A Dissertation on

"ANALYSIS OF AGNOR COUNT AND SAPA SCORE IN FNAC OF BREAST NEOPLASMS"

Dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

CHENNAI-600032

In Partial fulfillment of the regulations

Required for the award of

M.D.Degree in PATHOLOGY (BRANCH III)

DEPARTMENT OF PATHOLOGY

COIMBATORE MEDICAL COLLEGE

MAY 2020

Registration Number: 201713257

DECLARATION

I hereby declare that the dissertation entitled "**ANALYSIS OF AGNOR COUNT AND SAPA SCORE IN FNAC OF BREAST NEOPLASMS**" is a bonafide research work done by me in the Department of Pathology, Coimbatore Medical College during the period from **JULY 2017 TO JUNE 2019** under the guidance and supervision of **Dr. B. SUDHA, MD.,** Senior Assistant Professor, Department of Pathology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to The Tamilnadu Dr.MGR Medical University, Chennai towards the partial fulfilment of the requirements for the award of M.D., Degree (Branch III) in Pathology. I have not submitted this dissertation on any previous occasion to any University for the award of any Degree.

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CERTIFICATE OF APPROVAL

To
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Dear **Dr.Shiny Latha P**

The Institutional Ethics Committee of Coimbatore Medical College, reviewed and discussed your application for approval of the proposal entitled **"Analysis of AgNOR Count and SAPA Score in FNAC of Breast Neoplasms."**No.060/2017.

The following members of Ethics Committee were present in the meeting held on 24.11.2017.conducted at MM - II Seminar Hall, Coimbatore Medical College Hospital Coimbatore-18.

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We approve the Proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

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CERTIFICATE

This is to certify that the dissertation entitled "**ANALYSIS OF AGNOR COUNT AND SAPA SCORE IN FNAC OF BREAST NEOPLASMS**" is a bonafide work done by **Dr. P. SHINY LATHA,** a postgraduate student in the Department of Pathology, Coimbatore Medical College, Coimbatore under the guidance and supervision of **Dr. B. SUDHA MD.,**Senior Assistant Professor, Department of Pathology, Coimbatore Medical College and submitted in partial fulfilment of the regulations of The Tamilnadu Dr. MGR Medical University, Chennai towards the award of M.D. Degree (Branch III) in Pathology.

Guide

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CERTIFICATE

This is to certify that this dissertation work titled "**ANALYSIS OF AGNOR COUNT AND SAPA SCORE IN FNAC OF BREAST NEOPLASMS**" of the candidate Dr. P. SHINY LATHA with registration number 201713257 for the award of M.D degree in the branch of PATHOLOGY. I personally verified the urkund.com website for the purpose of plagiarism check I found that the uploaded thesis file contains from introduction to conclusion pages and result shows Two percentage (2%) of plagiarism in the dissertation.

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ACKNOWLEDGEMENT

To begin with, I thank the Almighty God for bestowing his blessing on me in completing this dissertation a successful one.

I wish to thank our beloved Dean Prof.**Dr.B.ASOKAN, M.S.,M.Ch., (Plastic surgery)** and vice principal **Dr.C.LALITHA MD.,(Pathology)** Coimbatore Medical College and Hospital for permitting me to conduct this study.

I express my sincere gratitude to **Dr. A. DHANALAKSHMI, M.D.,** Professor and Head, Department of Pathology, Coimbatore Medical College for her able guidance and support and also for providing all facilities to carry out this study.

It's a great pleasure to express my humble gratitude to my guide **Dr. B. SUDHA M.D**., Senior Assistant Professor, Department of Pathology for having suggested this topic for dissertation and for having rendered her valuable support and encouragement without which this project work would not have been feasible.

I also wish to record my sincere thanks to all my Associate and Assistant Professors of Department of Pathology, Coimbatore Medical College, for their constant support and encouragement throughout the work.

I extend my heartfelt thanks to all my colleagues and friends for their timely help, comments and support.

I thank all the technical staffs in the Department of Pathology, Coimbatore Medical College, for their sincere and timely technical assistance.

I express my heartfelt thanks to Department of Surgery, Coimbatore Medical College, for their constant support throughout the course of this study.

I express my heartfelt thanks and gratitude to my parents and to my sister for their extreme patience, constant support, encouraging words and source of strength all the way through this endeavour.

CONTENTS

LIST OF TABLES

LIST OF CHARTS

LIST OF COLOUR PLATES

INTRODUCTION

Breast Carcinoma is one of the most common neoplasms in women and is a leading cause of cancer related deaths worldwide. In recent years, improved diagnostic tools have made it possible to detect breast cancers at early, even pre-invasive stages leading to a significant decrease in breast cancer mortality rates over the past decades.

Nucleolar Organizer Regions (AgNORs) are specific portions of DNA that code for the transcription of ribosomal RNA (rRNA). rRNA is responsible for protein synthesis of the cell. Protein synthesis is a necessary step in the process of cell proliferation. Therefore a relation between NORs and cell proliferation is suggested. NORs can be selectively visualized by silver staining in routinely processed histological samples and in cytology smears. Argyrophilic Nucleolar Organizer Region (AgNOR) technique has a potential value in differentiating benign and malignant tumors. Counting the AgNOR is comparatively difficult as the dots are aggregated as a cluster within the nucleolus which are of small size and are overlapping.

Fine needle aspiration (FNA) is a rapid method for diagnosing breast lesions as an outpatient procedure. FNA has a sensitivilty of 87%, while specificity and positive predictive value of 98%, and negative predictive value of 60%.

Though we have good techniques, it is difficult to distinguish benign and malignant neoplasms in some breast lesions. Malignant neoplasms show enhanced proliferative activity. Nucleolar Organizer regions (NORs) is the earliest proliferation marker, which are increased in malignant neoplasms compared to benign neoplasms. The purpose of the study was to evaluate the role of mean Argyrophilic Nucleolar Organizer Region (AgNOR) count and Subjective Argyrophilic Nucleolar Organizer Region Pattern Assessment (SAPA) Score and comparison in Fine needle Aspirates of Breast neoplasms.

AIMS AND OBJECTIVES

- To analyse the Clinical and Cytomorphological features of Breast Neoplasm
- To assess the AgNOR count in FNAC of Breast neoplasms
- To assess the SAPA score in FNAC of Breast neoplasms
- To assess the cytological grade of breast carcinoma and correlate with AgNOR count & SAPA score.
- To compare the AgNOR count and SAPA score in Benign and Malignant breast lesions in FNAC
- To correlate the AgNOR count and SAPA score of Breast neoplasms in FNAC with Histopathology

REVIEW OF LITERATURE

The breasts form the secondary sexual organ in females while they are rudimentary in males. The breasts are the site of malignant change in as many as one in ten females. It extends vertically from second to the sixth rib and transversely from sternal edge medially to midaxillary line laterally. The superolateral quadrant of breast projects through the deep fascia forming the Axillary tail of Spence[1].

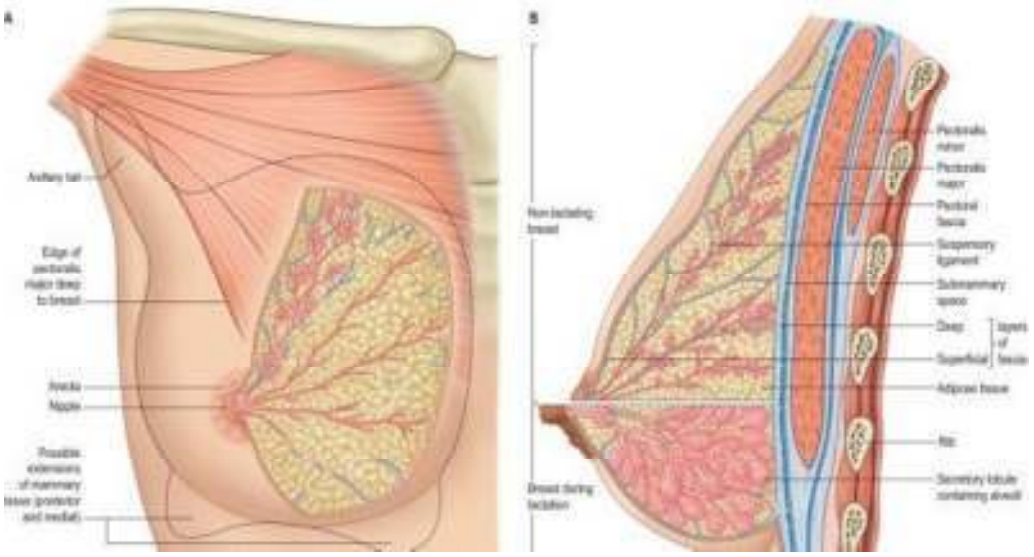


Figure 1: Anatomy of the Breast[1]

The breasts lies upon the deep pectoral fascia. The nipple projects from the centre of the breast anteriorly. The level of the nipple varies depending upon the size and shape of the breast, but it overlies the fourth intercostal space in most young females.[1]

Breasts develops from mammary ridges also known as milk line. They are nothing but the thickening of the epidermis. The mammary ridges extends

from axillary region to the medial side of thigh. These ridges disappears during the fetal development except in the ventral surface which later forms the breast[2].

The breasts are composed of lobes containing a network of glandular tissue with branching ducts and terminal secretory lobules in a connective tissue stroma. The connective tissue stroma surrounding the lobules is dense and fibrocollagenous, whereas intralobular connective tissue has a loose texture that allows rapid expansion of secretory component during pregnancy. The terminal duct lobular unit (TDLU) is the functional secretory component of milk in the breast. The glandular tissue has 10-15 lobes and each lobe drain into the collecting duct forming subareolar dilatation at the nipple called lactiferous sinuses. Benign and malignant neoplasm of breast arises from both the glandular and the stromal component [3].

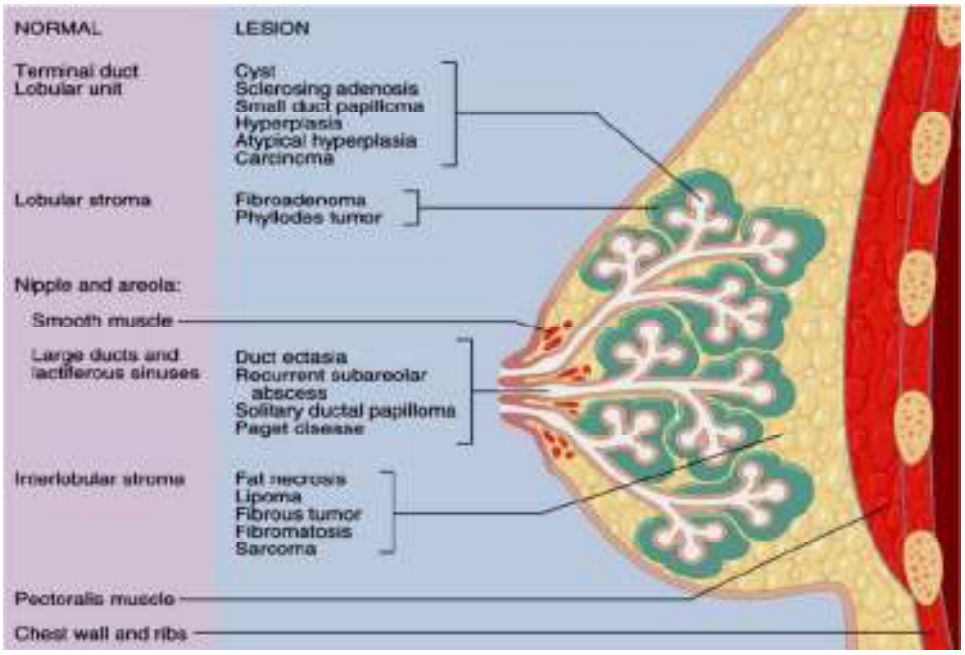


Figure 2- Anatomic origin of Breast lesions[3]

Ducts and TDLU are lined by a layer of cuboidal to columnar epithelium surrounded myoepithelial cells. Dermal lymphatics penetrates pectoralis major to join channels that drain the parenchymal tissues, and then follow the vascular channels to the axillary nodes and terminate in the subclavicular lymph nodes. Axillary nodes receive more than 75% of the lymph from the breast There are 20–40 nodes, grouped as pectoral (anterior), subscapular (posterior), central and apical[4]. There is no discernible variation between the male and female breast tissues from the birth until puberty.

At puberty, female breasts exhibits branching and lengthening of their ducts along with lobular development and proliferation of fibrous stroma and adipose tissues[5,6]. These breast changes occurs under the influence of cyclical estrogen and progesterone secretion during menstrual cycle accompanied by the action of insulin, glucocorticoids and growth hormone. During menopause, there is decrease in cellularity, number of lobules sparing the myoepithelial cells and collagenisation of intralobular stroma[7]. These physiological changes at various age groups give different histological appearances.

EPITHELIAL BREAST LESION[3]:

Epithelial breast lesions arises both from the ducts and lobules. They are

1. Non-proliferative changes
2. Proliferative breast disease without atypia
3. Proliferative breast disease with atypia

NON-PROLIFERATIVE CHANGES:

Non-proliferative lesions of breast includes

1. Duct Ectasia
2. Cysts
3. Apocrine changes
4. Mild hyperplasia
5. Adenosis
6. Fibroadenoma without complex features

Cytology smears of Cysts and Fibrocystic changes of Breast reveals low to moderately cellular smears composed of sheets of ductal epithelial cells, apocrine cells, cyst macrophages and dispersed bipolar nuclei[4]. Cytology smears of Adenosis reveals moderate cellularity with small groups of uniform epithelial cells in microacinar appearance and myoepithelial cells. Welling and Alpers[12] published Apocrine metaplasia are seen in breasts of more than 30 years of age whereas those of 13 to 19 years of age showed no apocrine metaplasia[12].

PROLIFERATIVE BREAST LESIONS:

Proliferative Breast lesions can be grouped as Proliferative Breast disease with or without atypia.

Proliferative Breast disease without Atypia includes

1. Moderate to florid hyperplasia
2. Sclerosing adenosis
3. Papilloma
4. Complex sclerosing lesions
5. Fibroadenoma with complex features

Cytology smears of Epithelial hyperplasia reveals low to moderate cellular smears with small to large sheets of cohesive ductal epithelial cells without nuclear atypia in a background of bare bipolar nuclei, apocrine cells and macrophages.

SCLEROSING ADENOSIS:

Sclerosing adenosis are usually misdiagnosed as carcinoma. They retain their architecture as rounded, lobulocentric configuration and are cellular more centrally than peripherally. The proliferating tubules are elongated, compressed and are lined by epithelial cells and also peripheral myoepithelial layer. The

risk of invasive carcinoma for sclerosing adenosis is the same as for proliferative breast disease without atypia.

Cytology smears of Sclerosing lesions reveals variable cellularity of cohesive ductal epithelial cells without recognizable myoepithelial cells in a background of apocrine cells, histiocytic cells, fibroblasts and macrophages.

Cytology smears of Papilloma reveals moderate to high cellularity with small clusters and dispersed epithelial cells having mild anisonucleosis in a background of debris, inflammatory cells, apocrine cells and macrophages.

