by the end of first trimester.[20] The main mammary bud then grows and shifts from a more dorsal to a ventral location.[21] There are six indentations along its basolateral edge, which will serve as the locations of any subsequent secondary mammary outgrowths[20]. This cell core continues to evaginate into the underlying stroma, surrounded by a more cellular zone of fibroblast-like cells within a collagenous mesenchyme.[21]

SECOND TRIMESTER:

The mesenchyme that surrounds the parent bud is formed vertically by each secondary lial bud, which has a slender stalk and bulbous end.[22] In order to form secondary buds,

which in turn give birth to lactiferous ducts, the secondary epithelial sprouts canalize and gather.[23]

The gland's fundamental structure is set by the time a baby is six months old. At this stage, a bed of thick fibroconnective tissue stroma with a well-defined tubular architecture is noted.[23] This is also the point at which both boys' and girls' breast tissue may be visible.[24]

THIRD TRIMESTER:

The third trimester is when the secondary epithelial buds repeatedly branch and canalise.[15,25] On the ultimate structure of breast at birth, there is disagreement. Some claim that the breast at birth shows no signs of lobular formation, simply ductal elements with the surrounding stromal components, despite the fact that most authors agree that these secondary process finish in rudimentary lobular elements.[15,25. 26]

The loose fibroconnective tissue stroma becomes more vascular in the latter stages of pregnancy. Limited secretory activity in the late-term fetus and newborn kid may result from a complex, as yet unexplained combination of maternal, placental, and fetal hormones.[23,26]

Each of the 15 to 20 lobes of glandular tissue that have formed at term contains a lactiferous duct. The mammary pit is where these ducts emerge onto the breast tissue. The skin covering the breast and the Cooper's fibrous suspensory ligaments, which connect the breast to the pectoralis major fascia, support the breast.[23,26]

INFANT BREAST:

Some features of breast development and involution take place within the first two years of life.[26,27] From two years of life until puberty, the typical gland is dormant. [26,28] The newborn's breast is often palpable at delivery, with varied amounts of tissue and no obvious gender differences. [29] As many as 70% of term newborns have transitory milk production and/or unilateral or bilateral breast augmentation as a result of pituitary gland releasing prolactin due to stimulation by maternal estrogens in the newborn.[27,30]

PREPUBERTAL DEVELOPMENT OF BREAST IN FEMALES:

ANATOMICAL GROSS CHANGES (TANNER STAGES):

Tanner described the most well-known macroscopic stages of breast development during adolescence.[30] These profound structural changes first manifest during stage1, the preadolescent period with just elevation of the papilla. The stroma and parenchyma have finished developing and are no longer in their infancy. The first secondary sexual trait to manifest is breast development, which typically occurs 6 months before pubic hair growth.[31] Puberty is the initial catalyst for mammary development, and estrogen's impact is reliant on the presence of pituitary growth hormone and growth hormone's ability to stimulate the production of insulin-like growth factor-1 (IGF-I) in the mammary gland.[32]

Stage 2 in tanner's entails development of breast bud along with the elevation of nipple, the production of a tiny bit of breast, and growth of diameter of the aerola.[30] tanner stage 3, which is acquired at a median age of 12.5 years, is marked by more amount of growth of areola and breast. The contours are not yet separated, as far as is known.[30,31] A pubertal girl's breast size difference is frequently observed between the stages 3&2 and tends to lessen in stages 4 &5. [33] Reconstructive surgery may be an option if there is chronic significant breast asymmetry, usually after Tanner 5 breast maturity is attained.[30,33]

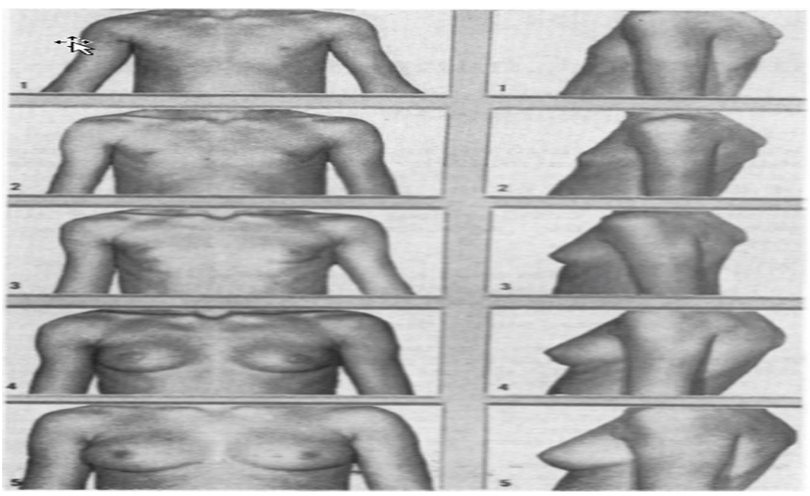


Figure 2: Tanner's Staging [19]

ANATOMY[34-36]:

To comprehend the diseases that breast gets affected with and create the planning which is required for surgical procedure, a thorough understanding of the anatomical structure of the breast is necessary. Most breasts exhibit some degree of asymmetry when examined. Kyphosis, scoliosis, and various pectus deformities are some other deformities.

Most of the breast tissue is formed by glandular and fatty components. However, each person has a different ratio of fatty tissue to glandular tissue. The sex hormone estrogen has a significant impact on breast development. Estrogen levels fall as menopause approaches, which also causes the glandular tissues to shrink.

Early in life, the breast organs will be there from 2nd to 6th ribs; as the breast ages and sags, it may, however, extend to below the sixth rib. The base of the breast or the posterior wall is formed by pectoralis major muscle. The Cooper ligaments hold the breast to the pectoralis major fascia. However, because of their flexibility, these ligaments permit breast movements. The Cooper ligaments in the majority of women stretch with time and ageing, eventually leading to a ptotic breast. Gravity makes the lower pole of the breast fuller than the upper pole. The Spence tail extends in the lateral edges of the breast and axilla.

The nipple is often located slightly above the inframammary crease& is seen in at 4th rib in the midclavicular line.

GLANDS:

The breast's underlying tissue is formed by glandular and fatty components. The fat to glandular component ratio keeps on changing due to factors such as age, menopausal status, parity status, as menopause approaches, a drop in estrogen levels causes glandular tissue to shrink and fatty tissue to expand.

STRUCTURE OF NIPPLE:

breastfeeding, the nipple is crucial. For effective nursing, a nipple must be at least millimeters long. However, the nipple's topography varies greatly; it might be flat, or even inverted, which can make it difficult for certain women to nurse.

NERVES:

The intercostal nerves T3-T5's branches provide the breast with sensory type of innervation. The cervical lower plexus is one of an additional nerves that offer sensory innervation. The lateral cutaneous branch of the T4 nerve is where the nipple's sensation comes from.

BLOOD SUPPLY:

The deep underlying arterioles that supply the breast parenchyma connect with the subdermal plexus, which is responsible for supplying blood to the breast surface.

The breast receives blood from:

1.Thoracoacromial artery

2.Internal mammary perforators (2nd to 5th)

3.Lateral thoracic artery

4.Thoracodorsal artery

5.Terminal branches of internal perforators (3rd to 8th).

At least 60% of the blood flow overall comes from the internal mammary artery's superomedial perforators.

THE LYMPHATIC SYSTEM:

Breast contains considerable lymphatic drainage that extends throughout the breast both superficially and deeply. The areolar and subareolar plexus make up the superficial lymphatics. The axillary lymph nodes are ultimately reached by the superficial lymphatics as they progress posteriorly and medially.

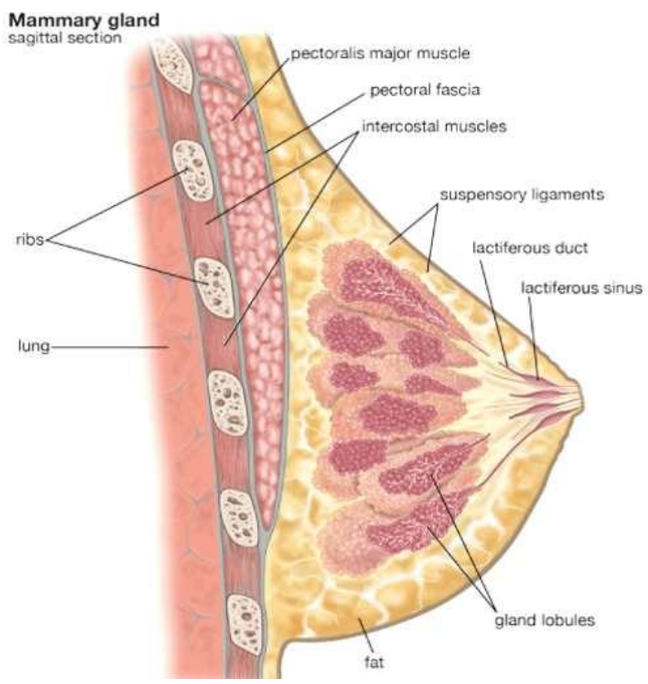


Figure 3: Normal breast anatomy [37]

NORMAL HISTOLOGY OF BREAST:

The breast's normal histology is made of acini and ducts which are arranged in the form of lobules and the stromal component comprising of predominantly adipose along with fibrous components. The two major constituents are stromal and epithelial elements. The dual layered epithelial lining by lobular systems and the ducts, which is rested on basement membrane is surrounded by stromal tissue. Columnar to cuboidal cells make the inner layer of the ducts and outer layer is formed by the myoepithelial cells. The ductules, ducts and the acini are surrounded by the basement membrane.[38]

The lobular units of terminal ducts are composed of:

1. Terminal ductules, the epithelium of which is differentiated into secretory acini which is seen in lactation and pregnancy.

2.Collecting ducts (Intralobular)

3.Intralobular stroma (specialized).

e lobes drain into their own lactiferous ducts which finally opens into nipple.[39]

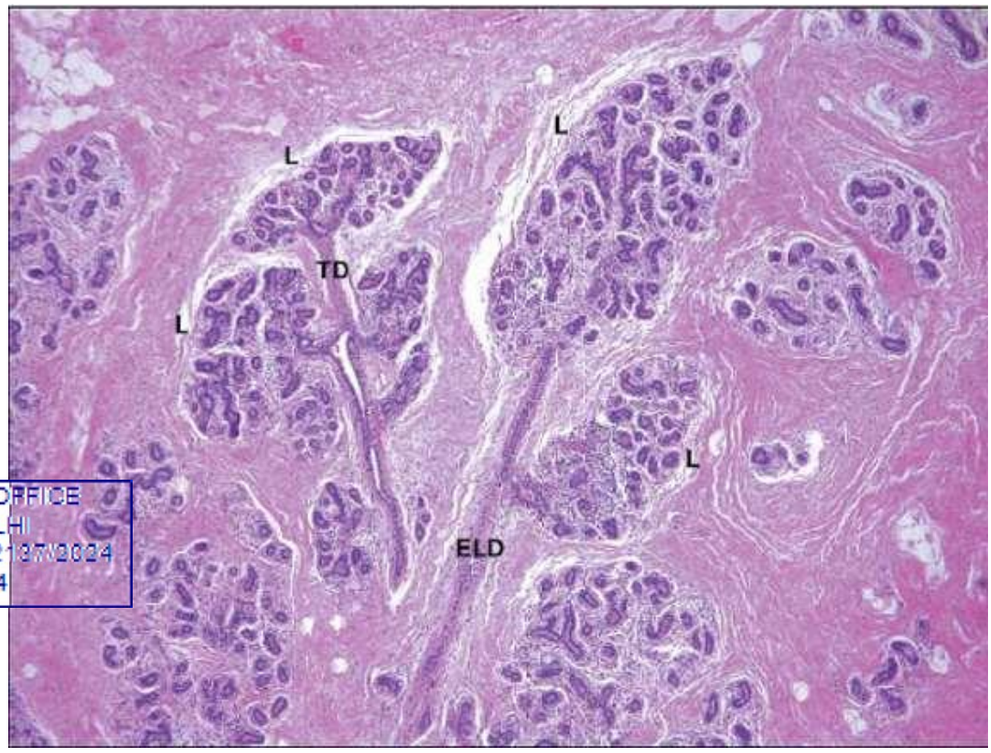


Figure 4: Normal histology of breast. (ELD – Extralobular ducts, TD – Terminal ducts, L – Lobules)[40]

ETIOLOGY AND RISKFACTORS:[41,42]

Many factors are there which play role in development of carcinoma breast. Some of the important factors are:

1. Geographical place: Western population is seen to be more affected than in Indian population.
2. Familial history- 5-10% of carcinoma breast cases are seen to show autosomal dominant pattern of inheritance.
3. Endogenous hormones: Late first pregnancy (>35 years), early menarche, delayed menopause, nulliparous women, non-lactational women show increased risk of breast cancer.
4. Molecular genetics: Mutations in genes such as PTEN, P53, BRCA1 & BRCA2 shows increased risk for breast cancer.

   Lifestyle patterns: Obesity, lack of physical exercise, smoking & alcohol intake show increased incidence for breast cancer development.

6. Benign lesions: Patients who are previously diagnosed with any benign breast lesion are at increased risk of developing malignancy.
7. Environmental risk factors: Prolonged exposure to harmful ionizing radiation.
8. Hormone therapy: Women who are on medical contraceptive pills, who are put on hormone replacement therapy also show increased risk.

ETIOPATHOGENESIS:[41,43]

There is increased rate of carcinoma breast cases worldwide. Most commonly it is seen affecting postmenopausal women. Carcinoma of breast can occur in women who have mutations in their genes or it can also be seen sporadically. Environmental factors are seen to play role in hereditary forms of breast cancer where as both environmental and genetic factors play role in development of carcinoma in sporadic cases. Developed countries when compared to developing countries show higher incidence (sixfold) of developing breast cancer.

Genetic mutations in genes such as PTEN, P53, BRCA1& BRCA2 show higher risk and chances of developing breast cancer. It is a huge task in cases of breast cancer to know the etiopathogenesis, detection in initial stages, decision for therapy and to know its outcome. The identification of most susceptible genes playing role in development of breast cancer has a chief role in understanding the etiopathogenesis of both sporadic and familial forms. Various types of factors will increase the risk and the chance of breast cancer which includes, environmental factors, lifestyle variations, hormonal changes, genetic factors.

MOLECULAR MECHANISM OF CARCINOGENESIS:[44]

Carcinoma breast shows diversity in its molecular mechanisms which has multiple processes that ultimately will result in the initiation, progression of the disease and the metastatic nature. There are three major groups into which carcinoma of the breast can be divided into which are the luminal subtypes along with positivity in hormone receptors (HR+), oncogene HER2 (HER2+), and the triple negative variant. New subtypes are added recently. With the help of this additional genes and the mutations, they give the molecular mechanisms and the pathway leading to tumerogenesis.[44]

/ oncogenes which are responsible for carcinogenesis are seen to play major role in ogenesis and metastatic ability in breast cancer. Resistant phenotypes are seen

emerging due to mutations and dysregulation of apoptotic pathway which are seen in driver oncogenes, which will ultimately affect the survival and therapy. Hence, targeting of the drivers and downregulating them is pursues in various cancers, including carcinoma of the breast. In patients with HER2 positive subtypes, targeted endocrine therapies are given, which are showing good outcome.[44]

New targeted therapies based on molecular mechanisms are recently developed which are the inhibitors in the DNA repair, which are seen in breast carcinomas with BRCA mutation, CDK4/6 inhibitor for both hormone receptor positive and HER2 negative variants of cancer breast cases.[44]

CLINICAL FEATURES:

Most common complaint among women is lump in the breast, which is slow growing and may or may not be associated with pain. Many of the times, they are identified in screening programs. Patient can also present with complaints such as discharge from the nipple, dimpling of the skin, puckering of skin, retraction of the nipple, eczematous changes. Bloody nipple discharge most commonly leans towards malignancy whereas skin involvement showing flaky, crusting of skin is seen in Paget's disease. Peau d' orange appearance is seen if the underlying lymphatics are involved. Fungating lesion or ulcerated lesion usually indicates advanced stage of the disease.

Sometimes very rarely patients can also present with lymphadenopathy which is seen in axilla and supraclavicular region with absence of breast lesion. Metastasis to other organs will the presenting complaint of patients with advanced disease. The symptoms depend on the site of metastasis and organ involved. Most common bone metastasis is seen to vertebra (lumbar). Patients show pathological fractures in underlying bine is involved by the tumor. Other manifestations include ascites, which is seen as a later complication. Ovarian involvement is seen via trans coelomic spread of the tumor. Similar complaints can be seen in benign breast lesions as well, hence radiological workup has to be done in all patients with lump in the breast especially in elderly women, followed by cytological evaluation and/or histopathological sampling.

WHO CLASSIFICATION OF CARCINOMA BREAST:[45]

Table1: WHO classification of breast carcinoma.

Epithelial tumors	Micro invasive carcinoma
Invasive breast carcinoma	Invasive breast carcinoma of no special type Pleomorphic carcinoma Carcinoma with osteoclast-like stromal giant cells Carcinoma with choriocarcinomatous features Carcinoma with melanotic features Invasive lobular carcinoma Classic lobular carcinoma Solid lobular carcinoma Alveolar lobular carcinoma Pleomorphic lobular carcinoma Tubuloalveolar carcinoma Mixed lobular carcinoma Tubular carcinoma Cribriform carcinoma Mucinous carcinoma Carcinoma with medullary features Medullary carcinoma Atypical medullary carcinoma Invasive carcinoma with NST with medullary features Carcinoma with apocrine differentiation Carcinoma with signet ring cell differentiation Invasive micropapillary carcinoma Metaplastic carcinoma of no special type Low-grade adenosquamous carcinoma Fibromatosis-like metaplastic carcinoma Squamous cell carcinoma Spindle cell carcinoma Metaplastic carcinoma with mesenchymal

	differentiation Chondroid differentiation Osseous differentiation Other types of mesenchymal differentiation Mixed metaplastic carcinoma Myoepithelial carcinoma
Rare types	Carcinoma with neuroendocrine features Neuroendocrine tumor, well-differentiated Neuroendocrine carcinoma, poorly differentiated Carcinoma with neuroendocrine differentiation Secretory carcinoma Invasive papillary carcinoma Acinic cell carcinoma Mucoepidermoid carcinoma Polymorphous carcinoma Oncocytic carcinoma Lipid rich carcinoma Glycogen rich, clear cell carcinoma Sebaceous carcinoma Salivary gland/skin adnexal type tumors Cylindroma Clear cell hidradenoma
Epithelial-myoepithelial tumors	Pleomorphic adenoma Adenomyoepithelioma Adenomyoepothelioma with carcinoma Adenoid cystic carcinoma
Precursor lesions	Ductal carcinoma in situ Lobular neoplasia Lobular carcinoma in situ Classic lobular carcinoma in situ Pleomorphic lobular carcinoma in situ Atypical lobular hyperplasia
aductal proliferative lesions	Usual ductal hyperplasia

Papillary lesions	Intraductal papilloma Intraductal papilloma with atypical hyperplasia Intraductal papilloma with ductal carcinoma in situ Intraductal papilloma with lobular carcinoma in situ Intraductal papillary carcinoma Encapsulated papillary carcinoma Encapsulated papillary carcinoma in situ
Benign epithelial proliferation	Sclerosing adenosis Apocrine adenosis Microglandular adenosis Radial scar/complex sclerosing lesion
Adenomas	Tubular adenoma Lactating adenoma Apocrine adenoma Ductal adenoma
Mesenchymal tumors	Nodular fasciitis Myofibroblastoma Desmoid-type fibromatosis Inflammatory myofibroblastic tumor Benign vascular lesions Haemangioma Angiomatosis Atypical vascular lesions Pseudoangiomatous stromal hyperplasia Granular cell tumor Benign peripheral nerve sheath tumors Neurofibroma Schwannoma Lipoma Angiolipoma Liposarcoma

	Angiosarcoma Rhabdomyosarcoma Osteosarcoma Leiomyosarcoma
Fibroepithelial tumors	Fibroadenoma Phyllodes tumor Benign Borderline Malignant Periductal stromal tumor, low grade Hamartoma
Tumors of nipple	Nipple adenoma Syringomatous tumor Paget's disease of the nipple
Malignant lymphoma	Diffuse large B cell lymphoma Burkitt's lymphoma T-cell lymphoma Anaplastic large cell lymphoma, ALK-negative Extranodal marginal zone B cell lymphoma of MALT type Follicular lymphoma
Metastatic tumors	
Tumors of the male breast	Gynaecomastia Carcinoma Invasive carcinoma In situ carcinoma
Clinical patterns	Inflammatory carcinoma Bilateral breast carcinoma

HISTOLOGICAL SUBTYPES:

INVASIVE/INFILTRATING DUCTAL CARCINOMA OF BREAST:

Invasive/Infiltrating ductal carcinoma is the largest group among breast cancers. This category has group of tumors which don't show any specific type of histology such as lobular variant or tubular variant. Other terminologies for this include – invasive ductal carcinoma, invasive ductal carcinoma not otherwise specified, infiltrating ductal carcinoma. These tumors show invasion into surrounding stroma and tissues and may show the tendency to metastasize.[45,46]

GROSS FEATURES:

Macroscopic features vary among different cases. The size of the tumor may range widely from 1 cm to 10 cms. The contours may be regular/irregular/nodular/showing stellate configuration. Sharp demarcation between tumor borders and surrounding stroma may not usually be seen. These tumors will be firm to hard in consistency on palpation. Sometimes there can be gritty feel while cutting with a knife. Cut surface is grey white in color.

Fig 5A: Photograph showing gross image – Mastectomy specimen

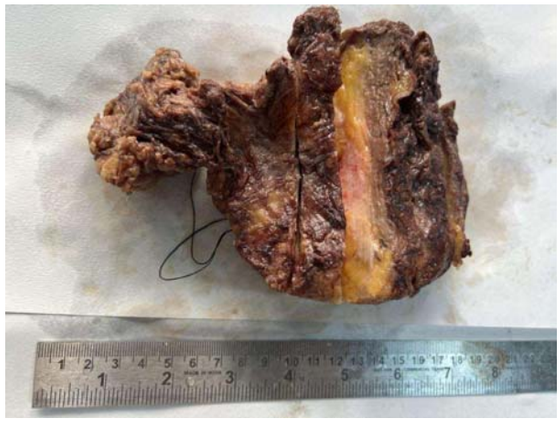
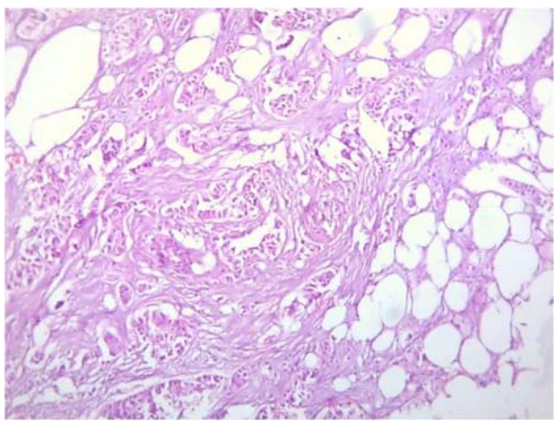


Figure 5B: Photograph showing gross image – cut section showing grey white tumor

MICROSCOPY:

The cells of the tumor are seen typically in trabecular pattern, cords & in clusters. These will show predominantly solid and sometimes syncytial pattern of infiltration into adjacent stroma. Individual tumor cells show abundant amount of eosinophilic cytoplasm, nucleus is regular, uniform, pleomorphic & showing prominent nucleoli. Mitotic figures can be seen at places. Many times an associated ductal carcinoma insitu (DCIS) component can also be seen. Stroma shows proliferation of fibroblastic tissue, also noted are areas of connective tissue and hyalinization. Necrosis is also noted at places.



gure 6: Microphotograph showing microscopy image – H&E – 10X - infiltrating ductal carcinoma breast

LOBULAR CARCINOMA:

This entity comprises of about 5-15% of breast cancers. Usually seen as focal tumor with insitu lobular component. Grossly they appear irregular with poorly defined margins. Individual tumor cells are small, and are arranged in Indian file pattern.[45,46]

TUBULAR CARCINOMA:

This entity comprises 2% of breast cancers and they are small in size of <2cms. These tumors show better prognosis and are less aggressive. Majority of the tumors show ER positivity. Characteristic microscopic feature is the lumina are lined by epithelial cells arranged in one single layer.[45,46]

CRIBRIFORM TYPE OF CARCINOMA:

One of the types of invasive malignancy with an intraductal cribriform pattern is called invasive cribriform carcinoma (ICC). 50% of the tumor may show a tubular pattern. It constitutes about 0.3%–0.8% of breast cancers and consists of a cribriform pattern in >90% of the lesion. The tumor has angulated islands, in which bridges of cells form a well-defined sieve-like pattern. The tumor, which has a majority of cribriform patterns and few tubular patterns, is also an invasive cribriform carcinoma. A mixed variant of invasion type of cribriform carcinoma is a tumor composing of <50% of other types of patterns other than tubular carcinoma. It metastasizes very rarely to the axillary lymph nodes and carries a good prognosis.[47,48]

CARCINOMA WITH MEDULLARY FEATURES:

It is a broad category that has medullary type of cancers (MC), atypical type of medullary cancers, and no special type subset of invasive carcinomas. Common features are pushing type of borders, growth pattern like a syncytium, cells, nuclei showing high grade & a dense infiltration by lymphocytes. They represent about <1% of all breast carcinomas.[49]

METAPLASTIC CARCINOMA:

The incidence of metaplastic carcinomas is just 0.3% of all of the invasive carcinoma. They omposed of other cellular components apart from the glandular component. The natous components vary from spindle cell component, myxoid, bone, and cartilage.

