DISSERTATION ON EVALUATION OF SIGNIFICANCE OF COMPUTERISED NUCLEAR MORPHOMETRY IN BENIGN AND MALIGNANT BREAST ASPIRATES

Dissertation submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY, CHENNAI

Submitted for

M.D. (PATHOLOGY)

MAY 2021 EXAMINATIONS

UNIVERSITY REGISTRATION NUMBER : 201813052

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ACKNOWLEDGEMENTS

To begin with, I thank GOD for his blessings and guidance in all my activities.

First and foremost, I bestow my high regards and gratitude to our respectable Dean, **Prof. Dr. R. Balaji,** MS., FRCS.,Ph.D.,FCLS Government Stanley Medical College and Hospital, Chennai for his encouragement and permission to conduct this study.

My heartfelt thanks and gratitude to my guide and mentor **Prof. Dr. Nalli. R. Sumitra Devi., M.D.,** Professor of Pathology, Department of Pathology, Government Stanley Medical College for her kind words, keen interest, valuable suggestions, guidance, constant encouragement and support for this study.

My sincere and heartfelt gratitude to our Professor and Head of the Department **Prof. Dr. P. Arunalatha. M.D.,** respectable Professors, **Prof. Dr.K.Valarmathi, M.D., Prof.Dr.S.Gomathi. M.D., Prof. Dr. R. Sathyalakshmi, M.D. Prof. Dr. M. Sureka, M.D., and Prof. Dr.T. Subachitra, M.D.,** for their invaluable suggestions, encouragement and support throughout their study.

It gives me immense pleasure to thank **Dr.Yogambal, M.D., Dr. Usha, M.D., Dr. Sangeetha, M.D., Dr.Francis Asir Joseph, M.D., Dr. Shanmugam, M.D., Dr.Rohinipriya, M.D., Dr.Arun Prabhakaran, M.D., Dr. Dougul Regis, M.D, Dr. Arthi, M.D., Dr. Maheshwaran, DCP,** Assistant Professors, Department of Pathology,

Government Stanley Medical College for their constant support and valuable suggestions.

My sincere thanks to my colleagues **Dr. I. Perachiselvi, Dr. Anantha Narayini, Dr. Nanda and my Juniors, Dr. Pushpa, Dr. Thivya, Dr. Sudeshana, Dr. Nandhini, Dr. Aafrin, Dr.Abinaya and Dr. Surendar** who has lent me their support and advice for the entire duration of this study.

My special thanks and heartfelt gratitude to our lab technicians Mr. Cheralathan, M**rs. Logeshwari, Mrs. Kalaivani, Mrs. Padmajothi, Mrs. Jayalakshmi and Mrs. Latha** for their patient, timely and selfless help during this study.

I am grateful to all the faculty members, my colleagues, lab technicians and support staff of the Department of Pathology, Government Stanley Medical College, Chennai for their constant support and kind help during the study.

On a personal level, I thank my father **Mr. M. Syed Meera Lebbai**, my husband **Mr. Mohamed Abbas,** my mother **Mrs. Rasheedha Begum,** myson **M. Jahran** and my daughter **M. Ayesha** for their constant support and encouragement.

Dr. S. HIMANA NISHARA

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LIST OF ABBREVIATIONS

WHO	-	World Health Organisation
FNAC	-	Fine Needle Aspiration Cytology
SHBG	-	Sex Hormone Binding Globulin
CIS	-	Carcinoma In Situ
HRT	-	Hormone Replacement Therapy
BMI	-	Body Mass Index
RBC	-	Red Blood Cells
RLN	-	Regional Lymph Node
RT PCR	-	Real Time – Polymerase Chain Reaction
IHC	-	Immunohistochemistry

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Document Information

Analyzed document	THESIS - 2.docx (D87414770)
Submitted	12/1/2020 5:53:00 PM
Submitted by	Himana Nishara
Submitter email	himananishara@gmail.com
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TITLE OF THE WORK : TO EVALUATE THE SIGNIFICANCE OF COMPUTERISED NUCLEAR MORPHOMETRY IN BENIGN AND MALIGNANT BREAST ASPIRATES.

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The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

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4. You should abide to the rules and regulation of the institution(s).
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6. You should submit the summary of the work to the ethical committee on completion of the work.

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INTRODUCTION

"IT IS TO EARLIER DIAGNOSIS THAT WE MUST LOOK FOR ANY MATERIAL IMPROVEMENT IN OUR CANCER CURE" - JOHN LOCKHART MUMMERY, 1926.

Cancer is still a major public health problem worldwide. Cancer refers to the uncontrolled growth and propagation of cells. It appears in almost any part of the body when a cell accumulates a set of mutations, generally over a period of years.[1] Among females, breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death, followed by colorectal and lung cancer[2]. Breast cancers affects both women and, very rarely, men (less than 1% of all breast cancer cases) [3]

In order to improve breast cancer outcomes and survival, early detection is crucial. The goal is to increase the proportion of breast cancers diagnosed at an early stage, allowing for more effective treatment to be used and reducing the risk of death. Early detection strategies include screening and early diagnosis.

Screening is the testing of women to identify cancers before the onset of any symptoms. Various tools are available for screening, of which the important and the effective ones include mammography, clinical breast examination and self-breast examination. Mammorgraphy is the use of low energy x-rays to identify the abnormalities in the breast. Clinical breast examination is the examination of both breasts by a trained health care

provider[4]. Mammography is the gold standard for breast imaging and cancer diagnosis[4]. However due to some limitations associated with mammography, such as low sensitivity especially in dense breasts, other modalities like ultrasound and magnetic resonance imaging can provide additional information. When a lump is diagnosed clinically or by imaging techniques, Fine needle Aspiration cytology is performed to aid in the diagnosis.

Fine needle aspiration cytology (FNAC) has been routinely used in assessment of the breast lesions. FNAC is an outpatient procedure in which a small amount of breast tissue or fluid is taken from the suspicious area and is checked for the presence of cancer cells in it. FNAC is a cost effective procedure and can prevent unnecessary surgery[5].

The cytological diagnosis is based on the subjective evaluation of cellularity, the morphology of the cells, the morphology of the nucleus and the presence of mitotic activity. This subjective evaluation can sometimes result in “grey zones” with false positive and false negative results. The incidence of this grey zones is estimated to be around 6.9 to 20% in the literature[6].

Normal cells gradually transform to form cancer cells through several changes. Nuclear changes during these transformational steps can be assessed quantitatively. Morphometry is the quantitative description of biological structures. Quantitative measurement of nuclear features like nuclear area, nuclear perimeter and nuclear diameter in cytological aspirates of breast lesions has been suggested to improve the diagnostic sensitivity and specificity.

Nuclear morphometry is capable of detecting changes that are too small to be visually perceived. When combined with the cytology impression, nuclear morphometry can help to resolve cases with diagnostic dilemma especially in the areas of grey zone[7].Our study is aimed at assessing the utility of nuclear morphometric parameters in cytological breast smears in categorising the benign and malignant breast diseases.

AIMS AND OBJECTIVES

Aim of the study:

1. To study the nuclear morphometric parameters in benign and malignant breast aspirates
2. To assess its role in differentiating between benign and malignant breast lesions
3. To compare the nuclear parameters like,
    - Nuclear diameter and radius
    - Perimeter
    - Nuclear area
    - Nuclear compactness

among four groups – Fibroadenoma, Fibrocystic change, Proliferative breast disease and Malignancy.

REVIEW OF LITERATURE

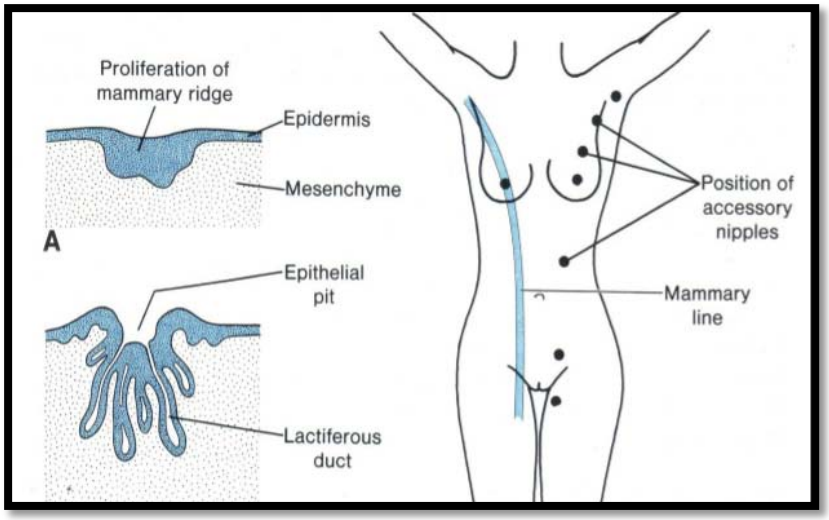
A knowledge on breast anatomy and development would provide a foundation for understanding the benign and malignant breast diseases.

DEVELOPMENT OF BREASTS

Breasts or the mammary glands are modified sweat glands. They first appear as bilateral bands of thickened epidermis called mammary lines or mammary ridges. In a 7-weeks old embryo, these lines extend on each side of the body to form the base of the forelimb to the region of the hind limb. A major portion of the mammary line disappears and a small portion in the thoracic region persists and penetrates the underlying mesenchyme. Here it forms 16 to 20 sprouts, which in turn give rise to small, solid buds.

By the end of prenatal life, the epithelial sprouts canalise and form lactiferous ducts. Initially the lactiferous ducts open into a small epithelial pit. Shortly after birth this pit is transformed into the nipple by proliferation of the underlying mesenchyme. At birth, lactiferous ducts have no alveoli and therefore no secretory apparatus. At puberty, however, increased estrogen levels stimulate branching from ducts to form alveoli and secretory cells[8].

Fig 1: Development of Breast



ANATOMY AND HISTOLOGY OF BREASTS

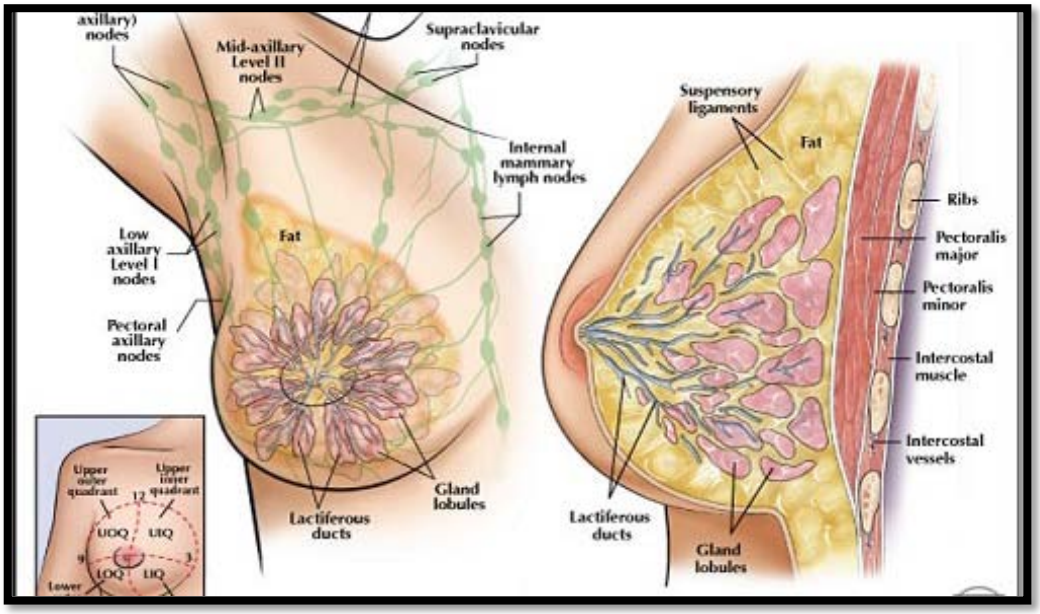
The breast lies on the anterior chest wall over the pectoralis major muscle and extends from the second to the sixth rib in vertical axis and from the sternal edge to the mid axillary line in the horizontal axis. Breast tissue also projects into the axilla as the tail of Spence. The breast extends laterally over the serratus anterior muscle and inferiorly over the external oblique muscle and the superior rectus sheath, the deep surface abuts the pectoralis fascia. It can range from 30 g to more than 1000 g.

The breasts are supplied by branches of the axillary, internal thoracic and intercostal arteries. The axillary artery supplies via the superior thoracic artery, the pectoral branches of the thoraco-acromial artery, the lateral thoracic artery and the subscapular artery. The internal thoracic artery supplies perforating branches to the anteromedial part of the breast. The second to fourth anterior intercostal arteries supply perforating branches laterally in the

anterior thorax. The second perforating artery is usually the largest and supplies the upper region of the breast, the nipple, areola and adjacent breast tissue.

Blood drains from the circular venous plexus around the areola and from the glandular tissue of the breast into the axillary, internal thoracic and intercostal veins.

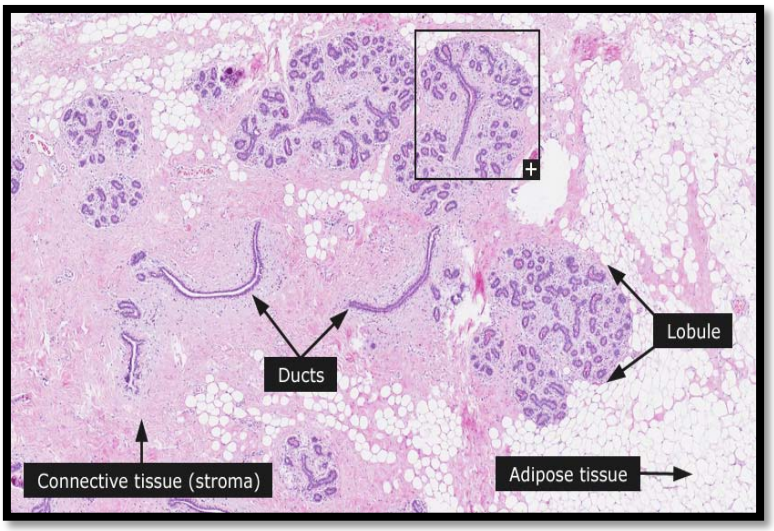
Fig 2: Anatomy of Breast



Human breasts are composed of parenchymal tissues consisting of a branching ductal system radiating from the nipple. The breast parenchyma consists of 15-20 lobes. Each lobule drains to nipple by lactiferous duct. They are separated from one another by interlobar connective tissue. Just before entering the nipple, each of the 15-20 main ducts expands into a dilated segment called the lactiferous sinus. Each lobe consists of 30-80 lobules, which contain the milk-producing elements of the breast.

The lobules in turn are composed of 20-40 terminal units or acini, which are surrounded by hormonally responsive intralobar connective tissue[9]. The proportion of fat, fibrous and parenchymal tissue vary greatly between individuals and with menopausal status, weight, number of live births and genetic factors[10].

Fig 3: Histology of Breast



CONCEPTS OF BENIGN AND MALIGNANT BREAST DISEASES

Anatomic and histologic structures of the breast undergo substantial change during the period from early adolescence to menopause[11]. The normal histologic appearance represents a spectrum ranging from a predominance of ducts, lobules, and intra- and inter-lobular stroma to patterns with a predominance of fibrous change and cyst formation, a process formerly called fibrocystic change. The term “fibrocystic changes” is now preferred since up to 50 to 60 percent of normal women may have this pattern histologically[12]. This

new term implies that women with lumpy breasts or non-discrete nodules do not have breast disease. Importantly, fibrocystic changes detected clinically incur no increased risk of breast cancer.

Specific changes in the breast, relating to stromal, ductal and glandular tissue occur as a function of age. During the early reproductive years, stromal hyperplasia may occur and produces juvenile breast hypertrophy[13] or rarely, the more significant problems of unilateral or bilateral macromastia (enlargement of breast tissue beyond what is considered normal)[14]. Changes in glandular and ductal tissue occur uncommonly. In the middle reproductive years, glandular breast tissue continues to undergo changes in response to cyclic increments in plasma levels of estradiol and progesterone and, if substantial, is called adenosis. Ductal changes remain uncommon while stromal hyperplasia may occur resulting in areas of ill-defined fullness ("lumpy-bumpy" consistency) on physical examination or in firm areas requiring biopsy.

The commonly encountered breast diseases are fibroadenoma, fibrocystic change, proliferative breast disease with or without atypia and malignancy, especially invasive carcinoma of no special type – ductal.

FIBROADENOMA OF BREAST:

Fibroadenomas are the most common benign tumours of the breast that usually present as a single breast mass in young women[15].They are multiple in about 20% of cases. They are assumed to be aberrations of normal breast development or the product of hyperplastic processes, rather than true

neoplasms[16]. Fibroadenomas comprise about 50% of all breast biopsies, and this rate rises to 75% for biopsies in women under the age of 20 years[17].

Fibroadenomas usually form during menarche (15 –25 years of age), a time at which lobular structures are added to the ductal system of the breast. Hyperplastic lobules are common at that time, and may be regarded as a normal phase of breast development. Hyperplastic lobules were shown to be histologically identical with fibroadenomas.

Analyses of the cellular components of fibroadenomas by means of polymerase chain reaction demonstrated that both the stromal and the epithelial cells are polyclonal, supporting the theory that fibroadenomas are hyperplastic lesions associated with aberration of the normal maturation of the breast, rather than true neoplasms[11]. Fibroadenomas are stimulated by estrogen and progesterone, and by lactation during pregnancy, and they undergo atrophic changes in menopause[18].There is 1.3 to 2.1 increased risk of breast cancer in women with fibroadenomas compared with the general population[11].