There are three categories that fibrocystic breast disease fall into. They are

- No or mild Usual Ductal Hyperplasia- No increased risk of invasive carcinoma
- Moderate or florid hyperplasia- (Proliferative Breast disease without atypia) 1.5 to 2 times the risk
- Atypical ductal hyperplasia / Atypical Lobular hyperplasia: 4 to 5 times the risk[55].

Proliferative Breast disease with Atypia includes

1. Atypical Ductal hyperplasia
2. Atypical Lobular hyperplasia

ATYPICAL DUCTAL HYPERPLASIA:

Atypical ductal/ lobular hyperplasia resembles low-grade ductal carcinoma in situ/ lobular carcinoma in situ due to high cellular proliferation[14].

The currently accepted definition for Atypical Ductal hyperplasia is that they are monomorphic cells having ovoid to rounded nuclei with micropapillae formation, and also tufts, fronds, bridges, solid and/or cribriform patterns within the involved space[55].

Cytology reveals high cellularity with increased crowding and overlapping within the cohesively arranged mild atypical epithelial cells and occasional bare bipolar nuclei[13].

ATYPICAL LOBULAR HYPERPLASIA:

Acute lobular hyperplasia are monomorphic proliferation of atypical epithelial cells with round nuclei and indistinct nucleoli. These cells are dyscohesive and contains intracytoplasmic lumina[55].

STROMAL TUMORS:

1. Fibroadenoma

2. Phyllodes tumor

FIBROADENOMA:

Fibroadenoma is more common benign neoplasm that occur in the age group of 20 to 35 years. They are often single, but multiple lesions can also be seen. Grossly, fibroadenoma are sharply demarcated , firm mass. Cut surface appears solid, gray white, bulging with a whorled appearance. Slit like spaces are often seen[55].

Fibroadenoma shows mixed epithelial and stromal proliferation, giving rise to the pericanalicular and intracanalicular patterns. Former due to stromal proliferation around the ducts without compression of the ductal elements and the latter due to compression of the ductal elements by the proliferating stromal component into slit like spaces.

Cytology smears of Fibroadenoma reveals antler horn like branched cohesive clusters of ductal epithelial cells admixed with fibromyxoid stroma in a background of bare bipolar nuclei.

Morphologic variations in fibroadenoma are of greater significance such as

- Hyalinisation, calcification and ossification of the stroma
- Multinucleated giant cell in the stroma
- Presence in the stroma of mature adipose tissue, smooth muscle or metaplastic cartilage
- Prominent myxoid change
- Hypercellular stroma
- Hemorrhagic infarct
- Ill-defined edge that blends with breast parenchyma
- Complex fibroadenoma- Sclerosing adenosis, cysts > 3mm, calcifications, papillary apocrine changes
- Squamous metaplasia
- Lactational changes
- Young patients, large tumor size and hypercellularity[55]

PHYLLODES TUMOR:

Phyllodes tumor is another fibroepithelial lesion. The term "Cystosarcoma phyllodes" (phyllo in Greek for leaf) means leaf like pattern. The tumor arises from periductal stroma with sparse lobular elements. Cytology reveals cellular stromal fragments with low to moderate cellularity of ductal elements in a background of bare oval to spindle cell nuclei.

Grossly, the tumor is round, circumscribed and firm. Cut surface is solid, gray white with cleft like spaces. Necrosis, cystic degeneration and hemorrhage can also be seen.

Microscopically, stroma is hypercellular with benign glandular elements. The amount of stroma determines whether the tumor is benign, borderline or malignant. In Benign Phyllodes, the stroma is fibroblastic in appearance with minimal stromal atypia. In malignant Phyllodes, there is higher degree of stromal cellularity, marked stromal nuclear atypia , numerous mitosis. Tumor necrosis has a poorer prognosis. The criteria of malignancy is overgrowth of glands by the malignant stroma so that low power views of the tumor shows stroma only with no epithelial components[55].

BREAST CARCINOMA:

Adenocarcinoma is the usual tumor that arise from duct and lobules. Invasive ductal carcinoma is the largest group of malignant tumors of breast comprising 75%. A generic term used is Invasive ductal carcinoma, not otherwise specified (NOS) or no special type (NST). This term reveals the distinction between most of the Invasive ductal carcinoma from special forms of ductal carcinoma such as tubular, medullary, metaplastic, mucinous, secretory, papillary and adenoid cystic carcinoma. The origin of Ductal carcinoma and Lobular carcinoma is Terminal Ductal Lobular Unit (TDLU)[7].

Majority of breast carcinoma are seen in the postmenopausal women. Sometimes it can occur in any age groups. Breast carcinoma is the most common malignant tumor and is the second most common cause of death in female population[55].

The risk factors in the development of carcinoma breast are as follows

- Postmenopausal age
- Country of birth
- Family history of women with first degree relative having breast cancer
- Early menarche and late age at first birth
- Intraductal proliferative breast lesions
- Exogenous estrogens

- Contraceptive agents
- Exposure to radiation
- Breast augmentation procedure

Breast cancer can be diagnosed by

- Clinical examination
- Mammography
- Breast ultrosonography
- Magnetic Resonance Imaging
- Cytology
- Core needle biopsy[55]

INVASIVE DUCTAL CARCINOMA

FNAC reveals highly cellular smears with dyscohesive sheets and singly dispersed malignant ductal epithelial cells in a background of necrotic debris and blood cells[7]. Most of the breast carcinomas show moderate to abundant cellularity, with dyscohesiveness of the cells. This is due to lack of cell to cell adhesion. The isolated cells have preserved cytoplasm in contrast to the naked nuclei of the benign lesions. In invasive carcinomas, there is no myoepithelial cells but there is nuclear pleomorphism, prominent nucleoli, irregular nuclear membrane and mitotic figures. Background usually shows nuclear debris, necrosis and inflammatory cells[57].

CARCINOMA AND ITS VARIANTS[57]:

Invasive Lobular carcinoma:

- Paucicellular smear
- Subtle atypia and rare single intact epithelial cells
- Cells form small chains in the aspirates
- Nuclei- eccentric, round or oval with dispersed chromatin
- Small distinct nucleoli
- Cytoplasm- scanty, clear or vacuolated

Tubular carcinoma:

- Variable cellularity
- Many cohesive clusters of uniform bland epithelial cells
- Cells are arranged in tubular structures with an angular appearance or comma like pattern
- Tubular structures appears three dimensional with central lumen
- Minimal cytological atypia

Invasive Cribriform carcinoma:

- Cohesive sheets and three dimensional cribriform clusters of bland looking and mitotically active ductal cells
- Ductal cells have round to oval nuclei, dispersed chromatin, inconspicuous nucleoli, small amount of ampophilic cytoplasm
- No myoepithelial cells seen

Mucinous carcinoma:

- Gelatinous material
- Variable cellularity
- Three dimensional group of cells surrounded by abundant extracellular mucinous material
- Linear strands of filmy, wispy material
- Cell groups are tightly cohesive cell balls, flat sheets, loosely cohesive clusters
- Ductal cells- small to medium sized with round to eccentric nuclei, with minimal nuclear pleomorphism
- Myoepithelial cells may be present

Carcinoma with medullary features:

- Definitive diagnosis is given by requirement of tissue sections
- Cellular aspirates
- Large pleomorphic tumor cells
- Background of lymphocytes and plasma cells
- Large cells- dispersed in clusters, syncytial groups or individually
- Cytoplasm- homogenous or granular
- Nuclei- irregular with clumped chromatin
- Macronucleoli

Metaplastic carcinoma:

- Homologous- squamous and spindle cells
- Heterologous- chondroid, osseous, rhabdoid elements
- Liquid necrotic aspirates
- Proteinaceous or myxoid background
- Neoplastic cell types- ductal, spindle shaped, squamous cells

Apocrine carcinoma:

- High tumor cellularity
- Tumor cells- singly or in syncytial tissue fragments
- Cells and nuclei- enlarged and pleomorphic
- Papillary clusters
- Absence of bare bipolar nuclei

Secretory carcinoma:

- Globular structures of small centrally located mucoid material
- Globular structures- uniform in size
- Nuclei- eccentric, ovoid with no atypia
- Prominent intracytoplasmic vacuolization
- Abundant colloid like material and cracking artifact

Acinic cell carcinoma:

- Acinic cells- small and uniform nuclei
- Abundant granular cytoplasm
- Tendency to form glandular structures

Glycogen rich carcinoma:

- Presence of clear cells filled with glycogen
- Cellular aspirate
- Tumor cells in groups, clusters or isolated cells
- Cytoplasm- ample, clear and fragile
- Moderate to marked pleomorphic nuclei

Lipid rich carcinoma:

- Moderately cellular
- Loosely cohesive tumor cells
- Well demarcated cytoplasm with many large vacuoles

Adenoid cystic carcinoma:

- Rare variant
- Clusters of cohesive small, uniform cells arranged around hyaline globules; associated with tubular structures covered with uniformly arranged epithelial cells
- Individual cells- small, round or ovoid nuclei with narrow rim of cytoplasm

FINE NEEDLE ASPIRATION CYTOLOGY

Fine Needle Aspiration Cytology (FNAC) is a minimally invasive and cost effective outpatient procedure with high diagnostic accuracy. This technique is safe, enables immediate reporting and provides high sensitivity and specificity for differentiating benign and malignant tumors. FNAC forms the part of triple assessment of breast lesions: clinical, imaging and morphology[8].

Fine Needle Aspiration Report must have the statement of

- Adequacy of the specimen
- The degree of cellularity
- Cytological description
- Specific diagnosis
- Benign or Malignant
- Code for overall categorization and management of the lesion

There are five codes used for clarity and quality assurance to facilitate the communication between Cytologists and clinicians. The codes are

- ✓ Code 1- Insufficient material
- ✓ Code 2- Benign
- ✓ Code 3- Atypical, probably benign
- ✓ Code 4- Suspicious, probably insitu carcinoma or malignant
- ✓ Code 5- Malignant[56]

ADEQUACY[56]:

The cellularity of the smear is determined by the

- Operator experience
- Number of passes
- Size and nature of the lesion

Grant studied 18 cases of cytology of Breast lesions and provided the following statistics in the year 1986. The study reveals 99% specificity, 92.5% sensitivity, 96.5% accuracy, 99.7% positive predictive value and 94.2% negative predictive value[15].

Dutta et al (2001) studied 51 cases of FNAC of Breast lesions. 28 cases were malignant, while remaining cases were benign constituting fibroadenoma, fibrocystic changes and mastitis. FNAC revealed a diagnostic accuracy of 90.2%[17].

APPROACH TO REPORT BREAST CYTOLOGY[56] :

Low power Assessment

- Cellularity- Scant cellularity is defined as 7 to 10 tissue fragments each of more than 20 cells, which gives tissue fragments to allow assessment of architecture
- Pattern of tissue fragments and dispersed cells
- Architecture of tissue fragments
- Presence or absence of other fragments like stroma, smearing artifact

High power Assessment

- Confirm features seen at low power
- Assess the types of dispersed cells and tissue fragments
- Degree of epithelial nuclear atypia in the tissue fragments and dispersed cells

NATIONAL CANCER INSTITUTE – GUIDELINES[16]:

The National Cancer Institute (NCI) provided the guidelines for uniform approach to breast FNAC. Use 22-25G needle and create negative pressure using syringe plunger and advance in a forward and backward movement towards the center of breast lesion. Aim the needle at the periphery of lesion, when suspecting necrotic and cystic lesions. Typically, 30-50 excursions with the needle are made over a period of 10-20 seconds[16].

CYTOLOGICAL GRADING:

In 1991, Robinson et al[45] suggested a protocol for the cytological grading of ductal carcinoma of breast. This grading system is simple and easily reproducible method. There are six cytological parameters to be considered in this grading system. They are

1. Cell dissociation
2. Uniformity of the cell
3. Cell size
4. Nucleolus
5. Nuclear margin
6. Nuclear chromatin

A score of 1-3 is provided to each of these parameters and by adding up all the scores, the lesion is graded[46].

TABLE 1: ROBINSON'S CYTOLOGIC GRADING SYSTEM[45,46]

Criteria	Score		
	1	**2**	**3**
Cell dissociation	Mostly in Clusters	Mixture of single cells and cells in clusters	Mostly single cell
Cell uniformity	Monomorphic	Mildly pleomorphic	Pleomorphic
Cell size	1-2 times RBC Size	3-4 times RBC size	>5 times RBC size
Nuclear margin	Smooth	Folds	Buds and clefts
Nucleoli	Indistinct	Noticeable	Abnormal
Chromatin	Vesicular	Granular	Clumped and cleared

Total score ranges from 6 – 18 and are graded as follows

Grade I : Score 06 – 11

Grade II : Score 12 - 14

Grade III : Score 15 - 18

Robinson's cytological grading showed an accuracy of 83%, 77.33% of true positivity and 11.33% of false negativity[47].

The other cytological grading systems used were

1. The Moriquand's grading with 77% accuracy, 69.33% true positivity and 15.33% false negativity
2. The Hunt's grading system has 70.66% accuracy, 70.66% true positivity and 29.33% false negativity
3. The Howell (SBR) Grading System has an accuracy of 53.89%, 40% true positivity and false negativity of 31.25%[46].

AgNORs: ARGYROPHILIC NUCLEOLAR ORGANIZER REGIONS:

Nucleolar Organizer Regions are used as a tool for the study of chromosomal disorder by the Cytogeneticists[32]. The Nucleolar Organizer Regions are DNA loops into the nucleoli of the cells. These loops are located in the chromosomes 13, 14, 15, 21 and 22, which are acrocentric chromosomes. NORs are seen in pairs on acrocentric chromosomes and at the metaphase of nuclei -20 NORs could be seen[18]. These are rDNA (ribosomal DNA) that uses RNA (ribonucleic acid) polymerase-1 enzyme and codes the transcription of ribosomal RNA (rRNA).Thus protein synthesis occurs in the cell. NORs codes the ribosomal RNA, which is an important step in synthesis of proteins and thereby related to proliferative activity of the cell[19].