Gross features vary from well-defined lesions to irregular masses with speculated margins. Microscopically there are two main subtypes: monophasic "sarcomatoid," also known as spindle cell carcinoma with squamous component or without squamous components, and the other one is biphasic "sarcomatoid" carcinoma. The tumor probably is derived from myoepithelial cells. Based on the myoepithelial cell's presence or absence, metaplastic carcinoma differentiates into epithelial and mesenchymal elements.[50,51]

THE AMERICAN JOINT COMMITTEE ON CANCER (AJCC) STAGES FOR BREAST CANCER:[52]

Table2: T – Primary tumor (pT):

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
Tis	(DCIS) Ductal carcinoma in situ
Tis	(LCIS) Lobular carcinoma in situ
Tis	(Paget) Paget disease of the nipple not associated with invasive carcinoma and/or carcinoma in situ (DCIS and/or LCIS) in the underlying breast parenchyma.
T1	T1mi Micro invasion 0.1 cm or less in greatest dimension
T1a	More than 0.1 cm but not more than 0.5 cm in greatest dimension
	More than 0.5 cm but not more than 1 cm in greatest dimension
T1b	More than 1 cm but not more than 2 cm in greatest dimension
T1c	Tumour more than 2 cm but not more than 5 cm in greatest dimension
T2	Tumour more than 5 cm in greatest dimension
T3	Tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or skin nodules)
T4a	Extension to the chest wall (does not include pectoralis muscle invasion only)
T4b	Ulceration, ipsilateral satellite skin nodules, or skin edema (including peau d'orange)
T4c	Both 4a and 4b, above
	Inflammatory carcinoma

Table3: N – Regional lymph nodes (pN):

pNX	cannot be assessed
pN0	No regional lymph node metastasis histologically
pN0(i-)	no regional lymph node metastasis by histology or immunohistochemistry
pN0(mol+)	pN0(i+) : isolated tumor cells (cluster ≤ 0.2 mm and < 200 cells)
pN1mi	RT-PCR positive but negative by light microscopy
pN1a	micrometastasis (tumor deposit > 0.2 mm and ≤ 2.0 mm or ≤ 0.2 mm and > 200 cells)
pN1b	metastasis in 1 - 3 axillary lymph nodes with at least 1 tumor deposit > 2.0 mm
pN1c	metastasis in internal mammary sentinel lymph node with tumor deposit > 2.0 mm
pN2a	pN1a and pN1b
pN2b	metastasis in 4 - 9 axillary lymph nodes with at least one tumor deposit > 2.0 mm
pN3a	metastasis in clinically detected internal mammary nodes with pathologically negative axillary nodes
pN3b	metastasis in ≥ 10 axillary lymph nodes with at least one tumor deposit > 2.0 mm or metastasis to infraclavicular lymph node
pN3c	positive internal mammary node by imaging with pN1a or pN1b
pNX	metastasis in ipsilateral supraclavicular lymph node

Table 4: Distant metastasis (M):

M0	No distant metastases
M1	Distant metastases

Table5: Stage grouping:

Stage 0	Tis	N0	M0
Stage IA	T1	N0	Mo
Stage IB	T0, T1	N1	M0
Stage IIA	T0, T1	N1	M0
	T2	N0	M0
Stage IIB	T2	N1	Mo
	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

MICROSCOPIC GRADE:

Considering both architecture and cytology have been found to correlate with prognosis, Elston and Ellis modified the original Bloom and Richardson and Bansal et al.[53] grading schemes based on tubule formation and nuclear degree atypia. This is the Modified Bloom-Richardson grading system (MBR) (Annexure - 3). It also incorporates the mitotic activity to the previous classification. The grade is calculated by summing the numbers obtained for formation of tubules, nuclear pleomorphic features and count of the mitotic activity.[54]

Table 6: Modified Bloom Richardson Grading of the tumor:[54]

Criteria	**Score 1**	**Score 2**	**Score 3**
Tubule formation	> 75%	10 to 75%	< 10%
Nuclear pleomorphism	Minimal variation in nuclear size and shape	Moderate variation in nuclear size and shape	Marked variation in nuclear size and shape
Mitotic counts per 'F	0-5	5-10	More than 11

all grade

- Grade 1(score 3,4 or 5)
- Grade 2(score 6 or 7)
- Grade 3(score 8 or 9)

Grading is advocated for all, regardless of morphological type, as it serves to prognosticate the metastasis and survival, independent of the lymph node's status, and predicts chemotherapy response.

NOTTINGHAM PROGNOSTIC INDEX.[55]

Table 5: Nottingham prognostic index in breast cancer

NPI	Score	5 year survival	Prognosis
I	≤2.4	96%	Excellent
II	>2.4 - ≤3.4	93%	Good
III	>3.4-5.4	78%	Moderate
IV	>5.4	44%	Poor

NPI = (0.2 X S) + N +G

Lymph nodes = number of lymph nodes, 0=1, 1-3 = 2, >3 = 3

PROGNOSTIC & PREDICTIVE FACTORS:[51]

1. Tumor size- It is the largest measured diameter of the tumor. An increase in tumor size is associated with more chances of distant metastasis rate and poor survival.
2. Histological type - Infiltrating ductal carcinoma is the commonest breast carcinoma constituting 22%. Inflammatory carcinoma has lower survival rates among different histological types, but with systemic chemotherapy, the prognosis is better, with 25 to 50% survival rates.
3. Presence of necrosis – Necrosis is an independent prognostic factor. Central necrosis and fibrosis were observed in large tumors with higher T stage and negligible in early

breast cancers. They significantly lack hormone receptors and are associated with a higher grade.

4. Inflammatory cell infiltrates – The presence of intratumor and peritumor mononuclear inflammatory cell infiltrate reflects the host defense mechanism against the tumor cells and is associated with better prognosis irrespective of their hormone receptor status, grade, and other clinic-pathological characteristics. Macrophages proved to be beneficial in fighting cancer cells.
5. Lymphatic invasion – This is associated with higher chances of lymph node metastasis and a higher tumor stage and guides the clinician in considering adjuvant treatment decisions in chemotherapy contraindicated patients.
6. Vascular invasion – Defined as "penetration by the tumor cells into the lumen of an artery or vein." It is associated with distant metastasis, larger tumor size, higher grade, and lower survival. The patients who have a systemic disease or metastatic disease will have a vascular invasion.
7. Perineural invasion – This is associated with lymphovascular invasion and a higher grade of the tumor.
8. Stromal characteristics – Tumors with minimal stromal reaction usually have a higher histological grade and higher nuclear grade. In contrast, tumors with an excellent stromal response like fibrosis and desmoplasia are stellate shaped, circumscribed, low grade, and are likely to be hormone receptor-positive.
9. Axillary node status is commonly associated with disease-free and overall survival rate. Tumors with higher grade, histological type, stage, and lymphovascular invasion have increased risk of axillary lymph node metastasis.

TUMOR STROMA:

Breast carcinoma is a diverse illness. Clinically, it has been categorized according to the degree of ER, PR, and HER2 neu expression. With a better understanding of illness features and consequences, molecular classification with many subtypes has recently been proposed. The complex tissue microenvironment in which cancer develops promotes metastasis, invasion, and persistent growth. Instead of being a cell-autonomous process, the development of cancer is co-mediated by the tumor microenvironment and cancer cells.[56,57]

MOLECULAR CLASSIFICATION :[52,58]

Table 8 – Molecular classification of breast carcinoma

MOLECULAR SUBTYPE				
	LUMINAL A LIKE	LUMINAL B LIKE	HER2 ENRICHED	BASAL LIKE
Gene expression Pattern, Clinical and biologic	-Expression of luminal (low-molecular-weight) cytokeratin's, and high expression of hormone receptors and associated genes - ~60% of invasive breast cancers ER/PR positive HER2 negative Low proliferation rate	-Expression of luminal (low-molecular-weight) cytokeratin's and moderate to weak expression of progesterone receptor and associated genes -~10% of invasive breast cancers ER positive, PR low positive HER2 expression variable (positive or negative) Intermediate or high proliferation rate (Ki-67 high) Luminal B tends	-High expression of HER2 and other genes in amplicon on 17q12 Low expression of ER and associated genes - ~15% of invasive breast cancers ER/PR negative HER2 positive (though not all HER2 enriched by molecular subtype are HER2+ by clinical definition) High proliferation rate TP53 mutation common	-High expression of basal epithelial genes, basal cytokeratin's Low expression of ER and associated genes Low expression of HER2 related genes - ~15% of invasive breast cancers Most ER/PR and HER2 negative ("triple negative") High proliferation rate TP53mutation common; BRCA1 dysfunction (germline,

		to be higher histologic grade than luminal A	More likely to be high grade and node positive	sporadic) Particularly common in African–American women
Histologic correlation	Tubular carcinoma Cribriform carcinoma Low grade invasive ductal carcinoma NST Classic lobular carcinoma	Invasive ductal carcinoma NST Micropapillary carcinoma	High-grade invasive ductal carcinoma NST	High-grade invasive ductal carcinoma NST Metaplastic carcinoma Carcinoma with medullary features

LEPTIN IN HUMAN PHYSIOLOGY:

As a result of the enthusiasm surrounding the discovery of leptin fifteen years ago, this prototype adipocyte-secreted protein/cytokine was given the name leptin, which is derived from the Greek for thin, "leptos." This study also says that adipose tissue is one of the most active organ with endocrine function and not only an energy storing organ. However, further clinical trials revealed that leptin was not of much benefit for treating obesity, which caused some initial disappointment.[59]

Leptin, which is a 167-amino-acid by-product of the human leptin gene, was discovered as a result of positional cloning of ob/ob mice, a mouse strain of obesity that was unintentionally identified at Jackson Laboratories.[60] These mice, who had a homozygous mutation of the leptin gene, had infertility, hyperphagia, severe obesity, diabetes, neuroendocrine abnormalities, and considerable weight gain.

Its levels are seen associated with the body fat content & are mostly released by white adipose tissue.[61] Leptin secretion is pulsatile, like that of many other hormones, and it varies significantly during the day, peaking in the nights and early hours of morning.[62,63]

Factors promoting leptin secretion:[62,63]

Excess energy stored as fat
Glucose
Insulin
Glucocorticoids
Estrogens
Inflammatory cytokines

Factors inhibiting leptin secretion:[62,63]

Low energy states with decreased fat stores
Fasting states
Catecholamines and adrenergic agonists
Thyroid hormones
Androgens
Inflammatory cytokines.

FACTORS THAT CONTROL THE AMOUNTS OF CIRCULATING LEPTIN:

Specific leptin receptors (ObRs), which are present in both peripheral tissues and the brain, bind to leptin to modulate its actions. The ObR gene can be spliced to produce several isoforms. Leptin is believed to be transported via blood-brain barrier through the ObR an iso form[64]. The hypothalamus, a crucial location for control of energy metabolism and function of neuroendocrine system, significantly expresses the ObRb iso form, which facilitates signal' transmission [65].

LEPTIN'S CONTRIBUTION TO ENERGY HOMEOSTASIS:

The main nervous system (central) is instructed to checkup on intake of food & spending of es in accordance with the level of circulating leptin, which acts as a marker for energy es. Leptin acts on the brain to control hunger, with immediate effects. Leptin controls

hunger by activating a complex neuronal circuit made up of orexigenic (i.e., appetite-stimulating) and an orexigenic (i.e., appetite-diminishing) neuro peptides in hypothalamus by binding to the ObRb-receptor. Leptin influences satiety outside of the hypothalamus by interacting with the mesolimbic dopamine system, which is involved in the motivation and reward of food, as well as the nucleus of the solitary tract of the brainstem.[66]

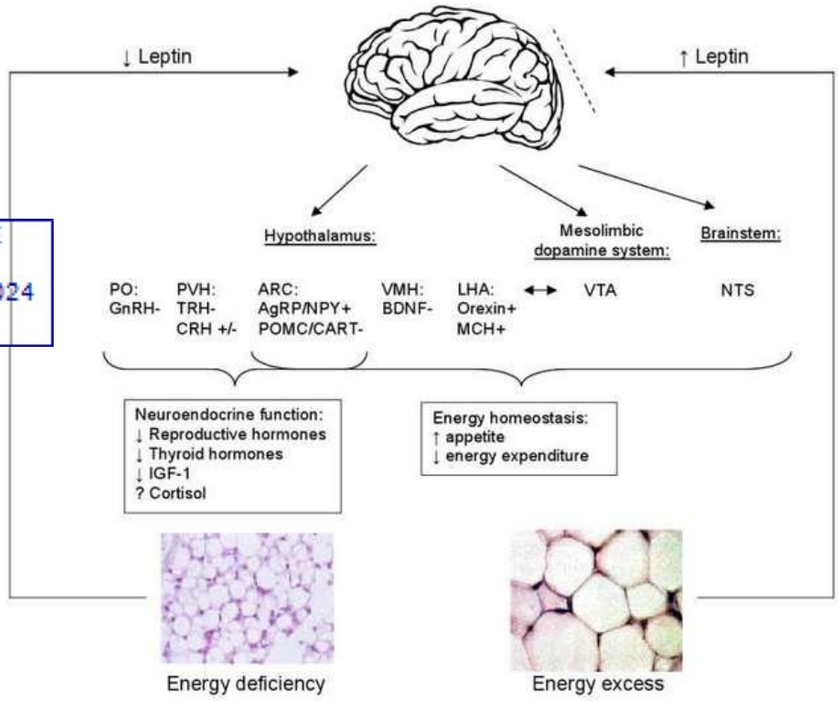


Figure 7: Schematic photograph showing leptin pathway[67]

THE ROLE OF LEPTIN IN REGULATING NEUROENDOCRINE FUNCTION:

The neuroendocrine response to acute calorie restriction takes place[69] as a result of the rapid reduction in leptin levels that occurs after a fast, prior to any changes in fat mass and out of proportion to those changes[68]. Reduced levels of reproductive hormones prevent pregnancy, which is an energy-intensive process; decreased thyroid hormone levels slow metabolism; increased growth hormone levels may release stored energy; and decreased insulin-like growth factor-1 (IGF-1) levels may slow growth-related processes all contribute to this response in mice and humans[70]. It seems that the interactions between leptin and the growth hormone and adrenal axis are less significant in humans than in animal models since people

with congenital leptin deficiency demonstrate normal linear growth and adrenal function, unlike mice[71].

SIGNIFICANCE OF LEPTIN IN METABOLIC SYNDROME, INSULIN RESISTANCE & WEIGHT GAIN:

Congenitally leptin-deficient individuals, ob/ob mice, db/db mice, and animals with a leptin receptor mutation all exhibit insulin resistance and other symptoms of the metabolic syndrome. Leptin therapy in the ob/ob mouse strain lowers hyperglycemia and hyperinsulinemia prior to weight loss[72]. Leptin therapy has been shown to lower triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels in persons with congenital leptin insufficiency in conjunction to hyperinsulinemia.[71] We currently understand the mechanisms through which central and peripheral activity mediate these effects.

LEPTIN IN CARCINOMA BREAST:

Leptin and its receptor expression were studied in breast cancer (n=205) and in the normal tissue of the breast (30) by Turkish researchers **Atalay Karacay I et al. [73] (2022),** who also examined the correlation between leptin and its receptor expression and clinico-pathological characteristics in breast malignancies. Leptin and its receptor were much more expressed in tumors of the breast than in healthy breast tissues. Leptin receptor expression and leptin expression shown a strong association (r = 0.6). Leptin expression levels and ER (r = 0.3) and PR (r = 0,3) expression levels were correlated positively. Leptin-positive tumors had reduced HER2 positivity rates. Expression of leptin levels and the grade(histology) were not correlated (r = -0.1). Invasive ductal carcinoma NSTs had higher percentages of leptin receptor positive than invasive lobular carcinomas.

In order to assess the blood level of leptin and association with the prognostic variables in the patients with cancer of the breast, Iranian researchers **Hajati A et al.[74] (2022)** conducted a case control study. Breast cancer patients' serum leptin levels were substantially higher than those of the control group (21.6 vs. 11.8). ER, PR, and HER2 expressions did not significantly correlate with plasma leptin levels. Additionally, no correlations between leptin levels and illness stage or grading were found.

et al.[75](2021) from Germany examined potential associations between time-varying , adiponectin, and resistin with all-cause mortality & risk of recurrence in a sizable

cohort of postmenopausal breast cancer patients. They also looked at the role of circulating adipokines in long-term prognosis. Overall, their investigation found no evidence of links between adipokines and any result. Adiponectin levels in the highest vs. lowest quintile were substantially correlated with higher breast cancer-specific mortality in ERPR-negative tumors (HR 2.51). Adipokines following breast cancer diagnosis were generally not linked to positive long-term outcomes. High concentrations of adiponectin can be associated to higher mortality in breast cancer patients with ERPR negative tumors and demand additional research.

In 58 cats with breast cancer, **Gameiro A et al.**[76] from Portugal in 2021 compared the levels of leptin and the leptin receptor (ObR) expression in tumor tissues to those of healthy animals. The results showed that, particularly in cats with luminal B and HER2-positive tumors, cats with mammary carcinoma have significantly lower serum leptin levels as well as a lower free leptin index. Interestingly, ulcerating tumors and shorter disease-free survival were associated with blood leptin concentrations over 4.2 pg/mL ($p = 0.0005$).

Chinese researchers **Liang X et al.**[77] **(2018)** done a study for looking into the function of leptin in breast cancer development. Leptin expression was shown to be higher in breast cancer tissues in their investigation when compared to nearby healthy tissues. BC patients had considerably greater serum levels of the leptin protein than healthy controls. A leptin inhibitor therapy significantly reduced the promoting effects of leptin on the multiplication and proliferation of breast cancer cells. Leptin increased multiplication of cancer cells and also activates catenin/wnt pathway. According to their research, leptin may accelerate the progression of cancer of breast by triggering catenin/wnt pathway.

China's **Gu L et al.**[78] **(2018)** performed a metanalysis on 43 papers that satisfied the criteria. Serum leptin levels were often substantially higher in BC patients than in controls (SMD = 0.6). When ethnicity and menstruation status were the only variables included in subgroup analysis, increased blood leptin - plasma/serum concentrations were likewise seen in individuals with the BC. Furthermore, serum leptin was noticeably higher in BC individuals with body mass index >25(SMD = 1.4). Additionally, the blood leptin content was noticeably greater in the BC patients who had lymph node metastases (SMD = 0.5).

pers were used in a metanalysis by **Pan H et al.**[79] **(2017).** In individuals who are eight or obese, a subgroup study of BMI found a relationship between BC and serum levels. Additionally, a postmenopausal woman's menopausal status revealed a

significant correlation. Furthermore, we discovered a substantial correlation between blood leptin levels and BC in Chinese women.

The relationship between the clinicopathological elements in the BC and a leptin phenotype was examined by **Khabaz MN et al.**[1] (Saudi Arabia) in **2017**. Leptin cytoplasmic immunohistochemistry staining was seen in 83.7% of BC patients and 92.6% of controls. Age, grade, histotypes, the stage, the lymph node involvement, the hormone receptor phenotypes, tumor recurrence, the HER2 and ER expression were all substantially linked with leptin immunostaining. All subgroups of clinicopathological characteristics had a reasonable number of patients with modest staining scores, with the exception of the ER-negative, PR-positive HER2-receptor phenotype and the mucinous carcinoma, which had a high degree of the leptin immunoreactivity. Additionally, the results of the Log Rank test showed that the survival distributions for various types of immunohistochemistry leptin scores were noticeably different. Unfavorable survival is associated with negative leptin immuno-staining.

Rodrigo C et al.[80] (2017) conducted a study in Sri Lanka to ascertain if serum visfatin, plasma leptin, soluble leptin receptor, free leptin index, and particular LEP and LEPR polymorphisms are risk factors for sporadic breast cancer. The levels of leptin, leptin/BMI, free leptin index, visfatin, and soluble leptin receptor were all considerably higher in the patients. The K109R A/G polymorphism in the LEPR gene increased the risk of breast cancer (odds ratio: 4.1). According to multivariate analysis, leptin, soluble leptin receptor, free leptin index, and the G109 (R109) allele of the LEPR gene K109R polymorphism are all recognized risk factors for breast cancer.

A research on the effects of metabolic syndrome on leptin and the receptor of it also in the cancer of the breast was conducted by **Carroll PA et al.**[81] **(2011)** from Ireland. Individuals with MetS had considerably greater expression of Ob in MAT and ObR in matching tumour tissue than patients with cancer who were merely obese or of normal weight. Individual MetS characteristics, but not obesity indicators, linked with Ob and ObR expression. In fat tissue and matching tumor samples, respectively, the mRNA expression of leptin ObR and Ob appears to be related to the presence of obesity in breast cancer. This greater Ob/ObR expression is primarily characterized by increased insulin resistance.

009 study, Korean scientists **Kim HS et al.**[82] looked at the expression of leptin and the receptor in human BC and how that impacted breast cancer patients' prognoses. Leptin

had positive cytoplasmic immunoreactivity in 39% of the patients, whereas Ob-R had positive cytoplasmic immunoreactivity in 79% of the patients. Breast cancer leptin expression was correlated with a high Ki-67 labelling index. The clinicopathologic variables with predictive relevance included the histologic grade, the T and N stages, the HER2 status, the expressions of Bcl-2, Ki-67, and p53, and others. Individuals with leptin-positive breast tumours and negative hormone receptor status had considerably longer overall survival.

A French investigation on the importance of leptin and leptin receptors in the development of cancer was conducted by **Jardé T et al.[83] in 2008.** ObR & leptin expressions were found in 85 &75%, respectively, of the primary BC patients examined. Leptin expression and the detection of Ob-R were substantially linked. In addition, oestrogen receptor expression and size of tumor were positively linked with Ob-R expression in primary BC. First, leptin works on breast tumor cells via an autocrine mechanism, as demonstrated by the co-expression of leptin & ObR and leptin in primary breast cancer. Second, the co-expression of Ob-R and oestrogen receptors raises the possibility that the estrogen & leptin systems interact to encourage the formation of breast cancer. Finally, the positive correlation between Ob-R expression and tumour size may indicate that Ob-R is a novel prognostic marker and that leptin functions as a growth factor.

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 are collected from Department of surgery and Department of Pathology from R.L. Jalappa Hospital and Research Center attached to Sri Devaraj Urs Medical College, Tamaka, and Kolar.

DURATION OF STUDY: 18 Months (January 2021 – June 2022).

INCLUSION CRITERIA**:** All fresh cases of primary Invasive Ductal Carcinoma of Breast diagnosed by FNAC or TRUCUT Biopsy and confirmed by Mastectomy.

EXCLUSION CRITERIA: Post Chemotherapy , Post Radiotherapy cases , recurrent cases, male breast cancer, secondary metastasis in breast or any other cancer in the patient, Patients on medication for Hyperlipidemia ,Pregnancy.

SAMPLE SIZE:

Sample size calculation done by using formula [73,74]

Formula:

To employ Fisher's arctanh transformation: $C(r) = \frac{1}{2}\log_e \frac{1+r}{1-r}$

Given a sample correlation r based on N observations that is distributed about an actual correlation value (parameter) ρ, then $C(r)$ is normally distributed with mean $C(\rho)$ and variance $\sigma^2 = 1/(N-3)$.

Under the null hypothesis, the test statistic is $Z = C(r)\sqrt{N-3}$ where $Z \sim N(0,1)$

ample size to achieve specified significance level and power is $N = \left(\frac{z_\alpha + z_\beta}{C(r)}\right)^2 + 3$

where z_p is the upper 100(1-p) percentile of the standard normal distribution.

Hypothesis: $H_0: \rho = 0$ *versus* $H_a: \rho = r \neq 0$

Data Input:

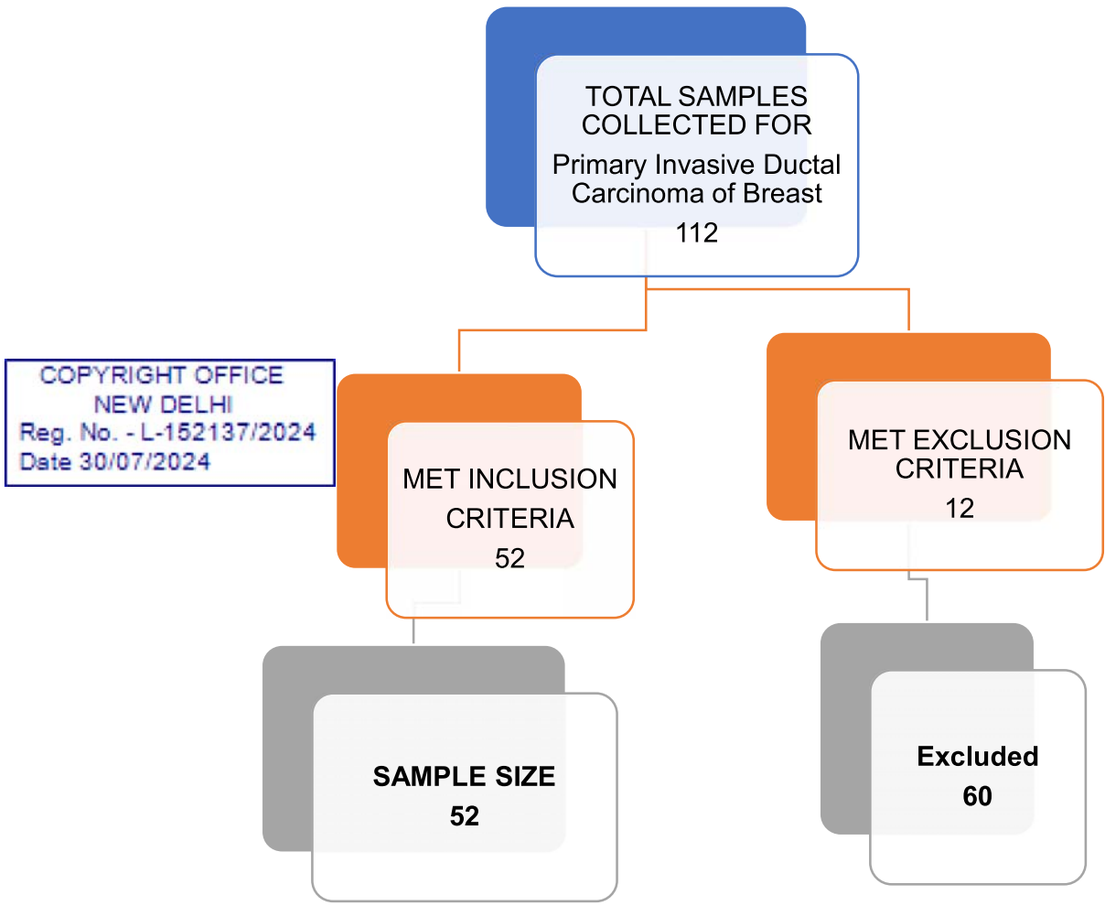
Input			Results	
α	0.01	Calculate		
β	0.05	Reset	N	33
r	0.644			

Variables	Descriptions		
α	Significance level (two sided test)	1%	0.01
1-β	Power of the test	β=95%	0.05
r*	Sample correlation	r value	0.644
N	Minimum Sample size needed	33	

* r value used in the calculation from a turkish by **Atalay Karacay I et al.** [73] in 2022.

Minimum sample size= 33

SAMPLE SIZE WITH JUSTIFICATION:-



METHODS:

Consent was taken/obtained from all the study participants before starting study. Ethical clearance obtained from institutional ethical committee, before conducting the study (IEC-571(a)/2020-21).

All freshly diagnosed primary Invasive Ductal Carcinoma of Breast cases by FNAC or TRUCUT biopsy and confirmed by mastectomy are included.