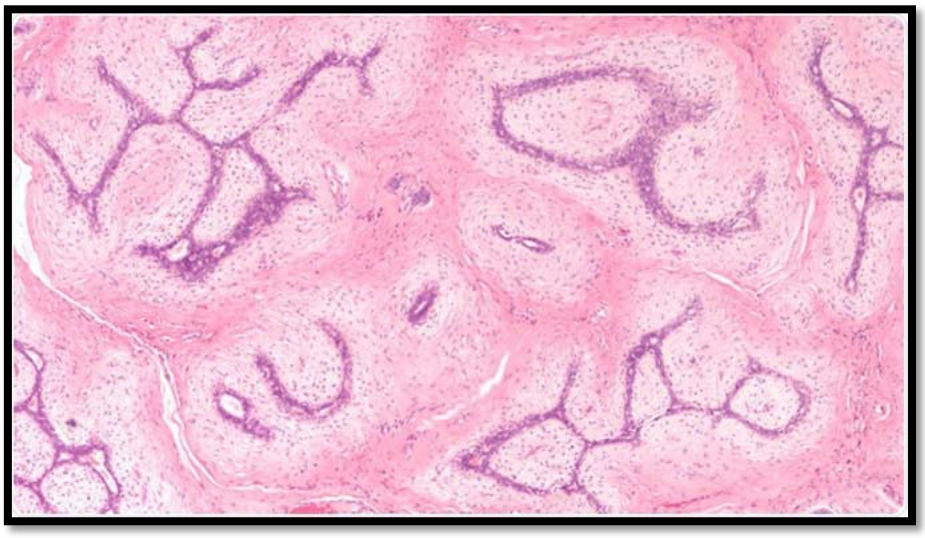
Histology[11]:

The microscopic appearance shows proliferation of both stromal and epithelial elements. It appears to arise in the lobule with proliferation of lobular stroma, enlarging by coalescence of adjacent lobular units to form a mass lesion. Classically, two patterns have been described: (a) pericanalicular, in which the stroma surrounds rounded epithelial elements and (b) intracanalicular, in which the epithelial elements are distorted, stretched, and

compressed by the proliferating stroma. The patterns frequently occur together and are not thought to convey clinical significance. The epithelial elements are composed of epithelial and myoepithelial layers, which are surrounded by basement membrane.

Several changes may occur within the epithelial elements, including apocrine metaplasia and varying degrees of epithelial hyperplasia. Secretory changes during pregnancy and lactation may affect the epithelium, whereas in fibroadenomas from elderly patients, the epithelium is frequently atrophic. Fibroadenomas that contain cysts larger than 3 mm, sclerosing adenosis, epithelial calcifications, or papillary apocrine change have been called complex fibroadenomas. Complex fibroadenomas were reported to be associated with a slightly greater risk for subsequent breast cancer than fibroadenomas lacking such changes.

Fig 4: Fibroadenoma of Breast – Histopathology, 10X



Variants of fibroadenoma[19]:

a. Usual/ adult type fibroadenoma
b. Myxoid fibroadenoma
c. Complex fibroadenoma
d. Juvenile fibroadenoma
e. Tubular adenoma

FIBROCYSTIC CHANGES OF BREAST

Cyst formation is one of the most common changes seen in breast tissue and is frequently seen in combination with other benign lesions. It occurs more commonly in women between 25 to 45 years of age. Reduced incidence is seen in postmenopausal women due to reduced serum estrogens.

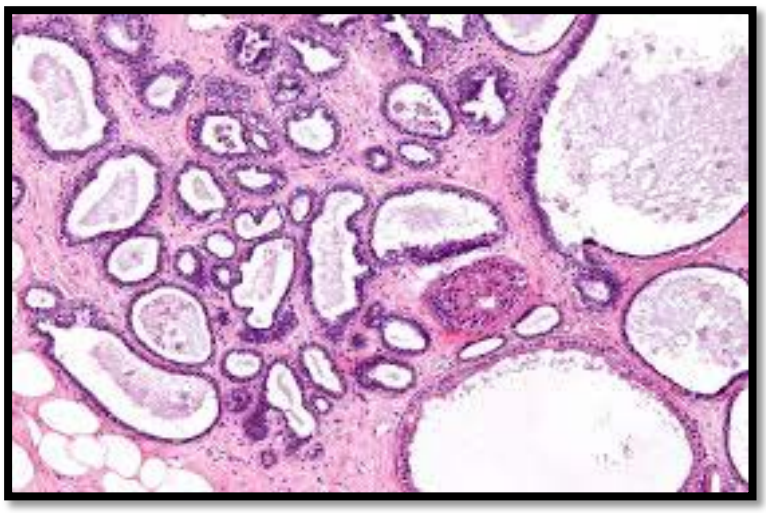
Cystic changes are more commonly seen in women with polycystic ovaries and are associated with Cowden syndrome (multiple hamartomas including trichilemmoma, high risk of breast, uterine and non-medullary thyroid cancer). They usually present as bilateral breast lumps although one breast may be affected more than the other.

Histology:

Microscopically, the cysts are lined by a double cell layer, and the luminal epithelium frequently shows apocrine metaplasia[11]. Cystic change does not usually cause major diagnostic problems; however, minor problems in interpretation may be encountered in the following situations,

1. In large cysts, the epithelial lining may be partially, if not entirely, lost.
2. In duct ectasia, an inflammatory reaction around a cyst is accompanied by fibrosis and plasma cells, which can be mistaken for infiltrating carcinoma on low-power examination.
3. An intense inflammatory reaction consisting mainly of foamy macrophages may be all that remains at the site of a cyst and may be mistaken for fat necrosis.
4. Elastic tissue can be demonstrated around ectatic ducts.
5. Benign cysts must be differentiated from cystic hypersecretory DCIS

Fig 5: Fibrocystic change, Histopathology, 10x



PROLIFERATIVE BREAST DISEASE

Usual Ductal Hyperplasia[20]

Usual ductal hyperplasia (UDH) is the preferred term of the WHO Working Group. The degree of proliferation is categorised as

a) Mild-three or four epithelial cells in thickness

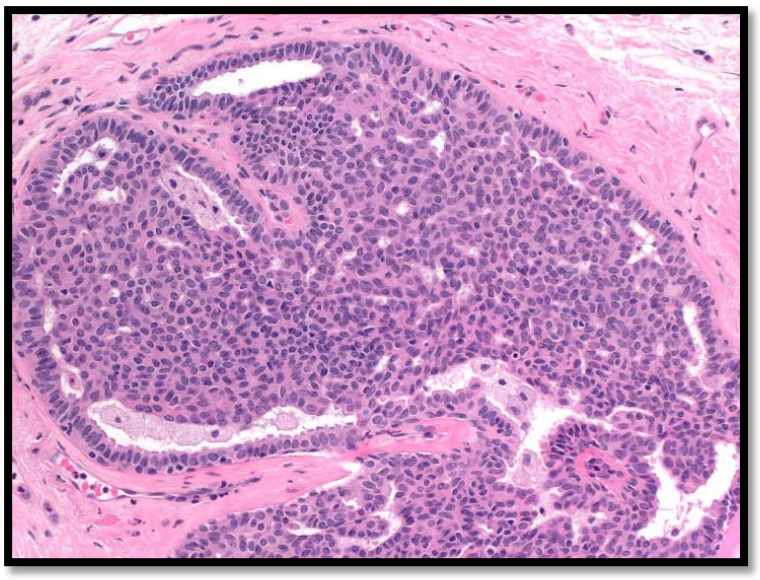
b) Moderate - more pronounced proliferation

c) Florid - the entire lumen is filled by the epithelial proliferation

The features most helpful in recognizing the benign nature of the proliferation are the following:

1. Nuclei that are oval (rather than round, except when cut transversely), normochromatic (rather than hyperchromatic), with nuclear grooves, and with slight overlap; small, single, indistinct nucleoli; occasional intranuclear inclusions; scant or no mitotic activity
2. Cytoplasm that is eosinophilic rather than pale and homogeneous.
3. Indistinct cytoplasmic borders, so that the nuclei seem to lie in a syncytial mass rather than within sharply outlined cell membranes.
4. Streaming effect, induced by the oval cells being vaguely arranged in parallel bundles
5. “Tufts” and “mounds” projecting into the lumen.
6. Presence of peripheral elongated clefts, bound on one side by a single layer of basally located cells and on the other by a solid intraluminal formation.
7. The intercellular lumina of UDH tend to be irregular in size, shape (elongated rather than round), and location (predominating at the periphery).

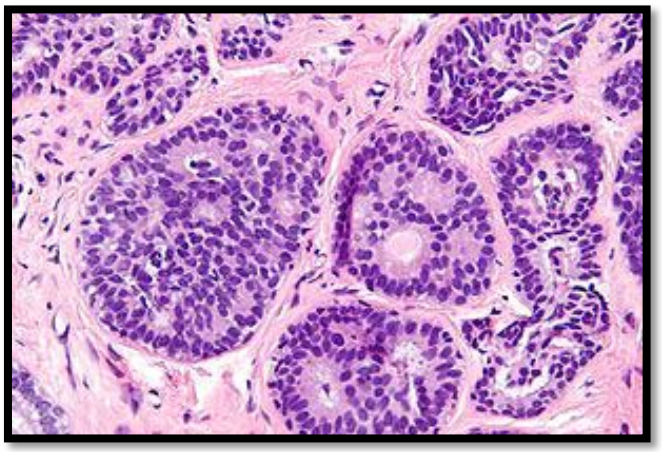
Fig 6: Usual ductal Hyperplasia, Histopathology, 10x



Atypical ductal and lobular hyperplasia:

There is a wide range in the degree of epithelial proliferation in benign breast disease. The terms ADH and ALH for proliferative lesions in which some but not all of the features of DCIS or LCIS, respectively, are present. The currently accepted definition of ADH is that of a lesion with cytologic and architectural features indistinguishable from those of low-grade DCIS, that is, monomorphic cells with ovoid to rounded nuclei and the formation of micropapillae, tufts, fronds, bridges, solid, and/or cribriform patterns within the involved space but either intimately admixed with UDH or showing only partial involvement of the TDLU. ALH is defined as a monomorphic proliferation of atypical epithelial cells with round nuclei and indistinct nucleoli. The cells are dyshesive and often have intracytoplasmic lumina.

Fig 7: Atypical ductal hyperplasia, Histopathology, 10x



CARCINOMA OF BREAST

Breast cancer develops due to uncontrolled growth of the epithelial cells at the junction of the terminal duct-lobular unit. It has been calculated that most breast cancers need about 5-10 years to develop from a single malignant cell to a tumor of 5-10 mm diameter [21]

Etiology and risk factors

Breast cancer is a multifactorial disease and a variety of factors contribute to the development of the disease. The genetic factors include changes in tumor suppressor genes, growth factor imbalances, enzyme production and telomerase activity. The non-genetic factors include environment, nutrition and other lifestyle risk factors leading to cancer. By identification of modifiable risk factors and controlling them, the risk of breast cancer can be lowered. Age >50 years, late age of menopause (>50 years), late age at first childbirth (>30 years), and high socioeconomic status were found to be major risk factors associated with breast cancer[22]. Any factor, such as

ovarian hormones and **growth factors** that increases cellular proliferation in breast epithelium raises the risk[23].

I. Reproductive risk factors:

A. Early menarche and late menopause:

Early age at menarche increases risk of breast cancer. For every one year delay, breast cancer frequency decreases by 10-20%. Breast cancer risk may be explained by effect of early menarche on estrogen level[24]. Women with surgically induced menopause have been shown to have reduced risks of breast cancers in comparison with women whose menopause occurs between the ages of 45 and 54. Women with late menopause after 55 years of age have a relative risk of 1.48. Increased risk of breast cancer in late menopause is due to long menstrual history and ovarian function[25].

B. Parity

Compared with never-pregnant women, an increasing number of full-term pregnancies was associated with greater **risk reduction** for both breast CIS and invasive breast cancer[26]. Women having four or more full-term pregnancies had a 31% lower breast CIS risk and 18% lower invasive breast cancer risk[16]

C. Age at first live birth

Early first pregnancy leads to maturation of terminal ductal lobular unit of breast thereby reducing risk of breast cancer. Hence, women who are more

than 35 years of age have 60% increased risk of breast cancer as compared to those who are less than 18 years of age at first pregnancy [27].

D. Breast feeding

Breastfeeding is of particular interest for breast cancer prevention because it is a modifiable risk factor. Breastfeeding not only reduces breast cancer risk but also confers other health benefits to the mother including reduced risk for endometrial and ovarian cancers and reduced risk for chronic conditions that are also risk factors for cancer, such as hypertension and diabetes. Women who had cumulatively breastfed for 12 months or longer had a 28% lower risk of breast cancer[28]

E. Hormones

Breast cancer risk is directly proportional to the levels of serum concentrations of sex hormones including total and free estradiol, androstenedione and testosterone[29]. In postmenopausal women, weight is directly proportional to plasma levels of estrone and estradiol, as well as unbound estradiol to SHBG. Hence postmenopausal obese women have greater risk of breast cancer development than in non-obese women.

F. Hormone replacement therapy

Breast cancer risk increases much more with the use of Hormone Replacement therapy. Risk increased consistently with increasing duration of use.[30] Women who received Combination HRT for more than 6 years have a 4.4-fold increased risk of breast cancer.[20]

II. Anthropometric risk factors:

The correlation between body weight and BMI with breast cancer risk differs based on the menopausal status. Obesity is associated with a lower risk of breast cancer in premenopausal women and an increased risk of breast cancer in postmenopausal women. Obese premenopausal women have decreased progesterone levels because of anovulation and a decreased progesterone secretion in the luteal phase. Also, leptin levels which increases with increasing fat stores, inhibit ovarian estrogen production, and thereby decreases breast cancer development.

Obesity increases breast cancer risk in postmenopausal women by increasing levels of endogenous estrogen[31]. The principal source of estrogen in postmenopausal female is the conversion of androstenodione to estrone in adipose tissue. Also the levels of sex-hormone-binding globulin fall with increasing BMI thus increasing the levels of free estradiol. In addition, obesity may increase the concentration of several circulating cytokines, which stimulate the activities of the enzymes, involved in the synthesis of estrogen.

III. Family history of breast cancer / genetic factors:

Family history of breast cancer is a well-established risk factor for breast cancer. Women who have first degree relatives with breast cancer have substantially increased risk of breast cancer. Risk is about 1.5-2 times more as compared to women with no such history. The risk may be further increased to 6 times if more than one first degree relative has been affected. Cancers

develop in this population at an earlier age. Also they have inherited DNA mutation of BRCA 1 or BRCA 2 gene that increases the risk of breast cancer.

Lynch distinguishes familial breast cancer from hereditary breast cancer. Familial breast cancer is defined as “Family having more than two first degree relatives with breast cancer in the absence of hereditary breast cancer”. Hereditary breast cancer is defined as “Pattern within a particular family having Mendelian segregation of breast cancer”. The former are probably events that may happen, by the laws of probability to cluster in a family, while the latter cancers are likely the results of inheritance of abnormal DNA[32].

Genetic factors have a role in approximately 5% of all breast cancer cases. But the risk percentage increases to 25% in cases below 30 years of age. Several genes are implicated in breast cancer development. BRCA 1 gene located on chromosome 17 and BRCA 2 present on chromosome 13 are associated with majority of inherited breast cancers. Around 2-5% of breast cancers are hereditary. BRCA 1 and BRCA 2 are the tumor suppressor genes with numerous important cell functions. It includes gene transcription, regulation of cell cycle check points and DNA repair.

Many genes other than BRCA are involved in breast cancer risk. Women with Li-Fraumeni syndrome have increased risk in development of early onset of many cancers including breast cancer. This syndrome is due to mutations in p53 tumor suppressor gene. In Ataxia telangiectasia, there is 100 fold increase in breast cancer risk in women. It is an autosomal recessive

syndrome due to DNA repair defect. Women with Cowden disease having mutation in the PTEN tumor suppressor gene develop breast cancer by 50 years of age[33].

IV. Other risk factors

A. Benign breast diseases

Certain types of benign breast diseases have an increased risk of breast cancer. There is 1.5 fold increased risk of breast cancer for those women with benign breast disease without hyperplasia compared to normal population. The risk of breast cancer among women with hyperplasia depends on the presence of atypia is present or not. Atypical hyperplasia is associated with 2.6 fold increased risk of breast cancer as compared to 1.8 fold increased risk in hyperplasia without atypia. Atypia in premenopausal women have higher relative risk of breast cancer than in post-menopausal women[34].

Table 1: Relative risk of invasive carcinomas associated with benign breast diseases

NO INCREASED RISK
Adenosis,other than sclerosing adenosis
Duct Ectasia
Fibroadenoma lacking complex features
Fibrosis
Mastitis
Cyst(gross/microscopic)
Simple apocrine metaplasia without associated adenosis
Squamous metaplasia
SLIGHTLY INCREASED RISK(1.5 – 2)
Complex Fibroadenoma
Moderate or florid hyperplasia without atypia
Sclerosing adenosis
Solitary papilloma without atypical hyperplasia
MODERATELY INCREASED RISK
Atypical ductal hyperplasia
Atypical lobular hyperplasia

B. Ionizing radiation

Ionizing radiations are associated with an increased risk of developing breast cancer. Increased incidence was observed among atomic bomb survivors or women exposed to radiation for diagnostic or therapeutic reasons. Relative risks vary from 1.2 - 2.4 and are related to both total dose and age at exposure[35].

C. Mammographic density

Mammographic density is a strong risk factor for breast cancer. Densities correspond to connective and epithelial tissues in the breast and the dark radiolucent areas represent fat. Women with higher mammographic densities are 4-6 times more likely to develop breast cancer[36].