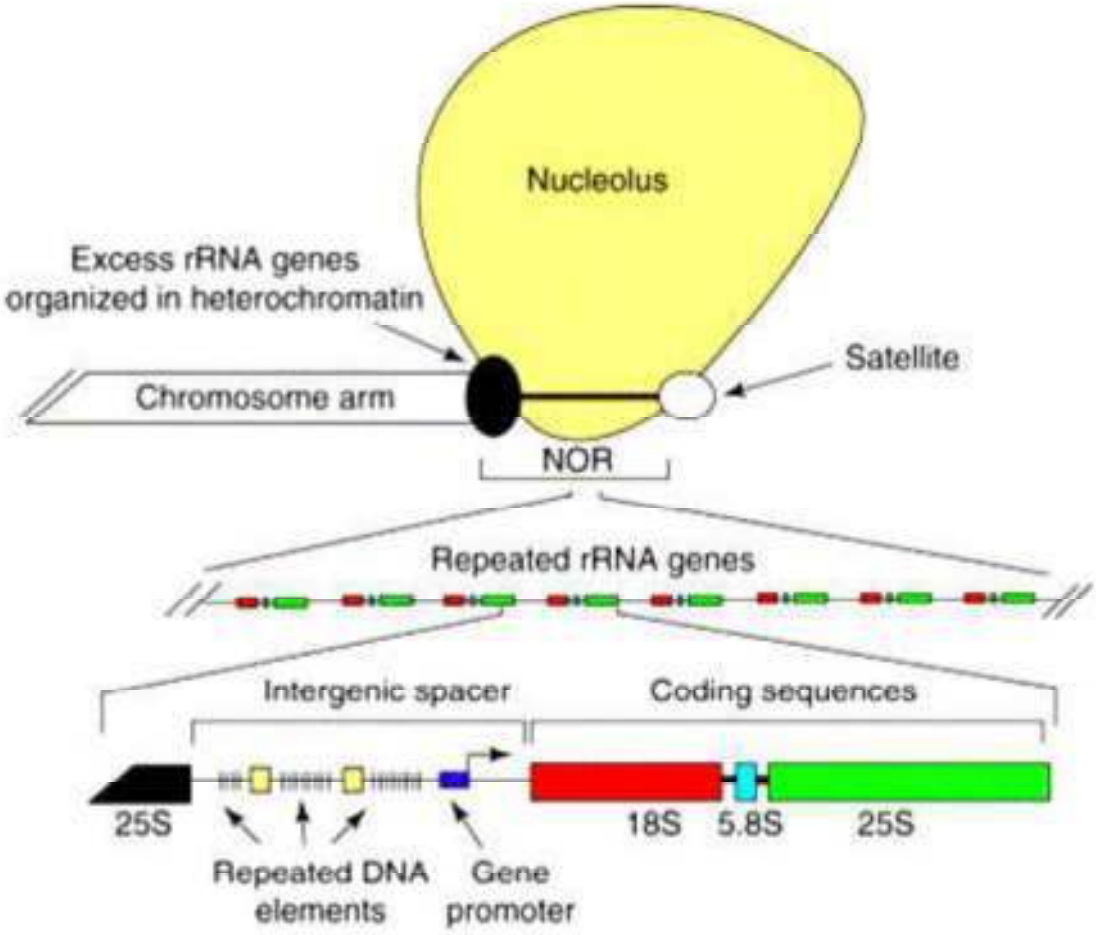


FIGURE 2 Organization of a typical Nucleolar Organizer Region[19]

PHYSIOLOGY OF AgNOR'S :

Human nucleoli constitutes fibrillar component, fibrillar center and granular component ultrastructurally. The fibrillar component are electron dense with 3-5nm fibrils. This sites processes the precursors of rRNA and stains with the antibody to 'Fibrillarin'. Fibrillarin is a protein related to small nuclear ribonucleoprotein (SnRNP) [19].

The fibrillar center contains ribosomal DNA, RNA polymerase I and topoisomerase and thereby forms the site for producing ribosomal RNA. By light microscopy, it parallels the interphase NOR.

The granular component constitutes the particle which forms ribosome precursors.

KINETICS OF THE CELL:

Tumor activity depends on Cellular kinetics. Proliferation rate determines the tumor activity. There are four phases in Cell cycle which depends on the activity of nuclear chromatin- such as S, G1, G2 and G0 phases. 'S' phase is the short resting phase of the cell which undergoes replication. The DNA content present at the end of 'S' phase forms the indicator of proliferative activity. Thereby, AgNOR detects the DNA content at this end of S phase[20]. The number, size and shape of the NORs vary according to the phase of the cell cycle and the nucleolar transcription.

In proliferating cells or tumor cells, the cell turnover and nucleolar transcription rate are relatively higher than normal cells. Thus, proliferative activity of the cell can be assessed by the quantity and morphology of the NORs. During prophase, the components of the fibrillar centre disperses and they are present in a particular position at metaphase on the short arm of chromosomes - 13, 14, 15, 21 and 22[18]. In a normal cell, these AgNORs are tightly aggregated within one to two nucleoli.

Factors determining the AgNORs:[21]

1. The stage of cell cycle

2. The transcriptional activity of the cell

3. In karyotype, the number of NORs bearing acrocentric chromosomes .

The AgNORs are dispersed throughout the nucleus , thereby easily seen by the cytologists in malignant lesions compared to the non-malignant ones. In interphase nuclei, the quantification of AgNORs is related more to their dispersion throughout the nucleus than to the actual number in the nucleus. Thus 'AgNOR count' in both benign and malignant lesions is a numerical index of dispersion of NOR within the nucleoplasm and not the absolute numbers. Therefore, the proliferative activity of the cell is nothing but the dispersion in itself. The current phase of transcription is indicated by the number of AgNORs. In 1975, NOR was first demonstrated by simple silver staining method that targets these argyrophilia-associated proteins which appear as brown/ black dots within the nucleoplasm of the cell[20].

Following are the conditions where mean AgNOR count is increased:

1. In active proliferation, the nucleolar dissociation is present in almost all cells. The AgNORs are seen throughout the nucleus.

2. A defect of the nucleolar association results in dispersion of AgNOR throughout the nucleus.

3. Increase in cellular ploidy increases the number of AgNOR bearing chromosomes.

4. Increased transcriptional activity increases the AgNOR activity

In the benign neoplastic cells, nucleus is relatively condensed and AgNORs are aggregated and shows 1-2 AgNOR per nucleus only, thereby visualizing NOR is difficult. In the malignant cells, where there is increased cellular proliferation, AgNORs are dispersed throughout the nucleus, thus the cytologist demonstrates them more easily. Hence, the quantification of AgNOR depends on the degree of disaggregation or dispersion of the number of AgNORs within the nucleus of the benign or malignant cells.

DEMONSTRATION OF NORs:

The Nucleolar Organizer Regions can be demonstrated by various methods which may either demonstrate the ribosomal DNA or the NOR associated proteins (NORAP).

TABLE 2: DEMONSTRATION OF NORs[19]

Reagent	**Target**
Silver colloid (AgNOR)	NORAP
Bismuth ions	100K NORAP
Radiolabelled rRNA	rDNA
Antibodies	NORAP epitopes

Among the above methods, the easier and the simpler method in identifying the NORs is the silver staining technique. This technique demonstrates the AgNORs (Argyrophilic Nucleolar Organizer Regions). This silver staining technique helps in visualizing the acidic NORAPs (Nucleolar Organizer Region Associated Proteins) which is associated with the RNA transcription.

AgNOR – TECHNICAL ASPECTS

The AgNOR staining technique is simpler compared to the other methods for identification of NORs as it is a one- step silver- staining technique. This method can also be used to demonstrate NORs on routinely done cytology smears and histology sections[22]. The main demerit is that it is time consuming in counting the little dots, and there is inter-observer variations.

Shortly, the one step silver-staining method constitutes the mixture of 50% silver nitrate solution and 1% formic acid in 2gms% of gelatin solution thereby acting as a colloid stabilizer[19]. These solutions are freshly prepared and used. Cytology smears are incubated in this mixture for 45 min to one hour followed by washing, dehydration, clearing and mount.

The NORs appear as black / brown dots in a background of pale yellow color at light microscopy and can be better appreciated by oil immersion lens. 50-100 neoplastic cells are usually counted and are expressed as a mean AgNOR count. Lymphocytes are used as internal controls. With minor

modification in this technique, total number of AgNOR per nucleus are counted rather than the sites counted. The intensity of the staining varies from one fixative to other. Alcohol fixatives, 95% ethanol and Carnoy's fixative provides better staining than mercury and dichromate fixatives[23].

PRINCIPLE:

The silver salts have high affinity for acidic NORAPs due to their high electron charge density and by their phosphate moieties.

AgNOR STAINING REACTION & PROBLEMS:

First and foremost common problem faced by silver staining method is the non-specific silver grain deposits seen in the background. By using clean glassware and deionised water background staining can be prevented.

Some minor modifications in staining method can also overcome this problem. They are:

1. In Inverted incubation technique, the slides are inverted into the staining solution. This maintains the contrast between the AgNORs and the background[24].

2. Immersion in 10% nitric acid solution after staining minimizes the background stain.

3. Replacing polyethylene glycol by gelatin as colloidal developer medium[25].

Second, is that the variations in staining time varies the intensity of AgNOR stain. If over-stained, clusters of AgNORs within nucleoli are obscured. If under-stained, they are too faint to assess.

Third, minor variations in thickness of the sections in histology sections has an effect on the number of AgNORs within nuclei. Cytology smears does not have this problem[26]. Thus, in cytological smears AgNOR count is far more superior than in histological sections[27].

ADVANTAGE:

One advantage of this technique is , previously stained cytology slides can be reused for silver staining method, thereby provides guide to the diagnosis.

DISADVANTAGE:

1. The reason for inaccuracy and inconsistency is inter-observer variations due to the manual counting procedures.
2. Overlapping of NORs within the nucleus leads to misjudged counts[20].

MODIFICATIONS IN THE AgNOR TECHNIQUE:

In 1986, Ploton first described the AgNOR technique. Following this, several modifications were made to improve the staining quality. Some of the modifications are

1. Combining Feulgen reaction and modified AgNOR staining technique. It enables the counting of NORs and evaluating the amount of DNA in the same cell nucleus by Feulgen reaction.

2. Combining cytofluorometric analysis on cell suspensions and AgNOR staining technique.

3. Using automatic image analysis software with AgNOR technique provides less subjective errors than traditional methods[29].

ENUMERATION OF AgNOR[30]

There are three groups of Nucleolar Organizer Regions within the nucleus. They are:

1. '**Aggregated AgNOR'** are the round, solitary structures that corresponds to the nucleolus of the cell. They are seen in resting cells and lymphocytes. The individual NORs is difficult to distinguish within the nucleus of these cells.

2. '**Nucleolar pattern'** is seen in the nucleus of the proliferating cells. NORs are dispersed within the nucleolus of the cell.

3. '**True AgNORs'** are dispersed throughout the nucleoplasm and are seen in malignant neoplastic cells.

These features can be better appreciated in cytological smears[30].

METHODOLOGY:

Enumeration of AgNORs based on their count, morphology and distribution. They are

1. Mean AgNOR count

2. AgNOR size variation grading

3. AgNOR distribution in the nuclei

4. Subjective AgNOR Pattern Assessment (SAPA)

Mean AgNOR count (mAgNOR):

Average or mean count of the number of NORs in the nucleus of 100 neoplastic cells. mAgNOR count correlates with mean DNA content of the cells which indicates the cell ploidy.

AgNOR size variation and distribution grading:

In 1991 – 1992 Ahsan et al used the criteria of variation in size and distribution of AgNORs within the nucleus. They demonstrated higher variation score of these parameters in malignant neoplasm compared to the benign neoplasms.

TABLE 3: AgNORs SIZE VARIATION GRADING

AgNOR Size Variation	Score
More or less uniform	0
Two different sizes	1+
More than two different sizes (but not those of 3+)	2+
All grades and sizes including too minute to be counted	3+

TABLE 4: AgNOR DISTRIBUTION IN THE NUCLEI

AgNOR distribution - nuclei	Score
Limited to nucleoli	0
Occasional dispersion outside nucleoli	1+
Moderate dispersion outside nucleoli	2+
Widely dispersed throughout the nucleus	3+

SUBJECTIVE AGNOR PATTERN ASSESSMENT:

Meehan et al proposed a method for scoring of Argyrophilic Nucleolar Organizer Regions called 'Subjective AgNOR Pattern Assessment (SAPA). The score is based on variation in the size and shape of the NORs and the morphologic patterns of NORs whether they appears scattered or aggregated[31].

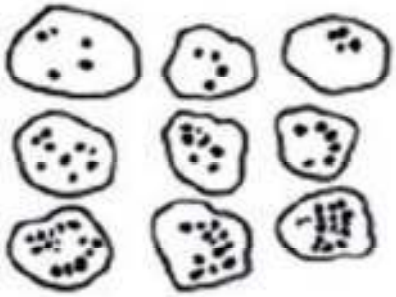
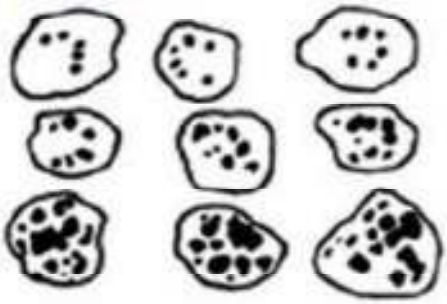
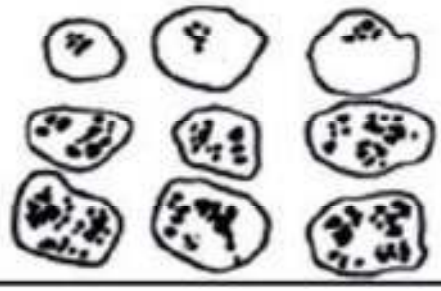
Dhakhwa R et al[32] conducted a study on 110 breast lumps and observed mean AgNOR count was 2.63 ± 1.36 and the SAPA score was 6.26 ± 1.19 in benign breast lesions. The mean AgNOR count was 8.42 ± 2.53 and SAPA score was10.05 ± 2.22 in malignant breast lesions. The cut off score for AgNOR count is considered as 6 for malignant neoplasm of breast, then the score provides 95.5% diagnostic accuracy , 88.9% specificity, 89.5% sensitivity, 82.2% positive predictive value and 98.5% negative predictive value. When the cut off value for SAPA score is considered as 8 for malignant neoplasm of breast, then there is 85.5% diagnostic accuracy, 83.3 % specificity, 89.5% sensitivity, 73.9 % positive predictive value and 93.8% negative predictive value.

TABLE 5: AgNOR Count and SAPA Score in Breast Lesions BY Dhakwa R et al[32]

Diagnosis	Number of cases	AgNOR Count	SAPA score
Fibrocystic changes	7	2.71+/-1.38	6+/-1.55
Fibroadenoma with fibrocystic changes	7	2.86+/-1.21	5.86+/-3.8
Intraductal papilloma	1	5	7
Infiltrating ductal carcinoma – NOS	32	8.31+/-2.6	9.94+/-2.2

In cases with diagnostic difficulties on Cytology smears, subjective AgNOR pattern assessment and AgNOR counting showed better accuracy in differentiating malignant from benign lesions. In few cases, this study when done separately may give contradictory results and thus it is more helpful when both are considered together.

TABLE 6: SUBJECTIVE AgNOR PATTERN ASSESSMENT SCORE

Parameter	Score	Illustration
Estimated number per cell		
Few (<5)	1	
Several (5-10)	2	
Many (>10)	3	
Variation satellite size and shape (score each)		
Uniform	1	
Moderate variation	2	
Marked variation	3	
Variation cluster size and shape (score each)		
Uniform	1	
Moderate variation	2	
Marked variation	3	

SAPA score is rapid, reproducible and minimal time consuming than counting the AgNOR dots[32]. The results of both SAPA score and AgNOR counts are similar in cytology smears[33].

Khanna AK et al[33] proposed the study, has found that SAPA score was very useful in distinguishing benign from malignant neoplasm of breast in cytology smears as well as histology specimens.