Case details are collected from the case files or interacting with the patient, which le – age, clinical presentation, physical examination findings including relevant

laboratory and radiological investigations. In local physical examination, site of lesion (right /left side and quadrant of breast), the size of the tumor, involvement of surrounding structures, No. of palpable Lymph Nodes including involvement of Nipple / Areola and skin changes. BMI of the patient are noted and the patient is classified as being the normal BMI / overweight to that age & geographic status / obese to that age& geographic status / severe obesity to that age& geographic status / morbid obesity to that age& geographic status /super obesity according to Asian BMI Criteria.

The breast tissue either TRUCUT or Mastectomy Specimen is fixed in Neutral Buffered Formalin – 10% – overnight and then grossed as per the SOP of the lab and representative bits are given from the tumor proper, resected margins including skin, nipple and areola. The tissue bits are processed as per the protocol of the lab. Tissue sections are stained with H & E stains. The tissue sections are screened and analysed for histomorphological features including histopathological type and grade of the tumor. The clinical stage of the tumor was noted . ER , PR , Her 2 neu , Ki 67 status was taken in whichever cases noted. Tissue sections were subjected to Leptin Immunohistochemistry.

ELISA:

6 ml of blood sample was taken in potassium EDTA vacutainer from the patient following confirmation of Diagnosis by FNAC or TRUCUT biopsy before the patient undergoes mastectomy since the Leptin levels might get altered after the removal of the tumor, and centrifuged at 1500 rpm for a time duration of 10 min, the plasma was separated, and was subjected to ELISA Leptin estimation.

ASSAY PROCEDURE:

Elisa kit used was taken from the company – Diagnostics Biochem Canada Inc.

All reagents were brought to room temperature before use. Calibrators, controls and specimen samples are assayed in duplicate.

1. Working solutions of the streptavidin-HRP conjugate and wash buffer were prepared.

µL of each calibrator, control and serum sample was pipetted into correspondingly belled wells in duplicate.

3. 80 µL of the monoclonal anti-leptin-biotin conjugate was pipetted into each well.

4. It was incubated on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.

5. Wells were washed 3 times with prepared wash buffer (300 µL/well for each wash) and the plate was tapped firmly against absorbent paper to ensure that it was dry.

6. 100 µL of prepared streptavidin-HRP conjugate was pipetted into each well.

7. It was incubated on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.

8. Wells were washed again in the same manner as step 5.

9. 100 µL of TMB substrate was pipetted into each well at timed intervals.

10. It was incubated on a plate shaker for 10-15 minutes at room temperature,

11. 50 µL of stopping solution was pipetted into each well at the same timed intervals as in step 9.

12. Plate was read on a microwell plate reader at 450nm within 20 minutes after addition of the stopping solution.

CALCULATIONS:

1. Mean optical density of each calibrator in duplicate was calculated.

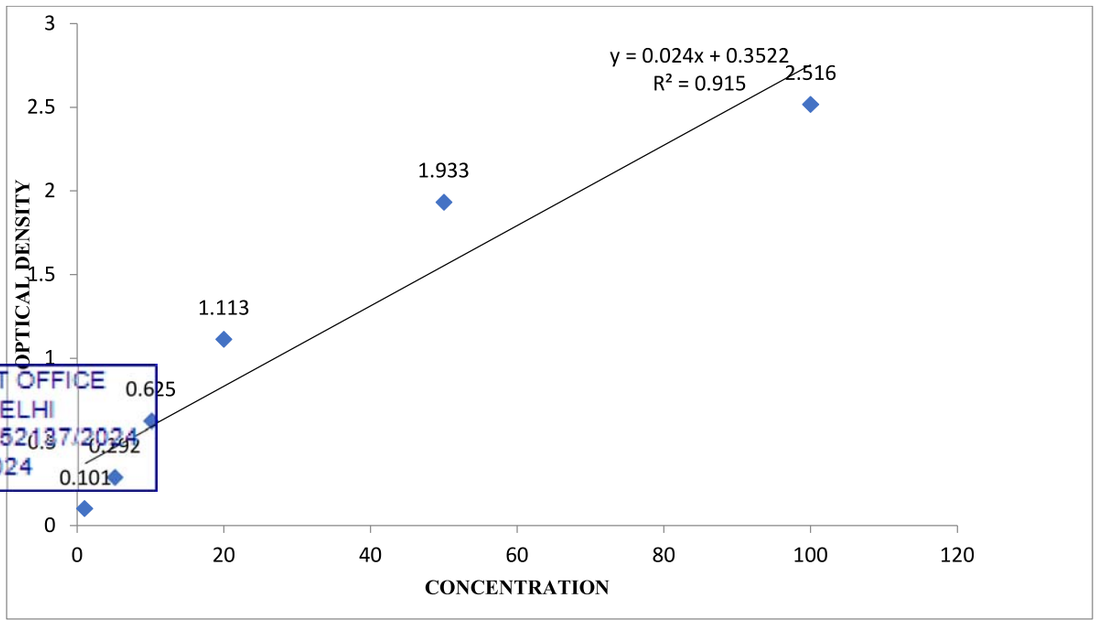
2. Calibrator curve was drawn on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. (If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended).

3. Mean optical density of each unknown duplicate was calculated.

4. Values of the unknowns were read directly off the calibrator curve.

5. If any sample read more than 100 ng/mL then it was diluted with assay buffer at a dilution of no more than 1:8. The result obtained was multiplied by the dilution factor.

Graph 1: Showing ELISA values of the subjects. X-axis – Concentration values. Y-axis – Optical density



ANALYSIS OF IMMUNOHISTOCHEMISTRY (IHC):

IHC – Primary antibody used was taken from Gene tex company.

Secondary antibody used was taken from Diagnostic Bio System company.

Polyclonal antibody – Rabbit – Reactivity – Human, Mouse.

PROCEDURE:

1.De-waxed sections were brought to distilled water.

2.These sections were washed briefly in distilled water for 1 – 2 minutes.

3.Antigen retrieval was done for15-20 minutes according to the standardization protocol in citrate buffer pH 6.0 and TRISEDTA pH 9 then were cooled for 5-10minutes.

4.Then they were washed in distilled water without letting the section dry out.

5.The section was endogenously per oxidized in 3% H2O2 for 10 minutes

6.Then the sections were washed in tris buffered solution (TBS) pH 7.4 for 2 minutes.

7.The sections were then covered with individual primary antibodies for 45 minutes to 1

slides were then washed for two times with TBS for 2 minutes

9.The sections were then covered with secondary antibody Horseradish Peroxidase (HRP) for 30 minutes

10.The slides were then washed for two times in TBS for 2 minutes

11.The sections were then covered with diaminobenzidine.

12.Tetrahydrochlodide (DAB) chromogen was used for 5 minutes.

13.Then the slides were washed with distilled water.

14.The sections were then covered with hematoxylin for 30 seconds.

15.The slides were washed with TBS followed by distilled water 2 times in 2 changes.

16.The sections were dehydrated by 3 changes of absolute alcohol & cleared with 2 changes of Xylene for 2 minutes.

17.Finally the slides were mounted with Dibutylpthalate Polystyrene Xylene (DPX).

QUALITY CONTROL PROCEDURES:

Known positive control slide (Tonsil) was stained along with testing slide.

The Immunohistochemistry Scoring for leptin was considered as:

0 – Negative Expression

1 – Expression less than that of a Normal Adipocyte

2 - Expression equal to that of a Normal Adipocyte

3 - Expression more than that of a Normal Adipocyte.[84]

All the data was entered in Microsoft XL sheet and statistical analysis was done using IBMM SPSS software version 22. The IHC leptin expression was correlated with plasma levels.

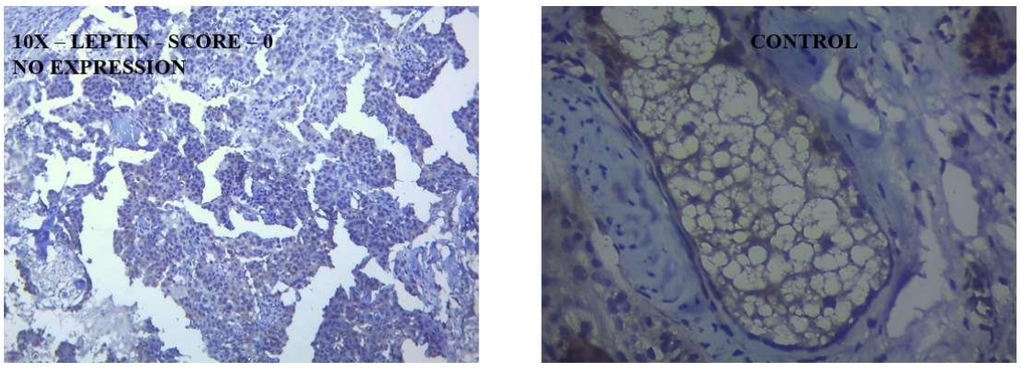


Fig 8: Microphotograph showing - score 0, No expression

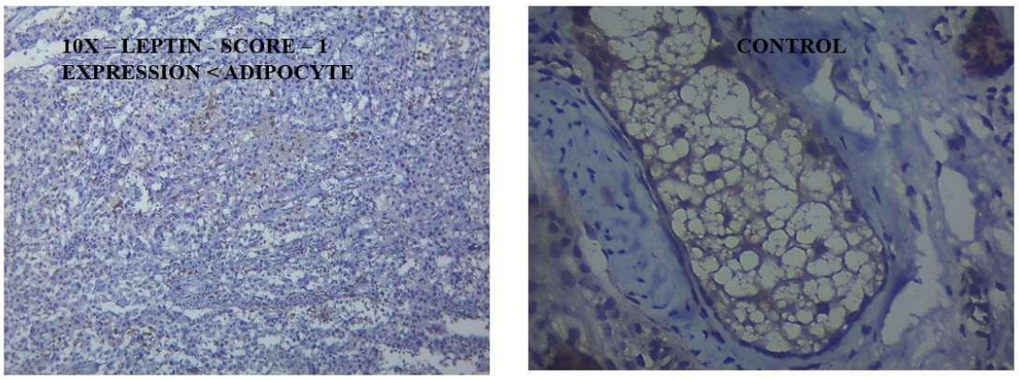


Fig 9: Microphotograph showing -score 1, Expression < Adipocyte

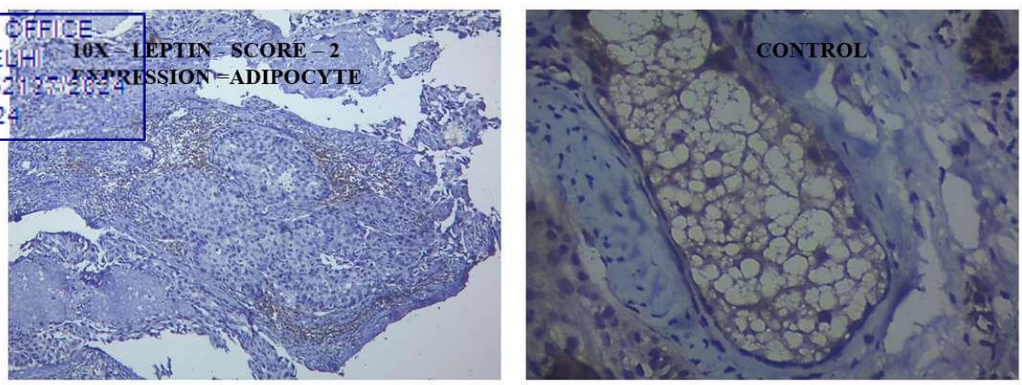


Fig 10: Microphotograph showing score 2, Expression = Adipocyte

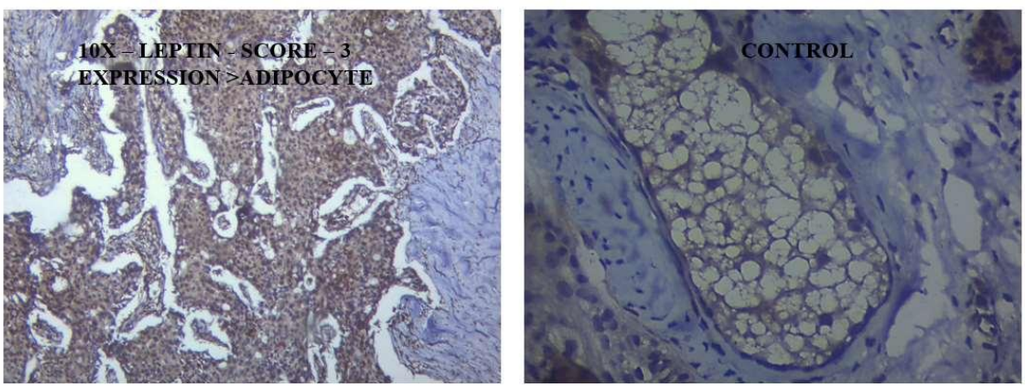


Fig 11: Microphotograph showing score 3, Expression > Adipocyte

METHODS:

Ethical committee approval

↓

Collected SAMPLES satisfying inclusion criteria

↓

Data collected from the samples

↓

Data entered into excel sheet

↓

Data compilation

↓

Statistical Analysis

↓

Conclusion

DATA VARIABLE:-

Age, Duration of lesion, Menopausal state, Family history, BMI, Tumor Size, Tumor Infiltrating Lymphocytes, Lymphovascular Invasion, Metastatic Lymph Nodes, Distant Metastasis, Grading, NPI, Clinical /Radiological Staging, TNM Staging, Immunohistochemistry- ER,PR,HER, K167, Leptin, Elisa –Leptin.

DATA ANALYSIS:

Data was entered in MS-excel 2007 and data - analysed using IBM SPSS (Statistical Package for the Social Sciences) software trail version 22. Nominal data analysis were presented in numbers & percentages. Continuous data were expressed as mean & standard deviation. Appropriate statistical tests were applied, (chi-square test) and < 0.05 p values considered as significant. Pearson's correlation was done.

r VALUE INTERPRETATION:

Table 9: r value interpretation

Coefficient Interval	Relation
0.00-0.199	Very low
0.20-0.399	Low
0.40-0.599	Medium
0.60-0.799	High
0.80-1.000	Very high

RESULTS:

Table10: Basic characteristics

BASIC CHARACTERISTICS		COUNT(N)	TABLE (%)
AGE CATEGORY	30 TO 39	1	1.9%
	40 TO 49	11	21.2%
	50 TO 59	22	42.3%
	60 TO 69	15	28.8%
	70 AND ABOVE	3	5.8%
MENOPAUSAL STATUS	PRE-MENOPAUSAL	13	25.0%
	POST-MENOPAUSAL	39	75.0%
PARITY	MULTIPARA	49	94.2%
	PRIMIPARA	3	5.8%
BMI	NORMAL	34	65.4%
	PRE-OBESE	4	7.7%
	UNDERWEIGHT	14	26.9%
TUMOR INFILTRATING LYMPHOCYTES	NO	35	67.3%
	YES	17	32.7%
LYMPHOVASCULAR INVASION	NO	51	98.1%
	YES	1	1.9%
METASTATIC LYMPH NODES	NO	49	94.2%
	YES	3	5.8%
DISTANT METASTASIS	NO	52	100.0%
NPI	MODERATE	12	23.1%
	GOOD	40	76.9%
ER	NEGATIVE	29	55.8%
	POSITIVE	23	44.2%
PR	NEGATIVE	31	59.6%
	POSITIVE	21	40.4%
HER2 NEU	NEGATIVE	37	71.2%
	POSITIVE	15	28.8%
KI67	<14%	22	42.3%
	>14%	30	57.7%

Table 11: Demographic data

PARAMETER	MINIMUM	MAXIMUM	RANGE	MEAN	STANDARD DEVIATION
AGE (YEARS)	35.00	72.00	35-72	56.29	9.03
PARITY	1.00	5.00	1-5	2.56	0.98
BMI	17.00	26.00	17-26	19.96	2.12
NPI (SCORE)	2.40	4.80	2.4-4.8	3.12	0.41
ELISA - LEPTIN (ng/ml)	13.21	79.54	13.2-79.5	40.92	20.05

Table 12: Age vs IHC leptin and Elisa leptin

AGE CATEGORY	IHC LEPTIN EXPRESSION								ELISA - LEPTIN (ng/ml)	
	0		1		2		3		Mean	SD
	N	%	N	%	N	%	N	%		
30 TO 39	0	0.0%	0	0.0%	1	1.9%	0	0.0%	26.17	
40 TO 49	0	0.0%	2	3.8%	5	9.6%	4	7.7%	37.96	18.21
50 TO 59	1	1.9%	2	3.8%	9	17.3%	10	19.2%	46.01	19.64
60 TO 69	2	3.8%	1	1.9%	4	7.7%	8	15.4%	37.98	21.55
70 AND ABOVE	1	1.9%	1	1.9%	1	1.9%	0	0.0%	34.06	26.77
TOTAL	4	7.7%	6	11.5%	20	38.5%	22	42.3%	36.43	20.05
P VALUE	0.566								0.59	

Chart 1: Age vs IHC leptin

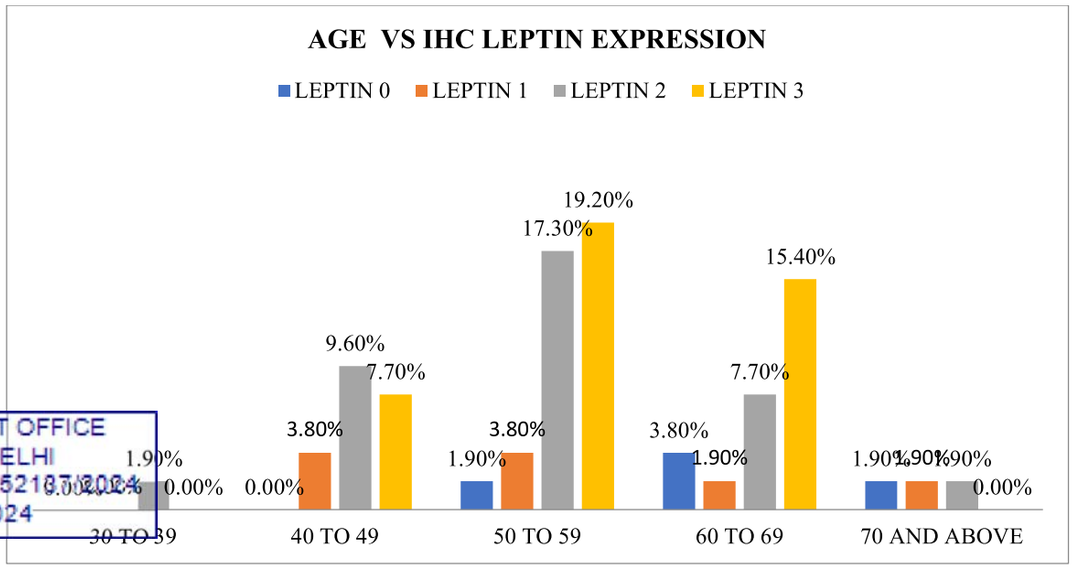
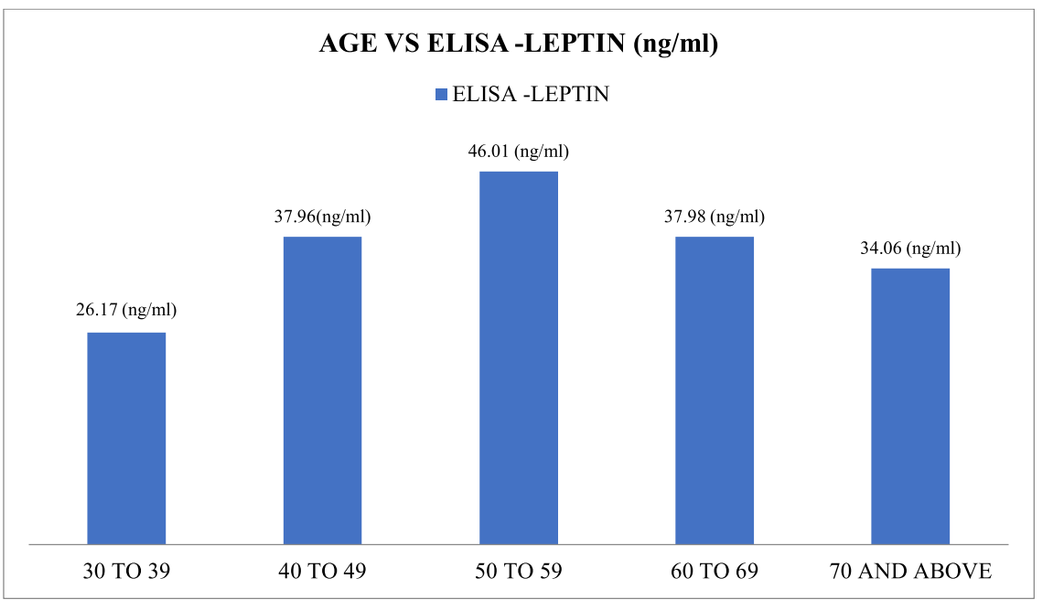


Chart 2: Age vs Elisa leptin



In present study, majority of the study population (42.3%) were belonging to 50 to 59 years oup and IHC leptin 3 scores were more prevalent in the same group . Elisa leptin was st among 50 to 59 years age group. But the difference between the groups was not to be significant.

Table 13: Menopausal status vs IHC leptin & Elisa leptin

MENOPAUSAL STATUS	IHC LEPTIN EXPRESSION								ELISA - LEPTIN (ng/ml)	
	0		1		2		3		Mean	Standard Deviation
	N	%	N	%	N	%	N	%		
PRE MENOPAUSAL	0	0.0 %	2	3.8%	7	13.5 %	4	7.7%	44.50	22.68
POST MENOPAUSAL	4	7.7 %	4	7.7%	13	25.0 %	18	34.6 %	39.73	19.27
Total	4	7.7 %	6	11.5 %	20	38.5 %	22	42.3 %	40.92	20.05
P VALUE	0.36								0.4	

Chart 3: Menopausal status vs IHC leptin

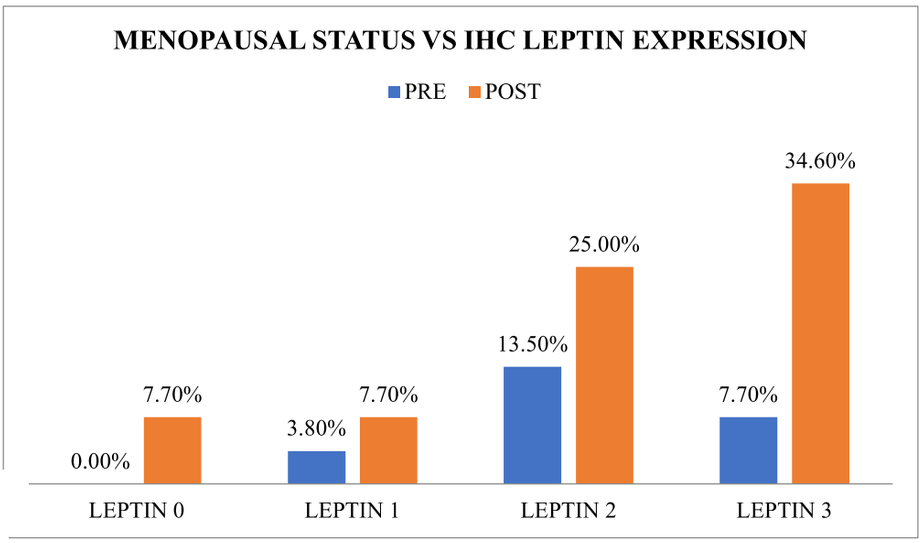
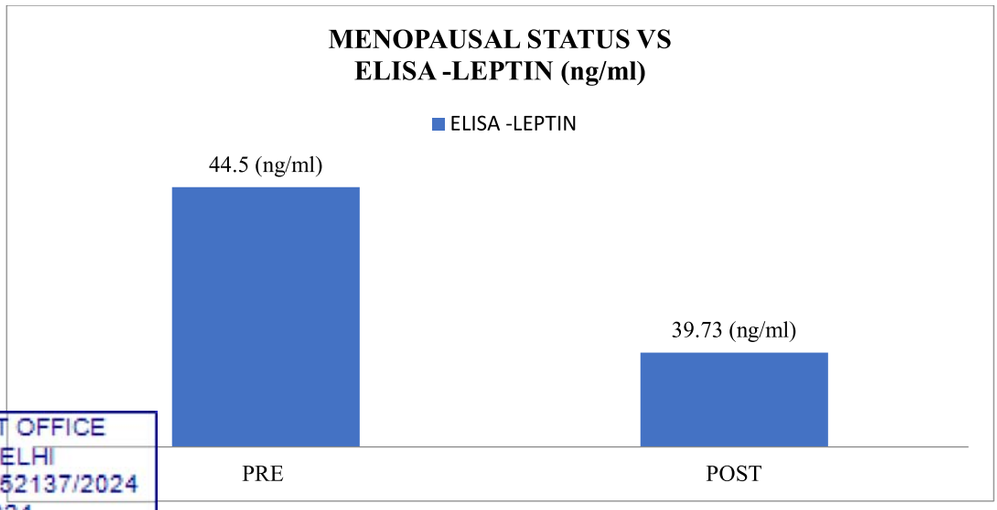


Chart 4: Menopausal status vs Elisa leptin



In present study, maximum number of the study population (75%) were belonging to post-menopausal group with IHC Leptin 3 score predominance among them. Elisa leptin was highest pre-menopausal women . But the difference between the groups was not found to be significant.