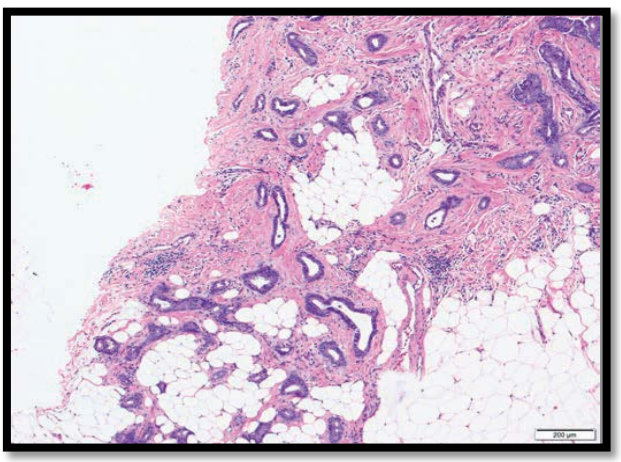
D. Socioeconomic Status

Higher socioeconomic status has a role in breast cancer. Developed countries have much higher incidence of breast cancer than developing countries. This correlation between breast cancer risk and socioeconomic status has appeared at both the individual as well as community level. The higher breast cancer risk among well-educated women appears to be attributable to greater exposure to breast cancer risk factors such as later age at first pregnancy, having few or no children, and more frequent use of oral contraceptives and hormone therapy.

Histology:

Microscopically the tumor can grow in diffuse sheets, well-defined nests, cords, or as individual cells. Glandular/tubular differentiation may be well developed, barely detectable, or altogether absent. The tumor cells vary in size and shape, the nuclei are large with varying degrees of pleomorphism, and nucleoli may be prominent. Mitotic figures vary from infrequent to more numerous. Areas of necrosis are unusual but may be identified in some cases. The amount of stroma ranges from none to abundant, and its appearance from densely fibrotic to cellular ("desmoplastic"). In cases with abundant stroma, it may be difficult to identify the tumor cells. Areas of "elastosis" may be present, which can involve the wall of the ducts and the vessels (mainly veins). Calcification has been reported in approximately 60% of cases, either as coarse or fine deposits or, rarely, as psammoma bodies; most often the calcifications are identified within the associated in situ component. A mononuclear inflammatory infiltrate of variable intensity may be present at the interface between tumor and stroma. Granulomatous inflammation is rarely seen.

Fig 8: Invasive Carcinoma No special type, Histopathology, 10x



DIAGNOSTIC MODALITIES IN IDENTIFYING BREAST DISEASES:

The choice of diagnostic method depends on the patient's state and stage, age of the individual and the density of the breast tissue.

A. Mammography

The current standard screening and diagnosis method is mammography imaging, which uses low energy 20 – 30 KeV X-rays. According to studies, the sensitivity (true positive) of this method is around 75%, but in middle – aged people whose breast tissues often have a higher mass density, the sensitivity is reduced to about 50%[37]. Screening mammography is used to detect cancer in asymptomatic women. Diagnostic mammography is used to evaluate,

1. Patients with breast symptoms or complaints such as nipple discharge or a palpable mass
2. Patient who had abnormal results on screening mammography
3. Patients who had underwent breast conservation therapy.

Digital mammography, also called full-field digital mammography (FFDM), is a mammography system in which the X-ray film is replaced by solid-state detectors that convert X-rays into electrical signals.

BIRADS diagnostic categories

The Breast Image Reporting and Data System (BIRADS), final assessment classification was developed by the American College of Radiology to standardize mammographic reporting. Follow up recommendation are made based on the final assessment category.

Table 2: Breast Imaging Reporting and Data system

Category	Description	Likelihood of malignancy
0	Incomplete : Need additional imaging evaluation or comparison with previous examinations	Unknown
1	Negative	Essentially 0 %
2	Benign	Essentially 0 %
3	Probably benign	Less than 2%
4	Suspicious of malignancy	12 – 25%
5	Highly suggestive of malignancy	Greater than 95%
6	Known malignancy	100%

B. Ultrasonography

Ultrasound helps to differentiate between solid and cystic breast masses that are mammographically detected or those that are palpated. If there is suspicious metastasis of nodes, ultrasound evaluation of axilla can be done to detect lymph nodes. Interventional procedures can be done for suspicious areas in breast or axilla under ultrasound guidance.

C. Breast MRI

The sensitivity of MRI for breast carcinoma is between 88 and 100 percent. Invasive breast cancer shows contrast enhancement on MRI. Owing to

higher sensitivity rates, preoperative MRI would estimate the extent of disease, more accurately than conventional imaging, thereby improving surgical planning.

D. PET (Positron Emission Tomography)

It is a non-invasive diagnostic procedure using 2-deoxy-2-fluoro [18F]-d-glucose (FDG) to obtain information about glucose metabolism. Malignant cells have increased rate of glycolysis than normal cells, and they overexpress GLUT1, which may be responsible for glucose accumulation. PET is effective in distinguishing benign and malignant tumors, Response of breast carcinoma to preoperative chemotherapy has been evaluated by PET.

E. Fine needle aspiration cytology

Fine needle aspiration cytology (FNAC) has been routinely used in assessment of the breast lesions. FNAC is an outpatient procedure in which a small amount of breast tissue or fluid is taken from the suspicious area and is checked for the presence of cancer cells in it. FNAC is a cost effective procedure and can prevent unnecessary surgery[5]

F. Biopsy

Core Needle biopsy

In core needle biopsy, tissue sample is obtained from the mass by using hollow needle. The advantages of core biopsy are low complication rate, avoidance of scarring and low cost.

Open biopsy

An open biopsy is recommended only in patients who have been appropriately investigated by imaging, FNAC, and or by core needle biopsy.

CLINICAL PRESENTATION

Patients present with hard, immobile breast lump probably with a short duration of disease. Sometimes they can present with nipple discharge, nipple inversion and skin retraction (peau de orange appearance)

WHO CLASSIFICATION OF INVASIVE BREASTCARCINOMAS:

- Microinvasive carcinoma
- Invasive breast carcinoma
- Invasive carcinoma of no special type (NST)
- 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
- Tubulolobular carcinoma
- Mixed lobular carcinoma

- Tubular carcinoma
- Cribriform carcinoma
- Mucinous carcinoma
- Carcinoma with medullary features
- Medullary carcinoma
- Atypical medullary carcinoma
- Invasive carcinoma 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 tumour, well-differentiated
- Neuroendocrine carcinoma, poorly differentiated (small cell carcinoma)
- 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 tumours
- Cylindroma
- Clear cell hidradenoma

HISTOLOGICAL GRADING OF BREAST CANCER

The commonly used grading system is modified Bloom Richardson scoring system also called as Elston-Ellis modification of the Scarff-Bloom-Richardson grading system or the Nottingham combined histologic grade. Histologic grading based on

1. Tubule formation

2. Nuclear pleomorphism,

3. Number of mitosis

Table 3: Modified Bloom Richardson grading criteria

PARAMETERS	**SCORE**
Tubule formation	
Majority of tumour (>75%)	1
Moderate degree (10 to 75%)	2
Little or no (<10%)	3
Nuclear Pleomorphism	
Small, regular uniform cells	1
Moderate increase in size and variation	2
Marked variation	3
Number of mitosis(Microscope Nikon 40x objective)	
Upto 7/10 hpf	1
8 – 14/10 hpf	2
More than equal to 15/10 hpf	3

FINAL GRADING

GRADE 1 – Total score, 3 – 5, well differentiated

GRADE 2 – Total score, 6 or 7, moderately differentiated

GRADE 3 – Total score, 8 or 9, poorly differentiated

STAGING

The most widely used staging system to stage breast carcinoma is TNM staging given by American Joint Committee on Cancer (AJCC) 8th edition.

This system provides information about the extent of cancer at the primary site (tumour or T), the regional lymph nodes (nodes or N), and spread to distant metastatic sites (metastases or M).

TNM STAGING

Primary Tumor (T)

TX	:	Primary tumor cannot be assessed
T0	:	No evidence of primary tumor
Tis	:	Carcinoma in situ; DCIS/LCIS/Paget‟s
T1	:	Tumor size (2 cm or less).
T1mi	:	less than 0.1cm micro invasion
T1a	:	more than 0.1 cm but less than 0.5 cm
T1b	:	more than 0.5cm but less than 1 cm
T1c	:	more than 1cm but less than 2 cm
T2	:	Tumor size 2-5 cm
T3	:	Tumor size more than 5cm
T4	:	Tumor of any size with direct extension to chest wall and or to the skin (ulceration or skin nodules)
T4a	:	Extension to chest wall, not including only pectoralis muscle invasion/adherence
T4b	:	Ulceration and/or ipsilateral satellite skin nodules and/or edema
T4c	:	Both of the above (T4a and T4b)
T4d	:	Inflammatory carcinoma

Regional lymph nodes (N)

NX	:	(RLN) cannot be assessed
N0	:	No regional lymph node metastasis
pN0(i-)	:	No RLN metastasis identified histologically, negative IHC
pN0(i+)	:	Malignant cells in RLN less than 0.2 mm (detected by H&E or IHC)
pN0(mol-)	:	No RLN metastasis histologically, negative molecular findings (RT-PCR)
pN0 (mol+)	:	Positive molecular findings (RT-PCR) but no RLN metastasis detected histologically or by IHC
pN1mi	:	Micrometastasis (greater than 0.2 mm and /or more than 200 cells but none greater than 2.0mm)
pN1a	:	Metastases in 1 to 3 axillary lymph nodes, at least one metastases greater than 2.0 mm
pN1b	:	Metastases in internal mammary nodes with micro metastases or macro metastases detected by sentinel lymph node biopsy but not detected clinically
pN1c	:	Metastases in 1 to 3 lymph nodes and in internal mammary nodes with micro metastases or macro metastases detected by sentinel lymph node biopsy but not detected clinically
pN2a	:	Metastases in 4-9 axillary lymph nodes (at least one tumor deposit greater than 2.0 mm).

pN2b : Metastases in clinically detected internal mammary nodes and in the absence of axillary LN metastasis

pN3a : Metastases in 10 or more axillary lymph nodes (at least one tumor deposit greater than 2.0mm); or metastases to the infraclavicular (level 3 axillary lymph nodes and in internal mammary lymph nodes) nodes

pN3b : Metastases in clinically detected ipsilateral internal mammary lymph nodes

Pathological classification (pN) is used only in conjunction with a pathological T assignment (surgical resection) (pT) and includes pathological evaluation of excised nodes from a sentinel lymph node biopsy and or lymph node dissection.

Distant metastases (M)

M1: Distant detectable metastasis as histologically proven larger than 0.2mm

FNAC IN BENIGN AND MALIGNANT BREAST DISEASES

The main purpose of FNAC of breast lumps is to confirm cancer preoperatively and to avoid unnecessary surgery in specific benign conditions. The role of FNAC in breast lumps include[38]:

1. The diagnosis of simple cysts
2. The investigation of suspected recurrence or metastasis in cases of previously diagnosed cancer
3. The confirmation of inoperable, locally advanced cancer

4. The preoperative confirmation of clinically suspected cancer

5. The investigation of any palpable lump, clinically benign or malignant, as a guide to clinical management

6. The ability to obtain tumour cells for special analysis and research e.g hormone receptor studies, DNA analysis, immunohistochemistry, cell kinetics and molecular studies.

Fine Needle Aspiration cytology reports are classified into 5 categories based on the **National Health Services Breast Screening Programme (NHSBSP) of Britain**[39]. The use of these standardized diagnostic categories is necessary to enhance communication within a multi-disciplinary team and for comparing results from other Centres. The diagnostic categories and their corresponding numerical codes are:

Table 4: Reporting categories of Breast cytology

NUMERICAL CODE	DIAGNOSTIC CATEGORY
C1	Inadequate/insufficient
C2	Benign
C3	Atypical/Indeterminate
C4	Suspicious of Malignancy
C5	Malignant

Fibroadenoma- FNAC:

FNAC diagnosis of fibroadenoma is highly accurate. Lopez-Ferrer reported a 79.3% predictive value out of 362 fibroadenoma aspirates with most diagnostic errors occurring in the older age group[40]. Cytologically, aspirates are hypercellular with characteristic monolayer sheets of benign-looking epithelial cells mixed with myoepithelial cells. These sheets are often described as "staghorn", having antler-like configuration on its edges[41].Cellular cohesiveness is often appreciated in the aspirate smear.

Accompanying the epithelial cells are the fibrillar stromal materials which may vary in cellularity and sometimes show myxoid change. Commonly, the background of the aspirate is composed of numerous naked/bipolar nuclei. This is one of the characteristic cytologic features of fibroadenoma. The added presence of large number of bipolar nuclei in the background of smear is a reliable feature in favor of fibroadenoma[42].

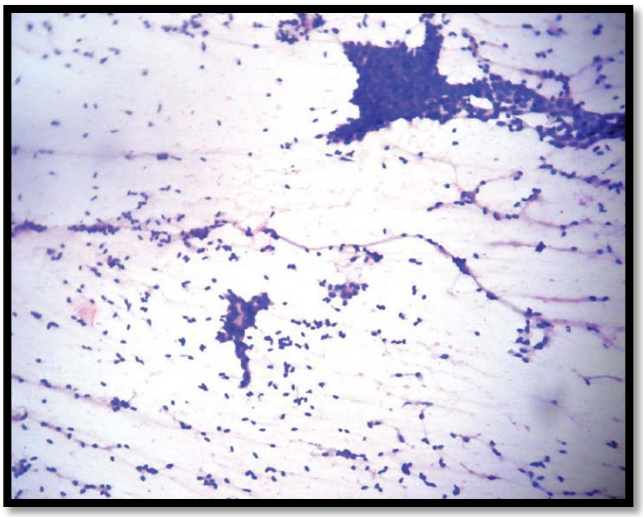
The commonly encountered cytological features of fibroadenoma are fibromyxoid stroma, staghorn clusters, and numerous single bare nuclei, being seen in 92.7%, 73.6%, and 73.6% of cases, respectively[43]. These findings constitute the diagnostic triad for fibroadenoma.

It is a known fact that fibroadenoma is difficult to distinguish from phyllodes tumor using aspiration cytology but there are some features that are more characteristic to phyllodes tumors that will support its diagnosis on cytology. A cellular aspirate with numerous plump and spindly nuclei,

pronounced of hypercellularity of stromal fragments, and presence of atypia are the key points that support a diagnosis of phyllodes tumor over fibroadenomas.

Fibroadenomas also need to be differentiated from papillomas, by virtue of the fact that the latter show presence of small cell balls or clusters, with either staghorn or papillary configurations in the smears[44].

Fig 9: Fibroadenoma of Breast, Cytology H&E,10X



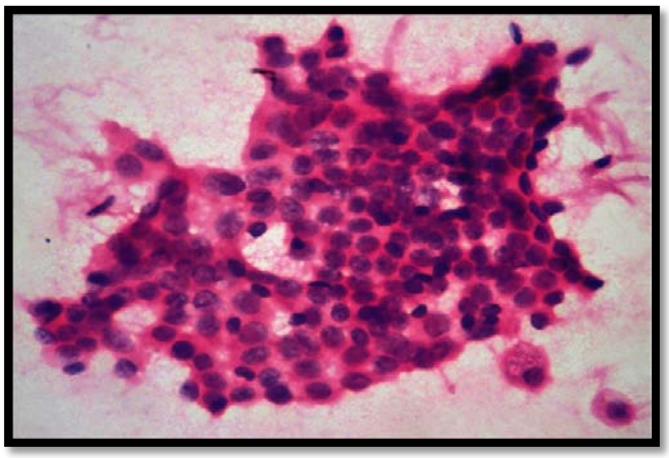
Criteria for diagnosis:

- Cellular smear with bimodal pattern containing epithelial and stromal fragments
- Large branching sheets of bland epithelial cells
- Numerous single, bare bipolar/oval nuclei
- Fragments of fibromyxoid stroma

Fibrocystic change - FNAC:

Fibrocystic changes may clinically cause an indistinct thickening or lump or an asymmetrical density in mammogram. Cytologically, it is a variant of common benign pattern in which cyst macrophages, apocrine metaplastic cells and sheets of duct epithelial cells are found in addition to the usual bimodal cell population of ductal epithelial cells and single bare oval nuclei.

Fig 10: Fibrocystic change of Breast, Cytology, H&E, 40X



Criteria for diagnosis:

- Complete disappearance of the lump after aspiration of the fluid
- Absence of altered blood or necrotic material in the aspirated fluid.
- Cyst macrophages more or less degenerate apocrine epithelial cells
- Inflammatory cells(polymorphs) variable

Proliferative breast diseases - FNAC:

The spectrum of epithelial proliferative process of breast include usual epithelial hyperplasia, atypical ductal hyperplasia, papilloma, radial scar and sclerosing adenosis. These entities are histologically well defined but there is certain overlap causing interobserver disagreement. The overlap is more important in FNAC smears and is often not possible to separate a particular case precisely within the spectrum. In such cases, definitive diagnosis is left to histology.