APPLICATIONS OF AgNORs:

AgNORs as a one step silver colloid staining technique was first used in the specimens of prostate. Followed by a variety of specimens which uses AgNOR staining to differentiate benign and malignant lesions. In malignant neoplasm, the tumor aggressiveness correlates with AgNOR count.

AgNORS IN BREAST:

Earlier cytogenetic workup studies performed in malignant breast lesions revealed unusual and ectopic NOR (Nucleolar Organizer Regions) patterns. This study has thrown light for the pathologist to explore the potentials of AgNORs in differentiating borderline breast lesions from the malignant ones. Many studies says, AgNOR values correlates well with the prognostic indices like tumor size, axillary lymph node status, MIB-1 index , Ki-67 index, and mitotic counts[34].

CYTOLOGY APPLICATIONS:

As AgNORs being the indicators of cellular proliferative activity correlates well with Ki-67 index, in a study conducted by Dervan PA, Gilmartin LG, Loftus BM, Carney DN[42] on 70 cases of malignant breast lesions and 27 cases of benign breast lesions. The correlation between AgNOR count and Ki-67 scores was significant. The view of these authors was also shared by Canepa M et al[43] who conducted a study on 53 cases of intra ductal breast carcinoma.

Kesari AL et al[44] evaluated 120 cases of intra ductal breast carcinoma and found a positive correlation between histological grading, AgNOR score and PCNA expression. Poorly differentiated carcinomas had a highly elevated AgNOR counts.

Our present study was aimed to find out whether there is any significant difference in the AgNOR values of benign and malignant neoplasm of the breast and also to find out if there is any significant change in the AgNOR values between the proliferative breast disease with atypia from without atypia.

MATERIALS AND METHODS

This prospective study is undertaken in the Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore during the study period of about two years (July 2017 to June 2019). The study includes Breast neoplasm cytology smears received from patients presented with clinically palpable Breast lumps for Fine Needle aspiration followed by staining for AgNOR and correlate with Histopathology. However, Histopathologic diagnosis is taken as gold standard. This study was conducted after obtaining clearance from the Institutional Ethical Committee. Patients presenting with clinically palpable breast lumps underwent fine needle aspiration of the lumps followed by Hematoxylin and eosin staining and AgNOR staining of the cytology smears. Histopathologic examination was done for the specimen received.

FINE NEEDLE ASPIRATION CYTOLOGY

FNAC was performed on patients who presented with clinically palpable breast lumps.

INCLUSION CRITERIA:

- Patients presenting with palpable breast lesions
- Females more than 16 years and less than 70 years

- Benign Breast Lesions- Fibroadenoma, Phyllodes tumour, Fibroadenosis
- Malignant BreastLesions- Carcinoma insitu, Invasive Carcinoma

EXCLUSION CRITERIA:

- Patient who refused FNAC procedure
- Females of Age less than 16 years and more than 80 years
- Patients in whom no definable breast mass can be detected
- Pregnant Females
- Males
- Inflammatory Breast Lesions

METHOD OF COLLECTION:

The FNA procedure is done as an outpatient procedure without anaesthesia in the cytology laboratory of our Pathology department. Before performing the procedure, consent was taken from the patient after explaining it. The consent form is in **Appendix I.** The history and clinical details of the patient is filled in a separate form as in **Appendix II**. Disinfection was done by scrubbing the skin with alcohol. A 23 gauge needle with 10ml disposable syringe is attached to the syringe holder. The clinically palpable breast lesion was first fixed between the thumb and index finger of one hand and the needle

was inserted to the estimated depth within the mass with the other hand. The negative pressure was created with 3-4 short passes in various directions and the material was aspirated. The needle is withdrawn after the negative pressure released. The material that was aspirated from the lesion is expressed on to glass slides and smeared. The smear is immediately fixed for 15 -20 minutes with 95% ethanol which is already kept in the coplin jar. The slides were stained with Hematoxylin and Eosin and AgNOR stain.

The silver-staining method constitutes the mixture of 50% silver nitrate solution and 1% formic acid in 2gms% of gelatin solution thereby acting as a colloid stabilizer[19]. These solutions are freshly prepared and used. Cytology smears are incubated in this mixture for 45 min to one hour followed by washing, dehydration, clearing and mounted for examination under the microscope.

STAINING PROTOCOL FOR AGNOR STAINING [54]:

AgNOR staining was performed using a one step silver – colloid technique.

PREPARATION OF STAINING SOLUTION:

Solution A: 2% gelatin in 1% formic acid

Solution B: 50% aqueous silver nitrate solution

WORKING SOLUTION:

One part of solution A mixed with two parts of solution B.

PROCEDURE:

1. The aspirated material is smeared onto the slides and is immediately fixed in 95% ethanol.
2. The slides are stained with the working solution (AgNOR stain).
3. The working solution of mixture A & B are layered over the slides and are kept in a dark room for a period of 45 – 50 minutes.
4. The silver colloid then washed with deionised water.
5. The smears are dehydrated through alcohol.
6. Clearing done by in Xylene.
7. Mounting by using DPX mounting medium.

THE STAINING PROTOCOL FOR HEMATOXYLIN AND EOSIN STAIN[54] IN FNAC is as follows.

1. The aspirated material is smeared onto the slides and immediately fixed in 95% ethanol.
2. Stain in alum haematoxylin for 7 min
3. Wash in running tap water.
4. Differentiate in acid alcohol – 1 dip
5. Wash in running tap water
6. Stain in 1% Eosin Y – 3 dip
7. Wash in running tap water for 5minutes
8. Dehydrate through graded alcohols
9. Clear in Xylene.
10. Mount using DPX mounting medium

THE STAINING PROTOCOL FOR HEMATOXYLIN AND EOSIN STAIN[54] IN HISTOPATHOLOGY is as follows:

1. Sections were deparafinized by immersing in xylene for 30 seconds.
2. Sections are then placed in Isopropyl alcohol for 15 minutes.
3. Wash in running tap water.
4. Sections are then stained with Ehrlich's Hematoxylin solution – 15 minutes.
5. Wash in running tap water
6. Differentiate with acid alcohol1% solution- 2 to 3 dips.
7. Blueing is done for 10 minutes
8. Counterstain is done with eosin 1% solution – 3 to 4 dips
9. Wash in tap water
10. Sections are dried
11. Dip in Xylene and mount in DPX

The Hematoxylin and eosin stained smears are analysed and concluded the cytological diagnosis. Another set of smears stained by AgNOR method were evaluated for mean AgNOR count and SAPA score using different variables as described above- such as estimated number per cell, variation in satellite size and shape and finally variation in cluster size and shape. Cytological grading of breast carcinoma was done according to the Robinson's cytological grading system which is a three-tier grading system, classifying carcinomas into grade 1, grade 2 and grade 3.

These smears were analysed and the results were combined by making a master chart with Cytology diagnosis, Robinson's grading system, AgNOR count and SAPA score and histopathology diagnosis for the available cases. Correlations between these grading systems along with Histopathological diagnosis were assessed. For this study, an Olympus microscope with 10X, 40X and 100X magnification objectives and 10X magnification eyepiece were used. The digital images of the selected stained smear preparations were photographed.

OBSERVATIONS & RESULTS

This study is a prospective study conducted in Department of Pathology, Coimbatore Medical College, Coimbatore for period of July 2017 to July 2019. This study includes sample size of 100 cases of Breast neoplasms in FNAC.

Out of these 100 cases of cytology smears of Breast neoplasms, in which Hematoxylin and eosin stain as well as AgNOR staining was done, 61 cases were operated and sent for Histopathology examinations which were received in our laboratory.

Table 7: Age Distribution of Breast neoplasms- Benign and Malignant

Age in groups	FNAC		
	Benign	**Malignant**	**Premalignant**
10-19	4(100.0%)	0(0.0%)	0(0.0%)
20-29	11(100.0%)	0(0.0%)	0(0.0%)
30-39	14(73.7%)	1(5.3%)	4(21.1%)
40-49	15(53.6%)	9(32.1%)	4(14.3%)
50-59	7(35.0%)	11(55.0%)	2(10.0%)
60-69	0(0.0%)	9(100.0%)	0(0.0%)
70-79	2(22.2%)	6(66.7%)	1(11.1%)

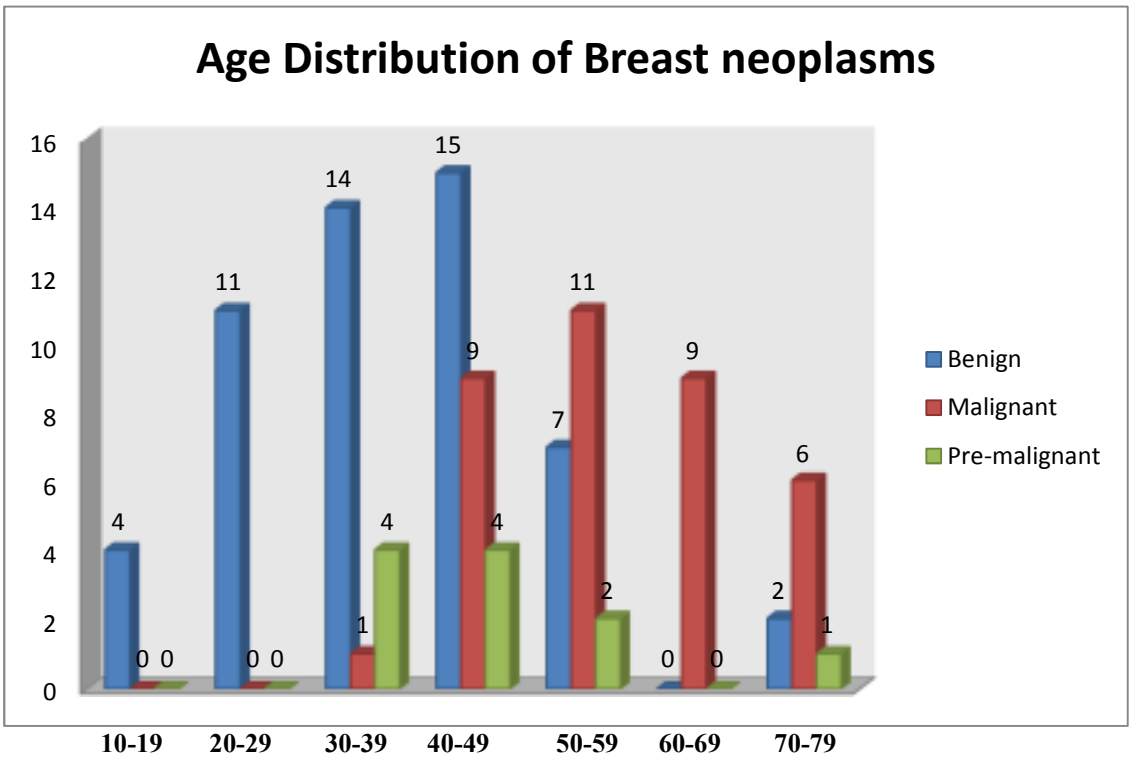


Chart 1: Age distribution of Breast Neoplasm

By dividing the age group of the female patients presenting with breast lumps, the benign breast neoplasms were common among the age group of 30 to 49 years, while the malignant breast neoplasms were 50 to 59 years. There is a overlap of these benign, malignant and premalignant lesions among the age group of 30 to 59 years. At the age group of 30 to 39 years, benign lesions were about 73.7%, followed by premalignant lesions of 21.1% and finally 5.3%. At the age group of 40 to 49 years, 53.6% were benign, 32.1% malignant and 14.3% premalignant lesions. At the age group of 50 to 59 years, 55% of the cases were malignant, 35% of cases benign and finally 10% premalignant. The youngest patient in our study with benign breast neoplasms was 16 years while the oldest was 75 years. The incidence of benign neoplasms were highest

among the age group of 30-39 years constituting 53.6% while malignant neplasms were maximum among 50-59 years constituting 55%. Premalignant lesions in FNAC were maximum among the age group of 30-39 years constituting 21.1%.

Breast lesions are more common on the left with a percentage of 57% and right side 43%. Both benign and malignant lesions were also common on the left side.

Among the four quadrants of breast, clinically palpable breast carcinomas are more common on the upper outer comprising 57%, followed by upper inner 28%, lower outer 15% and rarely lower inner quadrants.

Table 8:Frequency Distribution of Benign and Malignant Breast neoplasms in FNAC

FNAC	Frequency	Percentage (%)
Benign	53	53.0
Malignant	36	36.0
Total	100	100.0

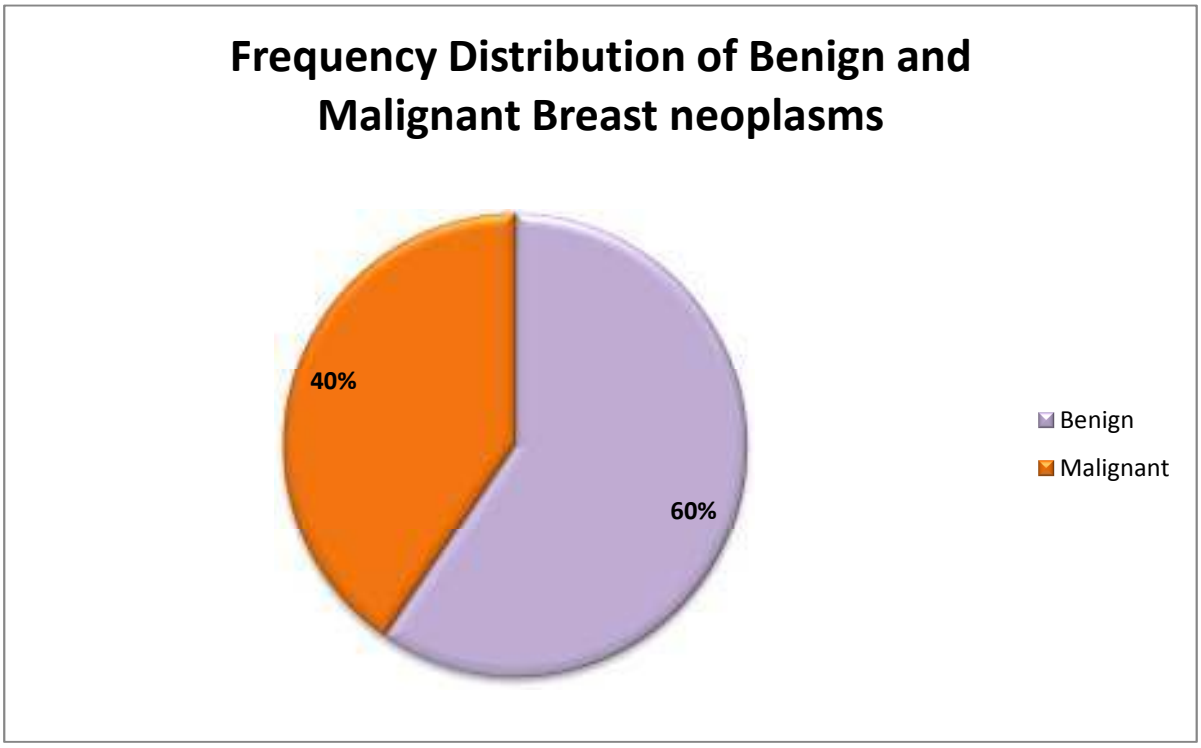


Chart 2: Frequency distribution of Benign and Malignant breast neoplasms

Out of 100 cytological smears, 53 cases were found to be benign with a percentage 53% and remaining 47 cases were malignant with a percentage 47%.