Table 14: Parity status vs IHC leptin & Elisa leptin

PARITY	**IHC LEPTIN EXPRESSION**								**ELISA - LEPTIN (ng/ml)**	
	0		1		2		3		Mean	SD
	N	%	N	%	N	%	N	%		
MULTIPARA	4	7.7%	6	11.5%	18	34.6%	21	40.4%	39.28	19.48
PRIMIPARA	0	0.0%	0	0.0%	2	3.8%	1	1.9%	67.63	3.33
TOTAL	4	7.7%	6	11.5%	20	38.5%	22	42.3%	40.92	20.05
P VALUE	0.2								0.04	

Chart 5: Parity status vs IHC leptin

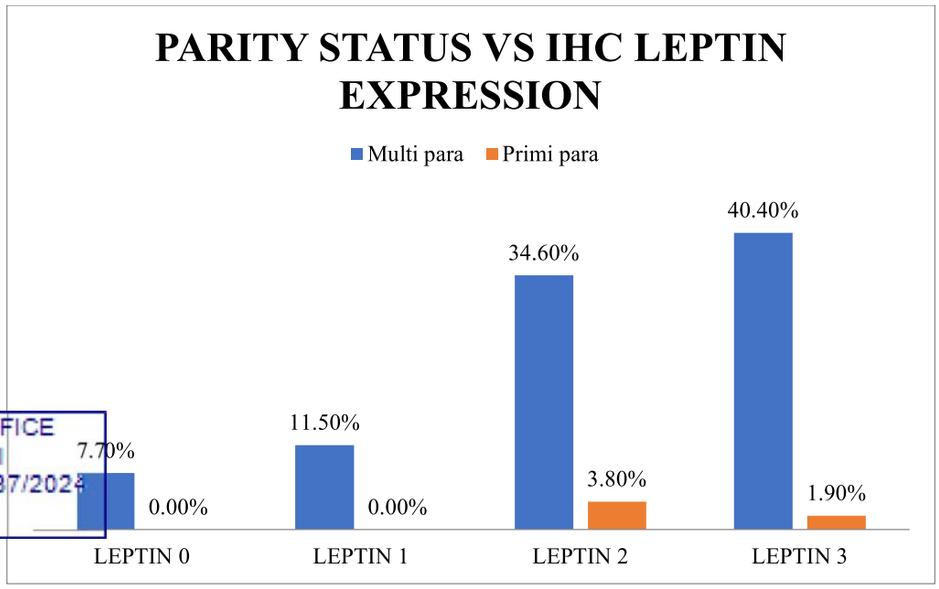
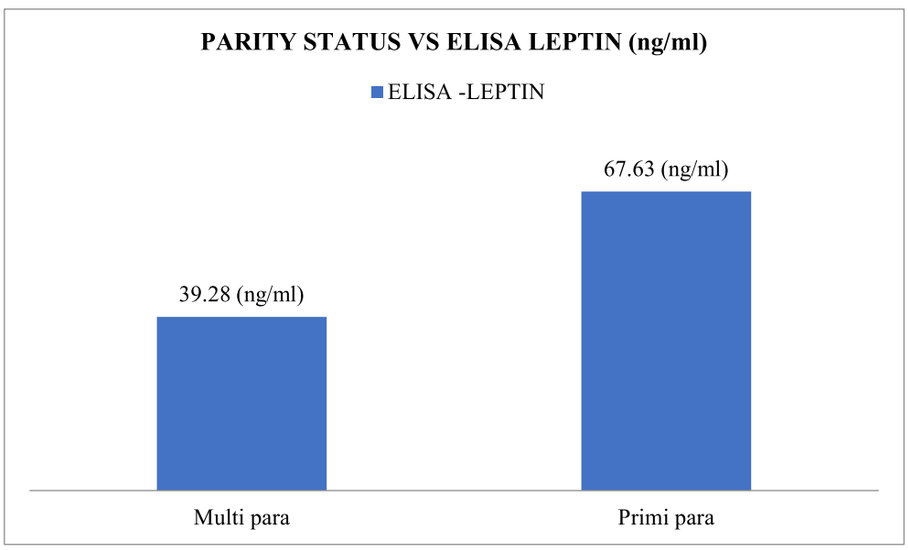


Chart 6: Parity status vs Elisa leptin



In present study, maximum number of study population (94.2%) were multiparous women HC Leptin 3 score predominance among them. But the difference between the groups ıot found to be significant. Elisa Leptin was significantly higher among primipara :n.

Table 15: BMI Status vs IHC leptin & Elisa leptin

BMI	IHC LEPTIN EXPRESSION								ELISA -LEPTIN (ng/ml)	
	0		1		2		3		Mean	Standard Deviation
	N	%	N	%	N	%	N	%		
normal	3	5.8%	2	3.8%	13	25.0%	16	30.8%	42.14	20.87
pre obese	0	0.0%	1	1.9%	2	3.8%	1	1.9%	38.81	20.35
underweight	1	1.9%	3	5.8%	5	9.6%	5	9.6%	38.54	19.08
[illegible]	4	7.7%	6	11.5%	20	38.5%	22	42.3%	40.92	20.05
P VALUE	0.69								0.8	

Chart 7: BMI Status vs IHC leptin

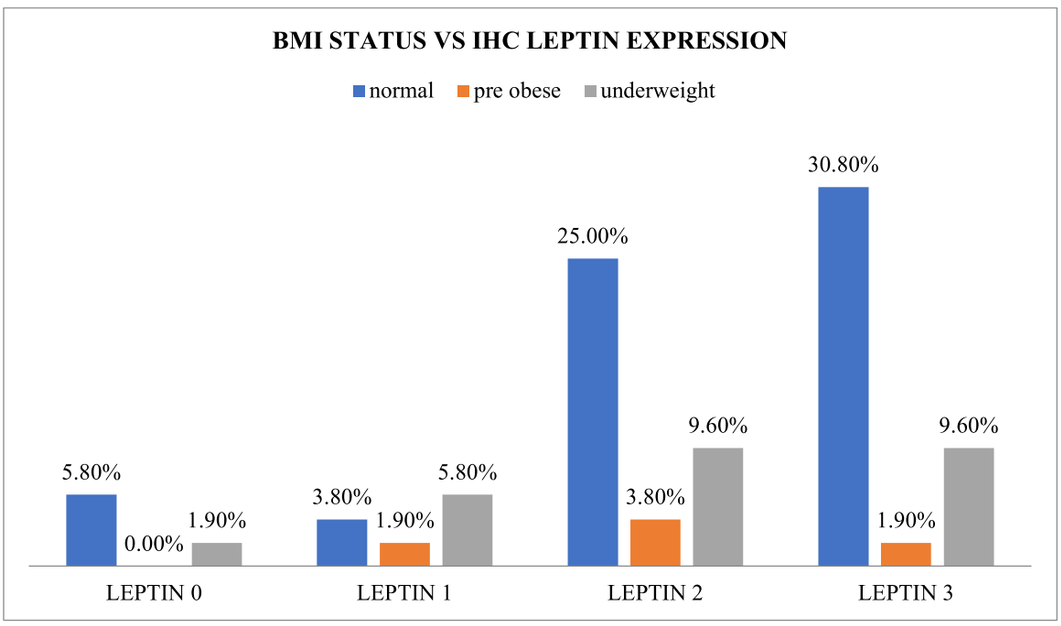
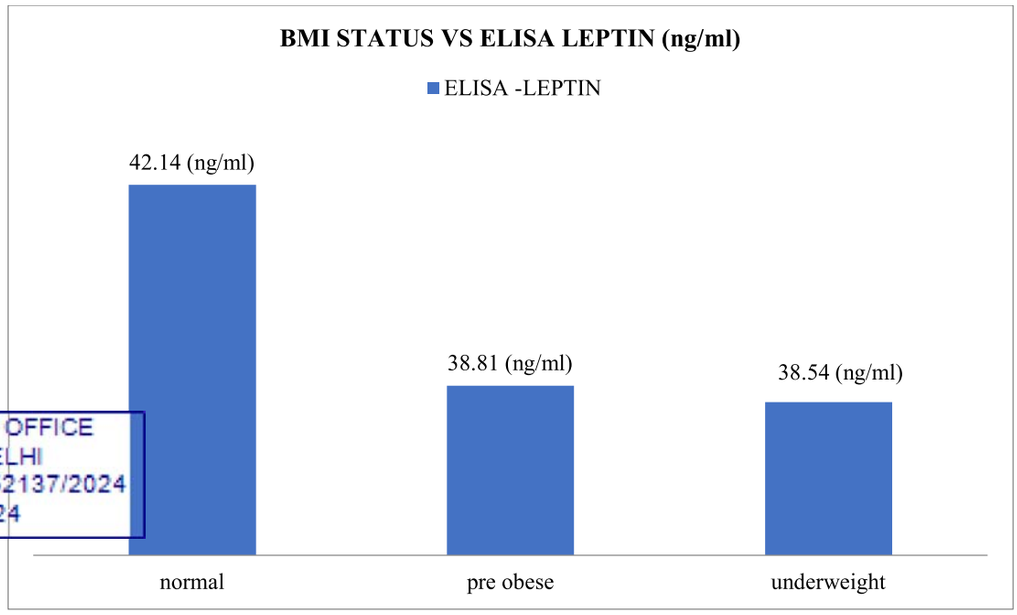


Chart 8: BMI Status vs Elisa leptin

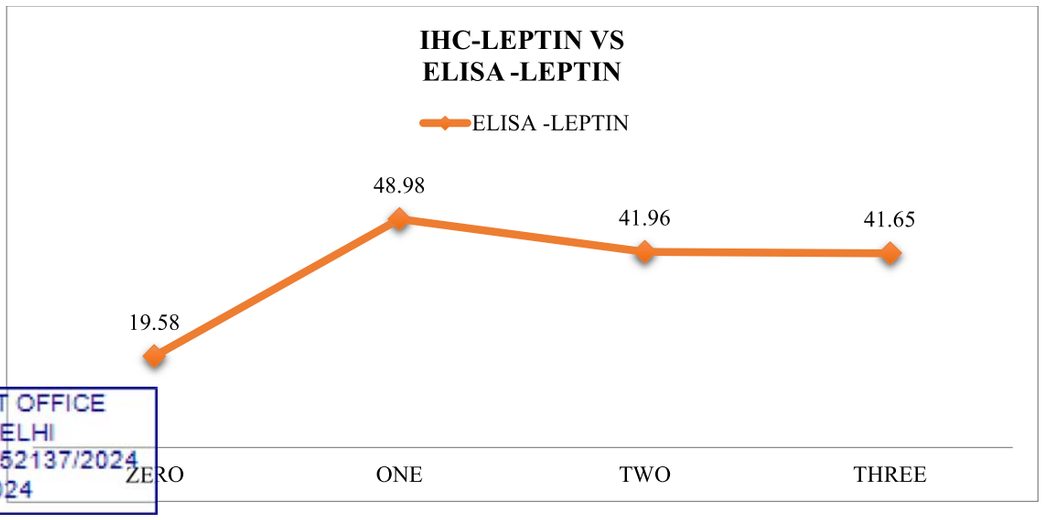


In present study, maximum of the study population (65.4%) were in normal BMI level with IHC Leptin 3 score predominance among them. Elisa Leptin was highest Normal BMI level. But the difference between the groups was not found to be significant.

Table 16: IHC leptin vs Elisa leptin association

IHC-LEPTIN EXPRESSION	ELISA -LEPTIN (ng/ml)		
	N	Mean	Std. Deviation
0	4	19.58	6.83
1	6	48.98	18.93
2	20	41.96	20.10
3	22	41.65	20.30
Total	52	40.92	20.05
P VALUE	0.1		

Chart 9: IHC leptin vs Elisa leptin association

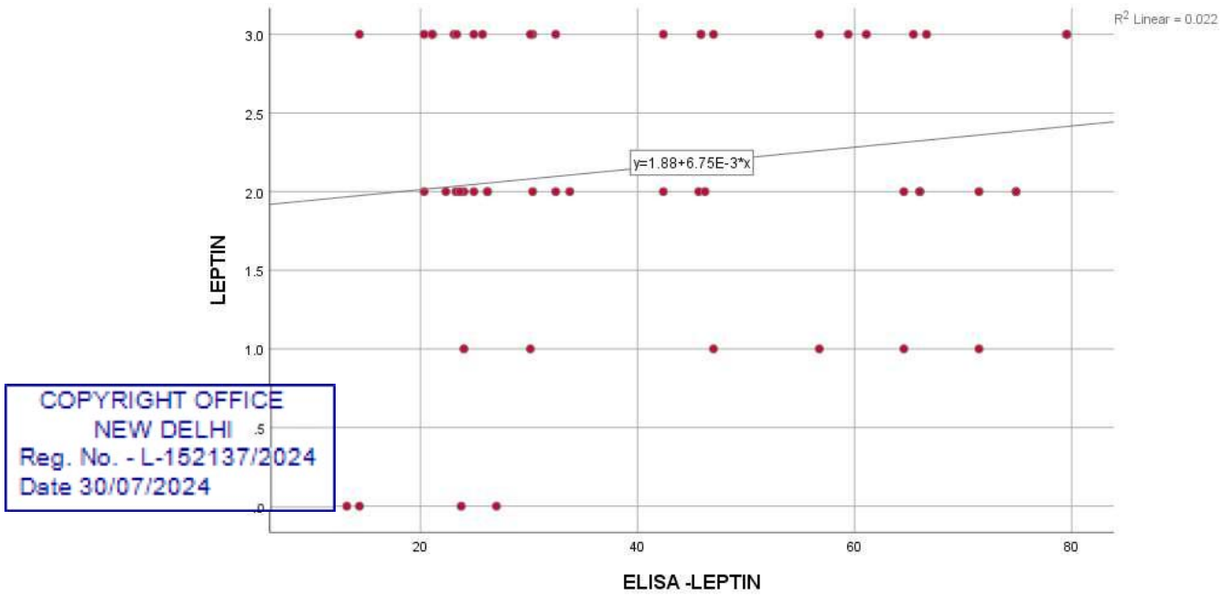


ELISA –Leptin mean levels with IHC- Leptin level zero had low and high at level one. But the difference between the means was not found to be significant.

Table 17: IHC leptin vs Elisa leptin correlation

Correlations			
		LEPTIN	ELISA -LEPTIN
LEPTIN	Pearson Correlation	1	0.148
	Sig. (2-tailed)		0.296
	N	52	52
ELISA -LEPTIN	Pearson Correlation	0.148	1
	Sig. (2-tailed)	0.296	
	N	52	52

Graph 2: IHC leptin vs Elisa leptin correlation



Correlation of ELISA –Leptin with IHC- Leptin levels were found to be weak positives and non-significant. Correlation of ELISA –Leptin with IHC- Leptin levels were found to be non-significant.

Table 18: IHC leptin expression among the study population

IHC LEPTIN EXPRESSION	TOTAL NO OF CASES (N,N%)
NEGATIVE	4 (7.69%)
POSITIVE	48 (92.3%)

Among the study population with sample size of 52, 48 (92.3%) cases show IHC leptin positivity.

Table 19: IHC leptin expression

	IHC LEPTIN EXPRESSION SCORE 0		IHC LEPTIN EXPRESSION SCORE 1		IHC LEPTIN EXPRESSION SCORE 2		IHC LEPTIN EXPRESSION SCORE 3	
CASES	N	%	N	%	N	%	N	%
	4/52	7.69%	6/52	11.5%	20/52	38.4%	22/52	42.3%

Among 52 cases studied, the maximum IHC leptin expression with score 3 is seen in maximum number of cases constituting 42.3% , and 7.6% of cases show negative IHC leptin expression.

Table 20: ELISA leptin concentration

	Minimum	Maximum	Mean±SD
ELISA Leptin (ng/ml)	13.21	79.54	40.92±20.05

In our study, the plasma leptin levels were recorded as the lowest being 13.21 ng/ml, highest being 79.54 ng/ml with the average of 40.92±20.05 ng/ml.

Table 21: Tumor size vs IHC leptin & Elisa leptin

TUMOR SIZE	IHC LEPTIN EXPRESION								ELISA - LEPTIN (ng/ml)	
	0		1		2		3		Mean	SD
	N	%	N	%	N	%	N	%		
T1	1	1.9%	2	3.8%	7	13.5%	3	5.8%	41.58	21.46
T2	2	3.8%	2	3.8%	12	23.1%	19	36.5%	39.80	19.89
T3	1	1.9%	1	1.9%	1	1.9%	0	0.0%	45.86	24.04
T4	0	0.0%	1	1.9%	0	0.0%	0	0.0%	56.75	23.5
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%	40.92	20.05
P VALUE	0.04								0.8	

Chart 10: Tumor size vs IHC leptin expression

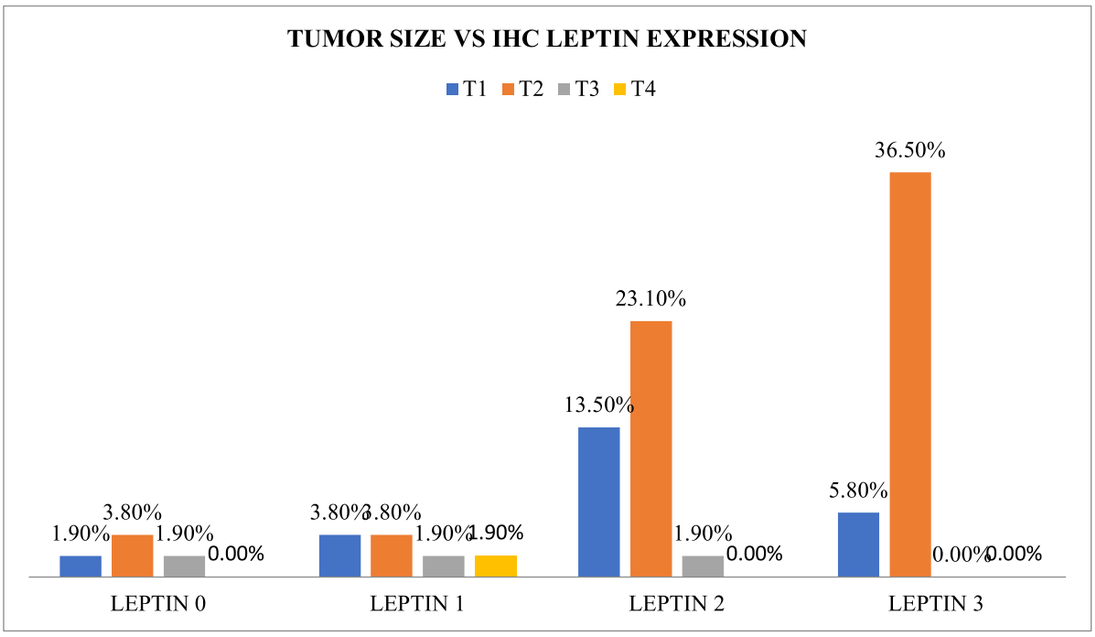
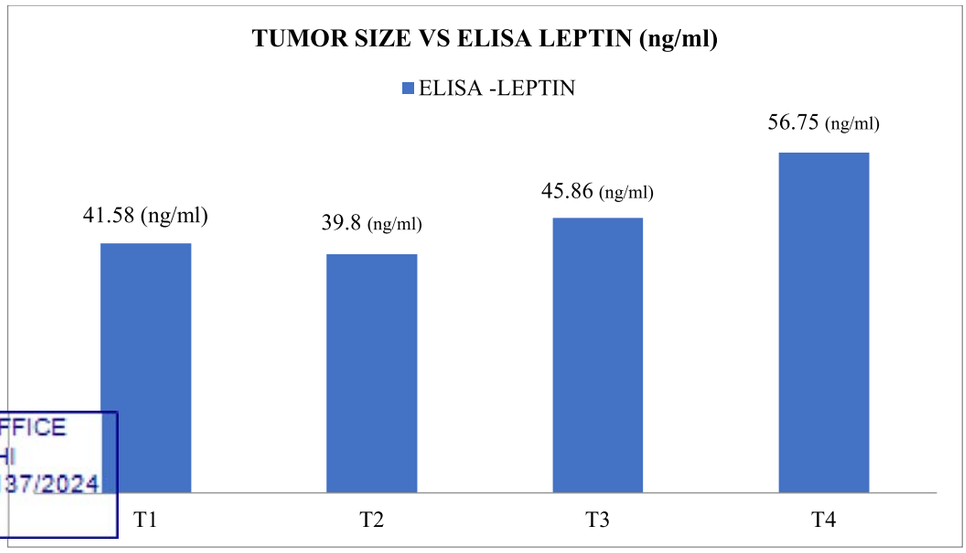


Chart 11: Tumor size vs Elisa leptin



In our study, maximum of the study population (67.3%) were in the tumor stage - pT2 with IHC Leptin 3 score showing predominance among them, which was statistically significant. Elisa leptin was highest tumor staging pT4 and the results were not statistically significant. But the difference between the groups was not found to be significant.

Table 22: Metastatic lymph nodes vs IHC leptin and Elisa leptin

METASTATIC LYMPH NODES	IHC LEPTIN EXPRESSION								ELISA - LEPTIN (ng/ml)	
	0		1		2		3		Mean	SD
	N	%	N	%	N	%	N	%		
NO	4	7.7%	6	11.5%	20	38.5%	19	36.5%	41.17	20.32
POSITIVE	0	0.0%	0	0.0%	0	0.0%	3	5.8%	36.74	17.66
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%	40.92	20.05
P VALUE	0.2								0.7	

Chart12: Metastatic lymph nodes vs IHC leptin

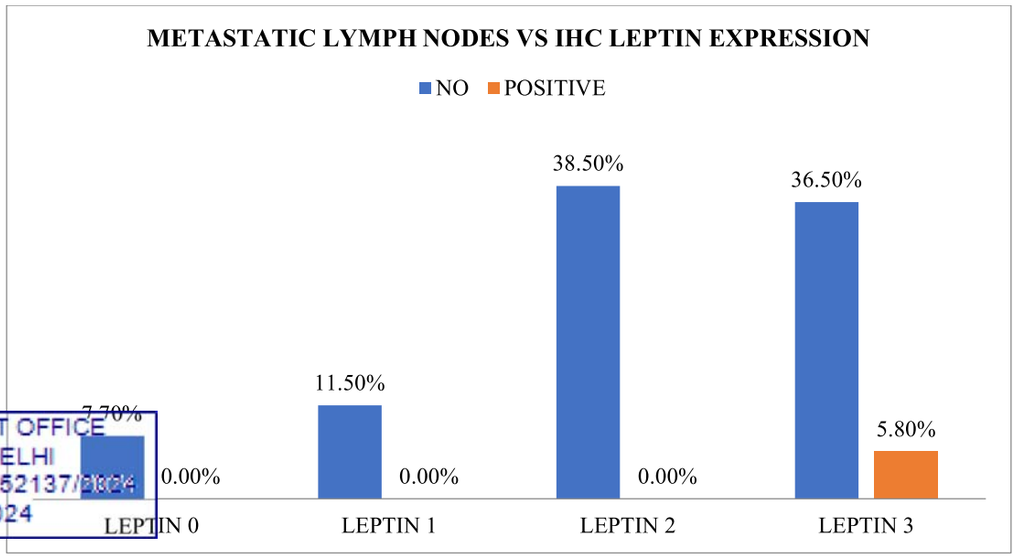
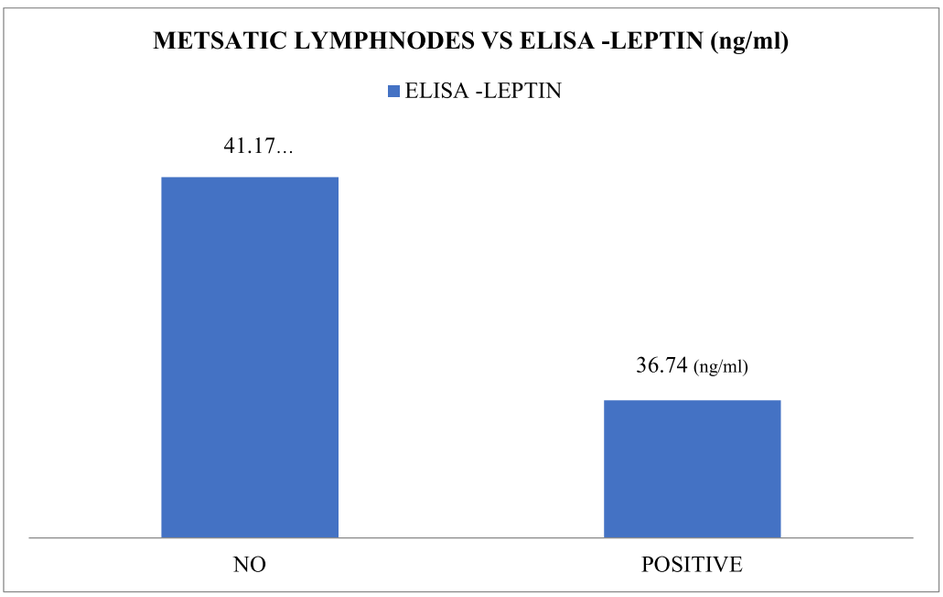


Chart 13: Metastatic lymph nodes vs Elisa leptin



In present study, most of the study population (94.2%) were not having metastatic lymph nodes/ lymph nodes showing tumor deposits with IHC Leptin 2 score predominance among them. Elisa Leptin was high among non-metastatic study population. But the difference en the groups was not found to be significant.

Table 23: Tumor infiltrating lymphocytes vs IHC leptin & Elisa leptin

TUMOR INFILTRATING LYMPHOCYTES	IHC LEPTIN EXPRESSION								ELISA -LEPTIN (ng/ml)	
	0		1		2		3		Mean	SD
	N	%	N	%	N	%	N	%		
NO	1	1.9%	6	11.5%	14	26.9%	14	26.9%	38.97	18.07
POSITIVE	3	5.8%	0	0.0%	6	11.5%	8	15.4%	44.94	23.71
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%	40.92	20.05
P VALUE	0.9								0.3	

Chart14: Tumor infiltrating lymphocytes vs IC leptin

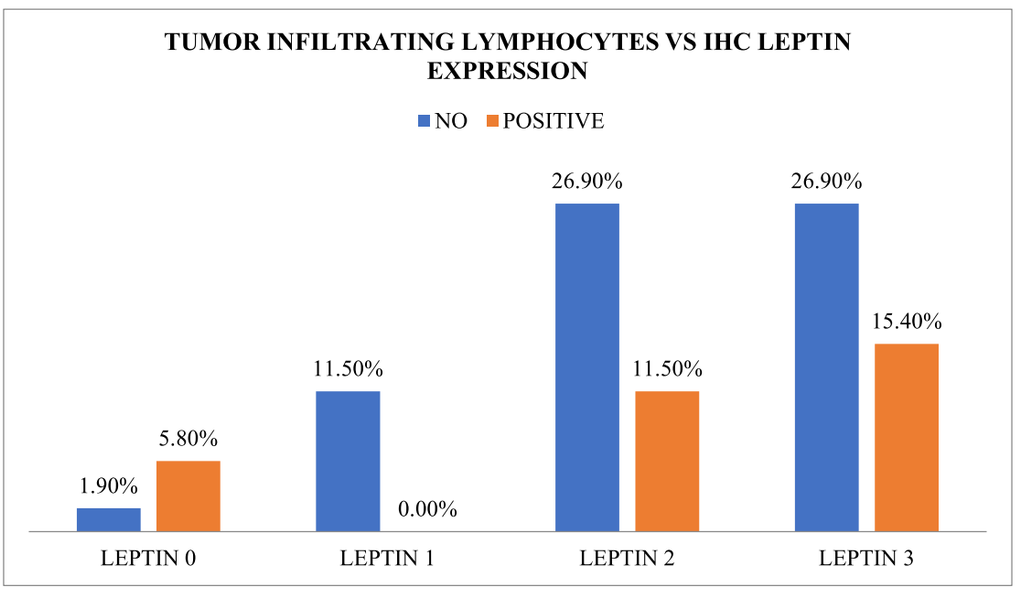
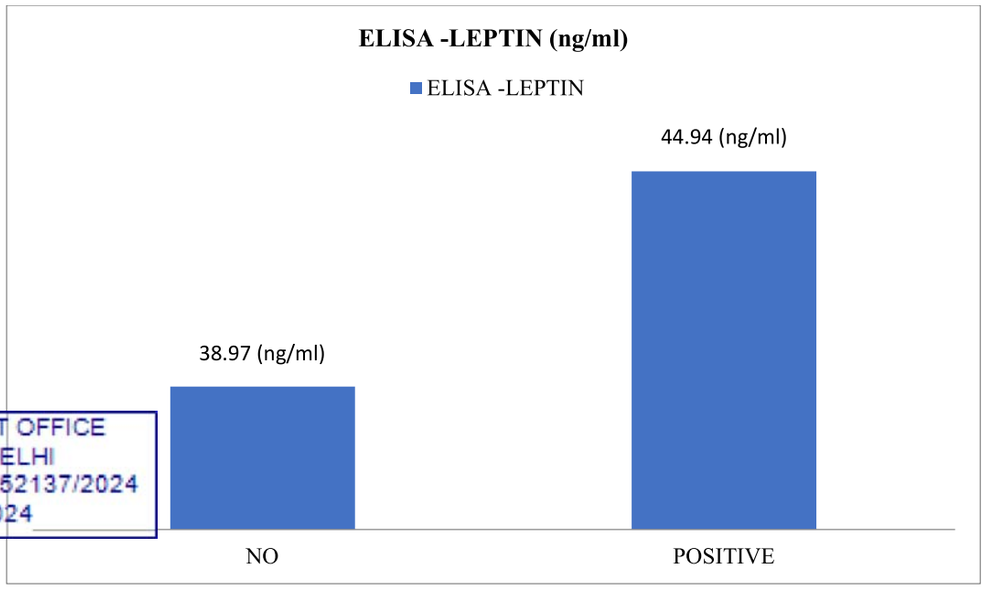


Chart 15: Tumor infiltrating lymphocytes vs Elisa leptin



In present study, predominant of the study population (67.3%) were not having Tumor Infiltrating Lymphocytes with IHC Leptin 2&3 score predominance among them. Elisa Leptin was high among the study population showing Tumor Infiltrating Lymphocytes. But the difference between the groups was not found to be significant.