Table 5: Cytological features of Proliferative Breast diseases

USUAL DUCTAL HYPERPLASIA	ATYPICAL DUCTAL HYPERPLASIA	LOW GRADE DCIS
Cell rich smears, large sheets of cohesive epithelial cells, few single cells	Cell rich smears, large sheets of cohesive epithelial cells, few single cells	Cell rich smears, large and small sheets of cohesive epithelial cells, few single cells
Cells often in a streaming pattern; focal crowding and overlapping of nuclei, rarely 'holes'	Focal crowding and overlapping of nuclei; 'holes' suggestive of cribriform pattern in some cases	Focal crowding and overlapping of nuclei; 'holes' suggestive of cribriform pattern common; some papillary cell groups.
Nuclear atypia absent or mild	Mild to moderate nuclear atypia	Mild to moderate nuclear atypia
Naked bipolar and myoepithelial nuclei present but may be few; clean background; calcium granules occasionally	Few naked bipolar and myoepithelial nuclei; debris and calcium occasionally present	Naked bipolar and myoepithelial nuclei absent; necrotic debris and calcium often but not invariably present

Fig 11: Usual ductal hyperplasia, Cytology, H&E, 10x

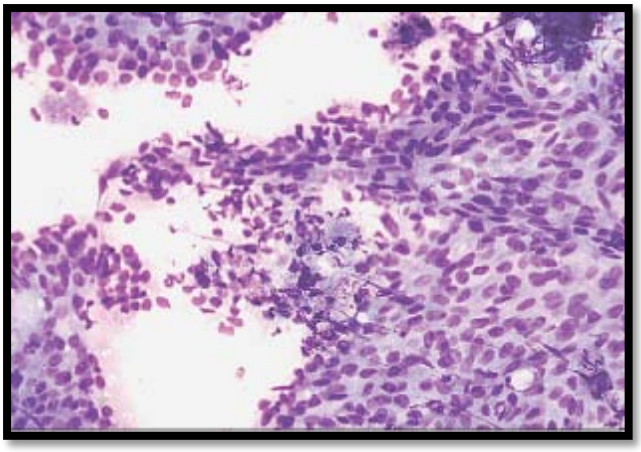
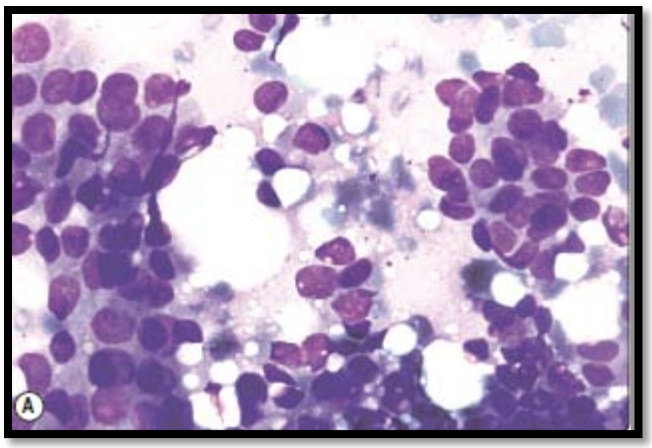


Fig 12: Atypical ductal hyperplasia, Cytology, H&E, 40x



Carcinoma of breast – FNAC:

Fine Needle aspiration of most cancers yield a larger number of cells than normal glandular breast tissue. The overall smear pattern (cellularity, presence or absence of bimodal cell population, cell cohesion, size and shape of cell aggregates, stromal components) is as important to the correct diagnosis as is the cytological detail.

Infiltrating ductal carcinoma of no special type:

Criteria for diagnosis:

- Moderately to highly cellular smear
- Single population of epithelial cells, no myoepithelial cells, no single bare bipolar nuclei
- Variable loss of cell cohesion- irregular cells and single cells
- Single epithelial cells with intact cytoplasm
- Moderate to severe nuclear atypia; enlargement, pleomorphism, irregular nuclear membrane and chromatin
- Fibroblasts and fragments of collagen (stromal desmoplasia) associated with atypical cells
- Intracytoplasmic neolumina in some cases
- Necrosis unusual, more suggestive of DCIS.

Table 6: Robinsons Cytological grading:

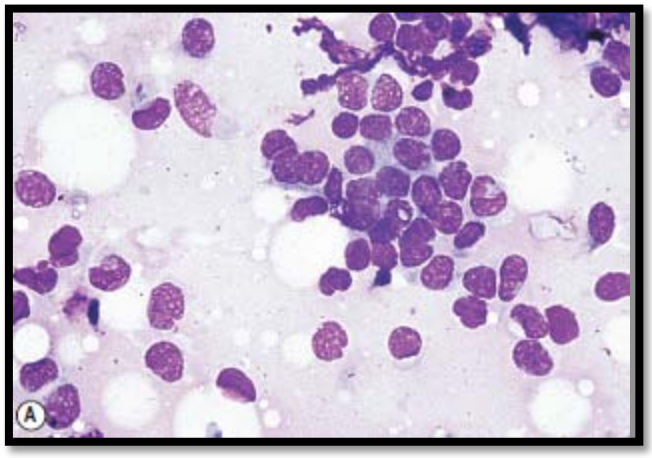
	Score 1	**Score 2**	**Score 3**
Dissociation	Cells mostly in clusters	Mixture of single and cell clusters	Cells mostly in singles
Cell size	1 - 2 x RBC	3 – 4 x RBC	>= 5 x RBC
Cell uniformity	Monomorphic	Mildly pleomorphic	Pleomorphic
Nucleoli	Indistinct	Noticeable	Prominent or pleomorphic
Nuclear margin	Smooth	Folds	Buds or clefts
Chromatin	Vesicular	Granular	Clumped and cleared

Grade 1 – score 6 – 11

Grade 2 – score 12 – 14

Grade 3 – score 15 – 18

Fig 13: Invasive duct carcinoma-NOS, Cytology, H&E, 40x



CONCEPT OF MORPHOMETRY:

Morphometry is the measurement of various cell parameters microscopically. In the last few decades there have been some studies based on computerised morphometry on benign and malignant cells, which can support the diagnosis in many cases and can improve sensitivity and specificity of diagnosis[45]. Morphometry is the quantitative description of structures such as cells, nuclei, and nucleoli.

Morphometric analysis was initially described by Jacobi in 1925, who found that the volume of a normal cell doubles before cell division. Heiberg and Kemp, in 1929 were the first to describe that cancer nuclei are larger than those of normal cells. In the 1950s, there was increased work on

morphological and stereological analysis. In the late 1970s and early 1980s, the application of morphometric analysis in cancer pathology became increasingly popular[46].

MORPHOMETRY – TYPES

- Planimetry- Measurement of geographic features of a cell in the 2-dimensional microscopical image.
- Stereotactic techniques - Estimating the fraction of various tissue components.

  Eg. Inner and outer surface density, as well as the shape and volume of a cell.

- Computer-assisted image analysis (morphometry) provides a new powerful tool for high-precision measurement of several variables characterizing the size and shape of cell nuclei, area measurement in conventional tissue sections.
- Image J software: "Image J" is a freely available java-based public-domain image processing and analysis program developed by the National Institutes of Health (NIH).
- Wayne Rasband is one of the authors of Image J who worked in the National

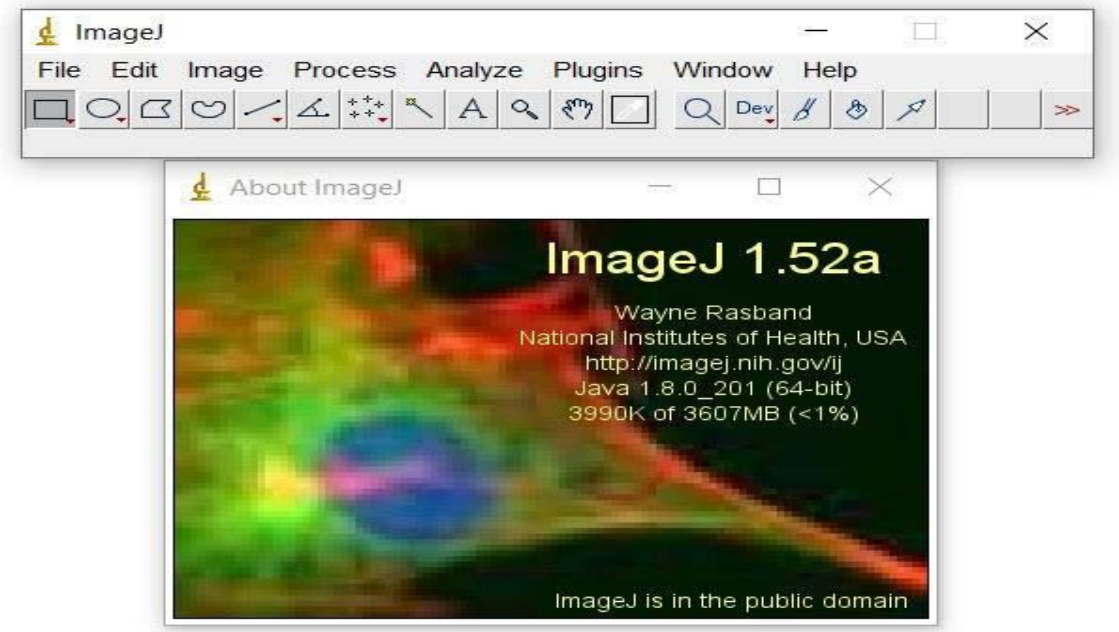
  Institute of Mental Health, Bethesda, Maryland, USA[47].

- He developed the Macintosh-based NIH Image
- Then he started Image J using the Java programming language.

- Various dimensions can be measured using this software. Some of them are perimeter, area.

In the digitalized world, there is a dramatic change in the computer-assisted analytical approaches to the diagnosis of histopathological slides. One such change is image analysis using computer software. Quantitative analysis is used not only in diagnosis but also for research applications. (to understand the pathogenesis of the disease process).

Fig 14: Image J software



Picture Courtesy: Rasband W.S , Image J ,U.S. National Institutes of Health, Bethesda, Maryland, USA.

UTILITY OF MORPHOMETRY ANALYSIS:

The first description of cell nucleus was given by Brown in 1833 and the first microscopic description of human malignant tumours by Muller in 1838[48]. Among the first to apply the microscope to the study of human cells was the French microscopist Donné, whose work culminated in an atlas published in 1845[49].

Many studies were conducted in analyzing the nuclear features by morphometry in other organs like breast, exfoliated buccal mucosal cells, squamous neoplasms, colon and thyroid.

As early as 1890, David von Hansemann postulated all cancers are characterised by asymmetrical cell division that ultimately leads to cancer[50].Sterobe in 1892 found asymmetrical mitoses in regenerative tissues and non-malignant tumours[51].

Currently, computer-assisted image analysis (nuclear morphometry) provides a new powerful tool for high-precision measurement of several variables characterising the size and shape of cancer cell nuclei in conventional tissue sections[52].

In 1982, Diamond and associates introduced nuclear morphometry to aid in prediction of prognosis among patients with prostate cancer[53]**.** He and his colleagues observed that nuclear roundness was very useful in separating long survivors among stage B patients from those who develop metastasis.

They observed no overlap in nuclear roundness between the two groups. Since then, many histological studies have used nuclear morphometry to predict prognosis in patients with prostate cancer[54].

Eichenberger and associates, in 1987, calculated 12 shape descriptors including nuclear roundness, ellipticity factors, and concavity factors. They used discriminate analysis to select the major morphometric parameters which best distinguished patients with good or poor prognosis. Elliptical shape measurement was found to be the best in this respect[55].

In 1989, Partin et al developed a morphometric evaluation system called Hopkin's Morphometry System and compared 15 different shape descriptors in stage A2 prostate cancer. These were analyzed by 17 different statistical tests. The best separation was provided by the lower quartile analysis of the ellipticity shape descriptor ($p<0.01$). These studies revealed that the elliptical shape of the nuclei is very important as a prognostic factor[56].

Buhmeida et al, in 2000, revealed that the nuclear size features are useful in distinguishing between different atypia groups of the prostate gland in fine needle aspiration biopsies, particularly if the sample-associated means of the size features (area, diameter and perimeter, short and long axes) are used for the interpretation of data. The study suggested if the upper range limit of sample-associated mean areas of nuclei is below 27µm2, it is most probable that we are dealing with benign cells. If the upper range limit is above 39µm2,

it is possible that there are malignant cells in the sample. However, values above 52µm2 represent malignant samples with certainty. Further studies will be necessary for associating nuclear size features with Gleason grades[57].

The results of the study by Martinez- Jabaloyas et al in 2002, revealed that mean nuclear area and other factors proved to have a prognostic value in the univariate analysis and concluded that nuclear morphometry in the primitive tumor provides independent prognostic information in survival analysis for patients with metastatic prostate cancer[58].

Morphometry has been used mainly to measure the surface area and subsequently the fractal dimension of liver lesions. Initially, morphometry was based on the manual determination of regions of interest before some procedures became automated. Dioguardi *et al.* in 2008,standardized metrical evaluation of the geometric properties of the parenchyma, inflammation, fibrosis, and alterations in liver tissues.[59]

Prasad H et al., studied morphologic and cytomorphometric analysis of exfoliated buccal mucosal cells in 50 diabetic patients with 5 controls. Smears were stained by Papanicoloau method and using a micrometer mean values of nuclear diameter, cell diameter, cytoplasmic diameter and nucleus: cytoplasmic ratio were obtained and found that diabetes produces definite morphological and morphometric changes in exfoliated buccal cells[60]

NUCLEAR MORPHOMETRY IN BREAST ASPIRATES

In 1987, Wittikend et al., studied the value of nuclear morphometry in the preoperative fine needle aspiration (FNA) cytologic diagnosis of mammary lesions, investigated and correlated with the lymph node status of the patients. The results suggested that nuclear perimeter can be used as an additional parameter not only for the FNA cytologic diagnosis of breast cancer, but also for the estimation of patients' prognosis[61].

Abdalla Fathi *et al.*[62] from their study in 2008 concluded a mean nuclear area of 64-82 μm^2 for benign cases and 72-163 μm^2 for malignant cases. Abdalla *et al.*also showed that clearly reduced cohesiveness was associated with larger nuclear size

Shivani Kalhan et al., in 2010, from their study concluded that nuclear morphometry successfully differentiated between benign and malignant aspirates and correlated significantly with cytologic grades. Morphometry was especially useful in the diagnosis of atypical ductal hyperplasia and ductal carcinoma *in situ*. Useful parameters were mean nuclear area, long axis, short axis and total run length. Cytohistologic correlation was 83.3%, 88.9% and 88.9% for cytological grades 1, 2 and 3 respectively[63].

In a study by Narasimha et al., in 2013, the nuclear area, perimeter, diameter, compactness, and concave points were found to be statistically significant ($P < 0.05$) parameters in differentiating benign, and malignant breast aspirates[64].

In 2015,Laishram S and Shariff S studied the nuclear morphology with regard to nuclear diameter; nuclear area; coefficient of variation of nuclear area; nuclear/cytoplasmic ratio and the ratio of largest to smallest nuclear diameter (L:S ratio) on 60 breast FNAC and found nuclear parameters to be significantly higher in the malignant lesions when compared to benign lesions[65].

Kashyap et al., in 2017, studied. Nuclear morphometry on cytology of benign and malignant breast lesions and found that nuclear morphometry could differentiate between benign and malignant aspirates with a gradually increasing nuclear size parameters like nuclear area, equivalent diameter, minimum feret, maximum feret, and perimeter[66].Cut-off values of 31.93 μm^2, 6.325 μm, 5.865 μm, 7.855 μm, and 21.55 μm for mean nuclear area, equivalent diameter, minimum feret, maximum ferret, and perimeter, respectively, were derived between benign and malignant cases, which could correctly classify 7 out of 8 ADH cases.

In the study by Parmer D and associates in 2015, mean nuclear area, perimeter, diameter, long axis, and short axis were highly significant in differentiating hyperplasia from carcinoma. These parameters were found to be statistically significant ($P < 0.0001$)[67].

In the same year, 2015, Yadav et al., studied that morphometric parameters revealed a progressive and statistically significant increase in values from benign to borderline to malignant cases. The morphometric parameters

studied were mean nuclear area, mean cytoplasmic area, perimeter and nuclear/cytoplasm ratio. On comparing benign with borderline and malignant, all the four parameters were found to be statistically significant with a p-value of less than 0.05 while on comparing borderline with malignant, two of the parameters, i.e. mean nuclear area and mean cytoplasmic area, were statistically significant.

MATERIALS AND METHODS

This study is a prospective and retrospective study of two years from July 2018 to July 2020. A total of 60 cases received in the Department of Pathology, Govt. Stanley Medical College were studied.

Study population: Patients from general population coming to Govt. Stanley medical college hospital for FNAC of breast masses

Sample size: 60 cases

Study duration: 2 year

Inclusion criteria: All benign breast neoplasms and ductal carcinomas with confirmed histopathological correlation.

Exclusion criteria: Lobular, medullary, and metaplastic carcinomas

Methodology:

- Informed consent was obtained from the patients for FNAC
- A concise clinical history, examination and details of relevant investigations were also obtained.
- Fine needle aspiration sample were obtained from cases of breast masses.
- Cytology was done to categorise breast aspirates into benign and malignant.
- 60 cases were selected and the cases were grouped under 4 categories as

1a – Fibroadenoma (20 cases)

1b – Fibrocystic change (13 cases)

1c – Proliferative breast diseases (7 cases)

1d – Malignancy (20 cases)

- A microscope with an × 2.5 ocular and an × 40 objective was used to visually select a field for analysis. A 640 × 400 pixel digital image of the field was produced by a camera.
- The images were analyzed using the Image J 1.44C morphometric software for image processing, and analysis (JAVA) developed by the National Institute of Health, USA.
- Around 50 nuclei were studied for each case.
- **ONLY CASES WITH CONFIRMED HISTOPATHOLOGICAL DIAGNOSIS WERE INCLUDED IN THE STUDY**

FNAC – Procedure:

1. The patients with palpable breast lump referred from general surgery department in the institution for FNAC were involved in the study.
2. The case history of the patient was recorded, including detail history of pain, nipple discharge, ulceration of nipple, and duration of lesion.
3. The examination of breast lump was done with recording of size and site of lump, consistency, fixation to skin and underlying tissue, and retraction of nipple along with regional lymph node involvement.