In FNAC, there were 53 benign cases among the sample size of 100. Of these, 22 cases were fibroadenoma with a percentage of 45.3%, 14 cases were Proliferative Breast disease without atypia of 26.4%, 11 were diagnosed to be fibrocystic disease of breast comprising 20.8%, and finally 4 cases were Benign Phyllodes comprising 7.5%.

Table 9: Frequency Distribution of Benign Breast neoplasms in FNAC

FNAC-Benign	Frequency	Percentage (%)
Benign Phyllodestumor	4	7.5
Fibroadenoma	24	45.3
Fibrocystic disease of Breast	11	20.8
Proliferative breast disease without atypia	14	26.4
Total	53	100.0

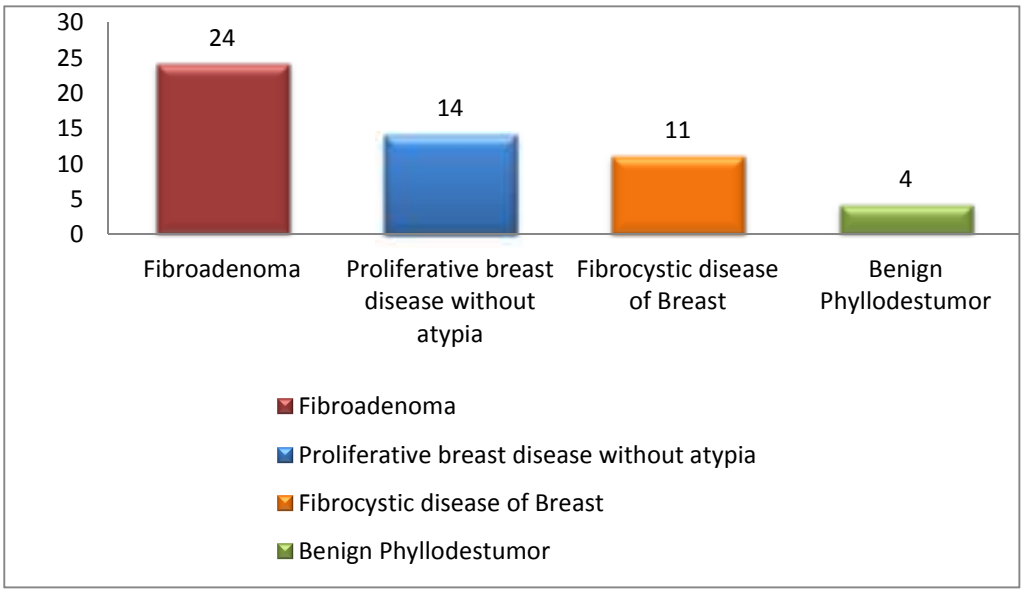


Chart 3: Frequency Distribution of Benign Breast neoplasms in FNAC

Premalignant lesions were Proliferative Breast disease with atypia were with a frequency of 11 cases out of 100 sample size. Therefore the percentage is 11%.

Table 10: Distribution of Malignant Breast neoplasms in FNAC

FNAC-Malignant	Frequency	Percentage (%)
Ductal carcinoma of Breast	34	94.4
Malignant Phyllodestumor	1	2.8
Suspicious of malignancy	1	2.8
Total	36	100.0

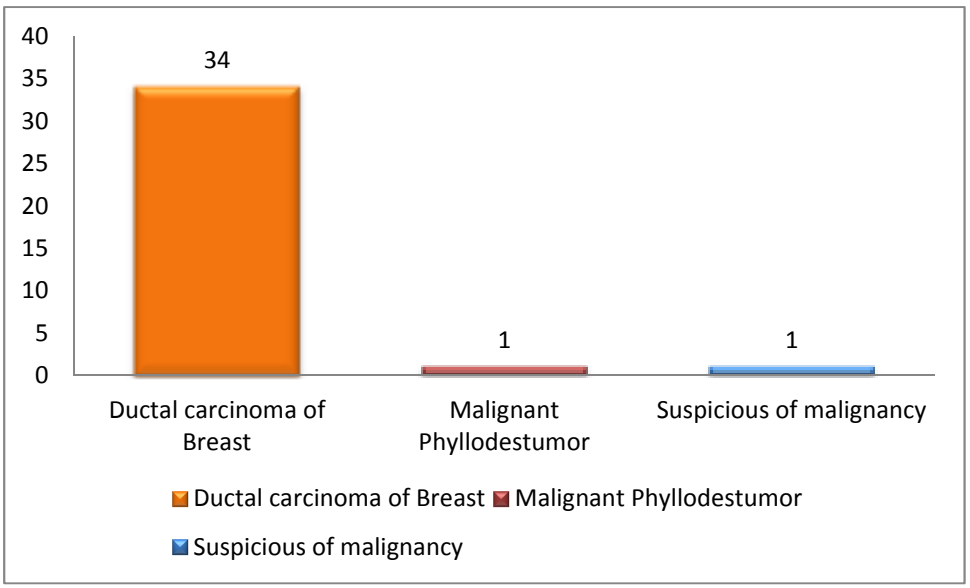


Chart 4: **Distribution of Malignant Breast neoplasms in FNAC**

Malignant lesions in FNAC were 36 cases out of 100. Among these 36 malignant lesions, they were subcategorized as Ductal carcinoma of breast which was 34 in number with a percentage of 94.4%, 1 case of Suspicious of malignancy comprising 2.8% and 1 case of Malignant Phyllodes tumor with 2.8%.

Table 11: Robinson's cytology grade distribution of Breast carcinoma

CYTOLOGY GRADE	Frequency	Percentage (%)
I	9	9.0
II	19	19.0
III	7	7.0
Total	35	100.0

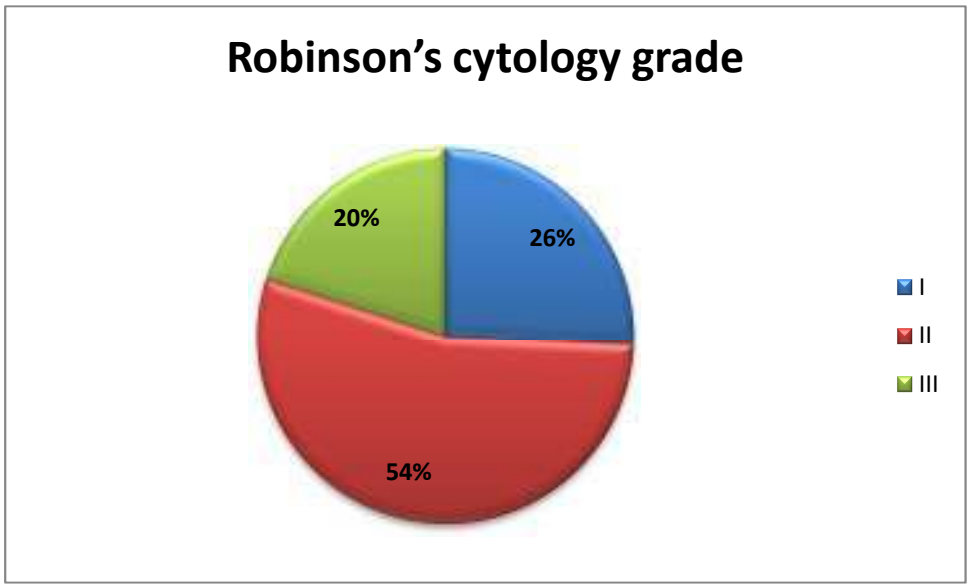


Chart 5: Distribution of Robinson's cytology grade among Ductal carcinoma

All cases of Breast carcinomas in FNAC are graded according to Robinson's Cytological grading into three categories. Out of 35 cases reported as Ductal carcinoma of breast in FNAC, 9 cases comes under grade 1 category having a score of 6-11 with a percentage of 25.7%. Grade 2 has a score of 12-14, of which 19 cases (54.28%) comes under this grade. 7 cases comes under grade 3 with a score of 15-18 having 20%.

Table 12: Association of FNAC with Mean Agnor

	FNAC	N	Mean	SD	P value
Mean Agnor	PREMALIGNANT	11	4.9182	1.51646	.001*
	BENIGN	53	3.6228	.91288	
	MALIGNANT	36	6.7872	2.10670	

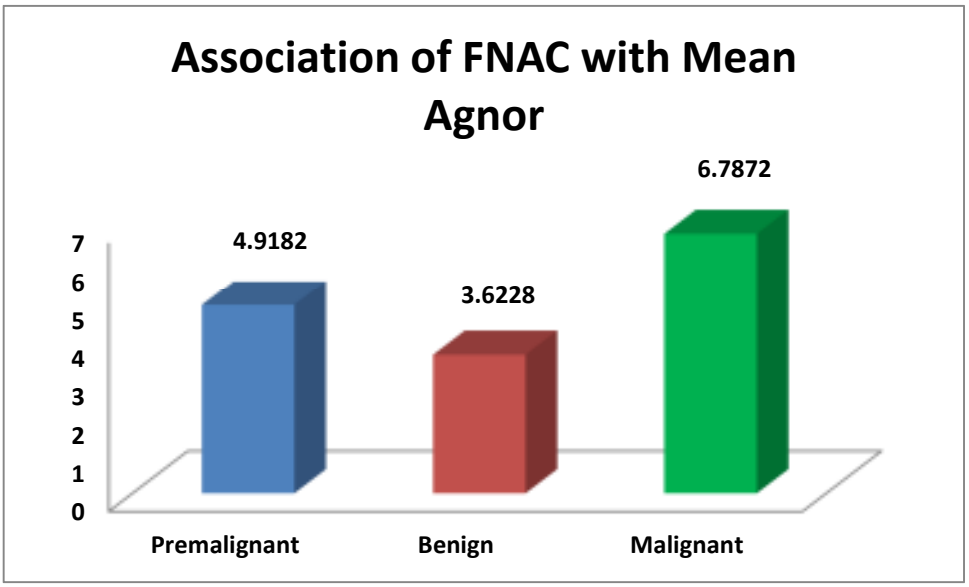


Chart 6: Association of FNAC with mean AgNOR

The AgNOR staining and analysis were done in 100 cytological smears , which revealed a mean AgNOR count of 3.62+/- 0.913 in benign neoplasms. In premalignant neoplasms, mean Agnor count of 4.92+/- 1.516 while malignant neoplasms were 6.78+/- 2.106. P value for mean AgNOR count among FNAC of both benign and malignant neoplasms were 0.001 (<0.05) which is statistically significant.

Table 13: Association of FNAC with Mean SAPA Score

	FNAC	N	Mean	SD	P value
Mean SAPA Score	PREMALIGNANT	11	10.27	1.954	.001*
	BENIGN	53	6.92	1.492	
	MALIGNANT	36	12.42	2.430	

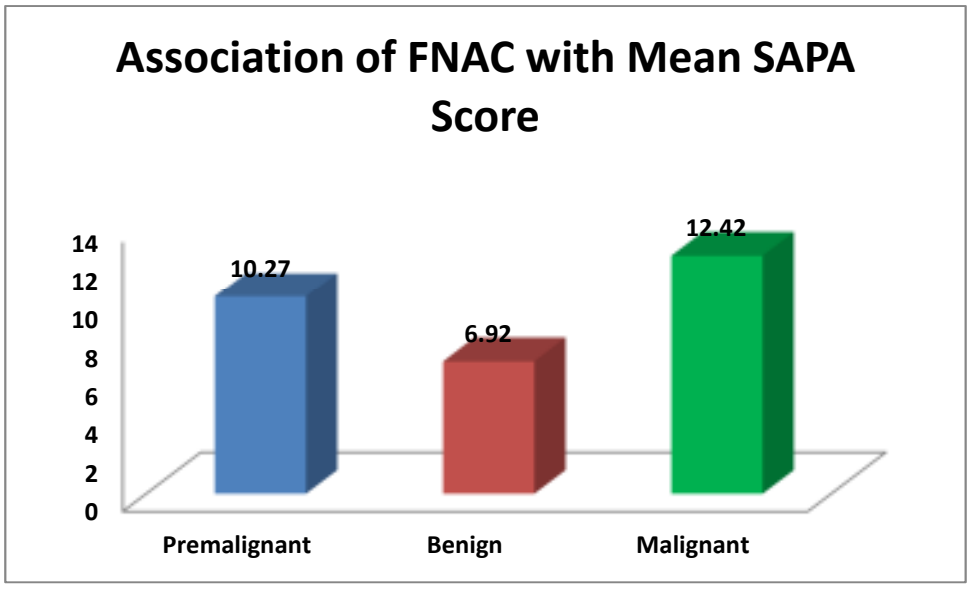


Chart 6: Association of FNAC with mean SAPA Score

SAPA Score was enumerated in the cytology smears under the variables of estimated number per cell, variation in cluster size and shape and in satellite size and shape. In benign neoplasms, SAPA score was 6.92 +/- 1.492, Premalignant lesions were 10.27 +/- 1.954 . Malignant neoplasms has a SAPA score of 12.42+/-2.430. P value for Subjective AgNOR Pattern Assesment (SAPA) score among FNAC of both benign and malignant neoplasms were 0.001 (<0.05) which is statistically significant.

Table 14: Distribution of Breast neoplasms in Histopathology

HISTOPATHOLOGY	NUMBER OF CASES (out of 61 cases)	PERCENTAGE
Benign	34	55.7%
Malignant	27	44.26%

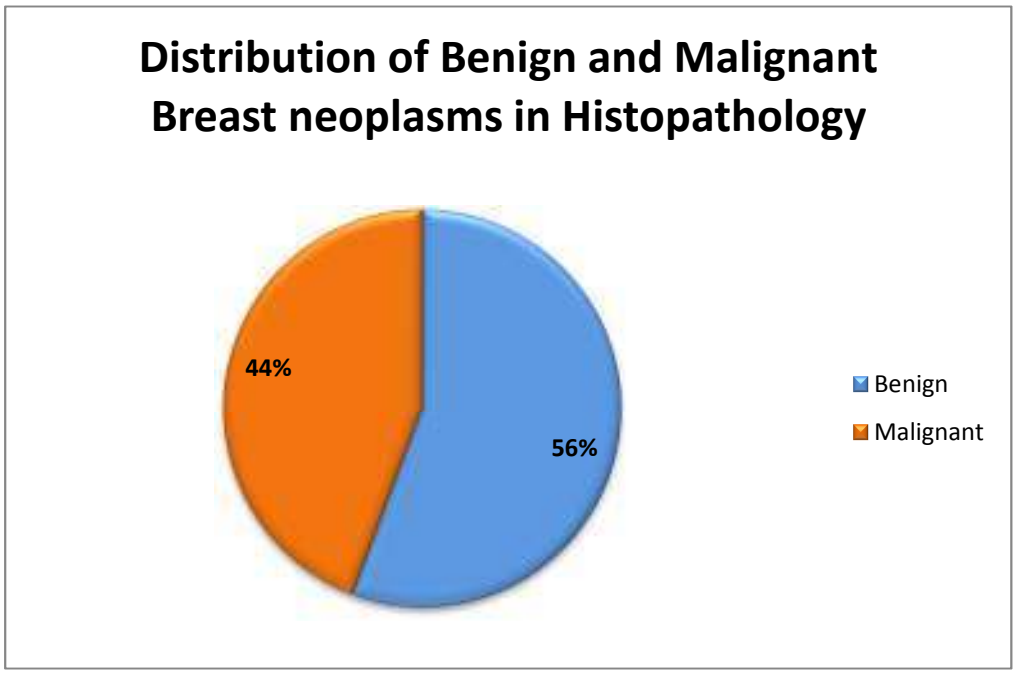


Chart 8: **Distribution of Breast neoplasms in Histopathology**

Out of 100 cases of cytology smears, 61 cases were operated and sent for histopathology. Among these cases, 34 cases came to be benign with a percentage of 55.7%, while 27 cases malignant with 44.26%.