Table 24: Lymphovascular invasion vs IHC leptin & Elisa leptin

LYMPHOVASCULAR INVASION	IHC LEPTIN EXPRESSION								ELISA-LEPTIN (ng/ml)	
	0		1		2		3		Mean	SD
	N	%	N	%	N	%	N	%		
NO	4	7.7%	6	11.5%	20	38.5%	21	40.4%	40.56	20.08
POSITIVE	0	0.0%	0	0.0%	0	0.0%	1	1.9%	59.42	23.5
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%	40.92	20.05
P VALUE	0.7								0.3	

Chart16: Lymphovascular invasion vs IHC leptin

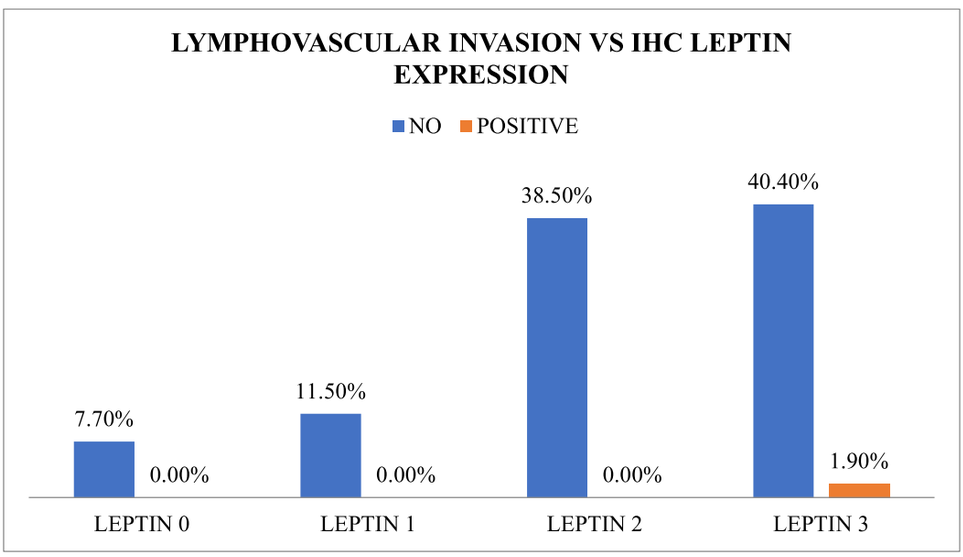
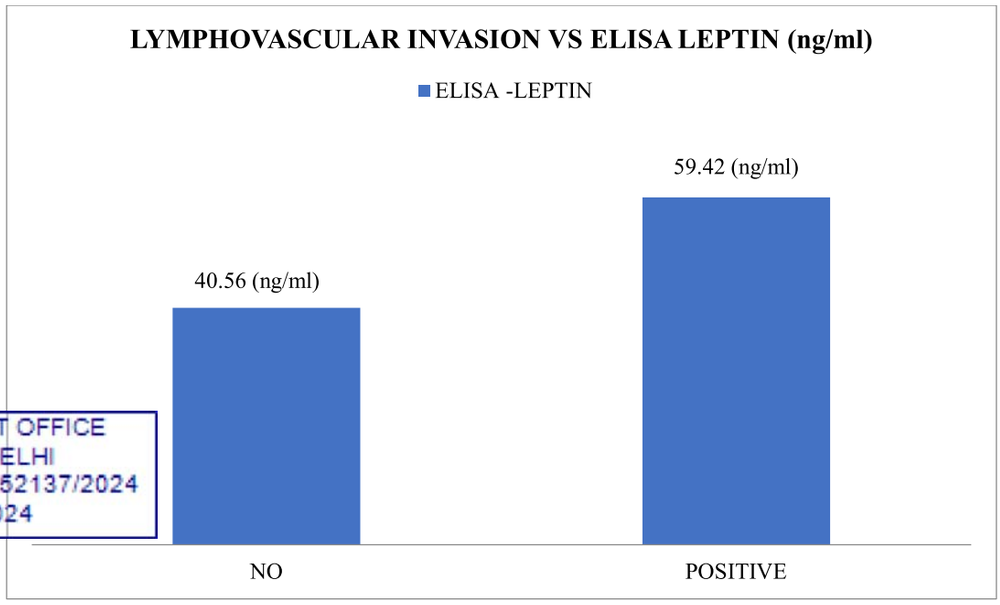


Chart 17: Lymphovascular invasion vs Elisa leptin



In present study, most of the study population (98%) were not having Lymphovascular Invasion with IHC Leptin 2&3 score predominance among them. Elisa Leptin was high among positive Lymphovascular Invasion study population. But the difference between the groups was not found to be significant.

Table 25: Staging vs IHC leptin & Elisa leptin

STAGE	IHC LEPTIN EXPRESSION								ELISA -LEPTIN (ng/ml)	
	0		1		2		3		Mean	Standard Deviation
	N	%	N	%	N	%	N	N%		
I	1	1.9%	2	3.8%	7	13.5%	2	3.8%	43.35	21.40
II	3	5.8%	3	5.8%	13	25.0%	20	38.5%	39.76	19.93
III	0	0.0%	1	1.9%	0	0.0%	0	0.0%	56.75	23.5
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%	40.92	20.05
P VALUE	0.05								0.6	

Chart 18: Staging vs IHC leptin

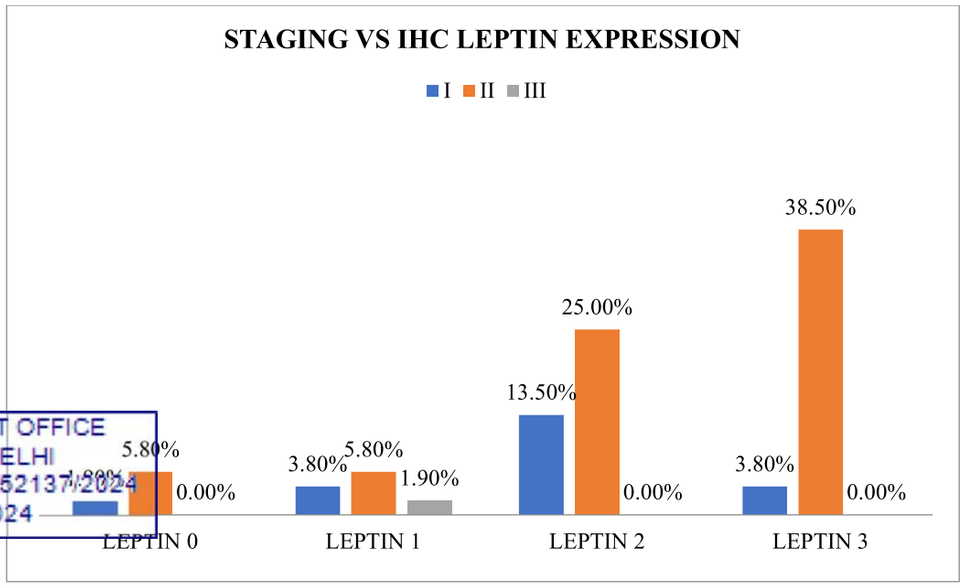
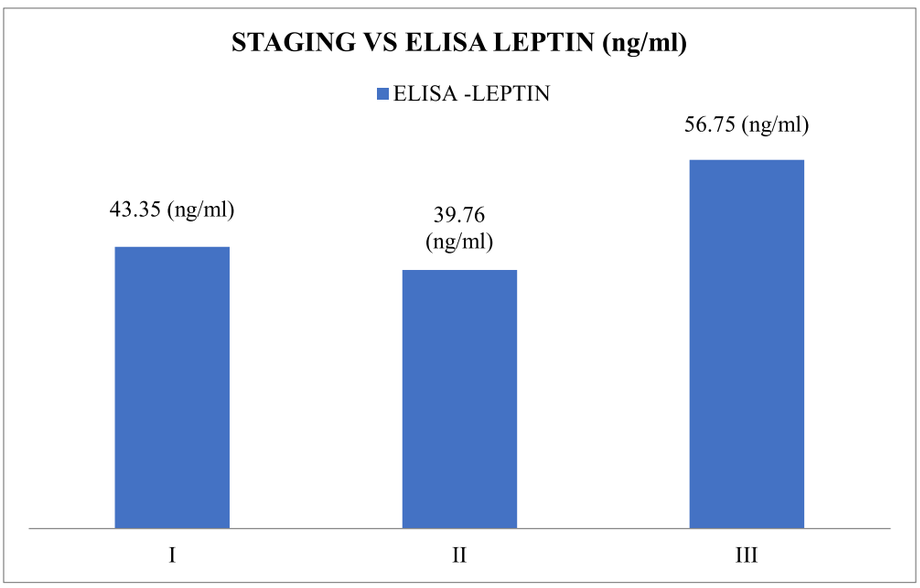


Chart 19: Staging vs Elisa leptin



In present study, predominant of the study population (75%) were in stage 2 with IHC Leptin scores predominance among them. Elisa Leptin was high among stage 3 group of study population. But the difference between the groups was not found to be significant.

Table 26: Modified bloom Richardson grading vs IHC leptin & Elisa leptin

MODIFIED BLOOM RICHARDSON GRADING	IHC LEPTIN EXPRESSION								ELISA - LEPTIN (ng/ml)	
	0		1		2		3		Mean	SD
	N	%	N	%	N	%	N	%		
1	0	0.0%	1	1.9%	6	11.5%	3	5.8%	41.15	19.72
2	2	3.8%	4	7.7%	9	17.3%	9	17.3%	40.60	23.05
3	2	3.8%	1	1.9%	5	9.6%	10	19.2%	41.21	16.77
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%	40.92	20.05
P VALUE	0.5								0.9	

Chart 20: Modified bloom Richardson grading vs IHC leptin

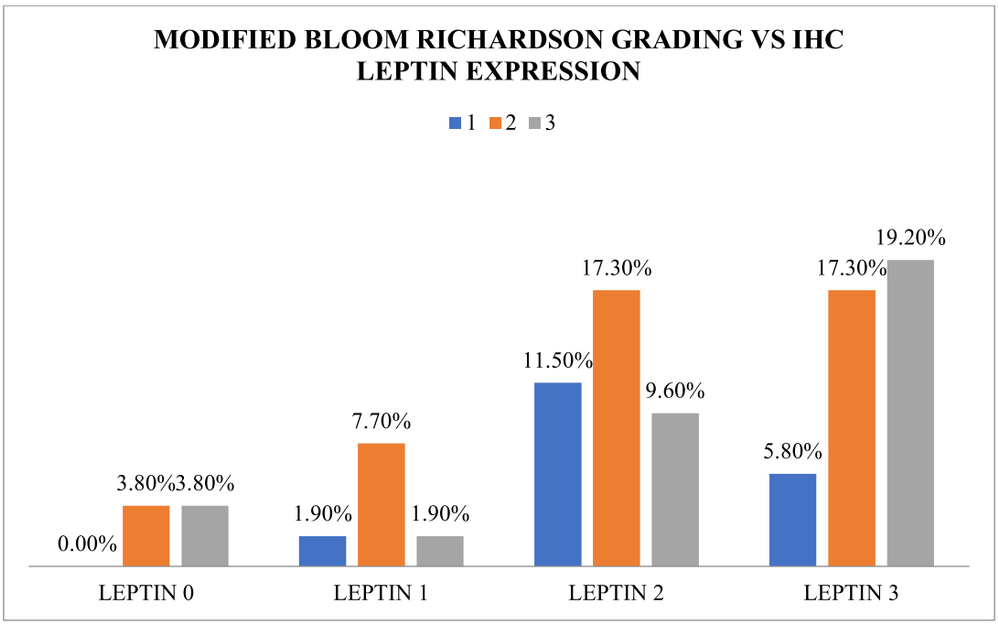
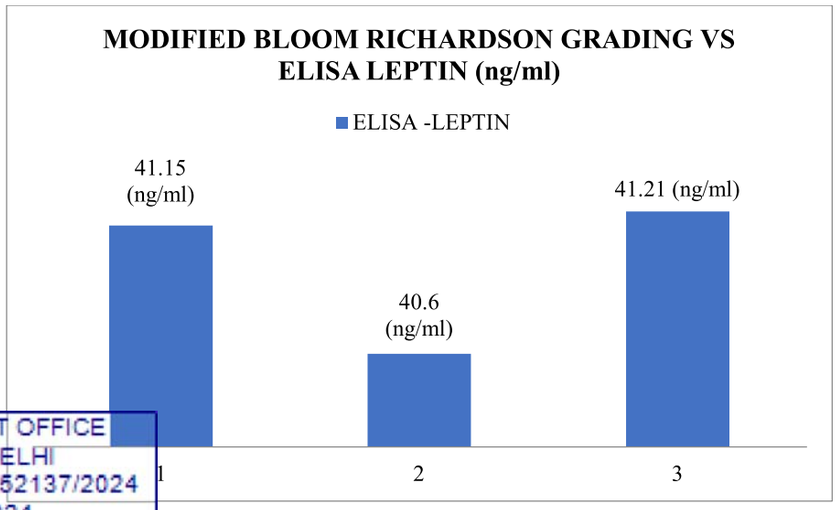


Chart 21: Modified bloom Richardson grading vs Elisa leptin



In present study, most of the study population (46.1%) were in grade 2 show high leptin expression with scores of 2 and 3. Elisa Leptin was high among population belonging to grade 2 and grade 3. But the difference between the groups was not found to be significant.

Table 27: NPI Prognostic score vs IHC leptin & Elisa leptin

NPI PROGNOSTIC SCORE	IHC LEPTIN EXPRESSION								ELISA - LEPTIN (ng/ml)	
	0		1		2		3		Mean	SD
	N	%	N	%	N	%	N	%		
GOOD	3	5.8%	4	7.7%	16	30.8%	17	32.7%	39.61	18.99
MODERATE	1	1.9%	2	3.8%	4	7.7%	5	9.6%	45.27	23.63
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%	40.92	20.05
P VALUE	0.9								0.3	

Chart 22: NPI Prognostic score vs IHC leptin

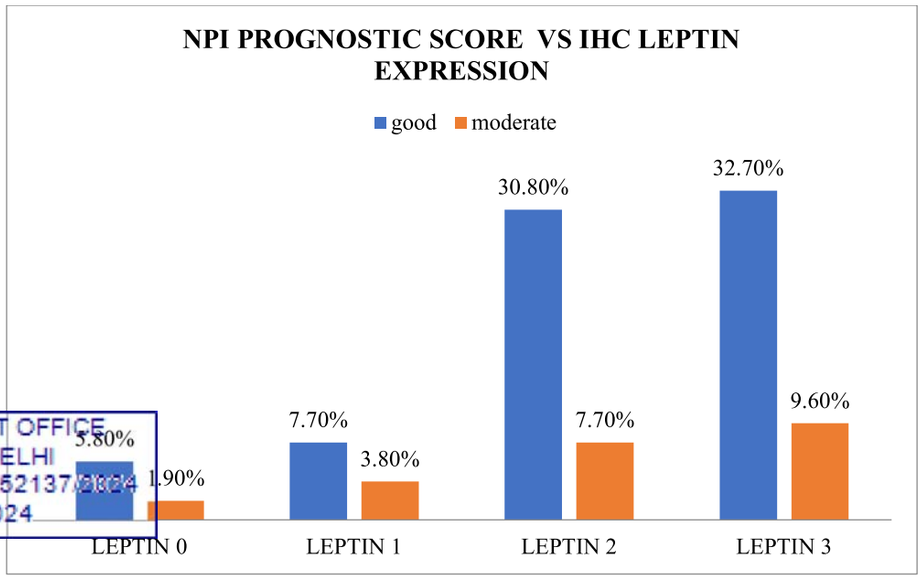
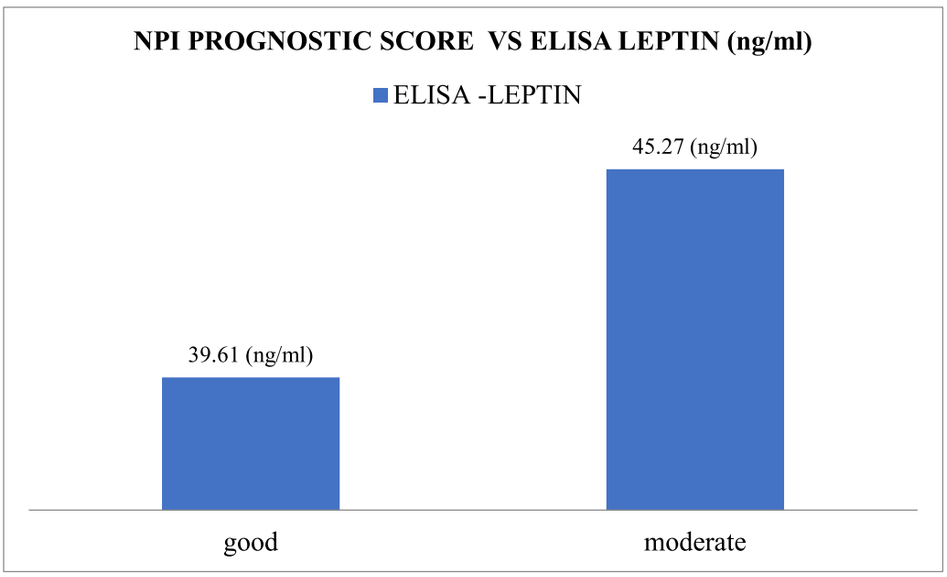


Chart 23: NPI Prognostic score vs Elisa leptin



In present study, predominance of the study population (76.9%) were having good prognosis according to NPI scoring system with IHC Leptin scores 2&3 seen predominantly among them Elisa Leptin was high among moderate prognosis study population according to NPI g system. But the difference between the groups was not found to be significant.

Table 28: ER expression vs IHC leptin & Elisa leptin

ER EXPRESSION	IHC LEPTIN EXPRESSION								ELISA-LEPTIN (ng/ml)	
	0		1		2		3		Mean	SD
	N	%	N	%	N	%	N	%		
Negative	4	7.7%	2	3.8%	10	19.2%	13	25.0%	34.80	16.66
Positive	0	0.0%	4	7.7%	10	19.2%	9	17.3%	48.64	21.61
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%	40.92	20.05
P VALUE	0.1								0.01	

Chart 24: ER expression vs IHC leptin

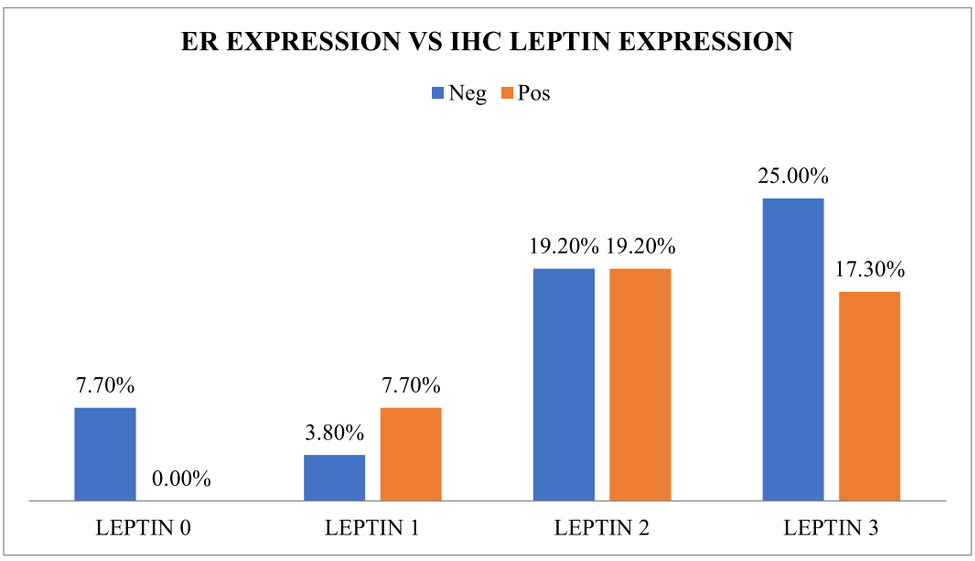
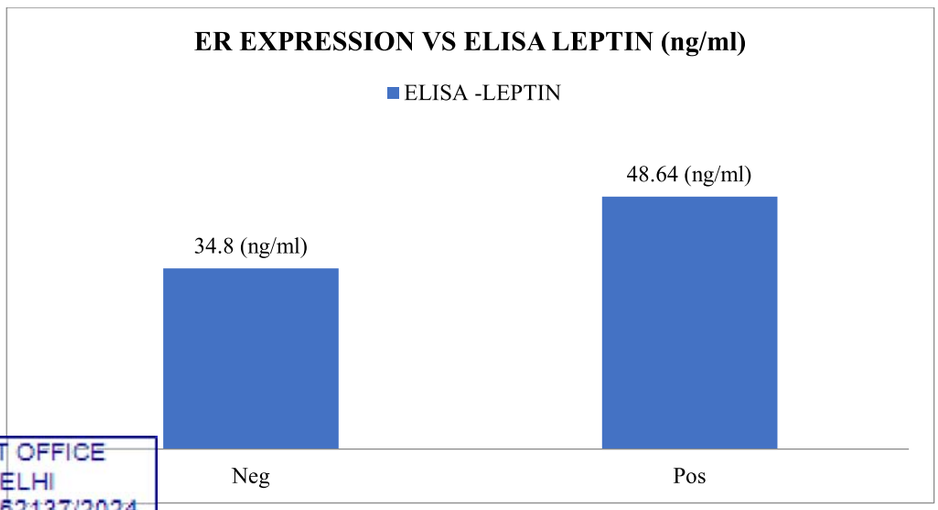


Chart 25: ER expression vs Elisa leptin



In present study, predominant of the study population (55.7%) were showing negative ER expression and among those, IHC scoring 2&3 is seen predominantly and the results obtained were not statistically significant. Elisa Leptin was high among cases showing positive ER expression and the difference between the groups was found to be significant. But the difference between the groups was not found to be significant.

Table 29: ER expression vs IHC leptin expression

		IHC LEPTIN				p value
		positive		negative		
		Count	Table N %	Count	Table N %	
ER	Neg	25	48.1%	4	7.7%	0.06
	Pos	23	44.2%	0	0.0%	

In this study, majority of the population (48.1%) are showing positive expression for immunohistochemistry leptin are showing negative immunohistochemical staining for estrogen receptor. And all the cases (44.2%) which are showing positive expression for ;en receptor immunohistochemistry are showing positive leptin immunohistochemical ssion also. The values obtained were not statistically significant.

Table 30: ER expression vs Elisa leptin

ER EXPRESSION	ELISA-LEPTIN (ng/ml)	
	Mean	SD
Negative	34.80	16.66
Positive	48.64	21.61
Total	40.92	20.05
P VALUE	0.01	

In this study, the study population showing positive immunohistochemical expression for estrogen receptor were showing average Elisa leptin values of 48.64±20.05 ng/ml and the study population showing negative immunohistochemical expression for estrogen receptor were showing average Elisa leptin values of 34.80±16.66 ng/ml and the values were statistically significant.