4. Consent was taken after due explanation of the procedure and its benefit to the patients.

5. Procedure was done using 24-gauge needle fitted on 10 ml disposable syringe in syringe holder.

6. The wet smear was fixed with iso propyl alcohol mixture and stained with hematoxylin and eosin

Staining procedure:

a) **Fixation:** The cytology smears are fixed in 95% ethyl alcohol or in other substitutes for a minimum of 15 minutes.

b) **Nuclear staining:** It is done by using haematoxylin stain. Harris haematoxylin or its modified form is used in Papanicolaou staining in regressive method, in which we deliberately over stain with haematoxylin and remove the excess stain by using a differentiating solution such as acid alcohol (0.05% HCl in 70% ethyl alcohol) or 0.05% aqueous solution of HCl alone. As haematoxylin is used in an acid pH, a pink colour will form and it is not stable. In order to make it stable, the compound is brought to alkaline pH (bluing) by treating with a weak alkaline solution. Running tap water which is slightly alkaline (pH 8) is used as bluing solution in small laboratories. In the present study the smears were stained using Hematoxylin

c) **Cytoplasmic staining:** Cytoplasmic stains are OG-6 and EA-36. Both are synthetic stains and OG-6 is a monochrome stain while EA-36 is a

polychrome stain. In the presents study the smears were stained in eosin for 30 seconds.

d) **Dehydration:** Rinse the smears in absolute alcohol for two or three changes for the removal of water. Smears left in rinses for long will lose too much stain.

Morphometry analysis:

Nuclear morphometric analysis was carried out using the Image J 1.44C morphometric software for image processing and analysis. Around 50 nuclei/ case were studied and the following nuclear features were analyzed:

- Nuclear area (the area within the outlined nuclear perimeter)
- Perimeter (the distance around the nuclear border)
- Diameter (diameter of the circle with the same area as the outlined nucleus.)
- Compactness of the cell nuclei calculated using the formula: Perimeter2/area.

The computer calculated the mean, standard deviation, and range for all the nuclear features.

Statistical Analysis:

Statistical analysis was done using SPSS software version 20. The following statistical analysis were done.

- Descriptive Statistics
- Percentages
- Chi Square test.
- Independent T-test

The results were considered significant at p-value <0.05

Ethical Consideration:

- The patient's confidentiality was maintained.
- There was no extra cost charged to the patient.
- For prospective cases, written informed consent was obtained

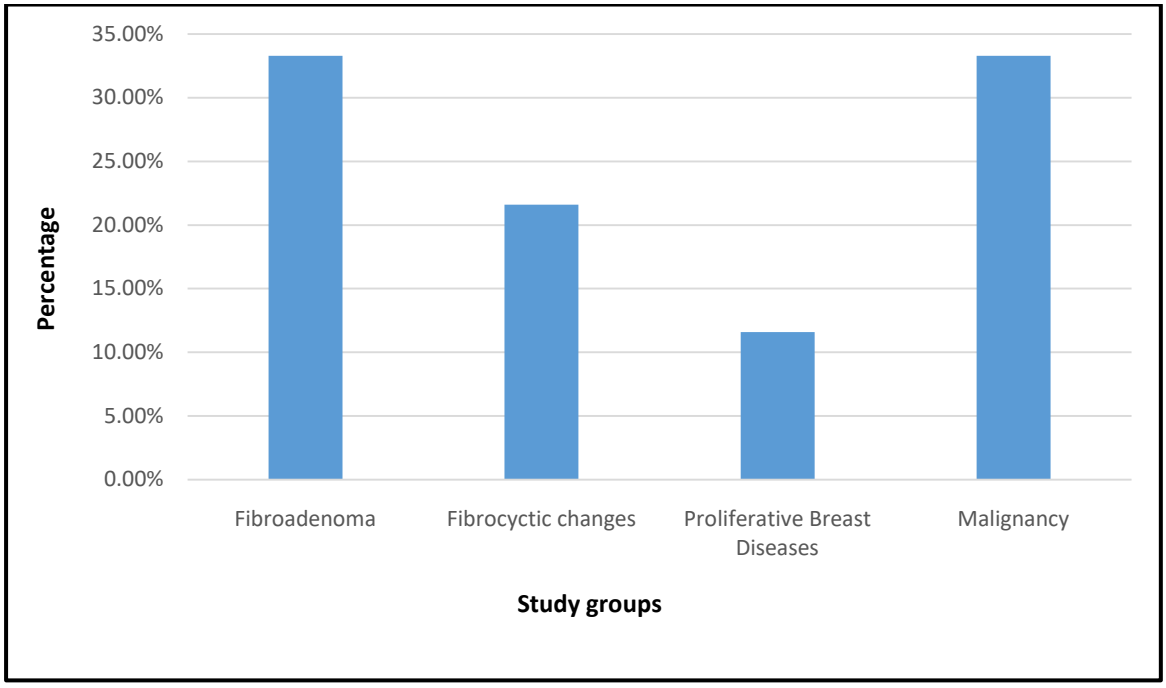
OBSERVATION AND RESULTS

In the present study, a total of 60 cytological smears of breast lumps were studies from July 2018 to July 2020, which included fibroadenoma (20 cases), fibrocyctic diseases (13 cases), proliferative breast diseases (7 cases) and malignancy (15 cases).

Table 7: Descriptive analysis of study groups in study population (N=60)

Study group	Frequency	Percentage
Fibroadenoma	20	33.3%
Fibrocystic change	13	21.6%
Proliferative Breast Diseases	7	11.6%
Malignancy	20	33.3%

Chart 1: Bar chart for study groups in the study population (N=60)

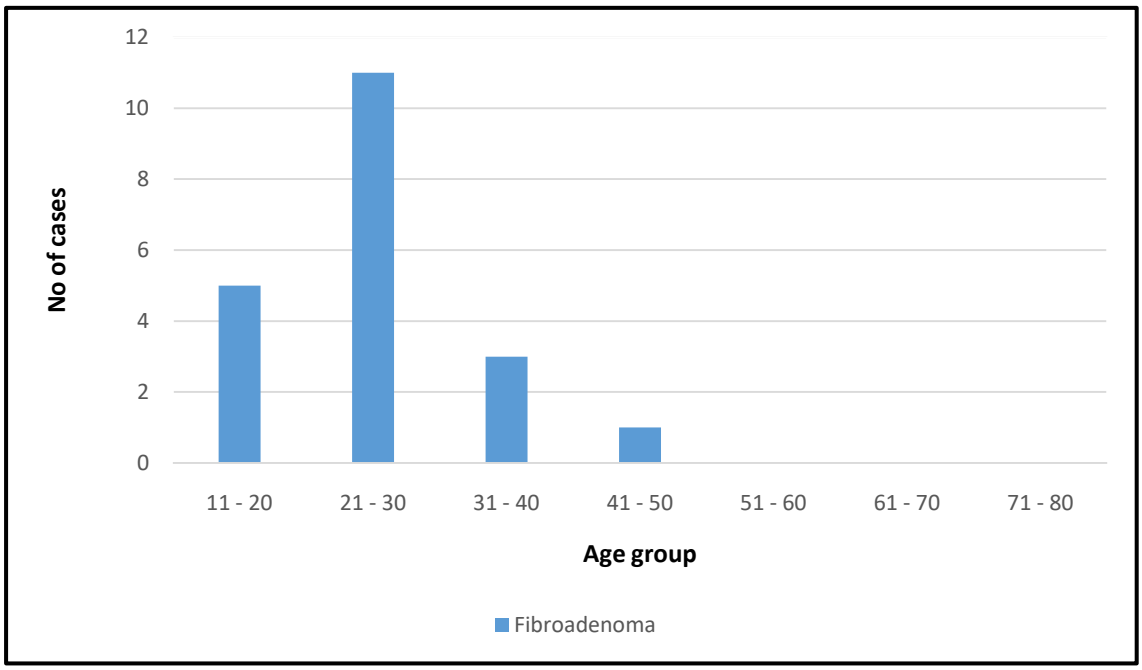


The age of the study population ranged from 16 years to 72 years. The age distribution among the study groups is as follows

Table 8: Age distribution in Fibroadenoma: (N = 20)

Age group (in years)	Frequency	Percentage
11 – 20	5	25%
21 – 30	11	55%
31 – 40	3	15%
41 – 50	1	5%
>50 years	0	0%
Total	20	100%

Chart 2: Age distribution in Fibroadenoma (N = 20)

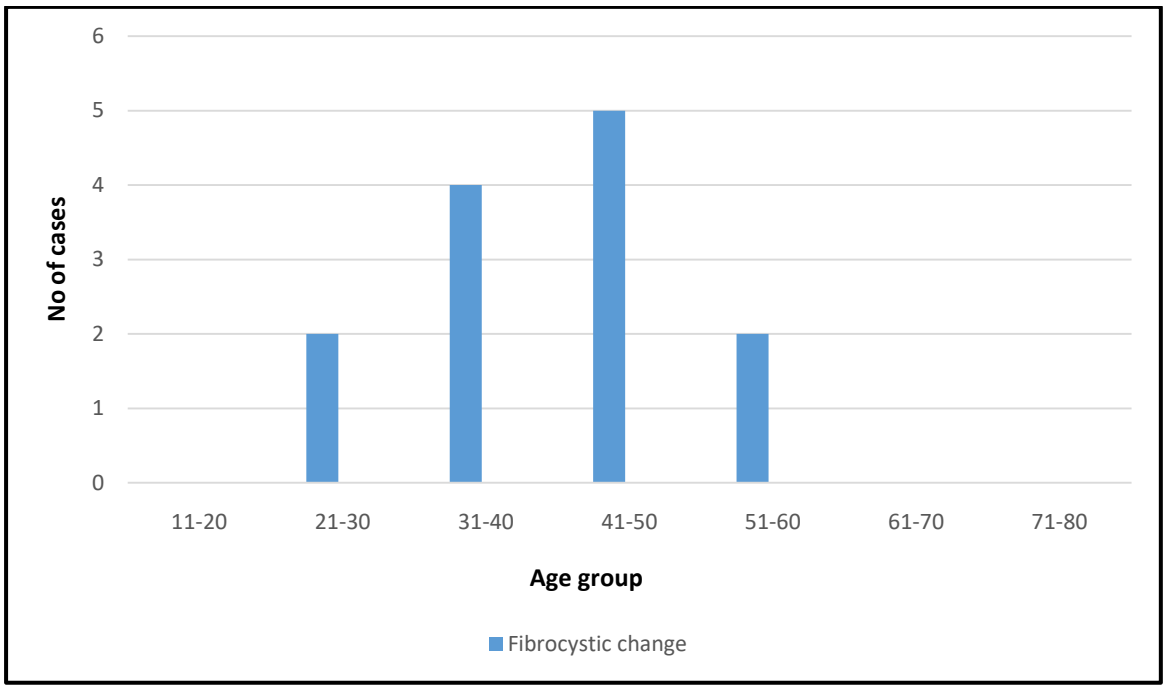


The chart shows age distribution in the fibroadenoma group. Fibroadenoma was predominantly found between 21 to 30 years of age.

Table 9: Age distribution in Fibrocystic change: (N = 13)

Age group (in years)	Frequency	Percentage
11 – 20	0	0%
21 – 30	2	15.38%
31 – 40	4	30.76%
41 – 50	5	38.46%
51 – 60	2	15.38%
>60 years	0	0%
Total	13	100%

Chart 3: Age distribution in Fibrocystic change (N = 13)

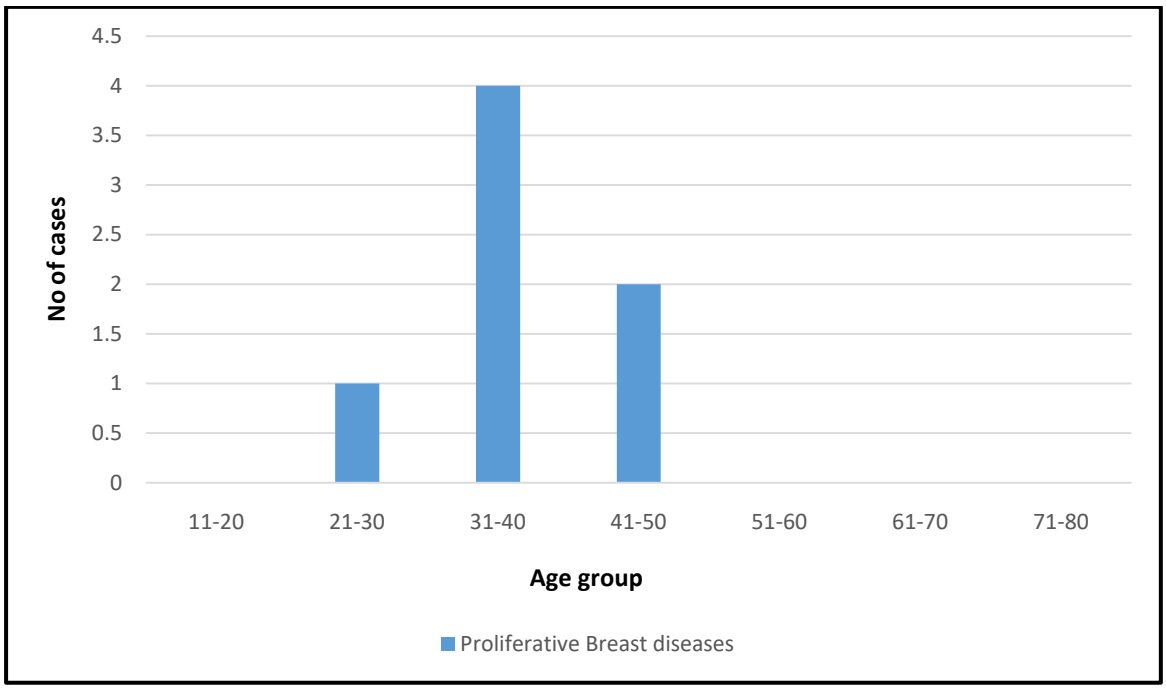


In the present study, Fibrocystic change was predominantly found in 41 to 50 years of age.

Table 10: Age distribution in Proliferative Breast diseases: (N = 7)

Age group (in years)	Frequency	Percentage
11 - 20	0	0%
21 – 30	1	14.28%
31 - 40	4	57.14%
41 - 50	2	28.57%
51 - 60	0	0%
>60 years	0	0%
Total	7	100%

Chart 4: Age distribution in Proliferative Breast diseases (N = 7)

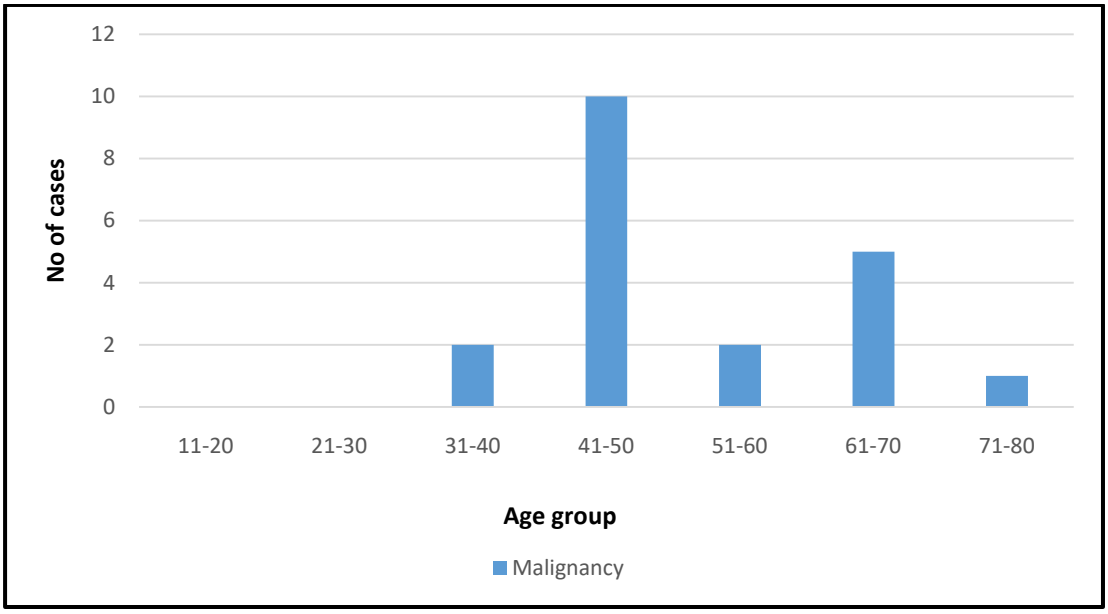


In the present study, proliferative breast diseases were predominantly found in 31 to 40 years of age.

Table 11: Age distribution in Malignancy: (N = 20)

Age group (in years)	Frequency	Percentage
11 - 20	0	0%
21 – 30	0	0%
31 - 40	2	10%
41 - 50	10	50%
51 - 60	2	10%
61 – 70	5	25%
71 - 80	1	5%
Total	7	100%

Chart 5: Age distribution in Malignancy (N = 20)

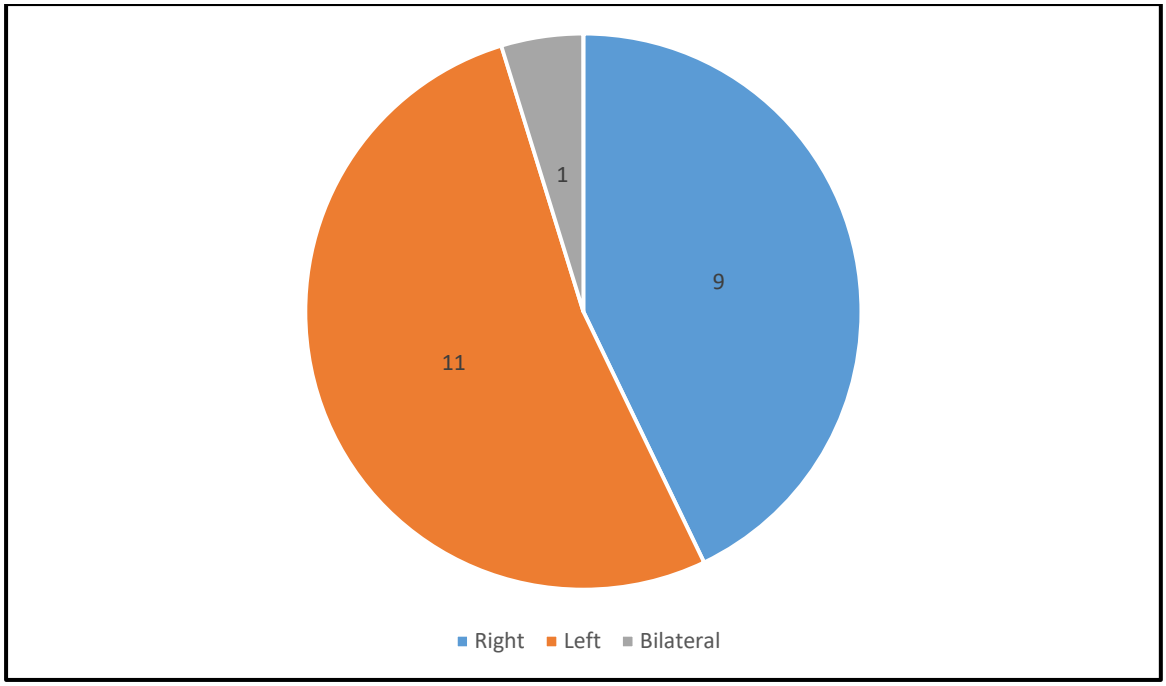


In the present study, malignancy was predominantly found in 41 to 50 years of age.