Table 15: Frequency Distribution of Benign Breast neoplasms in Histopathology

Histopathology-Benign	Frequency	Percentage (%)
Fibroadenoma	28	82.4
Fibroadenosis	5	14.7
Fibrocystic disease of Breast	1	2.9
Total	34	100.0

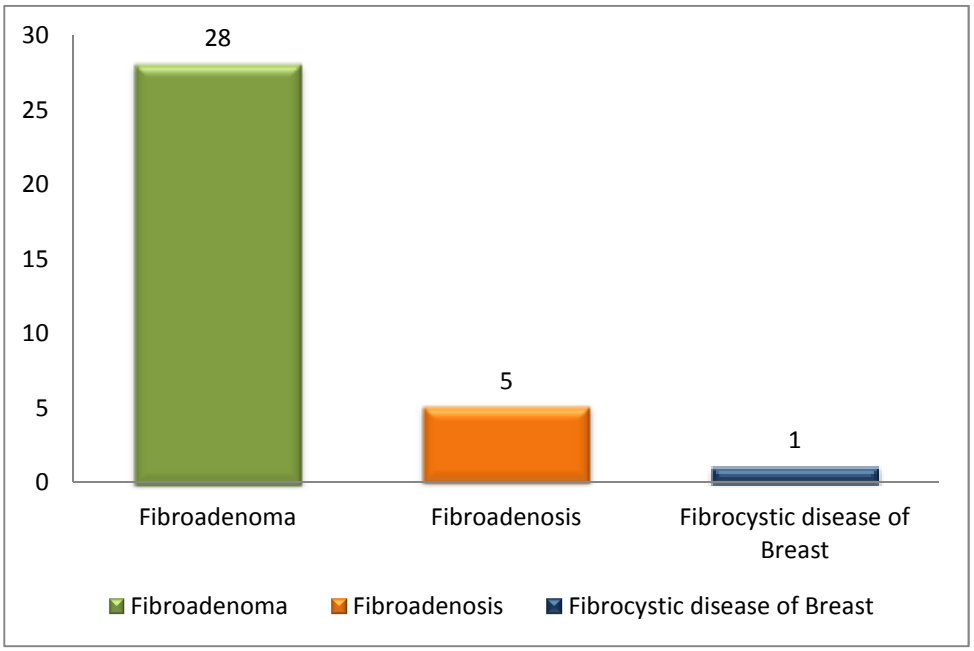


Chart 9: Frequency Distribution of Benign Breast neoplasms in Histopathology

These confirmed 34 histopathologic benign cases were subcategorized as Fibroadenoma with 28 cases comprising 82.35%, followed by 5 cases of fibroadenosis with 14.7% and finally 1 case of fibrocystic disease of breast with a percentage of 2.9%.

Table 16: Frequency Distribution of Malignant Breast neoplasms in Histopathology

Histopathology- Malignant	Frequency	Percentage (%)
Ductal carcinoma in situ	5	18.5
Invasive Carcinoma(Special types)	3	11.1
Invasive ductal carcinoma	16	59.3
Malignant Phyllodes tumor	3	11.1
Total	27	100.0

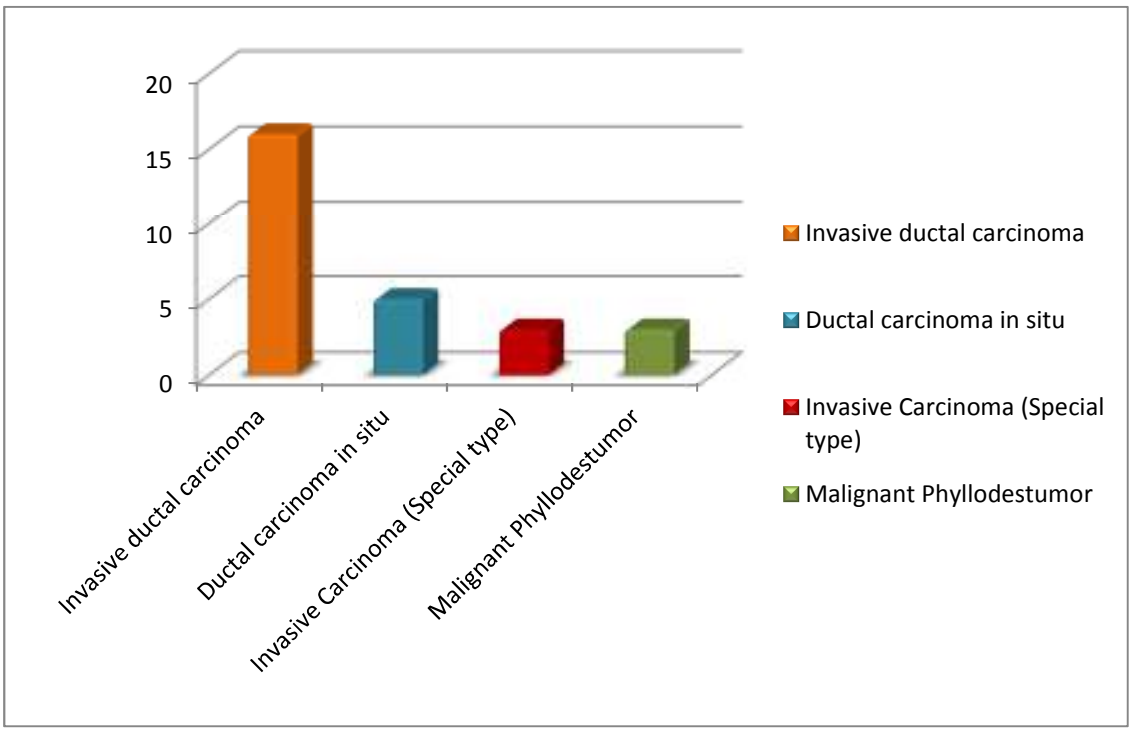


Chart 10: Frequency Distribution of Malignant Breast neoplasms in Histopathology

27 cases were diagnosed to be malignant in histopathology. Out of these 27 cases, Invasive ductal carcinoma were 16 in number with a percentage of 59.25%, other Invasive carcinomas- special types were 3 in number which includes microinvasive papillary carcinoma, lobular carcinoma and apocrine carcinoma with a percentage of 11.1%, Ductal carcinoma insitu were 5 in number with percentage of 18.5% and finally 3 cases of Malignant Phyllodes tumor comprising 11.1%.

Table 17:Association of FNAC with Mean Agnor in correlation with Histopathology

	Histopathology	N	Mean	SD	P value
Mean Agnor	BENIGN	34	3.4206	.90546	. 001*
	MALIGNANT	27	6.2526	1.76384	

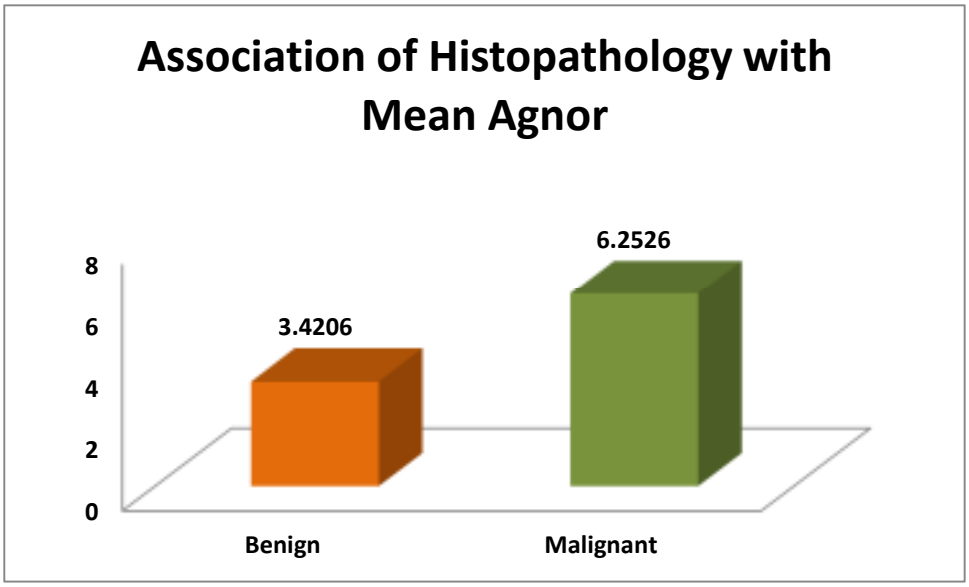


Chart 11: **Association of FNAC with Mean Agnor in correlation with Histopathology**

The AgNOR staining done in cytology smears correlated with histopathologic diagnosis of benign and malignant cases. The mean AgNOR count of 3.4206+/- 0.905 in benign neoplasms while in malignant neoplasms was 6.252+/- 1.763. P value of AgNOR was statistically significant with value of 0.001 (<0.05)

Table 18: Association of FNAC with Mean SAPA score in correlation with Histopathology

	Histopathology	N	Mean	SD	P value
Mean SAPA Score	BENIGN	34	6.82	1.585	001*
	MALIGNANT	27	11.33	2.253	

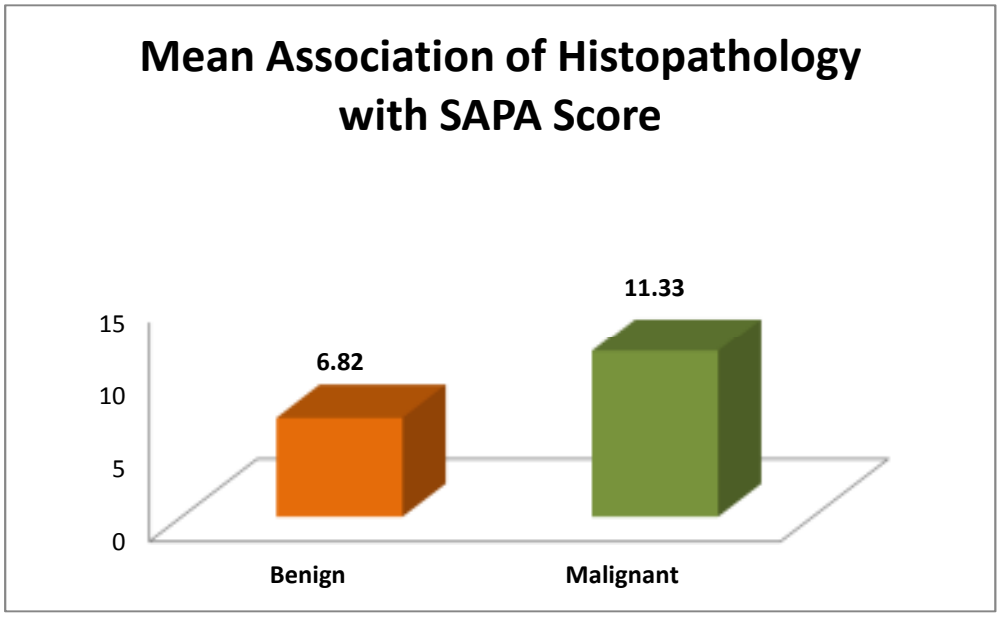


Chart 12: Association of FNAC with Mean SAPA score in correlation with Histopathology

SAPA Score was enumerated in the cytology smears correlating with histopathology diagnosis. In benign neoplasms, SAPA score was 6.82 +/- 1.585. Malignant neoplasms has a SAPA score of 11.33+/-2.253. P value of SAPA score was statistically significant with value of 0.001 (<0.05).

Table 19: Association of FNAC with HISTOPATHOLOGY

FNAC	HISTOPATHOLOGY		P Value
	Benign	Malignant	
Benign	32(91.4%)	3(8.6%)	.001*
Malignant	0(0.0%)	18(100.0%)	
Pre-Malignant	2(25.0%)	6(75.0%)	

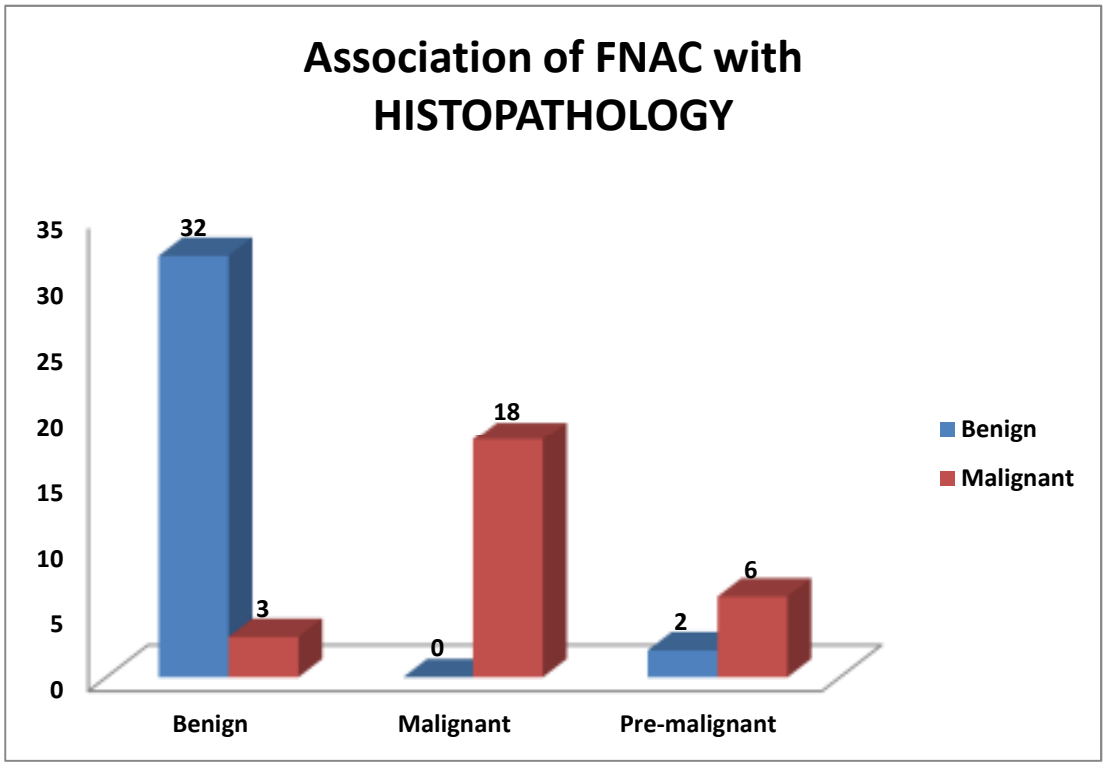


Chart 13: Association of FNAC with HISTOPATHOLOGY

FNAC diagnosis of 35 benign cases proved be 91.4% benign in Histopathology and remaining 8.6% to be malignant. P value is 0.001 (<0.05) and is statistically significant. Cytology diagnosis of all 18 malignant cases proved to 100% malignant in histopathology. In Premalignant breast lesions of 8 cases, 2 cases proved to be benign with percentage of 25% while 6 cases to be malignant with 75%. Association of FNAC diagnosis with Histopathology diagnosis of benign and malignant is statistically significant with a P value of 0.001(<0.05).