Table 31: PR expression vs IHC leptin & Elisa leptin

PR EXPRESSION	IHC LEPTIN EXPRESSION								ELISA - LEPTIN (ng/ml)	
	0		1		2		3		Mean	SD
	N	%	N	%	N	%	N	%		
Negative	4	7.7%	2	3.8%	10	19.2%	15	28.8%	34.71	16.42
Positive	0	0.0%	4	7.7%	10	19.2%	7	13.5%	50.08	21.75
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%	40.92	20.05
P VALUE	0.1								0.005	

Chart 26: PR expression vs IHC leptin

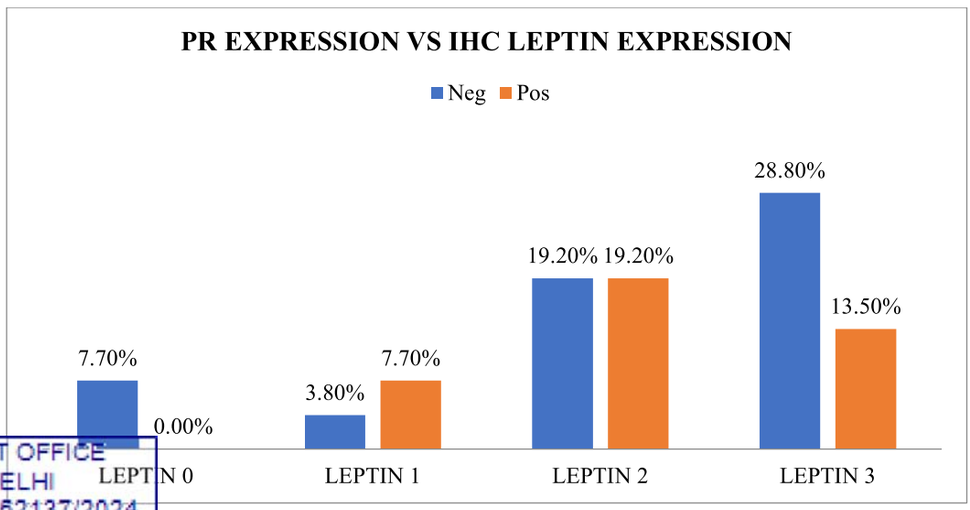
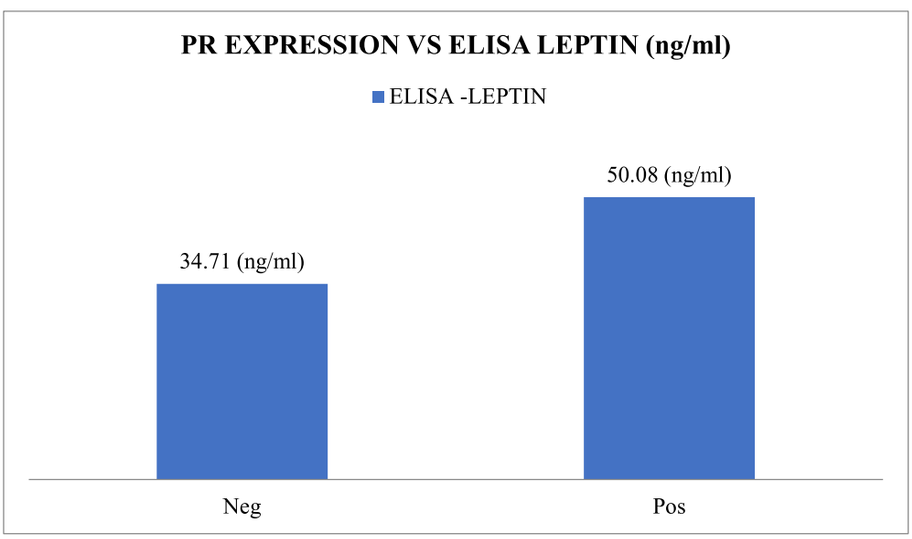


Chart 27: PR expression vs Elisa leptin



In this study, most of the study population (59.6%) were showing negative PR expression with IHC Leptin 2&3 scores predominantly among them and the results obtained were not statistically significant. Elisa Leptin was high among cases with positive PR expression and sults were found to be statistically significant.

Table 32: PR expression vs IHC leptin expression

		IHC LEPTIN				p value
		positive		negative		
		Count	Table N %	Count	Table N %	
PR	Neg	27	51.9%	4	7.7%	0.8
	Pos	21	40.4%	0	0.0%	

In the present study, majority of the population (51.9%) are showing positive expression for immunohistochemistry leptin are showing negative immunohistochemical staining for progesterone receptor. And all the cases (40.4%) which are showing positive expression for progesterone receptor immunohistochemistry are showing positive leptin immunohistochemical expression also. The values obtained were not statistically significant.

Table 33: PR expression vs Elisa leptin

PR EXPRESSION	ELISA-LEPTIN (ng/ml)	
	Mean	SD
Negative	34.71	16.42
Positive	50.08	21.75
Total	40.92	20.05
P VALUE	0.005	

In this study, the study population showing positive immunohistochemical expression for progesterone receptor were showing average Elisa leptin values of 50.08±21.75 ng/ml and the population showing negative immunohistochemical expression for progesterone or were showing average Elisa leptin values of 34.71±16.42 ng/ml and the values were ically significant.

Table 34: HER2 Neu expression vs IHC leptin & Elisa leptin

HER2 NEU EXPRESSION	IHC LEPTIN EXPRESSION								ELISA - LEPTIN (ng/ml)	
	0		1		2		3		Mean	SD
	N	%	N	%	N	%	N	%		
Negative	2	3.8%	4	7.7%	15	28.8%	16	30.8%	40.59	18.73
Positive	2	3.8%	2	3.8%	5	9.6%	6	11.5%	41.73	23.69
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%	40.92	20.05
p value	0.7								0.8	

Chart 28: HER2 Neu expression vs IHC leptin

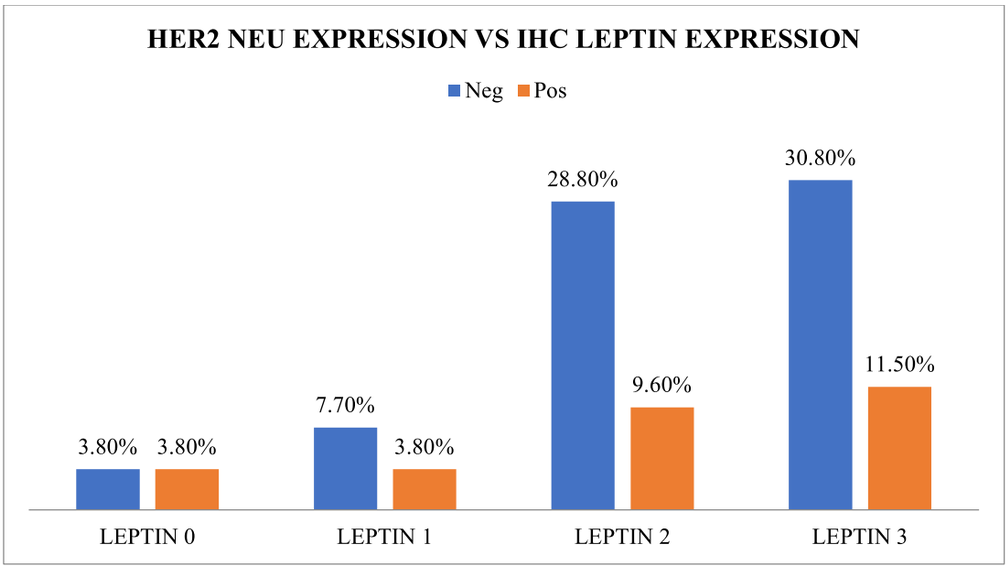
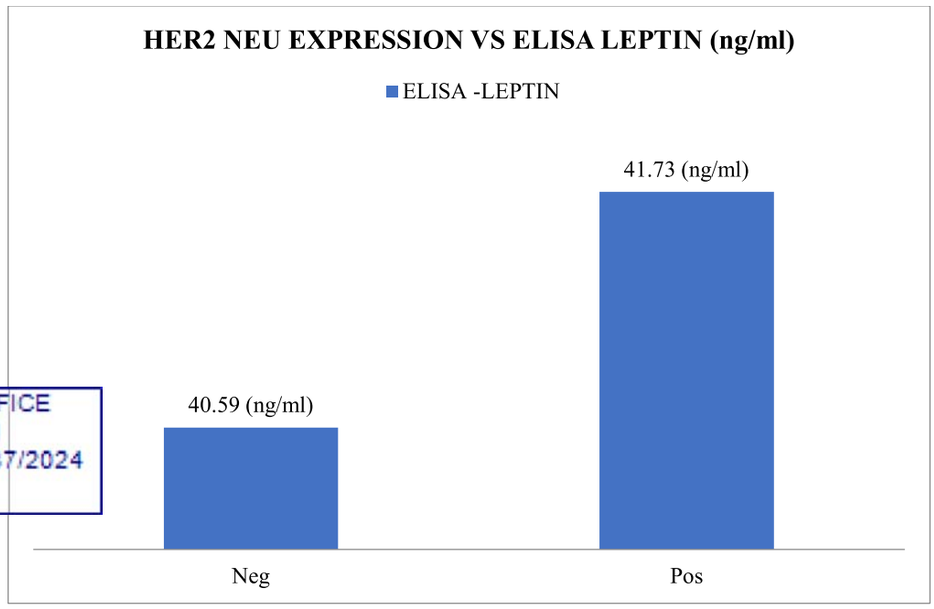


Chart 29: HER2 Neu expression vs Elisa leptin



In this study, most of the study population (71.1%) were showing negative HER2 NEU expression with IHC Leptin 2&3 score predominance among them. Elisa Leptin was high among cases showing positive HER2 NEU expression. But the difference between the groups was not found to be significant.

Table 35: HER2 Neu expression vs IHC leptin expression

		IHC LEPTIN				p value
		positive		negative		
		Count	Table N %	Count	Table N %	
HER2 NEU	Neg	35	67.3%	2	7.7%	0.3
	Pos	13	25.0%	2	0.0%	

In this study, majority of the population (67.3%) are showing positive expression for immunohistochemistry leptin are showing negative immunohistochemical staining for HER2 Neu receptor. And all the cases (25%) which are showing positive expression for HER2 Neu receptor immunohistochemistry are showing positive leptin immunohistochemical expression The values obtained were not statistically significant.

Table 36: HER2 Neu expression vs Elisa leptin

HER2 NEU EXPRESSION	ELISA -LEPTIN (ng/ml)	
	Mean	SD
Negative	40.59	18.73
Positive	41.73	23.69
Total	40.92	20.05
p value	0.8	

In this study, the study population showing positive immunohistochemical expression for HER2 Neu receptor were showing average Elisa leptin values of 40.59±18.73 ng/ml and the study population showing negative immunohistochemical expression for HER2 Neu receptor were showing average Elisa leptin values of 41.73±23.69 ng/ml and the values were not statistically significant.

Table 37: Ki67 expression vs IHC leptin & Elisa leptin

Ki67 expression	IHC LEPTIN EXPRESSION								ELISA-LEPTIN (ng/ml)	
	0		1		2		3		Mean	SD
	N	%	N	%	N	%	N	%		
<14%	0	0.0%	3	5.8%	10	19.2%	9	17.3%	44.43	19.69
>14%	4	7.7%	3	5.8%	10	19.2%	13	25.0%	38.34	20.25
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%	40.92	20.05
p-value	0.3								0.2	

Chart 30: Ki67 expression vs IHC leptin

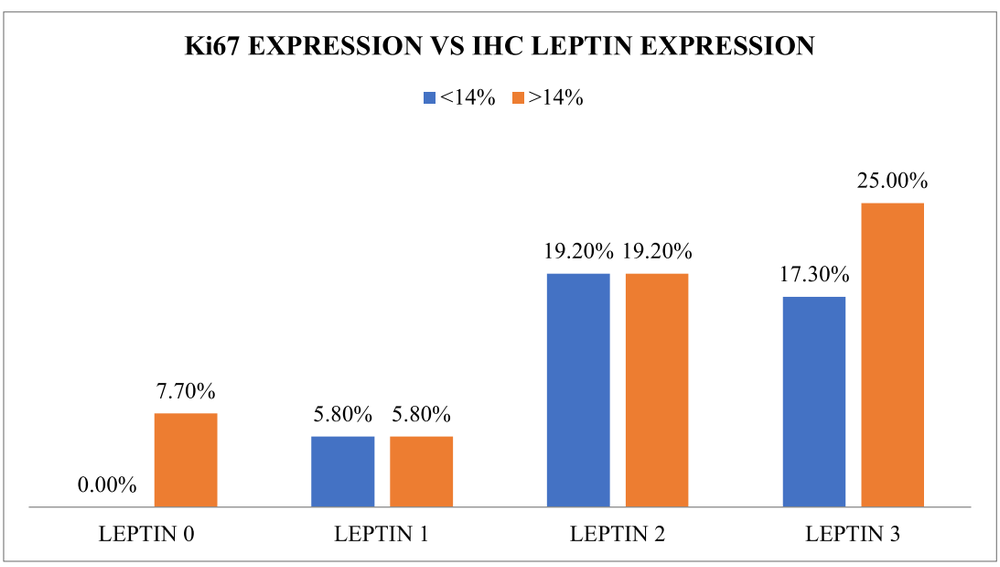
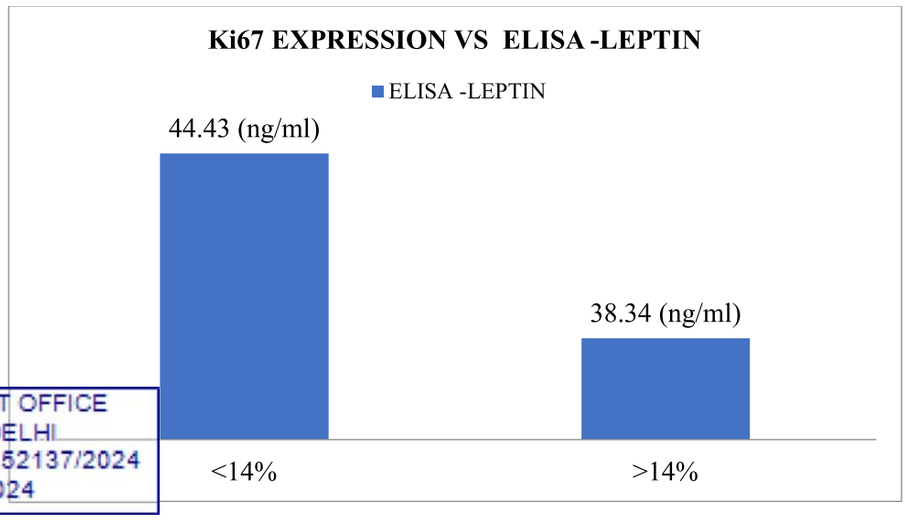


Chart 31: Ki67 expression vs Elisa leptin



In present study, majority of the study population (57.6%) were showing >14% KI67 expression with IHC Leptin 2&3 scores predominance among them. Elisa Leptin was high among cases showing <14% KI67 expression. But the difference between the groups was not found to be significant.

Table 38: Ki67 expression vs IHC leptin expression

		IHC LEPTIN				p value
		positive		negative		
		Count	Table N %	Count	Table N %	
KI67	<14%	22	42.3%	0	0.0%	0.7
	>14%	26	50.0%	4	7.7%	

In this study, most of the population (50%) were showing positive expression for immunohistochemistry leptin are showing>14% immunohistochemical staining for ki67. And all the cases (42.3%) were showing positive expression for leptin immunohistochemistry are ng <14% immunohistochemical expression of Ki67. The values obtained were not ically significant.

Table 39: Ki67 expression vs Elisa leptin

Ki67 expression	ELISA-LEPTIN (ng/ml)	
	Mean	SD
<14%	44.43	19.69
>14%	38.34	20.25
Total	40.92	20.05
p value	0.2	

In this study, the study population showing <14% immunohistochemical expression for Ki67 were showing average Elisa leptin values of 44.43±19.69 ng/ml and the study population showing >14% immunohistochemical expression for Ki67 were showing average Elisa leptin values of 38.34±20.25 ng/ml and the values were not statistically significant.

Table 40: IHC leptin in relation to molecular classification of breast

Molecular Classification	Total No of cases (%)	IHC LEPTIN SCORE 0	IHC LEPTIN SCORE 1	IHC LEPTIN SCORE 2	IHC LEPTIN SCORE 3	P Value
LUMINAL – A	9 (17.3%)	-	1	5	3	0.558
LUMINAL – B	14 (26.9%)	-	3	5	6	
HER2 ENRICHED	9 (17.3%)	2	1	3	3	
TRIPLE NEGATIVE	20 (38.4%)	2	1	7	10	

In this study, predominant number of cases constituting 38.4% were under triple negative
ory with IHC leptin score 3 preponderances among them, and the results were not
ically significant.

Table 41: Elisa leptin in relation to molecular classification of breast

Elisa leptin				
Molecular classification	N (%)	Mean	SD	P VALUE
HER2	9 (17.3%)	33.6	19.5	0.1
Luminal A	9 (17.3%)	48.3	22.7	
Luminal B	14 (26.9%)	48.8	21.7	
TRIPLE NEGATIVE	20 (38.4%)	35.3	15.8	
Total	52	40.9	20.1	

In the study, predominant number of subjects (38.4%) belong to triple negative category were showing the average Elisa leptin levels of 35.3±15.8 ng/ml, followed by luminal B (26.9%) showing average leptin values of 48.8±21.7 ng/ml, luminal A (17.3%) and Her 2 enriched (17.3%) showing average leptin levels of 48.3±22.7 ng/ml and 33.6±19.5 ng/ml respectively. But the obtained values were not statistically significant.

DISCUSSION:

The most crucial marker in relationship between breast cancer & obesity is leptin, which encourages the beginning, development, growth, and spread of tumours.[12]Through its interactions with some other molecules for signaling such Notch, growth factors, ER and inflammatory factors, leptin increases the risk of breast cancer.[13]

Many studies were done in which, the roles of plasma leptin and immunohistochemistry leptin in occurrence & prognosis of cancer of the breast were monitored separately. On each of the elements in the pathogenesis of invasive breast cancer, only few research have shed some insight.

Therefore, the current study's objective is to ascertain whether there is any association between the plasma ELISA Leptin levels of BC patients and the immunohistochemical expression of leptin in tissue sections.

The human body may transport leptin, a particular type of hormone, to take part in a number of physiological and biochemical activities. Previous research (**Wallace AM et al.[85]** , **Polyzos SA et al.[86])** has shown that human disorders such as cardiovascular disorders and fatty liver disease (non-alcoholic) typically develop in conjunction with elevated leptin expression levels.

Angiogenesis, reproduction, the immune system, energy balance, hunger regulation, and bone growth are all impacted by the pleiotropic molecule leptin. The proliferation of other cell types, including breast cells, is also impacted by leptin.[87,88]

AGE DISTRIBUTION:

The minimum age of presentation of the patients in study is 35years, the maximum age of presentation is 72years. The mean age of presentation in the present study is 56.29±9.03. Similarly, in a study done by Tayel S.I et al[89], the mean age group of presentation is 48.55±10.96 years. Another study done by Lee JS et al [90] and Atalay Karacay I et al[73] the mean age of presentation is 49.8±10.2 and 55 ± 12.6 respectively.

Table 42: Age distribution of the present study in comparison with other study

Study	Year	Mean±SD
Present study	2022	56.29±9.03
Tayel S.I et al[89]	2020	48.55±10.96
Lee JS et al [90]	2019	49.8±10.2
Atalay Karacay I et al[73]	2022	55 ± 12.6

Majority of the breast carcinoma study population 42.3% in present study were in 50 to 59 years age group. Population-based **cancer registry data[91]** from Delhi were utilised to explain the trend & epidemiology in breast cancer incidence in Delhi which supports present study.[91]

Breast cancer (BC) primarily affects middle-aged and older women, according to the **American Cancer Society[92]**. The typical age at breast cancer diagnosis is 62 years old. The average age at which breast cancer in women is found is therefore 62 years of age or less. Breast cancer diagnoses in women under 45 are incredibly uncommon.[91]

BMI OF THE POPULATION:

In present study, the minimum value of BMI is 17 (Kg/m2) and the maximum being 26 (Kg/m2). The mean BMI value is 19.96±2.12. In the study done by **Tayel S.I et al[89],** the values are seen significantly elevated with BMI of 28.63±3.87 (Kg/m2). The reasons for significant elevation in BMI levels could be due to geographic distribution as the study population in that study belongs to Egypt and along with that the lifestyle changes and diet practices play an important role. Another study done by **Lee JS et al [90] t**he mean BMI value is 23.5±9.4 in the study population. This study was done on Korean population and the lifestyle changes, high fatty diet could have contributed to elevated BMI levels in this population.

Table 43: BMI of the population in the present study in comparison with other study

Study	Year	Mean±SD
Present study	2022	19.96±2.12
Tayel S.I et al[89]	2020	28.63±3.87
Lee JS et al [90]	2019	23.5±9.4

In the present study, maximum number of study population comprising about 65.3% are under normal BMI category. The reason of maximum number of cases falling under normal BMI could be due to poor socioeconomic background, lack of knowledge, improper diet habits among low socioeconomic group as many of them are from semirural background in our study.

MENOPAUSAL STATUS:

Among the population included in this study, we made the population into two groups of premenopausal & postmenopausal, majority of the study population constituting 75% belong to postmenopausal group. Similar results are noted in the study done by **Tayel S.I et al[89]** , where majority of the patients belong to post-menopausal category constituting 57.5% of the study subjects.

Table 44: Postmenopausal and Premenopausal status in this study in comparison with other study

Study	Year	% Of post-menopausal women	% Of pre-menopausal women
Present study	2022	75%	25%
Tayel S.I et al[89]	2020	57.5%	42.5%

In the present study, only 25% of women are premenopausal and similarly, less number of :ts comprising 42.5% belong to premenopausal category who had developed breast r in study done by Tayel S.I et al[89].

The incidence of several cancers, including breast cancer, does, however, rise with advancing age. On the other hand, Breast cancer and endometrial cancer risk in women who enter menopause after age 55 is increased may be because of more exposure to higher estrogen.[93] Similarly in this study, the high incidence of cancer of the brest among postmenopausal women could have been due to high estrogen exposure.

PARITY:

In our present study, among the population studied, majority are multiparous women comprising 94.2%. Similar results are noted in the study done by **Tayel S.I et al[89]**, where majority of the patients belong to multiparous category comprising 95%. In the study done by **Lee JS et al [90]**, the maximum number of subjects are in multiparous category comprising 70.7% of the entire study population. The increase in the incidence among the subjects can be related to increased stress, improper lifestyle, and late pregnancy in the mothers.[89,90]

Table 45: Parity of study population in this study in comparison with other studies

Study	Year	% Of multiparous women
Present study	2022	94.2%
Tayel S.I et al[89]	2020	95%
Lee JS et al [90]	2019	70.7%

LEPTIN – IMMUNOHISTOCHEMISTRY IN TISSUE SECTIONS:

Table 46: Immunohistochemistry of leptin in tissue sections in present study in comparison with other studies

Study	Year	Score 0 (Negative staining)	Score 1 (Weak staining)	Score 2 (Strong staining)	Score 3 (Strong staining)	Total positivity
Present study	2022	7.69%	11.5%	38.4%	42.3%	92.3%
Khabaz MN et al[1]	2017	16.3%	61%	22.7%		83.7%
Ishikawa.M et al[84]	2004	-	7.89%	92.1%		100%
Atalay Karacay I et al[73]	2022	24.4%	49.3%	26.3%		75.6%

In the present study done on 52 subjects, 92.3% of population show positive leptin expression on immunohistochemistry. Among them, 7.6% show no leptin positivity, 11.5% shows leptin positivity of score 1, 38.4% shows leptin positivity of score 2, 42.3% shows leptin positivity of score 3. Similar results were obtained in other studies which are in concordance with the present study, done by Khabaz MN et al[1], Ishikawa.M et al[84]and Atalay Karacay I et al[73] where the positive expression for leptin is seen in 83.7%, 100% and 75.6% respectively. This suggests that the adipokine marker leptin shows a major part in tumorigenesis and progression of the tumor in breast carcinoma cases.

Leptin is over expressed in the majority of BC patients, and studies have shown that it plays a role in carcinogenesis and the development of BC.[94-97]

LEPTIN – ELISA IN PLASMA SAMPLES:

Table 47: Elisa – leptin levels in plasma levels in present study in comparison with other study

Study	Year	Mean±SD (Elisa – ng/ml)
Present study	2022	40.92±20.05
Tayel S.I et al[89]	2020	19.81±8.91

In the present study done on 52 subjects, the minimum value of Elisa leptin concentration is 13.2 ng/ml, maximum value is 79.54 ng/ml, range being 13.2 – 79.54 with the average of 40.92±20.05 ng/ml. In the study done by Tayel S.I et al[89], the average of plasma Elisa leptin concentration is 19.81±8.91. In our study, cutoff values for plasma Elisa leptin levels couldn't be derived as we didn't have control group. In the study done by Tayel S.I et al[89] the mean values of controls was given as 7.30±2.58 ng/ml. Leptin values were depended upon various parameters such as BMI, family history, lifestyle habits etc. The average values of leptin are elevated in the study group when we compare to the study done by Tayel S.I et al[89], the reason for this variance could be due to diet variations, lack of physical activity, family history, and also due to different kit (manufacturer) which was used to measure plasma leptin levels in the study subjects.

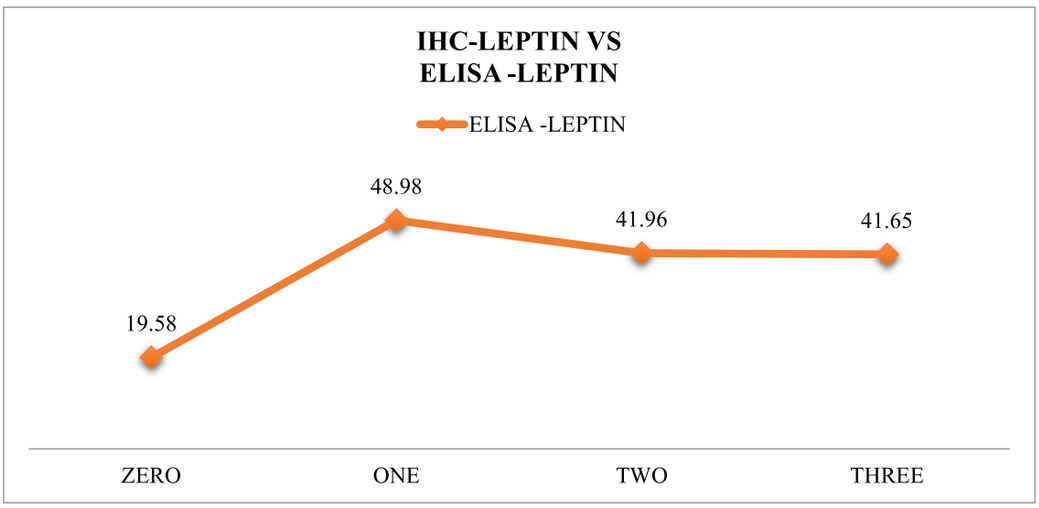
Surprisingly, no correlation between blood leptin levels and BC development has been seen in other investigations like **GU F et al.[98]** and **Aliustaoglu M et al.[99]**

CORRELATION &ASSOCIATION BETWEEN ELISA LEPTIN & IHC LEPTIN:

Table 48: IHC-leptin vs Elisa -leptin association

IHC-LEPTIN EXPRESSION	ELISA -LEPTIN (ng/ml)		
	N	Mean	Std. Deviation
0	4	19.58	6.83
1	6	48.98	18.93
2	20	41.96	20.10
3	22	41.65	20.30
Total	52	40.92	20.05
P VALUE	0.1		

Chart 32: IHC leptin vs Elisa leptin association

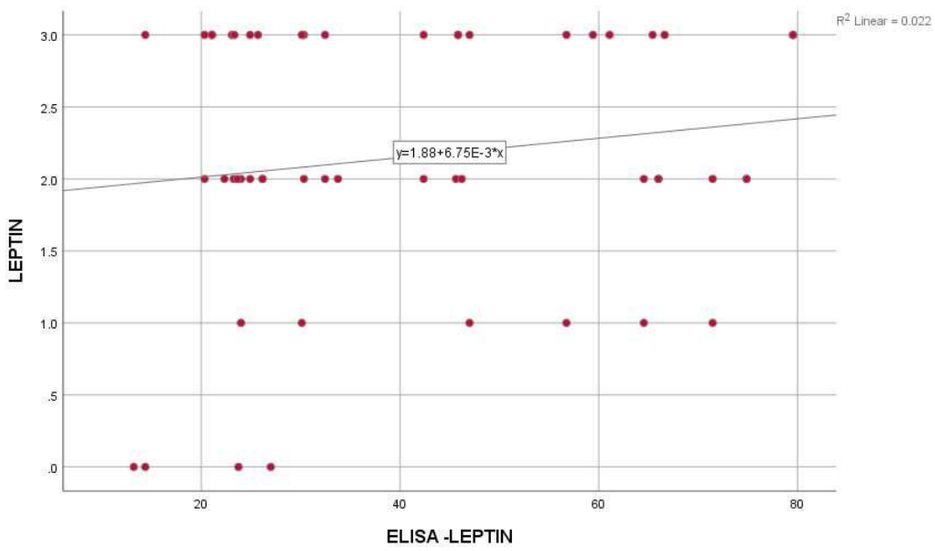


ELISA –Leptin mean levels with IHC- Leptin level zero had low and high at level one. But the difference between the means was not found to be significant.