Table 12: Laterality of the lesion in Fibroadenoma: (N=20)

Laterality	Frequency	Percentage
Right	9	45%
Left	11	55%
Bilateral	1	5%

Chart 6: Laterality of the lesion in Fibroadenoma: (N=20)

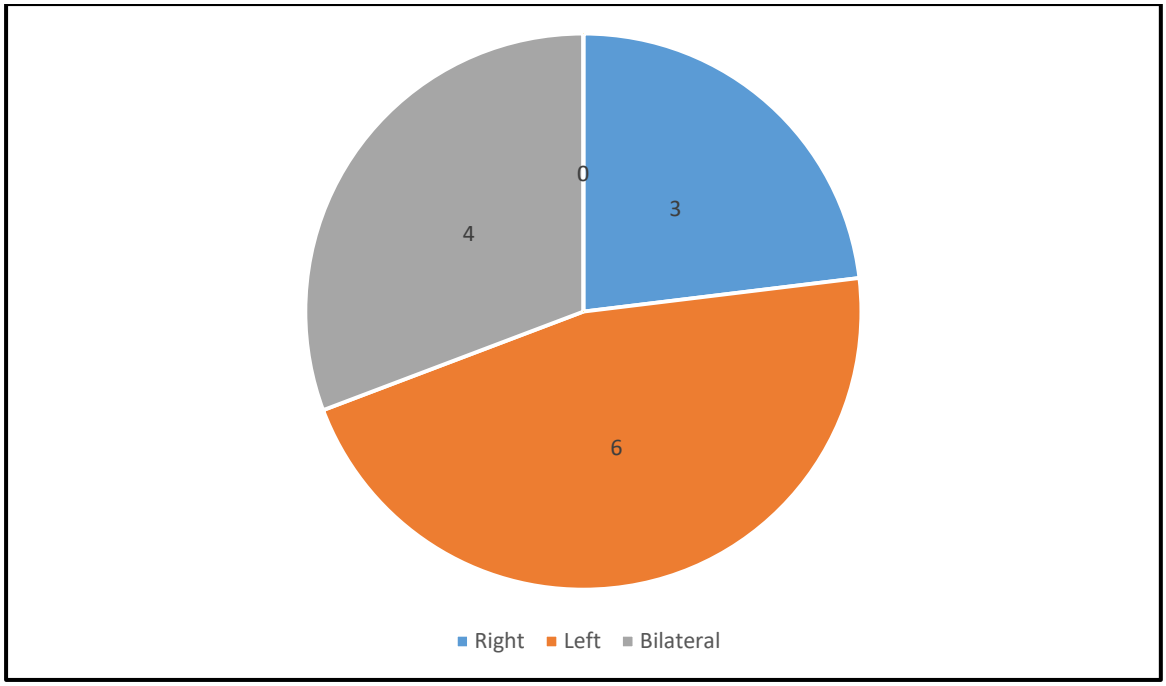


In the present study, Fibroadenoma was predominantly found in right breasts.

Table 13: Laterality of the lesion in Fibrocystic change: (N=13)

Laterality	Frequency	Percentage
Right	3	23.07%
Left	6	46.15%
Bilateral	4	30.76%

Chart 7: Laterality of the lesion in Fibrocystic change: (N=13)

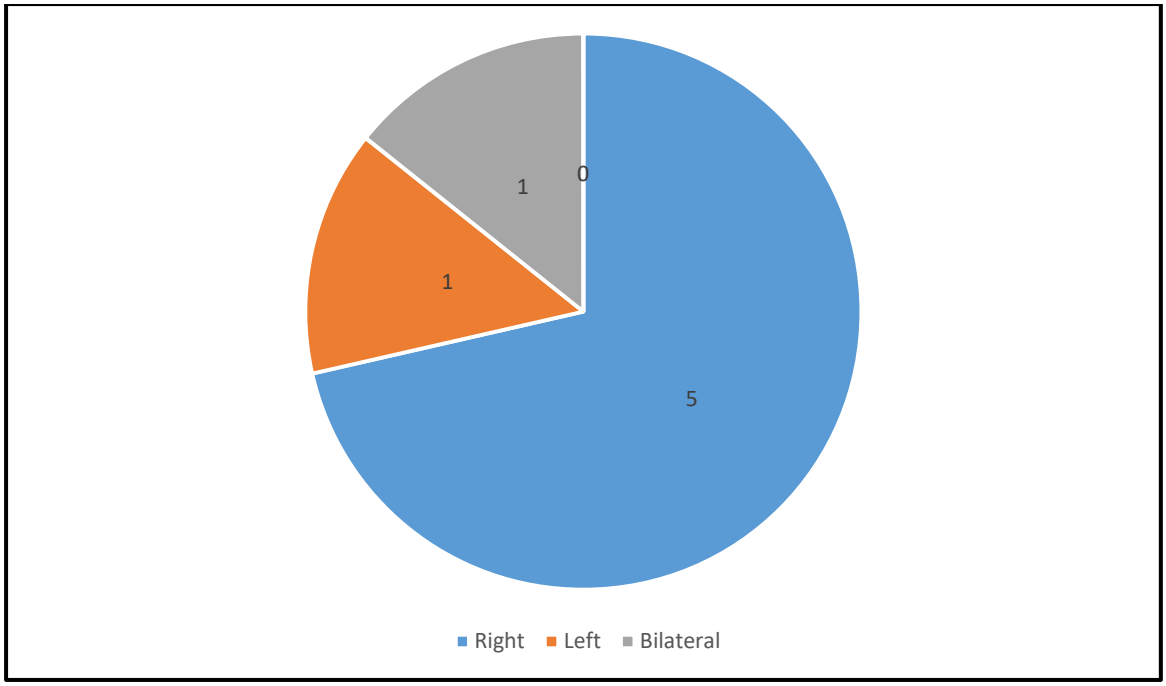


In the present study, Fibrocystic change was predominantly found in left breasts.

Table 14: Laterality of the lesion in Proliferative breast diseases: (N=7)

Laterality	Frequency	Percentage
Right	5	71.42%
Left	1	14.28%
Bilateral	1	14.28%

Chart 8: Laterality of the lesion in Proliferative Breast diseases: (N=7)

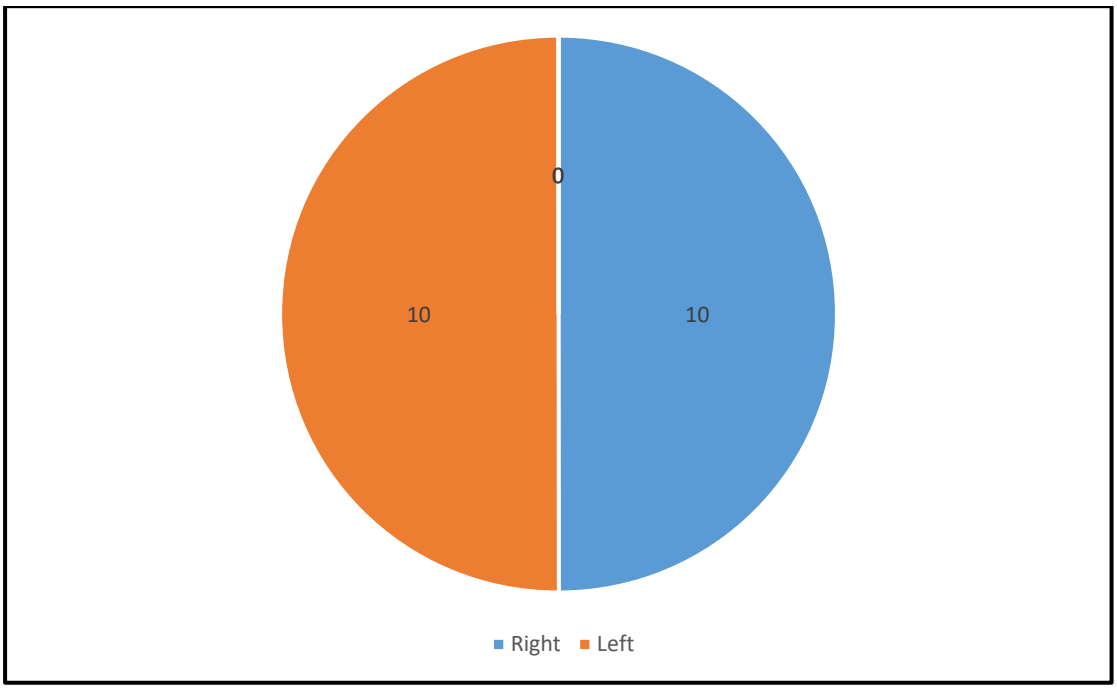


In the present study, proliferative breast diseases were predominantly encountered in right breasts.

Table 15: Laterality of the lesion in Malignancy: (N=20)

Laterality	Frequency	Percentage
Right	10	50%
Left	10	50%
Bilateral	0	0%

Chart 9: Laterality of the lesion in Malignancy: (N=20)



In the present study, malignancy was encountered equally in both the breasts.

Table 16: Atypia in proliferative breast diseases: (N =7)

Atypia	Frequency	Percentage
Present	**2**	**28.57%**
Absent	**5**	**21.42%**

Chart 10: Atypia in proliferative breast diseases: (N =7)

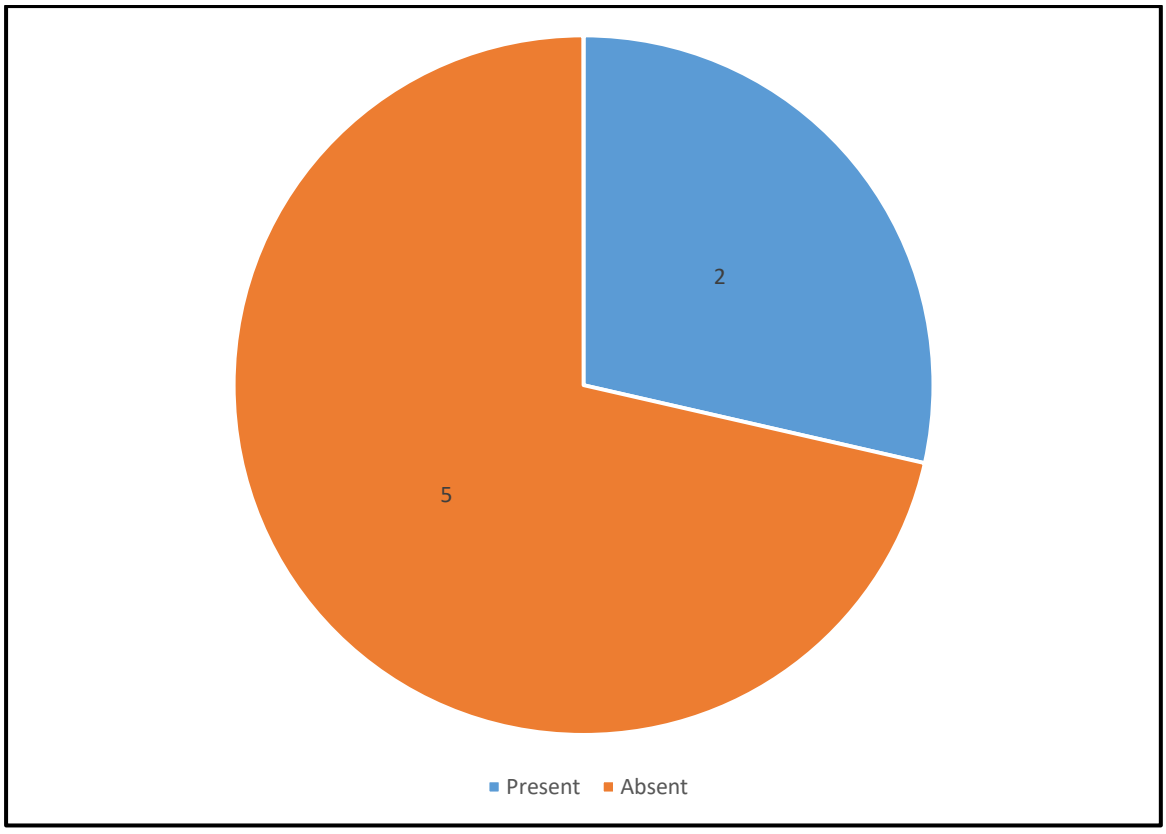
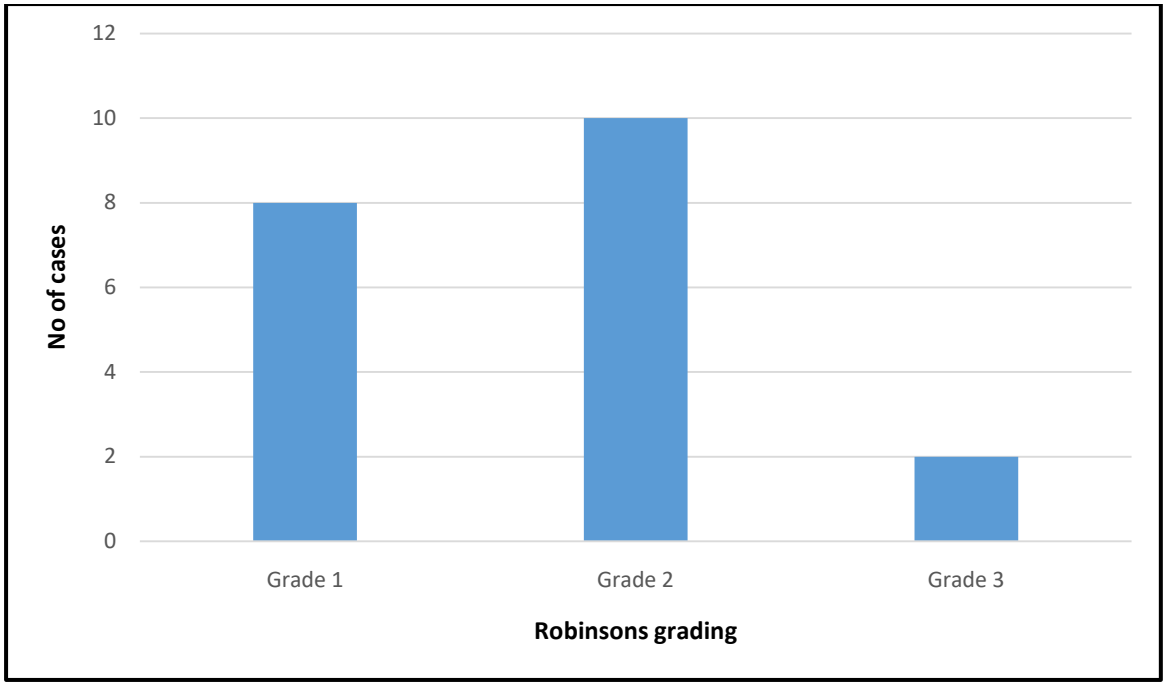


Table 17: Grade distribution of malignant cases: (N = 20)

Robinsons grading	Frequency	Percentage
Grade 1	8	40%
Grade 2	10	50%
Grade 3	2	10%

Chart 11: Grade distribution among malignant cases:



The nuclear parameters were calculated for 20 cases of fibroadenoma and mean values were calculated for all the four parameters.

Table 18: Nuclear parameters in Fibroadenoma: (N = 20)

Parameters	Minimum	Maximum	Range	Mean	Standard deviation
Area(μm²)	0.016	0.038	0.022	0.025	0.006789
Perimeter(μm)	0.481	0.717	0.236	0.607	0.066096
Diameter(μm)	0.180	0.282	0.102	0.225	0.028039
Compactness	10.05	18.78	8.73	15.20	14.776

The nuclear parameters were calculated for 13 cases of fibrocystic changes and the mean values were calculated for all the four parameters.

Table 19: Nuclear parameters in Fibrocystic change: (N= 13)

Parameters	Minimum	Maximum	Range	Mean	Standard deviation
Area(μm²)	0.025	0.048	0.023	0.03585	21.8996
Perimeter(μm)	0.080	0.847	0.767	0.66231	29.2969
Diameter(μm)	0.243	0.304	0.061	0.275	7.5951
Compactness	0.139	16.803	16.664	13.48	31.2233

The nuclear parameters were calculated for 7 cases of proliferative breast diseases and the mean values were calculated for all the four parameters.