Proliferative Breast disease with atypia is a challenging one for the surgeon to decide whether it is benign or malignant and thus the patient can be proceeded to Excision biopsy or Mastectomy. This study helps the Surgeon to reduce this dilemma as mean AgNOR count and SAPA score assist in differentiating malignant from benign neoplasms.

Total cases- (8 cases)	**Benign** - (2 cases)	**Malignant** (6 cases)	
Histopathology diagnosis	Fibroadenoma	Ductal carcinoma insitu	Invasive Ductal Carcinoma
Number of cases	2	2	4
Percentage	25%	25%	50%

Table 20: Frequency distribution of Benign and Malignant neoplasms among Proliferative Breast disease with atypia

Proliferative Breast disease with atypia has a total of 9 cases, of which 8 cases followed up with histopathology. In Histopathology diagnosis, 2 cases were benign and 4 cases were malignant. Among malignant cases, 2 cases came out to be Ductal carcinoma insitu and 4 cases to be Invasive ductal carcinoma.

Histopathology	N	Mean	Std. Deviation
BENIGN	2	3.4	2.12
MALIGNANT	6	5.4167	1.41

Table 21: Association of Histopathology with Mean Agnor count in Proliferative Breast disease with atypia

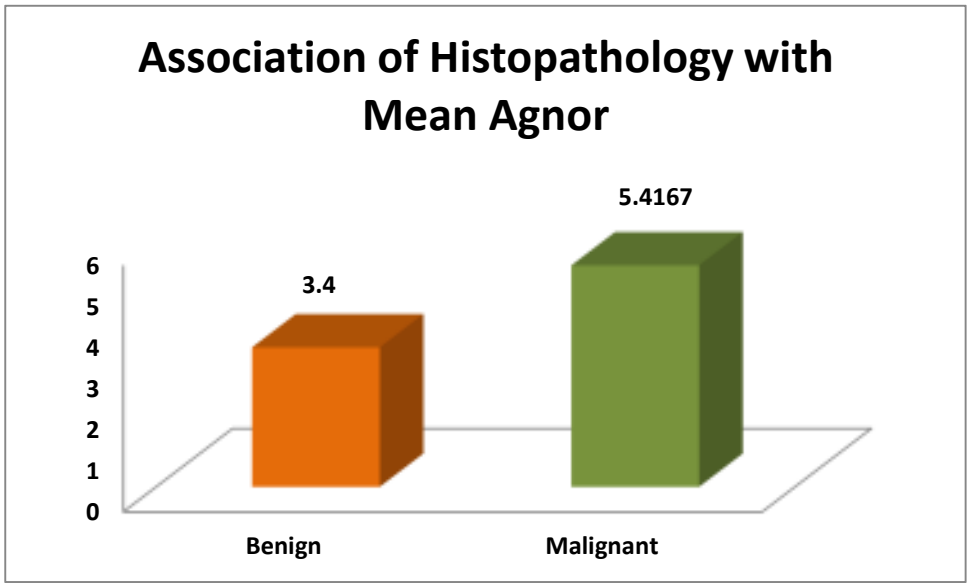


Chart 14: Association of Histopathology with Mean Agnor count in Proliferative Breast disease with atypia

Association of Mean AgNOR count in Cytology of Proliferative Breast disease with atypia with that of histopathology revealed 3.4 +/- 2.12 in Benign lesions and 5.416 +/-1.41 in Malignant diagnosis.

	Histopathology	N	Mean	Std. Deviation
Mean SAPA Score	BENIGN	2	9.00	4.24
	MALIGNANT	6	10.33	1.36

Table 14: Association of Histopathology with SAPA Score in Proliferative Breast disease with Atypia

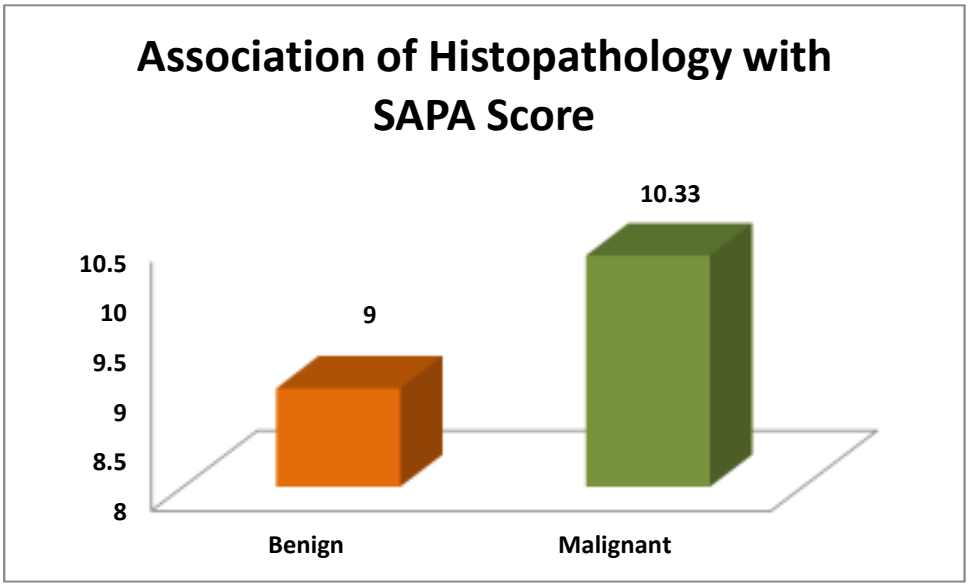
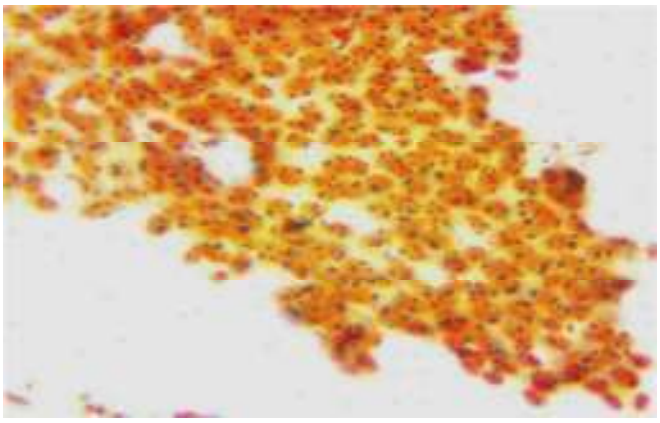


Chart 15: Association of Histopathology with SAPA Score in Proliferative Breast disease with Atypia

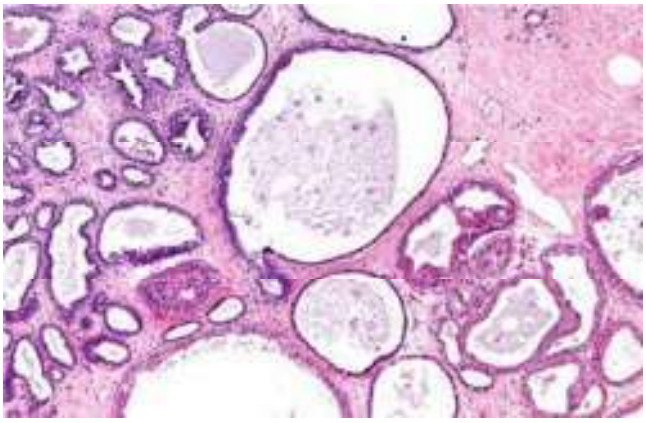
Association of SAPA Score in Cytology of Proliferative Breast disease with atypia with that of histopathology revealed 9.0 +/- 4.24 in Benign lesions and 10.33 +/-1.36 in Malignant diagnosis

COLOUR PLATE I

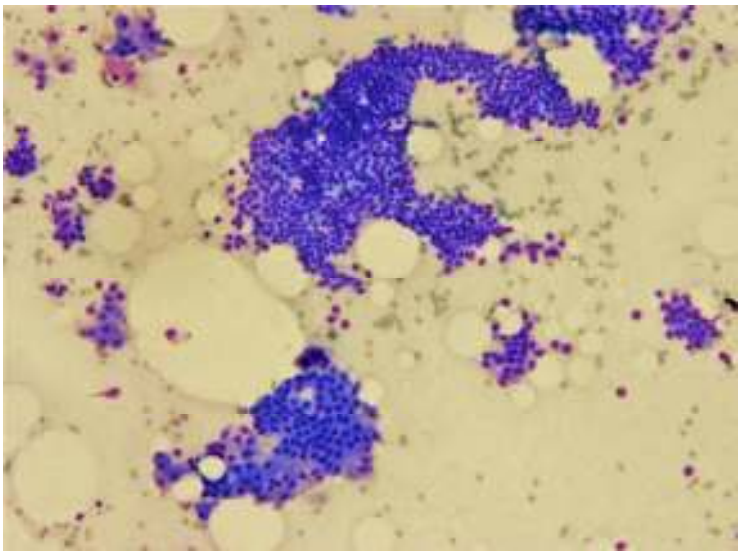
FIBROCYSTIC DISEASE OF BREAST



1A



1B

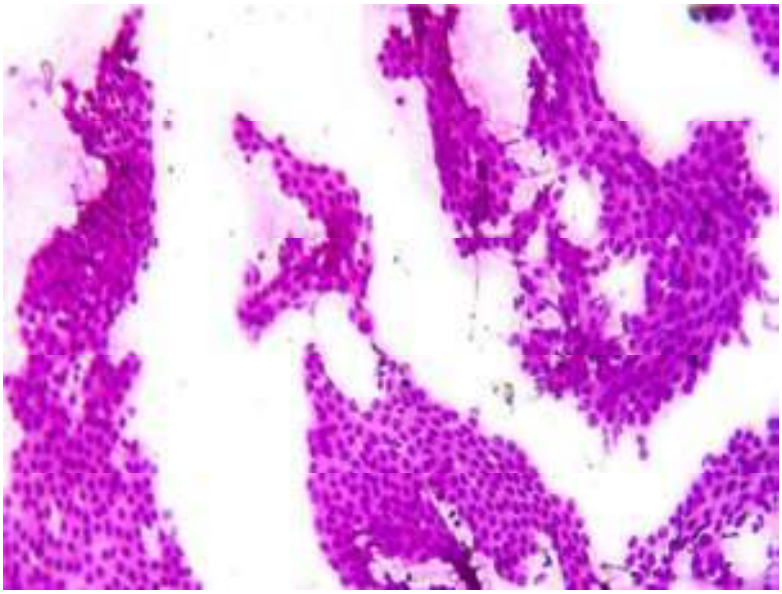


1C

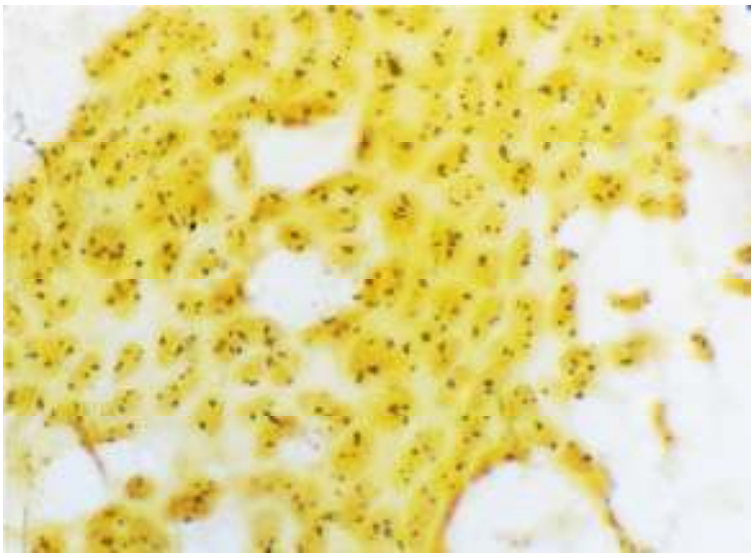
1. Fibrocystic disease of Breast (a) FNAC- AgNOR stain, 100X magnification (b) Histopathology- H&E stain, 40 X magnification (c) FNAC- H& E stain,10X magnification

COLOUR PLATE 2

PROLIFERATIVE BREAST DISEASE WITHOUT ATYPIA



2a

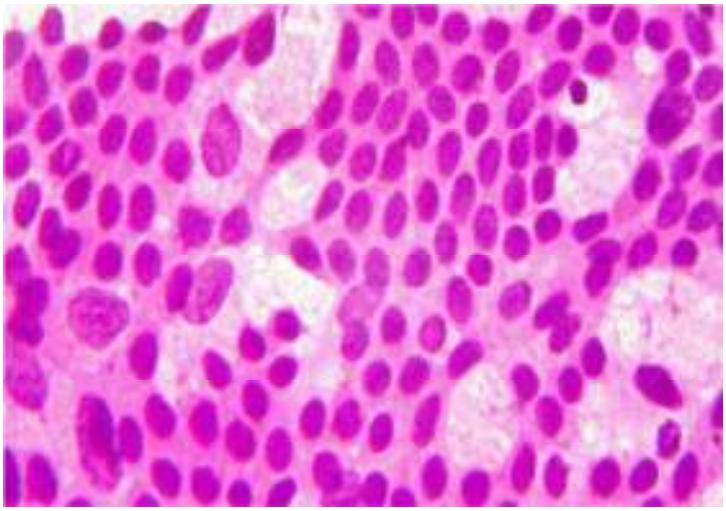


2b

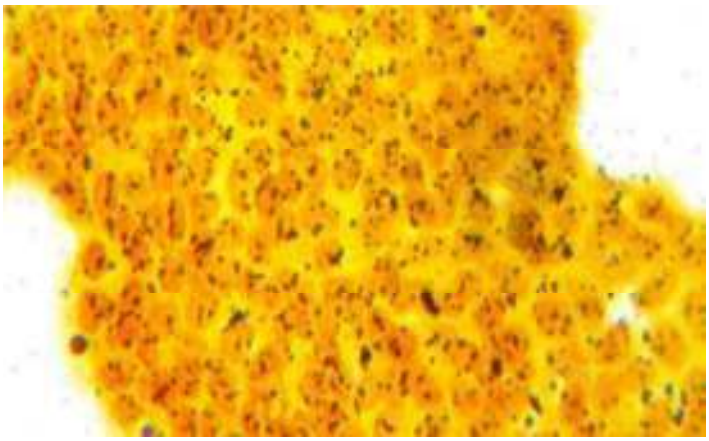
2. Proliferative breast disease without atypia (a) FNAC- H&E stain, 40X magnification (b) FNAC- AgNOR stain, 100X magnification