Table 49: IHC leptin vs Elisa leptin correlation

Correlations			
		LEPTIN	ELISA -LEPTIN
LEPTIN	Pearson Correlation	1	0.148
	Sig. (2-tailed)		0.296
	N	52	52
ELISA -LEPTIN	Pearson Correlation	0.148	1
	Sig. (2-tailed)	0.296	
	N	52	52

Graph 3: IHC leptin vs Elisa leptin correlation in the present study



Correlation of ELISA –Leptin with IHC- Leptin levels were found to be non-significant.

This study shows that the ELISA and IHC are to be taken as independent parameters in carcinoma breast cases and no association or correlation can be found in between these two entities. As of now, there is no published data comparing the association/correlation of leptin s in plasma and leptin expression in immunohistochemistry in carcinoma breast cases. , much data could not be taken into consideration.

AGE IN RELATION TO LEPTIN:

Table 50: Comparison of IHC leptin expression & age with other study

Study	Year	Age group	Expression of IHC Leptin	P Value
Present study	2022	50-59	High (score3)	0.56
Khabaz MN et al[1]	2017	50-59	High expression	0.023

In the present study, the highest expression of leptin is noted in age group of 50-59 (42.3%), Similarly, in the study done by **Khabaz MN et al[1]** , the population presented in age group of 50-59 showed high expression of leptin with a statistically significant p value.

Table 51: Comparison of Elisa leptin levels and age with other study

Study	Year	Mean age	P value
Present study	2022	56.29±9.03	0.59
Tayel S.I et al[89]	2020	48.55±10.96	0.975

The average age of patients in the present study is 56.29±9.03 with p value of 0.59. The maximum number of study population (42.3%) belong to 50-59 years age group, and the results are not statistically significant. Elisa leptin was highest among 50 to 59 years age group with average concentration of 46.01±19.64. Similarly in the study done by **Tayel S.I et al[89]** shows mean age group of presentation of 48.55±10.96 and the results were not statistically significant. The reason for majority of the subjects being in that age group could have been due to the hormonal changes which begin to happen in the peri/post-menopausal age group, stress related factors, lack of physical activity, neglecting food habits.[89]

BMI IN REALTION TO LEPTIN:

Table 52: IHC leptin expression in relation to BMI status

BMI	IHC LEPTIN EXPRESSION							
	0		1		2		3	
	N	%	N	%	N	%	N	%
normal	3	5.8%	2	3.8%	13	25.0%	16	30.8%
pre obese	0	0.0%	1	1.9%	2	3.8%	1	1.9%
underweight	1	1.9%	3	5.8%	5	9.6%	5	9.6%
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%
P VALUE	0.69							

In this study, most of the study population (65.4%) were in Normal BMI level with IHC Leptin 3 score predominance among them. But the results obtained were not statistically significant.

As per our knowledge, no study is available to compare BMI value among patients with immunohistochemistry leptin expression.

Table 53: Comparison of Elisa leptin levels and BMI status with other study

Study	Year	Mean±SD BMI	P Value
Present study	2022	19.96±2.12	0.8
Tayel S.I et al[89]	2020	28.63±3.87	<0.001

The mean BMI values in this study is 19.96±2.12 (Kg/m2). BMI in the present study is divided into underweight, normal and pre obese categories. 65.3% of study population were under normal BMI range in our study. Elisa Leptin was highest in normal BMI patients with verage concentration of 42.14±20.87. Also the plasma leptin values were not seen ically significant. In a study done by **Tayel S.I et al[89]** the mean BMI value among

breast cancer patients was 28.63±3.87 (Kg/m2) and the results are statistically significant, when compared with leptin values.

MENOPAUSAL STATUS IN RELATION TO LEPTIN:

Table 54: IHC leptin expression in relation to menopausal status

MENOPAUSAL STATUS	IHC LEPTIN EXPRESSION							
	0		1		2		3	
	N	%	N	%	N	%	N	%
PRE	0	0.0%	2	3.8%	7	13.5%	4	7.7%
POST	4	7.7%	4	7.7%	13	25.0%	18	34.6%
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%
P VALUE	0.36							

In this study, most of the study subjects (75%) were belonging to post-menopausal group with IHC Leptin 3 score predominance among them. But the results were not statistically significant.

As per our knowledge, no study is available to compare menopausal status among patients with immunohistochemistry leptin expression.

Table 55: Comparison of Elisa leptin levels & postmenopausal status with other study

Study	Year	% Of post menopausal women	P Value
Present study	2022	75%	0.4
Tayel S.I et al[89]	2020	57.5%	0.8

Table 56: Comparison of Elisa leptin levels & premenopausal status with other study

Study	Year	% Of pre-menopausal women	P Value
Present study	2022	25%	0.4
Tayel S.I et al[89]	2020	42.5%	0.8

In our study, we have divided the study population into premenopausal and post menopausal category, majority of the women (75%) belong to postmenopausal state and when evaluated for leptin values in the plasma, it is noted that higher values of plasma leptin levels are seen in plasma of premenopausal women with mean value of 44.5±22.68, however the results are not statistically significant. In comparison with the study done by **Tayel S.I et al[89]**, in which maximum population who is showing high leptin values in plasma belong to postmenopausal category and the results are in concordance with our study.

In the present study, only 25% of study population are under pre-menopausal category showing no statistical significance. Similar results are seen in study done by Tayel S.I et al[89]

Harris HR et al.[100] & Hu X et al.[101] studies have shown a negative corelatable status between the levels of the leptin in the blood & the incidence of BC in premenopausal women.

However, **Assiri AM et al.[102]** discovered a favorable link in the post-menopausal women but a negative correlation in pre-menopausal women between cancer of breast development and serum levels of the leptin.

PARITY IN RELATION TO LEPTIN:

Table 57: IHC leptin expression in relation to parity

PARITY	IHC LEPTIN EXPRESSION							
	0		1		2		3	
	N	%	N	%	N	%	N	%
MULTIPARA	4	7.7%	6	11.5%	18	34.6%	21	40.4%
PRIMIPARA	0	0.0%	0	0.0%	2	3.8%	1	1.9%
TOTAL	4	7.7%	6	11.5%	20	38.5%	22	42.3%
P VALUE	0.2							

In this study, most of the study population (94.2%) were multiparous women with IHC Leptin 3 score predominance among them. But the results obtained were not statistically significant.

As per our knowledge, no study is available to compare parity among patients with immunohistochemistry leptin expression.

Table 58: Comparison of Elisa leptin levels & parity with other study

Study	Year	% Of multiparous women	P Value
Present study	2022	94.2%	0.04
Tayel S.I et al[89]	2020	95%	1.00

In the present study, we have divided the study population into nulliparous and primiparous/multiparous population, maximum were multiparous women (94.2%) and the leptin levels are seen significantly elevated in primiparous women showing mean concentration of 67.63±3.33 with statistically significant values. In the study done by **Tayel S.I et al[89]**, where majority of the patients belong to multiparous category (95%), the p value ot found to be significant.

In present study parity didn't played much role as there was significant difference was found may due to the small sample size, lifestyle changes, dietary habits, socioeconomic background. Contrary to nulliparous women, parous women had a lower probability of developing ER+ breast cancer though no connection was seen for ER- cases according to **Fortner RT et al.**[103]

pT SIZE IN RELATION TO LEPTIN:

Table 59: Comparison between IHC leptin expression and pT size of tumor with other studies

Study	Year	pT (size)	Leptin Expression	P Value
Present study	2022	pT2	High expression (Score3)	0.04
Khabaz MN et al[1]	2017	pT2	High expression	0.57
Ishikawa.M et al[84]	2004	pT2	Strong expression	0.2

In our present study, maximum number of study population (67.3%) belong to pT2 category and are showing high leptin expression with scores of 2 & 3. The values in our study were noted as statistically significant. In other studies done by Khabaz MN et al[1] and Ishikawa.M et al[84], where we noted similar finding of the highest number of subjects belonging to pT2 category, but when compared along with IHC leptin expression, the values are not statistically significant.

Table 60: Elisa leptin levels in relation to pT size of tumor

TUMOR SIZE	ELISA -LEPTIN (ng/ml)		
	Mean ± SD		
	N		
T1	1	41.58	21.46
T2	2	39.80	19.89
T3	1	45.86	24.04
T4	0	56.75	23.5
Total	4	40.92	20.05
P VALUE	0.8		

In this study, most of the study subjects (67.3%) were in tumor stage - T2. Elisa leptin was highest tumor staging T4 with an average of 56.75±23.5 ng/ml. The results were statistically significant.

As per our knowledge, no study is available to compare pT size of tumor among breast cancer patients with Elisa leptin values.

pN – NODAL STATUS IN RELATION TO LEPTIN:

Table 61: IHC leptin in relation to nodal status

METASTATIC LYMPH NODES	IHC LEPTIN EXPRESSION							
	0		1		2		3	
	N	%	N	%	N	%	N	%
NO	4	7.7%	6	11.5%	20	38.5%	19	36.5%
POSITIVE	0	0.0%	0	0.0%	0	0.0%	3	5.8%
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%
P VALUE	0.2							

In this study, most of the study population (94.2%) were not having metastatic lymph nodes/ lymph nodes showing tumor deposits with IHC Leptin 2 score predominance among them. The results obtained were not statistically significant.

Table 62: Elisa leptin in relation to nodal status

METASTATIC LYMPH NODES	ELISA -LEPTIN (ng/ml)	
	Mean	SD
NO	41.17	20.32
POSITIVE	36.74	17.66
Total	40.92	20.05
P VALUE	0.7	

In this study, most of the study population (94.2%). Elisa Leptin was high among non-metastatic study population. The results obtained were not statistically significant.

As per our knowledge, no study is available to compare pN nodal status of tumor among breast cancer patients with IHC leptin expression and Elisa leptin values.

TUMOR INFILTRATING LYMPHOCYTES IN RELATION TO LEPTIN:

Table 63: IHC leptin expression in relation to tumor infiltrating lymphocytes

TUMOR INFILTRATING LYMPHOCYTES	IHC LEPTIN EXPRESSION							
	0		1		2		3	
	N	%	N	%	N	%	N	%
NO	1	1.9%	6	11.5%	14	26.9%	14	26.9%
POSITIVE	3	5.8%	0	0.0%	6	11.5%	8	15.4%
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%
P VALUE	0.9							

Table 64: Elisa leptin levels in relation to tumor infiltrating lymphocytes

TUMOR INFILTRATING LYMPHOCYTES	ELISA -LEPTIN (ng/ml)	
	Mean	SD
NO	38.97	18.07
POSITIVE	44.94	23.71
Total	40.92	20.05
P VALUE	0.3	

In this study, most of the study population (67.3%) were not having Tumor Infiltrating hocytes with IHC Leptin 2&3 score predominance among them. Elisa Leptin was high

among positive Tumor Infiltrating Lymphocytes study population. The results obtained for both IHC and Elisa values were not statistically significant.

As per our knowledge, no study is available to compare tumor infiltrating lymphocyte status of tumor among breast cancer patients with IHC leptin expression and Elisa leptin values.

LYMPHOVASCULAR INVASION IN RELATION TO LEPTIN:

Table 65: Comparison of IHC leptin expression & Lymphovascular invasion with other studies

Study	Year	Lymphovascular invasion	Leptin Expression	P Value
Present study	2022	Absent	High expression (Score2 & 3)	0.7
Khabaz MN et al[1]	2017	Absent	High expression	0.4
Ishikawa.M et al[84]	2004	Absent	Strong expression	1
Atalay Karacay I et al[73]	2022	Absent	Positive	0.2

In this study, predominant of the population (98%) showed no lymphovascular invasion and all those cases showed leptin expression scores of 2 & 3 on immunohistochemistry and the values are not statistically significant. In other studies done by Khabaz MN et al[1], Atalay Karacay I et al[73], Ishikawa.M et al[84], they also showed that the results are in concordance with values majority of the subjects showing no lymphovascular invasion and showing statistically not significant.

Table 66: Comparison of Elisa Leptin levels in relation to Lymphovascular invasion

LYMPHOVASCULAR INVASION	ELISA-LEPTIN (ng/ml)	
	Mean	SD
NO	40.56	20.08
POSITIVE	59.42	23.5
Total	40.92	20.05
PVALUE	0.3	

In this study, predominant of the study population (98%) were not having Lymphovascular invasion. Elisa Leptin was high among positive Lymphovascular Invasion study population. The results obtained were not statistically significant.

As per our knowledge, no study is available to compare lymphovascular invasion status of tumor among breast cancer patients with Elisa leptin values.

pTNM STAGING IN RELATION TO LEPTIN:

Table 67: Comparison of IHC Leptin expression & p TNM Staging of tumor with other studies

Study	Year	pTNM stage	Leptin Expression	P Value
Present study	2022	Stage 2	High expression (Score2 & 3)	0.05
Khabaz MN et al[1]	2017	Stage 2	High expression	0.02
Ishikawa.M et al[84]	2004	Stage 2	Strong expression	0.6
Atalay ıcay I et al[73]	2022	Stage 2	Positive	0.4

In the present study, maximum subjects (75%) belonged to stage 2 and showed high expression of leptin (score 2 & 3) and the values are noted as statistically significant. Similar results were obtained in study done by **Khabaz MN et al**[1], where the maximum population belonged to stage 2 and the p value is noted as statistically significant. In other studies done by **Ishikawa.M et al**[84] and **Atalay Karacay I et al**[73], showed similar population showing highest in stage 2 but the p values are not statistically significant.

Table 68: Elisa leptin levels in relation to pTNM staging of tumor

STAGE	ELISA -LEPTIN (ng/ml)	
	Mean	Standard Deviation
I	43.35	21.40
II	39.76	19.93
III	56.75	23.5
Total	40.92	20.05
P VALUE	0.6	

In this study, most of the study patients (75%) were belonging to stage 2. Elisa Leptin was high among stage 3 group of study population. The results obtained were not statistically significant.

As per our knowledge, no study is available to compare pTNM staging among breast cancer patients with Elisa leptin values.

MODIFIED BLOOM RICARDSON GRADING IN RELATION TO LEPTIN:

Table 69: IHC leptin expression in relation to modified bloom richardson grading

MODIFIED BLOOM RICHARDSON GRADING	IHC LEPTIN EXPRESSION							
	0		1		2		3	
	N	%	N	%	N	%	N	%
1	0	0.0%	1	1.9%	6	11.5%	3	5.8%
2	2	3.8%	4	7.7%	9	17.3%	9	17.3%
3	2	3.8%	1	1.9%	5	9.6%	10	19.2%
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%
P VALUE	0.5							

Table 70: Elisa leptin levels in relation to modified bloom richardson grading

MODIFIED BLOOM RICHARDSON GRADING	ELISA - LEPTIN (ng/ml)	
	Mean	SD
1	41.15	19.72
2	40.60	23.05
3	41.21	16.77
Total	40.92	20.05
P VALUE	0.9	

In this study, most of the study subjects (46.1%) were in grade 2 show high leptin expression with scores of 2 and 3. Elisa Leptin was high among population belonging to grade 2 and 3. The results obtained were not statistically significant.

As per our knowledge, no study is available to compare modified bloom Richardson grading among breast cancer patients with IHC leptin expression and Elisa leptin values.

NPI IN RELATION TO LEPTIN:

Table 71: IHC leptin expression in relation to nottingham prognostic index

NPI PROGNOSTIC SCORE	IHC LEPTIN EXPRESSION							
	0		1		2		3	
	N	%	N	%	N	%	N	%
GOOD	3	5.8%	4	7.7%	16	30.8%	17	32.7%
MODERATE	1	1.9%	2	3.8%	4	7.7%	5	9.6%
Total	4	7.7%	6	11.5%	20	38.5%	22	42.3%
P VALUE	0.9							

Table 72: Elisa leptin levels in relation to nottingham prognostic index

NPI PROGNOSTIC SCORE	ELISA -LEPTIN (ng/ml)	
	Mean	SD
GOOD	39.61	18.99
MODERATE	45.27	23.63
Total	40.92	20.05
P VALUE	0.3	

In this study, most of the study population were having good prognosis according to NPI scoring system with IHC Leptin scores 2&3 seen predominantly among them. Elisa Leptin was high among moderate prognosis study population according to NPI scoring system. The results obtained were not statistically significant.

As per our knowledge, no study is available to compare Nottingham prognostic index among cancer patients with IHC leptin expression and Elisa leptin values.

ER EXPRESSION IN RELATION TO LEPTIN:

Table 73: Comparison of IHC leptin expression & ER expression with other studies

Study	Year	ER Expression	ER Expression	P Value
Present study	2022	Negative	Positive	0.1
Khabaz MN et al[1]	2017	Negative	Positive	0.02
Ishikawa M et al[84]	2004	Negative	Positive	0.7
Atalay Karacay I et al[73]	2022	Negative	Positive	<0.01

In the present study, maximum number of study population showed ER negative expression comprising 55.7%, on the contrary to the studies done by Khabaz MN et al[1], Atalay Karacay I et al[73], Ishikawa.M et al[84] where the highest number of subjects were showing ER positivity.

The ER negative subjects in our study, showed strong IHC leptin expression with scores of 2&3, similar kind of results were noted in studies done by Khabaz MN et al[1], Atalay Karacay I et al[73], Ishikawa.M et al[84] but the values are not statistically significant.

In the present study, majority of the subjects with positive ER expression showed leptin expression positive scoring of 2 and 3, but the values were not statistically significant.

Table 74: Comparison of Elisa leptin & ER expression with other studies

ER EXPRESSION	ELISA-LEPTIN (ng/ml)	
	Mean	SD
Negative	34.80	16.66
Positive	48.64	21.61
Total	40.92	20.05
P VALUE	0.01	

In this study the study population showing positive immunohistochemical expression for estrogen receptor were showing average Elisa leptin values of 48.64±20.05 ng/ml and the study population showing negative immunohistochemical expression for estrogen receptor were showing average Elisa leptin values of 34.80±16.66 ng/ml and the values were statistically significant.

As per our knowledge, no study is available to compare ER expression among breast cancer patients with Elisa leptin values.

PR EXPRESSION IN RELATION TO LEPTIN:

Table 75: Comparison of IHC leptin expression & PR expression with other studies

Study	Year	PR Expression	PR Expression	P Value
Present study	2022	Negative	Positive	0.1
Khabaz MN et al[1]	2017	Negative	Positive	0.44
Ishikawa.M et al[84]	2004	Negative	Positive	0.96
y Karacay I et al[73]	2022	Negative	Positive	<0.01

In this study, maximum number of study population showed PR negative expression comprising 59.6%, on the contrary to the studies done by Khabaz MN et al[1], Atalay Karacay I et al[73], Ishikawa.M et al[84] where the maximum subjects were showed PR positivity.

The PR negative subjects in our study, showed strong leptin expression with scores of 2&3, similar kind of results were noted in studies done by Khabaz MN et al[1], Atalay Karacay I et al[73], Ishikawa.M et al[84] but the values are not statistically significant.

In the present study, majority of the subjects with positive PR expression (32.7%) shows leptin expression positive scoring of 2 and 3, but the values are not statistically significant.

Table 76: Comparison of Elisa leptin & PR expression with other studies

PR EXPRESSION	ELISA-LEPTIN (ng/ml)	
	Mean	SD
Negative	34.71	16.42
Positive	50.08	21.75
Total	40.92	20.05
P VALUE	0.005	

In this study, the study subjects showing positive immunohistochemical expression for progesterone receptor were showing average Elisa leptin values of 50.08±21.75 ng/ml and the study population showing negative immunohistochemical expression for progesterone receptor were showing average Elisa leptin values of 34.71±16.42 ng/ml and the values were statistically significant.

As per our knowledge, no study is available to compare PR expression among the cases of breast cancer patients with Elisa leptin values.

HER 2 NEU EXPRESSION IN RELATION TO LEPTIN:

Table 77: Her 2 neu expression in the relation to the IHC leptin expression

		IHC LEPTIN				p value
		positive		negative		
		Count	Table N %	Count	Table N %	
HER2 NEU	Neg	35	67.3%	2	7.7%	0.3
	Pos	13	25.0%	2	0.0%	

In this study, most of the population (67.3%) are showing positive expression for Immunohistochemistry leptin are showing negative immunohistochemical staining for HER2 Neu receptor. And all the cases (25%) which are showing positive expression for HER2 Neu receptor immunohistochemistry are showing positive leptin immunohistochemical expression also. The values obtained were not statistically significant.

Table 78: Her 2 neu expression in relation to Elisa leptin

HER2 NEU EXPRESSION	ELISA -LEPTIN (ng/ml)	
	Mean	SD
Negative	40.59	18.73
Positive	41.73	23.69
Total	40.92	20.05
p value	0.8	

In this study, the study population showing positive immunohistochemical expression for HER2 Neu receptor were showing average Elisa leptin values of 40.59±18.73 ng/ml and the study population showing negative immunohistochemical expression for HER2 Neu receptor were showing average Elisa leptin values of 41.73±23.69 ng/ml and the values were not ically significant.

As per our knowledge, no study is available to compare Her 2 neu expression among breast cancer patients with the IHC leptin expression and Elisa leptin values.

KI67 IN RELATION TO LEPTIN:

Table 79: Ki67 expression in relation to IHC leptin expression

		IHC LEPTIN				p value
		positive		negative		
		Count	Table N %	Count	Table N %	
KI67	<14%	22	42.3%	0	0.0%	0.7
	>14%	26	50.0%	4	7.7%	

In this study, most of the population (50%) are showing positive expression for immunohistochemistry leptin are showing>14% immunohistochemical staining for ki67. And all the cases (42.3%) which are showing positive expression for leptin immunohistochemistry are showing <14% immunohistochemical expression of Ki67. The values obtained were not statistically significant.

Table 80: Ki67 expression in relation to Elisa leptin levels

Ki67 expression	**ELISA-LEPTIN (ng/ml)**	
	Mean	SD
<14%	44.43	19.69
>14%	38.34	20.25
Total	40.92	20.05
p value	0.2	

In this study, the study population showing <14% immunohistochemical expression for Ki67 showing average Elisa leptin values of 44.43±19.69 ng/ml and the study population

showing >14% immunohistochemical expression for Ki67 were showing average Elisa leptin values of 38.34±20.25 ng/ml and the values were not statistically significant.

As per our knowledge, no study is available to compare Ki67expression among breast cancer patients with IHC leptin expression and Elisa leptin values.

MOLECULAR CLASSIFICATION IN RELATION TO LEPTIN:

Table 81: IHC leptin in relation to molecular classification of breast

Molecular Classification	Total No of cases (%)	IHC LEPTIN SCORE 0	IHC LEPTIN SCORE 1	IHC LEPTIN SCORE 2	IHC LEPTIN SCORE 3	P Value
LUMINAL – A	9 (17.3%)	-	1	5	3	0.558
LUMINAL – B	14 (26.9%)	-	3	5	6	
HER2 ENRICHED	9 (17.3%)	2	1	3	3	
TRIPLE NEGATIVE	20 (38.4%)	2	1	7	10	

In this study, maximum number of cases constituting 38.4% are under triple negative category with IHC leptin score 3 preponderance among them, and the results were not statistically significant.

Table 82: Elisa leptin in relation to molecular classification of breast

Elisa leptin				
Molecular classification	N (%)	Mean	SD	P VALUE
HER2	9 (17.3%)	33.6	19.5	0.1
Luminal A	9 (17.3%)	48.3	22.7	
Luminal B	14 (26.9%)	48.8	21.7	
TRIPLE NEGATIVE	20 (38.4%)	35.3	15.8	
Total	52	40.9	20.1	

In the present study, maximum number of subjects (38.4%) belong to triple negative category were showing the average Elisa leptin levels of 35.3±15.8 ng/ml, followed by luminal B (26.9%) showing average leptin values of 48.8±21.7 ng/ml, luminal A (17.3%) and Her 2 enriched (17.3%) showing average leptin levels of 48.3±22.7 ng/ml and 33.6±19.5 ng/ml respectively. But the obtained values were not statistically significant.

As per our knowledge, no study is available to compare molecular classification of breast among breast cancer patients with IHC leptin expression and Elisa leptin values.

CONCLUSION:

Among the study population, 92.3% cases show IHC leptin positivity. Plasma leptin levels were recorded with the mean of 40.92±20.05 ng/ml. Correlation of ELISA –Leptin with IHC-Leptin levels were found to be weak positives and non-significant.

Among the various parameters studied, the immunohistochemistry leptin expression in relation to size of the tumor & stage of the tumor were showing statistically significant value. Elisa leptin levels in relation to parity, estrogen receptor and progesterone receptor were showing statistically significant values.