Table 20: Nuclear parameters in Proliferative Breast diseases: (N=7)

Parameters	Minimum	Maximum	Range	Mean	Standard deviation
Area(μm²)	0.042	0.111	0.069	0.071	0.211
Perimeter(μm)	0.087	1.752	1.665	1.031	0.499
Diameter(μm)	0.299	0.478	0.179	0.369	0.054
Compactness	44.96	45.140	44.96	17.19	13.52

The nuclear parameters were calculated for 20 cases of malignancy and the mean values were calculated for all the four parameters.

Table 21: Nuclear parameters in Malignancy: (N = 20)

Parameters	Minimum	Maximum	Range	Mean	Standard deviation
Area(μm²)	0.147	1.059	0.912	0.377	0.2868
Perimeter(μm)	1.409	3.795	2.386	2.313	0.8232
Diameter(μm)	0.451	1.318	0.867	0.821	0.3097
Compactness	13.23	36.98	23.74	16.56	6.4933

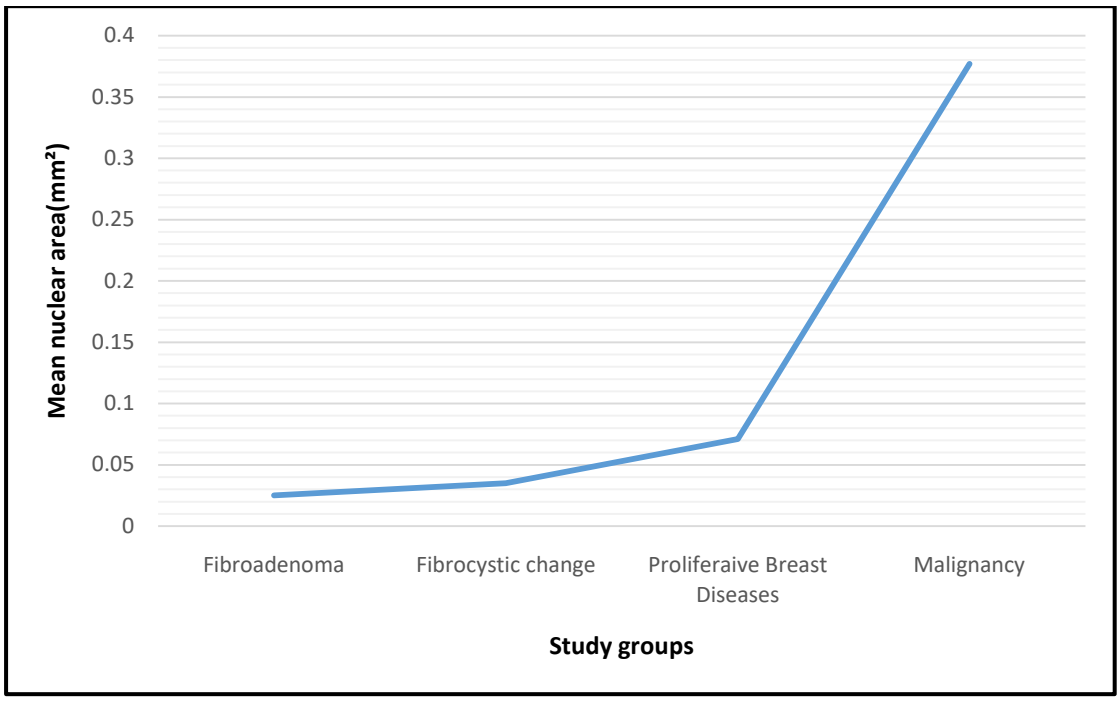
The mean nuclear area was compared between the four study groups.

Table 22: Comparison of nuclear area in between the study groups:

Study groups	Mean nuclear area(mm^2)
Fibroadenoma	**0.025**
Fibrocystic change	**0.035**
Proliferative Breast diseases	**0.071**
Malignancy	**0.377**

- The mean nuclear area increased in fibrocystic change as compared to fibroadenoma
- The mean nuclear area increased in proliferative breast disease as compared to fibrocystic change.
- There was a significant increase in nuclear area in malignancy as compared to proliferative breast diseases.

Chart 12: Comparison of nuclear area in between the study groups:



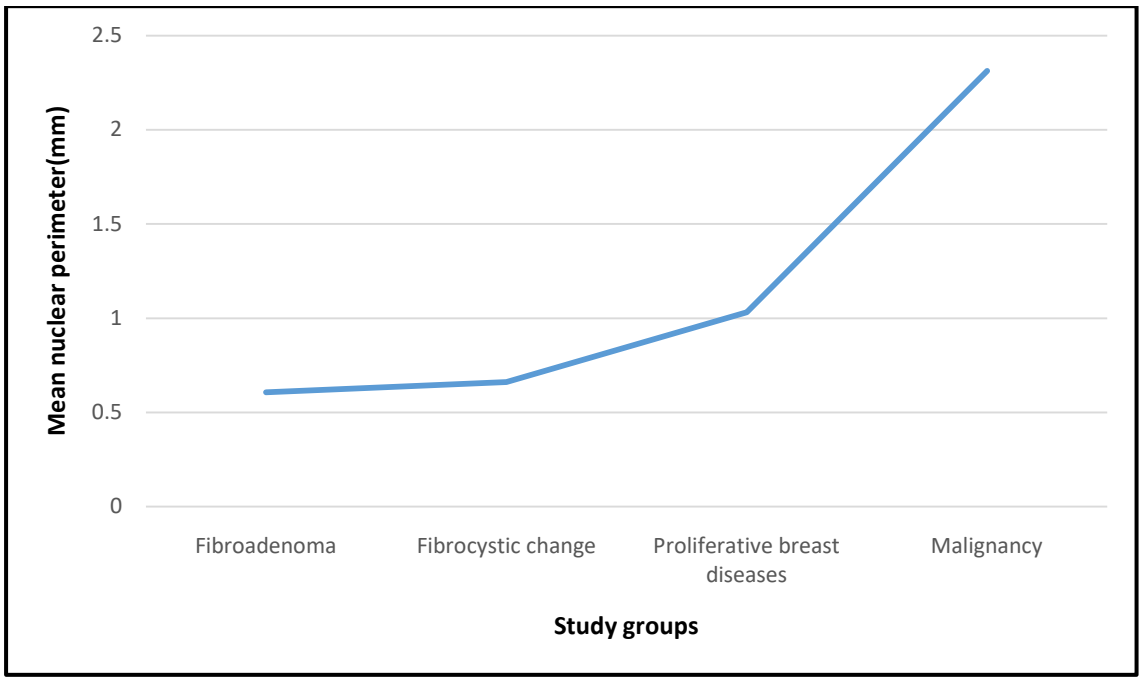
The chart shows a consistent increase in mean nuclear area from fibroadenoma to fibrocystic change to proliferative breast diseases and from proliferative breast disease to malignancy.

Table 23: Comparison of nuclear perimeter in between the study groups:

Study groups	Mean nuclear perimeter(mm)
Fibroadenoma	**0.607**
Fibrocystic change	**0.662**
Proliferative Breast diseases	**1.031**
Malignancy	**2.313**

- The mean nuclear perimeter increased in fibrocystic change as compared to fibroadenoma
- The mean nuclear perimeter increased in proliferative breast disease as compared to fibrocystic change.
- There was a significant increase in nuclear perimeter in malignancy as compared to proliferative breast diseases.

Chart 13: Comparison of nuclear perimeter in between the study groups:



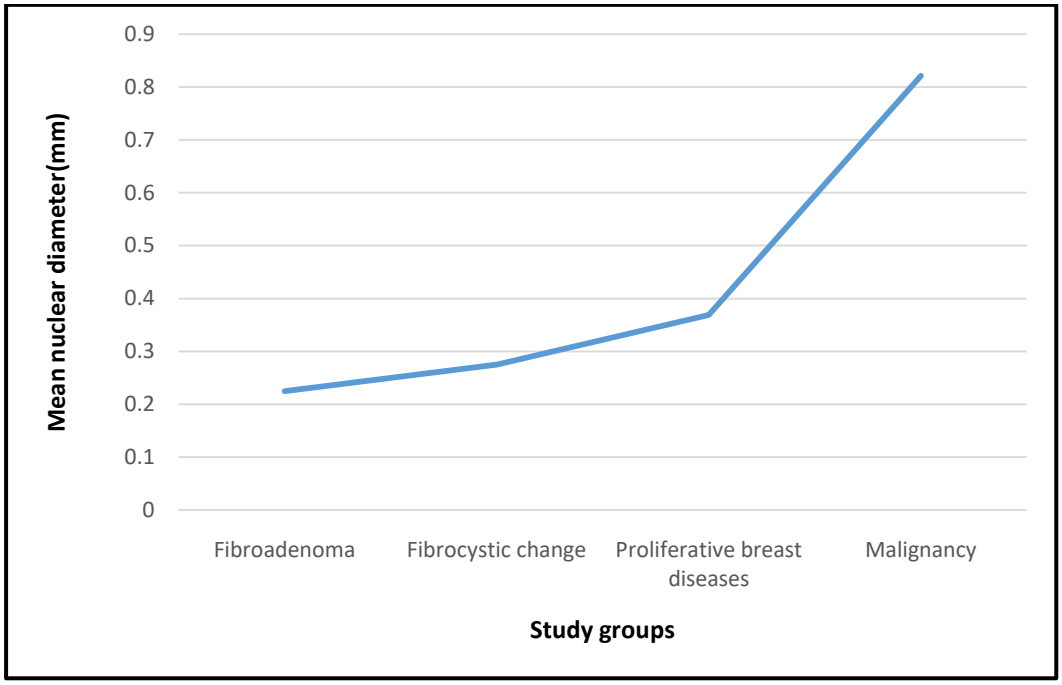
The chart shows a consistent increase in mean nuclear perimeter from fibroadenoma to fibrocystic change to proliferative breast diseases and from proliferative breast disease to malignancy

Table 24: Comparison of nuclear diameter in between the study groups:

Study groups	Mean nuclear diameter(mm)
Fibroadenoma	**0.225**
Fibrocystic changes	**0.275**
Proliferative Breast diseases	**0.369**
Malignancy	**0.821**

- The mean nuclear diameter increased in fibrocystic change as compared to fibroadenoma
- The mean nuclear diameter increased in proliferative breast disease as compared to fibrocystic change.
- There was a significant increase in nuclear diameter in malignancy as compared to proliferative breast diseases.

Chart 14: Comparison of nuclear diameter in between the study groups:



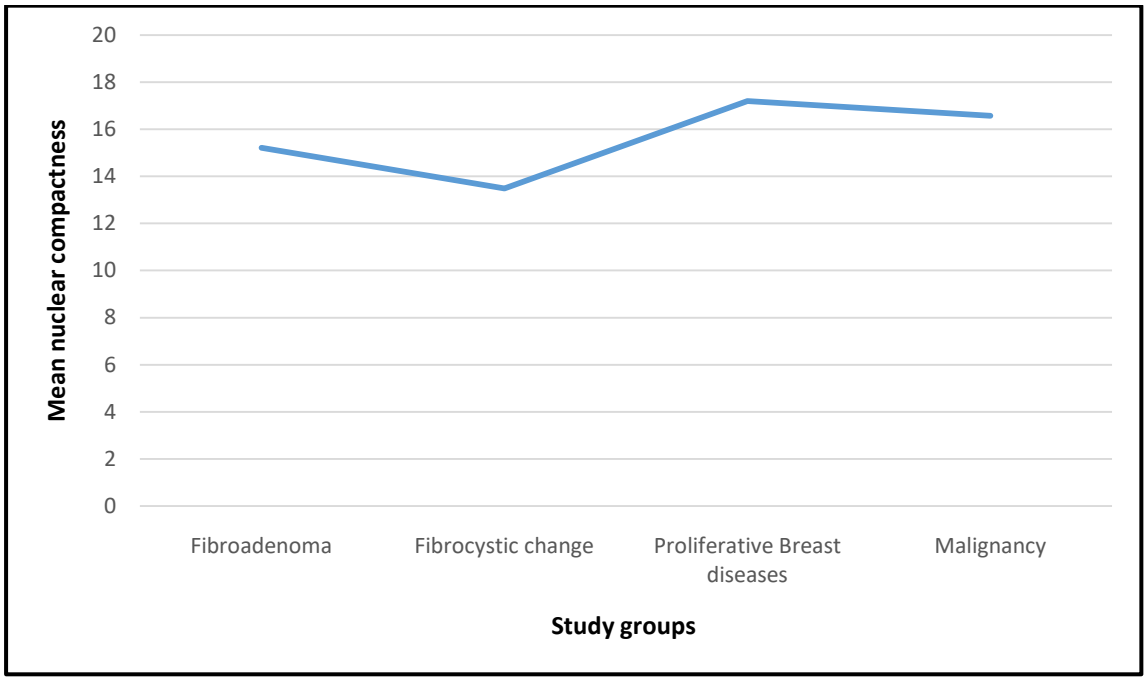
The chart shows a consistent increase in mean nuclear perimeter from fibroadenoma to fibrocystic change to proliferative breast diseases and from proliferative breast disease to malignancy

Table 25: Comparison of nuclear compactness in between the study groups

Study groups	Mean nuclear compactness
Fibroadenoma	**15.20**
Fibrocystic change	**13.48**
Proliferative Breast diseases	**17.19**
Malignancy	**16.56**

- The mean nuclear compactness decreased in fibrocystic change as compared to fibroadenoma
- The mean nuclear compactness increased in proliferative breast disease as compared to fibrocystic change.
- There was a decrease in nuclear perimeter in malignancy as compared to proliferative breast diseases.

Chart 15: Comparison of nuclear compactness in between the study groups:



There is no consistent increase or decrease in mean nuclear compactness in between the study groups.

Table 26: Comparison of nuclear parameters between fibroadenoma and fibrocystic change

Nuclear parameters	Fibroadenoma	Fibrocystic change	P value
Area(mm^2)	0.025	0.035	0.00*
Perimeter(mm)	0.607	0.662	0.342
Diameter(mm)	0.225	0.275	0.00*
Compactness	15.20	13.48	0.192

***Statistically significant**

All the four parameters are compared between fibroadenoma and fibrocystic change. Of the four parameters, nuclear area and nuclear diameter were found to be statistically significant.

Table 27: Comparison of nuclear parameters between fibrocystic change and proliferative breast diseases

Nuclear parameters	Fibrocystic change	Proliferative Breast disease	P value
Area(mm^2)	0.035	0.071	0.004*
Perimeter(mm)	0.662	1.031	0.102
Diameter(mm)	0.275	0.369	0.003*
Compactness	13.48	17.19	0.503

***Statistically significant**

All the four parameters are compared between fibrocystic change and proliferative breast diseases. Of the four parameters, nuclear area and nuclear diameter were found to be statistically significant.

Table 28: Comparison of nuclear parameters between proliferative breast disease and malignancy:

Nuclear parameters	Proliferative Breast disease	Malignancy	P value
Area(mm^2)	**0.071**	**0.377**	**0.004***
Perimeter(mm)	**1.031**	**2.313**	**0.102**
Diameter(mm)	**0.369**	**0.821**	**0.003***
Compactness	**17.19**	**16.56**	**0.503**

***Statistically significant**

All the four parameters are compared between proliferative breast diseases and malignancy. Of the four parameters, nuclear area and nuclear diameter were found to be statistically significant.