LIMITATIONS:

1. Small sample size.
2. Unicentric study.
3. No control group for comparison.

SUMMARY:

1. The present study was taken up to see association/correlation between immunohistochemistry leptin expression with plasma elisa leptin levels in invasive ductal carcinoma breast cases.
2. Majority of the study population (42.3%) of breast carcinoma were in the age group of 50 to 59 years.
3. Post-menopausal women predominance (75%) there in present study.
4. Multi para cases were predominant (94.2%) in present study.
5. Normal BMI population was predominant (65.3%).
6. Tumor Infiltrating Lymphocytes was seen in only 32.7% of the population.
7. Lymphovascular Invasion was seen in only one case (1.9%).
8. Metastatic Lymph Nodes were seen in only three cases (5.8%).
9. There were no cases (0%) of distant metastatis.
10. Majority (76.9%) had Good NPI Prognosis.
11. Around 40% of the cases were having ER, PR positive expression.
12. 28.8% of the cases only showed HER2 NEU positive expression.
13. High Ki67 index was seen almost 57.7% of the study population.
14. Among the study population with sample size of 52, 48 (92.3%) cases show IHC leptin positivity.
15. Among 52 cases studied, the maximum IHC leptin expression with score 3 is seen in maximum number of cases constituting 42.3%, and 7.6% of cases show negative IHC leptin expression.
16. In the present study, the plasma leptin levels were recorded as the lowest being 13.21 ng/ml, highest being 79.54 ng/ml with the average of 40.92±20.05 ng/ml.
17. ELISA –Leptin mean levels with IHC- Leptin level zero had low and high at level one. But the difference between the means was not found to be significant.
18. Correlation of ELISA –Leptin with IHC- Leptin levels were found to be non-significant.
19. IHC Leptin expression score 3 was seen predominantly in the population belonging to age group 50-59 (42.3%) years and Elisa leptin levels were also noted highest with the average value of 46.01±19.64 ng/ml, among that age group but the results obtained were not statistically significant.

20. IHC Leptin expression score 3 was seen predominantly in the post-menopausal women (75%) and Elisa leptin levels were noted highest in pre-menopausal women with the average value of 44.50±22.68 ng/ml, but the results obtained were not statistically significant.
21. IHC Leptin expression score 3 was seen predominantly in the multiparous women (94.2%) , the results obtained were not statistically significant and Elisa leptin levels were noted highest with the average value of 67.63±3.33ng/ml, among that primiparous women, and the results obtained were statistical correlation.
22. IHC Leptin expression score 3 was seen predominantly in the population with normal BMI (65.4%) and Elisa leptin levels were noted highest with the average value of 52.14±20.87ng/ml, among the same group, but the results obtained were not statistically significant.
23. Majority of the study population (67.3%) were in the tumor stage - T2 with IHC Leptin 3 score showing predominance among which was statistically significant. Elisa leptin was highest tumor staging T4 and the results were not statistical correlation.
24. Most of the study population (94.2%) were not having metastatic lymph nodes/ lymph nodes showing tumor deposits with IHC Leptin 2 score predominance among them. Elisa Leptin was high among non-metastatic study population. But the results obtained were not statistically correlation.
25. Majority of the study population (67.3%) were not having Tumor Infiltrating Lymphocytes with IHC Leptin 2&3 score predominance among them. Elisa Leptin was high among the study population showing Tumor Infiltrating Lymphocytes. But the results obtained were not statistical correlation.
26. Majority of the study population (98%) were not having Lymphovascular Invasion with IHC Leptin 2&3 score predominance among them. Elisa Leptin was high among positive Lymphovascular Invasion study population. But the results obtained were not statistically correlation.
27. Majority of the study population (75%) were in stage 2 with IHC Leptin 2&3 scores predominance among them and the results obtained were statistically significant. Elisa Leptin was high among stage 3 group of study population and the results were not statistically correlation.

Majority of the study population (46.1%) were in modified blood Richardson - grade 2 show high leptin expression with scores of 2 and 3. Elisa Leptin was high among

population belonging to grade 2 and grade 3. But the results obtained were not statistically correlation.

29. Most of the study population (76.9%) were having good prognosis according to NPI scoring system with IHC Leptin scores 2&3 seen predominantly among them. Elisa Leptin was high among moderate prognosis study population according to NPI scoring system. But the results obtained were not statistically significant.
30. Majority of the study population (55.7%) were showing negative ER expression and among those, IHC scoring 2&3 is seen predominantly and the results obtained were not statistically significant. Elisa Leptin was high among cases showing positive ER expression and the difference between the groups was found to be significant.
31. Most of the study population (59.6%) were showing negative PR expression with IHC Leptin 2&3 scores predominantly among them and the results obtained were not statistically significant. Elisa Leptin was high among cases with positive PR expression and the results were found to be statistically correlation.
32. Most of the study population (71.1%) were showing negative HER2 NEU expression with IHC Leptin 2&3 score predominance among them. Elisa Leptin was high among cases showing positive HER2 NEU expression and the results obtained were not statistically corretation.
33. Most of the study population (57.6%) were showing >14% Ki67 expression with IHC Leptin 2&3 scores predominance among them. Elisa Leptin was high among cases showing <14% Ki67 expression and the results were not statistically significant.
34. Maximum number of cases constituting 38.4% are under triple negative category with IHC leptin score 3 preponderances among them, and the results were not statistically correlation.
35. Maximum number of subjects (38.4%) belong to triple negative category were showing the average Elisa leptin levels of 35.3±15.8 ng/ml, followed by luminal B (26.9%) showing average leptin values of 48.8±21.7 ng/ml, luminal A (17.3%) and Her 2 enriched (17.3%) showing average leptin levels of 48.3±22.7 ng/ml and 33.6±19.5 ng/ml respectively. But the obtained values were not statistically correlation.

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PATIENT PROFORMA

Anonymized Sample No:

Chief complaint:

History of presenting illness:

Past history:

Personal history:

Menopausal State:

Premenopausal / post-menopausal

BMI:

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

Local examination:

Biopsy Number:

Gross:

Tumour size:

Microscopy:

static Lymph Nodes:

Lymphovascular Invasion:

Tumor Infiltrating Lymphocytes:

NPI prognostic score:

Histopathological diagnosis:

Modified bloom richardson grading:

Immunohistochemically Scoring:

0 – Negative Expression
1 – Expression less than that of a Normal Adipocyte
2 - Expression equal to that of a Normal Adipocyte
3 - Expression more than that of a Normal Adipocyte.

Estrogen Receptor:

Progesterone Receptor:

Her 2 Neu:

Ki 67:

INFORMED CONSENT FORM

STUDY TITLE: ASSOCIATION OF IMMUNOHISTOCHEMISTRY LEPTIN EXPRESSION WITH PLASMA ELISA LEPTIN 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 IMMUNOHISTOCHEMISTRY LEPTIN EXPRESSION WITH PLASMA ELISA LEPTIN LEVELS IN INVASIVE DUCTAL CARCINOMA BREAST**.**

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

The main aim of the study is to check ASSOCIATION OF IMMUNOHISTOCHEMISTRY LEPTIN EXPRESSION WITH PLASMA ELISA LEPTIN LEVELS IN INVASIVE DUCTAL CARCINOMA BREAST.

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 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.Y. Jahnavi Reddy
Phone number: 8985543069.

ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆ

ಜನಾವಿ ರೆಡ್ಡಿ

ಅಧ್ಯಯನ ಶೀರ್ಷಿಕೆ: ಇಮ್ಯುನೊಹಿಸ್ಟೊಕೆಮಿಸ್ಟ್ರಿ ಆಫ್ ಅಸೋಸಿಯೇಷನ್ ಲೆಪ್ಟಿನ್ ಅಭಿವ್ಯಕ್ತಿಯು ಪ್ಲಾಸ್ಮಾ ಎಲಿಸಾ ಲೆಪ್ಟಿನ್ ಮಟ್ಟಗಳು ಇನ್ವಾಸಿವ್ ಡಕ್ಟಲ್ ಕಾರ್ಸಿನೋಮಾ ಸ್ತನದಲ್ಲಿ

ಸ್ಥಳ: ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ, ಶ್ರೀ ದೇವರಾಜ ಅರಸು ವೈದ್ಯಕೀಯ ಕಾಲೇಜು (ಕೋಲಾರ).

ಈ ಅಧ್ಯಯನದ ಮುಖ್ಯ ಉದ್ದೇಶವು ಇಮ್ಯುನೊಹಿಸ್ಟೊಕೆಮಿಸ್ಟ್ರಿ ಲೆಪ್ಟಿನ್ ಎಕ್ಸ್ಪ್ರೆಶನ್ ಅನ್ನು ಪ್ಲಾಸ್ಮಾ ಎಲಿಸಾ ಲೆಪ್ಟಿನ್ ಮಟ್ಟಗಳನ್ನು ಆಕ್ರಮಣಶೀಲ ಡಕ್ಟಲ್ ಕಾರ್ಸಿನೋಮಾ ಸ್ತನದಲ್ಲಿ ಪರೀಕ್ಷಿಸುವುದು.

ನೀವು ಪ್ಯಾಥಲಜಿ ವಿಭಾಗದಲ್ಲಿ ನಡೆಸಿದ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವಂತೆ ನಿಮ್ಮನ್ನು ವಿನಂತಿಸಲಾಗಿದೆ. ಈ ಅಧ್ಯಯನವು ರೋಗಿಗಳ ಕಾರ್ಸಿನೋಮಾ ಮಾದರಿಗಳ ಮೇಲೆ ನಡೆಯುತ್ತದೆ. ಈ ಮಾದರಿಗಳನ್ನು ಕೋಲಾರದ ಶ್ರೀ ದೇವರಾಜ ಅರಸು ವೈದ್ಯಕೀಯ ಕಾಲೇಜಿನ ರೋಗ ಶಾಸ್ತ್ರ ವಿಭಾಗದಿಂದ ಸಂಗ್ರಹಿಸಲಾಗುವುದು.

ಈ ಅಧ್ಯಯನವನ್ನು ಸಾಂಸ್ಥಿಕ ನೀತಿ ಶಾಸ್ತ್ರ ಸಮಿತಿ ಅನುಮೋದಿಸುತ್ತದೆ. ಸಂಗ್ರಹಿಸಿದ ಮಾಹಿತಿಯನ್ನು ಕೇವಲ ಶೈಕ್ಷಣಿಕ ಮತ್ತು ಪ್ರಕಟಣೆಗಾಗಿ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ. ಇದರಲ್ಲಿ ಭಾಗವಹಿಸಲು ಯಾವುದೇ ಒತ್ತಾಯವಿಲ್ಲ. ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸ್ವಇಚ್ಛೆಯಿಂದ ಒಪ್ಪಿಕೊಂಡರೆ ಮಾತ್ರ ನೀವು ಸಹಿ ಮಾಡಲು / ಹೆಬ್ಬೆಟ್ಟಿನ ಗುರುತನ್ನು ನೀಡಲು ವಿನಂತಿಸಲಾಗುತ್ತದೆ.

ನಿಮ್ಮಿಂದ ಸಂಗ್ರಹಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿ ಇಡಲಾಗುತ್ತದೆ ಮತ್ತು ಯಾವುದೇ ಹೊರಗಿನವರಿಗೆ ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ನಿಮ್ಮ ಗುರುತನ್ನು ಬಹಿರಂಗಪಡಿಸುವುದಿಲ್ಲ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮಗೆ ಯಾವುದೇ ಆರ್ಥಿಕ ಪ್ರಯೋಜನಗಳು ದೊರೆಯುವುದಿಲ್ಲ.

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.

ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸಿದ್ಧರಿದ್ದರೆ, ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಬಯಸುವುದಾಗಿ ನೀವು ಒಪ್ಪಿಕೊಳ್ಳುವ ಮಾಹಿತಿಯುತ ಸಮ್ಮತಿ ನಮೂನೆಗೆ ಸಹಿ ಮಾಡುವಂತೆ ನಿಮ್ಮನ್ನು ಕೇಳಲಾಗುತ್ತದೆ ಮತ್ತು ಇಡೀ ಕಾರ್ಯವಿಧಾನವನ್ನು ಅಧ್ಯಯನ ವೈದ್ಯರು ನಿಮಗೆ ವಿವರಿಸುತ್ತಾರೆ. ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ವಿವರಣೆ ಇಲ್ಲದೆ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮ್ಮ ಸಮ್ಮತಿಯನ್ನು ಹಿಂತೆಗೆದುಕೊಳ್ಳಲು ನೀವು ಸ್ವತಂತ್ರರಿದ್ದೀರಿ ಮತ್ತು ಇದು ನಿಮ್ಮ ಭವಿಷ್ಯದ ಆರೈಕೆಯನ್ನು ಬದಲಿಸುವುದಿಲ್ಲ. ಈ ಮಾಹಿತಿಯಾನ್ನು ಪ್ರಕಟಣೆಗಾಗಿ ಬಳಸಿಕೊಳ್ಳಲಾಗುವುದು.

ಯಾವುದೇ ಸ್ಪಷ್ಟನೆಗಾಗಿ ನೀವು ತನಿಖಾಧಿಕಾರಿಯನ್ನು ಸಂಪರ್ಕಿಸಲು ಸ್ವತಂತ್ರರು.

ಮುಖ್ಯ ತನಿಖಾಧಿಕಾರಿ: ಡಾ.ವೈ. ಜಾಹ್ನವಿ ರೆಡ್ಡಿ

ದೂರವಾಣಿ ಸಂಖ್ಯೆ: 8985543069.

ಮಾಹಿತಿ ಸಮ್ಮತಿ ಪತ್ರ

ಅಧ್ಯಯನ ಶೀರ್ಷಿಕೆ: ಇಮ್ಯುನೊಹಿಸ್ಟೊಕೆಮಿಸ್ಟ್ರಿ ಆಫ್ ಅಸೋಸಿಯೇಷ ಲೆಪ್ಟಿನ್ ಅಭಿವ್ಯಕ್ತಿಯು ಪ್ಲಾಸ್ಮಾ ಎಲಿಸಾ ಲೆಪ್ಟಿನ್ ಮಟ್ಟಗಳು ಇನ್ವಾಸಿವ್ ಡಕ್ಟಲ್ ಕಾರ್ಸಿನೋಮಾ ಸ್ತನದಲ್ಲಿ.

ನಾನು, _____ ರೋಗಿಯನ್ನು ಓದಿದ್ದೇನೆ

ಮಾಹಿತಿ ಹಾಳೆ ಮತ್ತು ಅಧ್ಯಯನದ ಉದ್ದೇಶ, ಬಳಸಲಾಗುವ ಕಾರ್ಯವಿಧಾನ, ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಒಳಗೊಳ್ಳುವಿಕೆ ಮತ್ತು ಮಾಹಿತಿಯ ಸ್ವರೂಪಕ್ಕೆ ಸಂಬಂಧಿಸಿದ ಅಪಾಯ ಮತ್ತು ಪ್ರಯೋಜನಗಳನ್ನು ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ಸಂಗ್ರಹಿಸಿ ಬಹಿರಂಗಪಡಿಸಲಾಗುತ್ತದೆ.

ಅಧ್ಯಯನದ ವಿವಿಧ ಅಂಶಗಳಿಗೆ ಸಂಬಂಧಿಸಿದಂತೆ ನನ್ನ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ನನಗೆ ಅವಕಾಶವಿದೆ ಮತ್ತು ನನ್ನ ಪ್ರಶ್ನೆಗಳಿಗೆ ನನ್ನ ತೃಪ್ತಿಗೆ ಉತ್ತರಿಸಲಾಗುತ್ತದೆ.

ನಾನು, ಸ್ವಯಂಪ್ರೇರಿತವಾಗಿ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಒಪ್ಪುತ್ತೇನೆ ಮತ್ತು ಪ್ರಬಂಧಕ್ಕಾಗಿ ನನ್ನ ವೈಯಕ್ತಿಕ ಮಾಹಿತಿಯ ಸಂಗ್ರಹ ಮತ್ತು ಬಹಿರಂಗಪಡಿಸುವಿಕೆಯನ್ನು ಅಧಿಕೃತಗೊಳಿಸುತ್ತೇನೆ. ಈ ಅಧ್ಯಯನದಿಂದ ಯಾವುದೇ ರೀತಿಯ ಚಿಕಿತ್ಸೆಯು ಬದಲಾಗುವುದಿಲ್ಲ.

ಹೆಸರು ಮತ್ತು ಸಹಿ / ಹೆಬ್ಬೆಟ್ಟು ಗುರುತು ದಿನಾಂಕ:

ಸ್ಥಳ:

ಹೆಸರು ಮತ್ತು ಸಹಿ / ಹೆಬ್ಬೆಟ್ಟು ಗುರುತು ದಿನಾಂಕ:

ಸ್ಥಳ:

(ಸಾಕ್ಷಿ/ಪೋಷಕ/ ಪಾಲಕ/ ಪತಿ)

KEYS TO MASTER CHART

BMI	Body mass index

NPI	Nottingham Prognostic 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
Stage	TNM - stage
ER	Estrogen receptor
PR	Progesterone receptor
Her2 neu	Human epidermal growth factor receptor 2 neu
Molecular	Molecular classification of breast
Leptin	Immunohistochemistry leptin expression
Elisa leptin	Elisa leptin levels

Intellectual Property Office

Certificate of Registration for a UK Design

Design number: 6429408

Grant date: 07 May 2025

Registration date: 09 March 2025

This is to certify that,

in pursuance of and subject to the provision of Registered Designs Act 1949, the design of which a representation or specimen is attached, had been registered as of the date of registration shown above in the name of

Dr. Navuduri Suvarna Jyothi, Dr. Tathagata Dey, Dr. Karthik Muthusamy, Dr. Selvakumar Sambandan, Dr. Anil Kumar, Dr. Bagepalli Srinivas Ashok Kumar, Dr. Ponnudurai Kathiresan, Mr. Pradeep Kumar Lakshminaryana Murthy, Mr. Muhammad Mubassir, Dr. Udaybhan Yadav

in respect of the application of such design to:

Compact and Reusable Blood Toxicity Monitoring Kit with Foldable Design

International Design Classification:
Version: 15-2025
Class: 24 MEDICAL AND LABORATORY EQUIPMENT
Subclass: 01 APPARATUS AND EQUIPMENT FOR DOCTORS, HOSPITALS AND LABORATORIES

THE PATENT OFFICE OFFICIAL

Adam Williams

Adam Williams
Comptroller-General of Patents, Designs and Trade Marks
Intellectual Property Office
The attention of the Proprietor(s) is drawn to the important notes overleaf.

Intellectual Property Office

Certificate of Registration for a UK Design

Design number: 6423241

Grant date: 18 February 2025

Registration date: 12 February 2025

This is to certify that,

in pursuance of and subject to the provision of Registered Designs Act 1949, the design of which a representation or specimen is attached, had been registered as of the date of registration shown above in the name of

Dr. Saurov Mahanta, Dr. Rishi Kumar Verma, Dr. Rahul Bipinbhai Parmar, Dr. Hemant Dnyaneshwar Chandore, Dr. Bagepalli Srinivas Ashok Kumar, Mrs. Shani Dominic, Dr. Ranjan Singh, Dr. Amit Kumar Singh, Dr. Sonali Singh, Ms. Komal Kaistha

in respect of the application of such design to:

Artificial Intelligence Based Device for Herbal Medicine Analysis

International Design Classification:
Version: 15-2025
Class: 24 MEDICAL AND LABORATORY EQUIPMENT
Subclass: 01 APPARATUS AND EQUIPMENT FOR DOCTORS, HOSPITALS AND LABORATORIES

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Adam Williams
Comptroller-General of Patents, Designs and Trade Marks
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The attention of the Proprietor(s) is drawn to the important notes overleaf.

Certificate of Registration for a UK Design

Intellectual Property Office

Design number: 6415102

Grant date: 17 March 2025

Registration date: 01 January 2025

This is to certify that,

in pursuance of and subject to the provision of Registered Designs Act 1949, the design of which a representation or specimen is attached, had been registered as of the date of registration shown above in the name of

Dr. Preeti Mangala, Ms. Monika Sharma, Mrs. Anusha Jasmin, Mrs. Poonam Poonam, Mr. Mohammed Khalid, Ms. Nanjundaiya Sadashiva Disha, Dr. Anil Kumar Singh, Ms. Rupalee Manohar Dhane, Dr. Shivshankar Digambar Mhaske, Mr. Lokender Singh

in respect of the application of such design to:

Microscopic Device for the Preparation of Nano-Formulation Drugs for Cancer Therapy

International Design Classification:
Version: 15-2025
Class: 24 MEDICAL AND LABORATORY EQUIPMENT
Subclass: 01 APPARATUS AND EQUIPMENT FOR DOCTORS, HOSPITALS AND LABORATORIES

Version: 15-2025
Class: 16 PHOTOGRAPHIC, CINEMATOGRAPHIC AND OPTICAL APPARATUS
Subclass: 06 OPTICAL ARTICLES

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CERTIFICATE OF REGISTRATION

1230554

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Date d'enregistrement /
Date of registration
07 FÉVR / FEB 2025

Titre /
Title
ALKALOID-ENCAPSULATED NANOPARTICLES FROM HOODIA GORDONII: SYNTHESIS, CHARACTERIZATION, AND ANTIOXIDANT EFFECTS IN RECTAL CANCER

Catégorie /
Category
LITTÉRAIRE / LITERARY

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Canadian Intellectual Property Office

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DR. MADHU C.DIVAKAR,

DR. ANIL KUMAR,

DR. TANMAY GHOSH,

DR. RAGHAV DIXIT,

DR. SIMANCHAL PANDA,

DR. HARJEET SINGH

...publication /
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...de première publication /
Place of first publication

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सत्यमेव जयते

ORIGINAL
क्रम सं/ Serial No. : 193852

पेटेंट कार्यालय, भारत सरकार The Patent Office, Government Of India

डिजाइन के पंजीकरण का प्रमाण पत्र | Certificate of Registration of Design

डिजाइन सं. / Design No.	:	441051-001
तारीख / Date	:	18/12/2024
पारस्परिकता तारीख / Reciprocity Date*	:	
देश / Country	:	

प्रमाणित किया जाता है कि संलग्न प्रति में वर्णित डिजाइन जो *PORTABLE MEDICATION EXPIRY DATE SCANNER* से संबंधित है, का पंजीकरण, श्रेणी 14-02 में 1.Dr. Bhargav Kumar Nagalla 2. Dr. Meenakshi Jaiswal 3.Ms. Rutuja Ganesh Tandale 4.Dr. G. Anantha Lakshmi 5.Mr. Mohammed Khalid 6.Dr. Amit Kumar Singh 7.Ms. Snehal Sanjeev Rajora 8.Dr. Sreejith. K 9.Mr. Amit Verma 10.Mrs. V. Jhansi Lakshmi के नाम में उपर्युक्त संख्या और तारीख में कर लिया गया है।

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डिजाइन अधिनियम, 2000 तथा डिजाइन नियम, 2001 के अध्यधीन प्रावधानों के अनुसरण में।
In pursuance of and subject to the provisions of the Designs Act, 2000 and the Designs Rules, 2001.

जारी करने की तिथि : 06/03/2025
Date of Issue :

उमा शंकर पंडित
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ORIGINAL

क्रम सं/ Serial No. : 201695

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डिजाइन के पंजीकरण का प्रमाण पत्र | Certificate of Registration of Design

डिजाइन सं. / Design No.	:	439373-001
तारीख / Date	:	03/12/2024
पारस्परिकता तारीख / Reciprocity Date*	:	
देश / Country	:	

प्रमाणित किया जाता है कि संलग्न प्रति में वर्णित डिजाइन जो *ROGUE CELL REMOVAL ANTIMICROBIAL BANDAGE* से संबंधित है, का पंजीकरण, श्रेणी 24-04 में 1.Dr. Shakeel Ahmed Adhoni 2. Dr. Sharangouda J. Patil 3.Dr. Kiran. B 4.Dr. Meenakshi Jaiswal 5.Dr. Arun Manikrao Mahale 6.Dr. Kadanthottu Sebastian Joseph 7.Dr. Ashok Kumar BS 8.Dr. Chandrashekhar Unakal 9.Dr. Shivam Singh 10.Dr. Shailaja Sameer Harugade के नाम में उपर्युक्त संख्या और तारीख में कर लिया गया है।

Certified that the design of which a copy is annexed hereto has been registered as of the number and date given above in class 24-04 in respect of the application of such design to *ROGUE CELL REMOVAL ANTIMICROBIAL BANDAGE* in the name of 1.Dr. Shakeel Ahmed Adhoni 2. Dr. Sharangouda J. Patil 3.Dr. Kiran. B 4.Dr. Meenakshi Jaiswal 5.Dr. Arun Manikrao Mahale 6.Dr. Kadanthottu Sebastian Joseph 7.Dr. Ashok Kumar BS 8.Dr. Chandrashekhar Unakal 9.Dr. Shivam Singh 10.Dr. Shailaja Sameer Harugade.

डिजाइन अधिनियम, 2000 तथा डिजाइन नियम, 2001 के अध्यधीन प्रावधानों के अनुसरण में।

In pursuance of and subject to the provisions of the Designs Act, 2000 and the Designs Rules, 2001.

जारी करने की तिथि : 09/06/2025
Date of Issue :

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Date d'enregistrement /
Date of registration
23 AVR / APR 2025

Titre /
Title
MOLECULAR MIMICRY IN ARTIFICIAL BLOOD: DESIGNING SYNTHETIC OXYGEN-CARRYING SOLUTIONS WITH BIOCOMPATIBLE HEMOGLOBIN ANALOGUES

1232675

Numéro d'enregistrement
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Catégorie /
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ORIGINAL
क्रम सं/ Serial No. : 198618

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डिजाइन के पंजीकरण का प्रमाण पत्र | Certificate of Registration of Design

डिजाइन सं. / Design No.	:	447014-001
तारीख / Date	:	05/02/2025
पारस्परिकता तारीख / Reciprocity Date*	:	
देश / Country	:	

प्रमाणित किया जाता है कि संलग्न प्रति में वर्णित डिजाइन जो *DIGITAL AND PORTABLE DEVICE FOR ESTIMATION OF IRON IN BLOOD* से संबंधित है, का पंजीकरण, श्रेणी 24-01 में 1.Mrs. Battula Sowjanya Lakshmi 2. Dr. Aarti Dubey 3.Mrs. Pragya Mishra 4.Mrs. Shweta Yadav 5.Mr. Sushil Kumar Tiwari 6.Mr. Vinay Kumar 7.Dr. Sagar Sopanrao Muley 8.Mr. Ashok Kumar BS 9.Prof. (Dr.) E. SIVA Rami Reddy 10.Dr. Darekar Avinash Bhaskar के नाम में उपर्युक्त संख्या और तारीख में कर लिया गया है।

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डिजाइन अधिनियम, 2000 तथा डिजाइन नियम, 2001 के अध्यधीन प्रावधानों के अनुसरण में।
In pursuance of and subject to the provisions of the Designs Act, 2000 and the Designs Rules, 2001.

जारी करने की तिथि : 07/05/2025
Date of Issue :

उमा शं. भंडि

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