Fig 15: Measurement of nuclear area and nuclear perimeter using image j

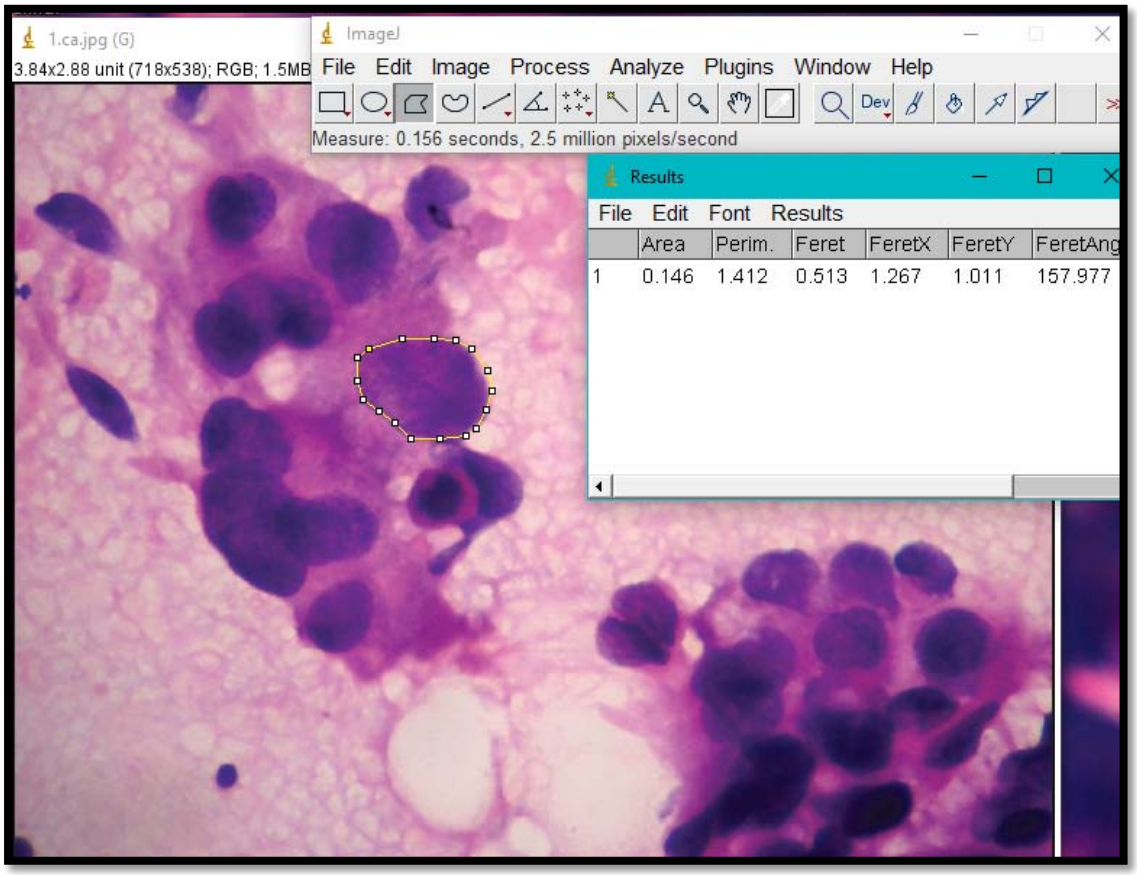
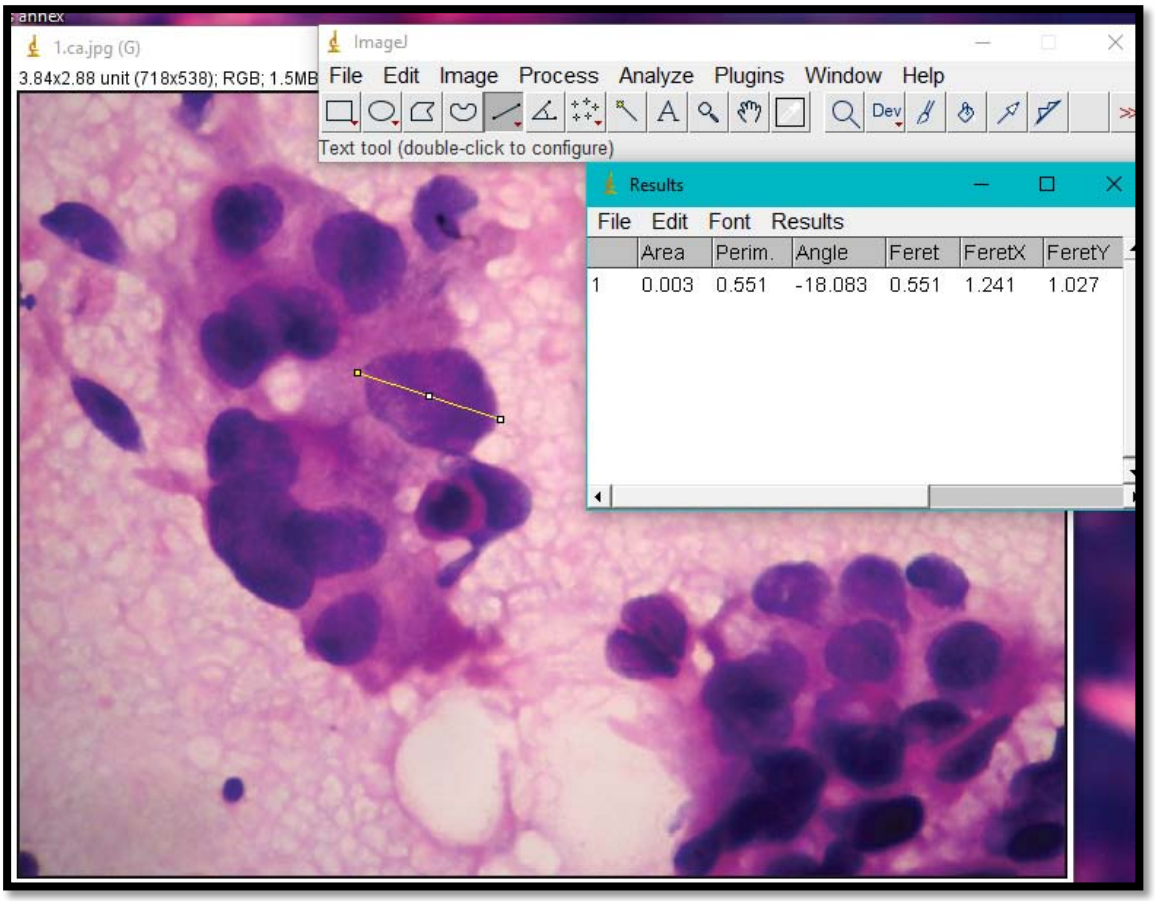


Fig 16: Measurement of nuclear diameter using image j



DISCUSSION

In this study, a total of cases of cytological aspirates from breast masses were studied. The study population included four study groups – Fibroadenoma (20 cases), Fibrocystic change (13 cases), Proliferative breast diseases (7 cases) and malignancy (20 cases) after confirmed histopathological diagnosis.

In the present study the nuclear parameters showed a gradual increase from benign to malignant cases except nuclear compactness. This was in consistent with study by Kashyap et al[66] .who also showed a gradual increase in all the parameters from benign to malignancy.

In differentiating between the four groups, mean nuclear area and mean nuclear diameter were found to be significant. This was in consistent with the study by Kashyap et al and abdalla et al.Abdalla et al also derived a cut off nuclear diameter for benign cases and malignant cases.

Shivani Kalhan et al. in their study correlated the nuclear parameters with the cytological grade in malignant cases. Usual parameters according to their study was found to be mean nuclear area, long axis, short axis and total run length. Cytohistologic correlation was 83.3%, 88.9% and 88.9% for cytological grades 1, 2 and 3 respectively. In the present study, mean nuclear area and nuclear diameter were found to be useful.

In a study by Parmer et al, the useful parameters mean nuclear area, perimeter, diameter, long axis, nuclear cytoplasmic features were found to be useful in differentiating hyperplasia from malignancy. In the present study

Mean nuclear area and mean nuclear diameter was found to be useful. Long axis, nuclear cytoplasmic ratio were not included in the present study.

Some studies have explored the correlation between morphometry and cytologic grading using various morphometric parameters. Most have found a significant association using multivariate analysis. In the present study, correlation with cytological grade was not included.

Yadav et al from their study showed that comparison of borderline with malignant category revealed a statistically significant role of mean nuclear area and mean cytoplasmic area while other parameters such as perimeter and N/C were found to be insignificant statistically. The present also showed a statistically significant role of nuclear area and perimeter was not found to be statistically significant.

The differences in the observed values of different morphometric parameters in various studies may be due to the application of different morphometric methods. However, a strictly standardized and uniform technique along with regular calibration of computerized morphometric analysers may augment the precision and accuracy, enhancing the reproducibility of results.

SUMMARY

In the present study, nuclear parameters of 60 cytological aspirate smears from breast masses were studied including 20 cases of fibroadenoma, 13 cases of fibrocystic changes, 7 cases of proliferative breast diseases and 20 cases of malignancy.

Of the 7 proliferative breast diseases 2 cases were present with atypia and other 5 cases with no atypia. Of the 20 malignant cases, 8 cases belonged to grade 1, 10 cases belonged to grade 2 and 2 cases belonged to grade 3.

The predominant age group in Fibroadenoma was 21 to 30 years and left breast was predominantly involved.

The predominant age group in fibrocystic change was 41 to 50 years of age and left breast was predominantly involved.

The predominant age group involved in proliferative breast diseases was 31 to 40 years of age and right breast was predominantly involved.

The predominant age group in malignancy was 41 to 50 years of age and both the breasts were equally involved.

Nuclear parameters were studied for all the 60 cases.50 nuclei/case were studied. The parameters studied were nuclear area, nuclear perimeter, nuclear diameter and nuclear compactness.

The mean nuclear area showed an increase from fibroadenoma to fibrocystic change, fibrocystic change to proliferative breast diseases, proliferative breast diseases to malignancy.

The mean nuclear perimeter showed an increase from fibroadenoma to fibrocystic change, fibrocystic change to proliferative breast diseases, proliferative breast diseases to malignancy.

The mean nuclear diameter showed an increase from fibroadenoma to fibrocystic change, fibrocystic change to proliferative breast diseases, proliferative breast diseases to malignancy.

The mean nuclear compactness showed no significant increase or decrease in between the groups.

In differentiating fibroadenoma and fibrocystic change, mean nuclear area and mean nuclear diameter were found to be statistically significant (p value<0.05).

In differentiating fibrocystic change and proliferative breast diseases, mean nuclear area and mean nuclear diameter were found to be statistically significant (p value<0.05).

In differentiating proliferative breast diseases and malignancy, mean nuclear area and mean nuclear diameter were found to be statistically significant.

CONCLUSION

Breast cancer is the commonly diagnosed cancer among women and the leading cause of cancer deaths among women. In order to improve breast cancer outcomes and survival, early detection is crucial. Fine Needle Aspiration cytology plays an important role in early detection of malignancy.

Nuclear morphometry has proved to be very useful in differentiating benign lesions from malignant ones on cytology. However, a study on large scale is needed for further evaluation on its role in “gray” zone in cytology.

Thus, after training, internal calibration, and standardisation, nuclear morphometry can prove to be a very useful tool in supplementing FNAC in differentiating between benign and malignant lesions for crucial decision on patient management, especially in cases with diagnostic dilemma.

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MASTER CHART

S.NO	CYTOLOGY NUMBER	AGE	LATERALITY	DIAGNOSIS	HPE DIAGNOSIS	NA	NP	ND	NC
1	CY 3578/19	36	LEFT	FIBROADENOMA	FIBROADENOMA	0.016	0.481	0.200	12.32
2	CY 3580/19	35	RIGHT	FIBROADENOMA	FIBROADENOMA	0.016	0.515	0.180	16.57
3	CY 3591/19	30	RIGHT	FIBROADENOMA	FIBROADENOMA	0.021	0.593	0.250	16.74
4	CY 3613/19	28	BILATERAL	FIBROADENOMA	FIBROADENOMA	0.022	0.618	0.241	17.36
5	CY 3625/19	20	LEFT	FIBROADENOMA	FIBROADENOMA	0.017	0.497	0.195	14.52
6	CY 3642/19	21	LEFT	FIBROADENOMA	FIBROADENOMA	0.032	0.717	0.213	16.06
7	CY 3651/19	21	RIGHT	FIBROADENOMA	FIBROADENOMA	0.020	0.586	0.212	17.16
8	CY 3658/19	19	LEFT	FIBROADENOMA	FIBROADENOMA	0.038	0.618	0.236	10.05
9	CY 3661/19	21	RIGHT	FIBROADENOMA	FIBROADENOMA	0.024	0.625	0.249	16.27
10	CY 3666/19	19	LEFT	FIBROADENOMA	FIBROADENOMA	0.021	0.628	0.200	18.78
11	CY 3669/19	24	LEFT	FIBROADENOMA	FIBROADENOMA	0.022	0.587	0.206	15.66
12	CY 3712/19	16	RIGHT	FIBROADENOMA	FIBROADENOMA	0.024	0.613	0.200	15.65
13	CY 51/20	45	LEFT	FIBROADENOMA	FIBROADENOMA	0.030	0.688	0.202	15.77
14	CY 54/20	23	LEFT	FIBROADENOMA	FIBROADENOMA	0.035	0.512	0.278	7.48
15	CY 60/20	26	LEFT	FIBROADENOMA	FIBROADENOMA	0.026	0.630	0.220	15.26
16	CY 65/20	22	RIGHT	FIBROADENOMA	FIBROADENOMA	0.031	0.672	0.212	14.56
17	CY 67/20	32	RIGHT	FIBROADENOMA	FIBROADENOMA	0.028	0.615	0.234	13.50
18	CY 110/20	18	LEFT	FIBROADENOMA	FIBROADENOMA	0.037	0.715	0.231	13.81
19	CY 127/20	24	RIGHT	FIBROADENOMA	FIBROADENOMA	0.019	0.623	0.210	20.42
20	CY 130/20	25	LEFT	FIBROADENOMA	FIBROADENOMA	0.023	0.615	0.261	16.44
21	CY 3565/19	60	RIGHT	FIBROCYSTIC CHANGE	FIBROCYSTIC CHANGE	0.028	0.587	0.261	12.30
22	CY 3632/19	44	BILATERAL	FIBROCYSTIC CHANGE	FIBROCYSTIC CHANGE	0.048	0.847	0.302	14.94
23	CY 3692/19	32	LEFT	FIBROCYSTIC CHANGE	FIBROCYSTIC CHANGE	0.030	0.710	0.300	16.80
24	CY 10/20	28	LEFT	FIBROCYSTIC CHANGE	FIBROCYSTIC CHANGE	0.025	0.582	0.262	13.54
25	CY 25/20	48	LEFT	FIBROCYSTIC CHANGE	FIBROCYSTIC CHANGE	0.025	0.590	0.267	13.92
26	CY 32/20	35	LEFT	FIBROCYSTIC CHANGE	FIBROCYSTIC CHANGE	0.033	0.696	0.257	14.67
27	CY 73/20	42	RIGHT	FIBROCYSTIC CHANGE	FIBROCYSTIC CHANGE	0.037	0.713	0.243	13.73
28	CY 86/20	39	BILATERAL	FIBROCYSTIC CHANGE	FIBROCYSTIC CHANGE	0.046	0.808	0.280	14.19
29	CY 105/20	54	LEFT	FIBROCYSTIC CHANGE	FIBROCYSTIC CHANGE	0.035	0.742	0.286	15.73
30	CY 141/20	45	BILATERAL	FIBROCYSTIC CHANGE	FIBROCYSTIC CHANGE	0.039	0.787	0.304	15.88
31	CY 148/20	29	RIGHT	FIBROCYSTIC CHANGE	FIBROCYSTIC CHANGE	0.047	0.818	0.295	14.23
32	CY 150/20	39	LEFT	FIBROCYSTIC CHANGE	FIBROCYSTIC CHANGE	0.034	0.740	0.280	16.10
33	CY 154/20	45	BILATERAL	FIBROCYSTIC CHANGE	FIBROCYSTIC CHANGE	0.039	0.718	0.248	13.21
34	CY 3576/19	46	LEFT	PROLIFERATIVE BREAST DISEASE WITH ATYPIA	PROLIFERATIVE BREAST DISEASE WITH ATYPIA	0.076	1.051	0.333	14.53

35	CY 3582/19	30	BILATERAL	PROLIFERATIVE BREAST DISEASE WITHOUT ATYPIA	PROLIFERATIVE BREAST DISEASE WITHOUT ATYPIA	0.061	0.986	0.365	15.93
36	CY 3633/19	34	RIGHT	PROLIFERATIVE BREAST DISEASE WITHOUT ATYPIA	PROLIFERATIVE BREAST DISEASE WITHOUT ATYPIA	0.064	0.940	0.372	13.80
37	CY 3705/19	39	RIGHT	PROLIFERATIVE BREAST DISEASE WITHOUT ATYPIA	PROLIFERATIVE BREAST DISEASE WITHOUT ATYPIA	0.079	1.117	0.478	15.79
38	CY 243/20	40	RIGHT	PROLIFERATIVE BREAST DISEASE WITHOUT ATYPIA	PROLIFERATIVE BREAST DISEASE WITHOUT ATYPIA	0.111	1.290	0.371	14.99
39	CY 274/20	40	RIGHT	PROLIFERATIVE BREAST DISEASE WITH ATYPIA	PROLIFERATIVE BREAST DISEASE WITH ATYPIA	0.068	1.152	0.366	19.51
40	CY 360/20	41	RIGHT	PROLIFERATIVE BREAST DISEASE WITHOUT ATYPIA	PROLIFERATIVE BREAST DISEASE WITHOUT ATYPIA	0.042	0.8887	0.299	18.73
41	CY 3609/19	72	RIGHT	MALIGNANCY	MALIGNANCY	0.147	1.409	0.520	13.50
42	CY 3623/19	50	RIGHT	MALIGNANCY	MALIGNANCY	0.158	1.446	0.483	13.23
43	CY 29/20	53	LEFT	MALIGNANCY	MALIGNANCY	0.154	1.500	0.451	14.61
44	CY 38/20	40	RIGHT	MALIGNANCY	MALIGNANCY	0.288	2.098	0.791	15.28
45	CY 43/20	48	RIGHT	MALIGNANCY	MALIGNANCY	0.205	1.714	0.604	14.33
46	CY 112/20	45	LEFT	MALIGNANCY	MALIGNANCY	1.059	3.794	1.275	13.59
47	CY 185/20	50	RIGHT	MALIGNANCY	MALIGNANCY	0.828	3.421	1.213	14.13
48	CY 191/20	50	LEFT	MALIGNANCY	MALIGNANCY	0.859	3.524	1.274	14.45
49	CY 206/20	48	RIGHT	MALIGNANCY	MALIGNANCY	0.704	3.316	1.314	15.61
50	CY 275/20	45	RIGHT	MALIGNANCY	MALIGNANCY	0.752	3.216	1.318	13.75
51	CY 279/20	35	LEFT	MALIGNANCY	MALIGNANCY	0.305	2.124	0.891	14.79
52	CY 288/20	70	RIGHT	MALIGNANCY	MALIGNANCY	0.256	1.944	0.572	14.76
53	CY 179/20	48	LEFT	MALIGNANCY	MALIGNANCY	0.148	1.448	0.615	14.16
54	CY 312/20	66	LEFT	MALIGNANCY	MALIGNANCY	0.212	1.689	0.498	13.45
55	CY 363/20	50	LEFT	MALIGNANCY	MALIGNANCY	0.151	1.448	0.529	13.88
56	CY 420/20	52	LEFT	MALIGNANCY	MALIGNANCY	0.315	2.224	0.916	15.70
57	CY 429/20	48	RIGHT	MALIGNANCY	MALIGNANCY	0.226	2.891	0.824	36.98
58	CY 469/20	65	RIGHT	MALIGNANCY	MALIGNANCY	0.290	3.124	0.868	33.65
59	CY 482/20	70	LEFT	MALIGNANCY	MALIGNANCY	0.319	2.304	0.924	16.64
60	CY 498/20	68	LEFT	MALIGNANCY	MALIGNANCY	0.184	1.626	0.592	14.36