COLOUR PLATE 3

PROLIFERATIVE BREAST DISEASE WITH ATYPIA



3a

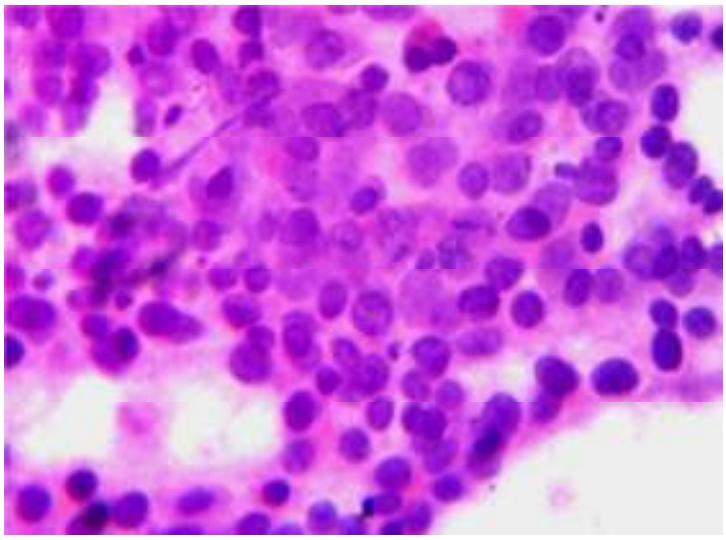


3b

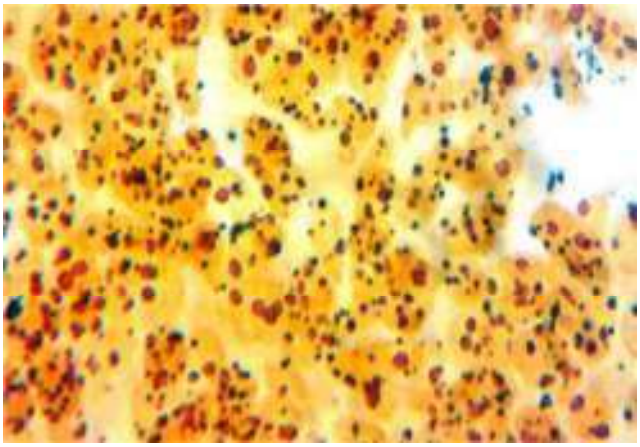
3. Proliferative breast disease with atypia (a) FNAC- H&E stain , 40X magnification(b) FNAC- AgNOR stain, 100X magnification

COLOUR PLATE 4

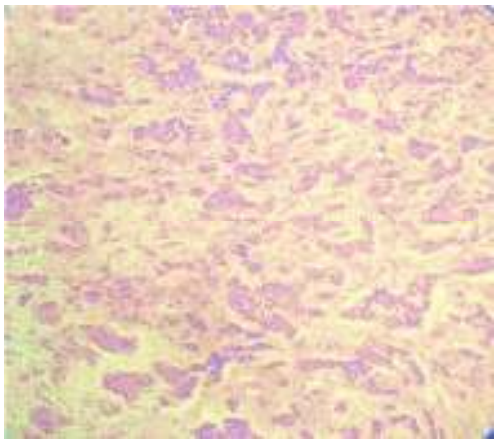
DUCTAL CARCINOMA BREAST



4A



4B

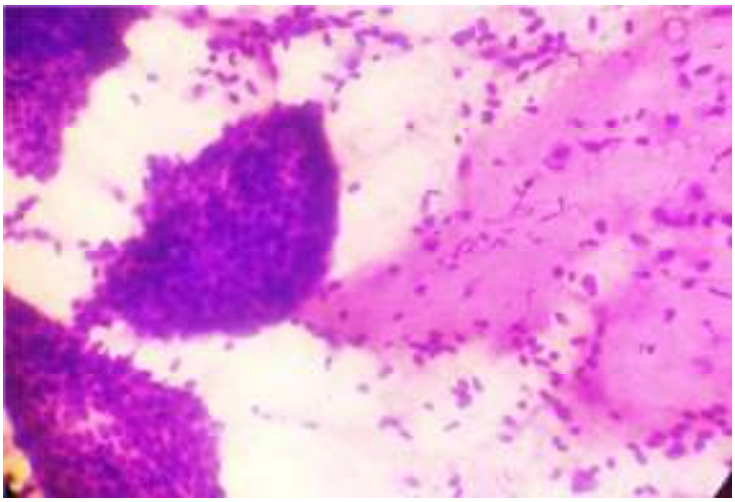


4C

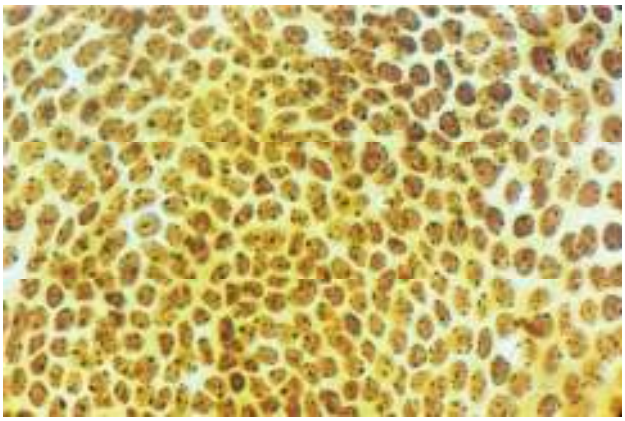
4. Ductal carcinoma of breast (a) FNAC- H&E stain, 40X magnification, (b) FNAC- AgNOR stain, 100X magnification, (c)Histopathology- H&E stain, 10X magnification

COLOUR PLATE 5

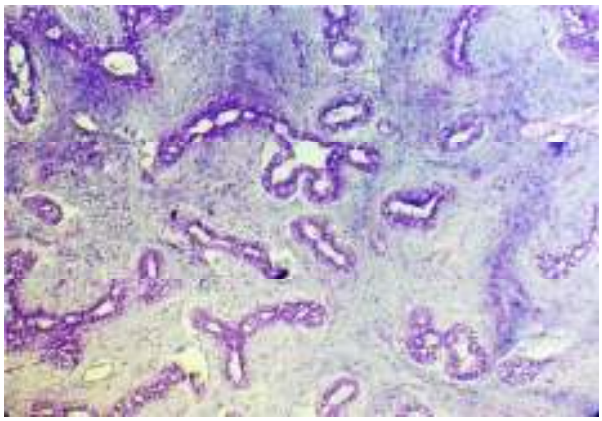
FIBROADENOMA



5A



5B

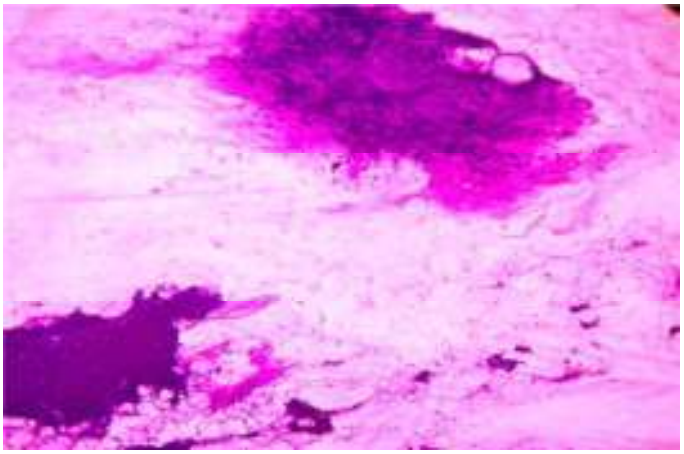


5C

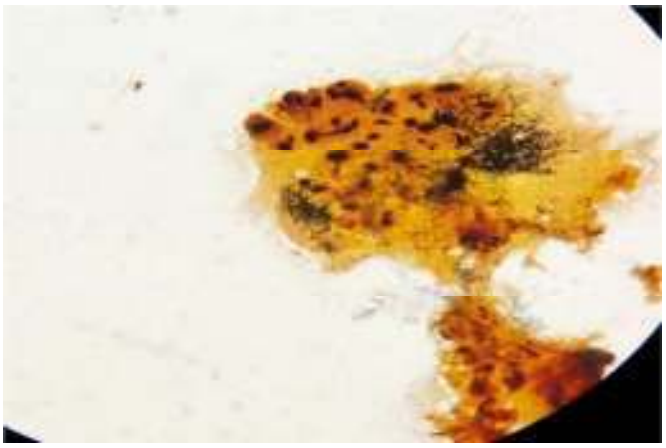
5. Fibroadenoma (a)FNAC- H&E stain, 40X magnification, (b)FNAC-AgNOR stain, 100X magnification, (c) Histopathology- 40X magnification

COLOUR PLATE 6

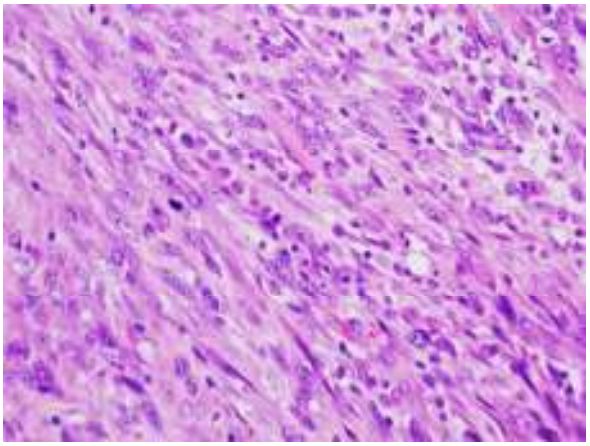
MALIGNANT PHYLLODES TUMOR



6A



6B

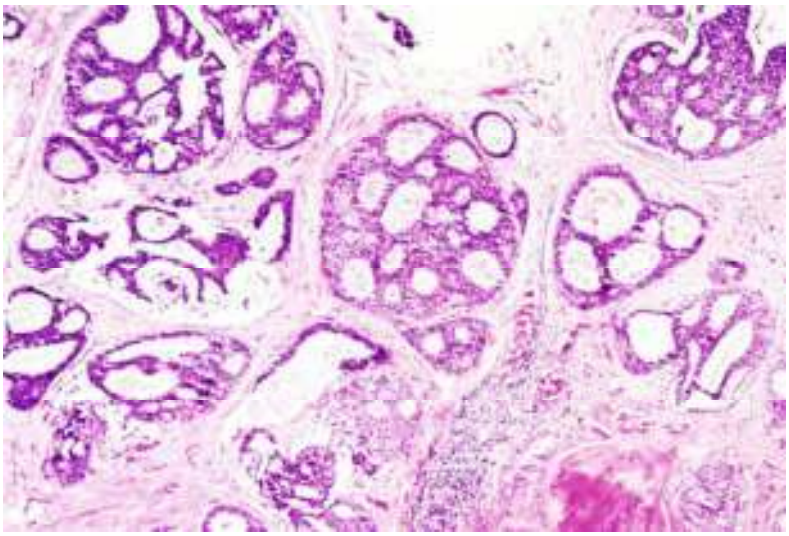


6C

6. Malignant Phyllodes tumor (a) FNAC- H&E stain, 10X magnification, (b)FNAC-AgNOR stain, 100Xmagnification, (c) Histopathology- H&E stain, 10X magnification

COLOUR PLATE 7

DUCTAL CARCINOMA INSITU



7. Ductal carcinoma insitu- Histopathology, H&E stain,40X magnification

DISCUSSION

Fine Needle Aspiration Cytology (FNAC) is rapid, simple and cost effective out-patient procedure that plays an important role in the management of different lesions of the breast. It also provides information related to the diagnosis and treatment of the patient[49]. FNAC also pose a challenge and dilemmas in diagnosis of certain situations. Mean Argyrophylic Nucleolar Organizer Region count and Subjective AgNOR Pattern Assessment provides exclusive details about the cellular proliferation. Mean AgNOR count as well as SAPA score provides a comparable diagnostic potential but the latter is more convenient and rapid for AgNOR evaluation. Thus, AgNOR count and SAPA score can be used in detecting malignancy. Moreover, AgNOR staining method is simple and cost effective. The AgNORs appear as clustered dots as black homogenous silver precipitates. These dots are scattered around the nucleus in satellites or in clusters. In benign lesions, the AgNOR dots appears homogenous, symmetric with regular contours while in malignant lesions, the AgNOR dots are asymmetric with irregular contours. They are seen as smaller, aggregrated and scattered dots. The number, frequency, and dispersion of AgNOR dots are high in malignant neoplasm as compared to the benign neoplasm of breast. Thus AgNORs reflects the aggressiveness of the lesion and act as a proliferative marker, thus having a great value in diagnosing cytoplogy[28].

Our present study was done on 100 cases of breast lesions involving both benign and malignant lesions of breast. The cytology smears was first stained with both Hematoxylin & Eosin and AgNOR stains. The AgNOR dot number and morphology is analysed tediously in every case and the results are tabulated. Similarly Robinson's Cytological grading was also calculated for each case and the findings are tabulated. The current study analyses the significance of AgNOR count and Subjective AgNOR Pattern Assessment (SAPA) score in distinguishing malignant from benign neoplasms of breast. The study also evaluates the significance of AgNOR score in relation to Robinson's score in grading Carcinoma of breasts.

Benign breast neoplasms were common among the age group of 30 to 49 years, while the malignant breast neoplasms were 50 to 59 years. The youngest patient in our study with benign breast neoplasms was 16 years while the oldest was 75 years who was malignant. The incidence of benign neoplasms were highest among the age group of 30-39 years while malignant neoplasms were 50-59 years constituting 55%. Premalignant lesions in FNAC were maximum among the age group of 30-39 years . In 2006, Mi-Jung Kim et al[50] revealed a mean age of 47.4 years for breast carcinomas, Gloria Piero et al. had a mean age of 54 years of breast carcinoma.

In our study, upper outer quadrant is more common followed by upper inner, lower outer and rarely lower inner quadrant. In studies proposed by Azzopardi and Weidner also revealed upper outer quadrant is the most

common quadrant of breast carcinoma. The least common quadrant is lower inner quadrant.

AgNOR AND SAPA SCORE – BENIGN LESIONS:

In FNAC, mean AgNOR count of benign lesion was 3.62 +/- 0.912 while SAPA score was 6.92 +/- 1.492. In Histopathologically confirmed benign neoplasms, the mean AgNOR count in its cytology smear was 3.42 +/- 0.905 and SAPA score was 6.82 +/- 1.585. The P value is statistically significant.

Simba M et al[51] proposed the study with mean AgNOR count of 1.8 for benign neoplasms of the breast. Dasgupta A et al[52] reported mean AgNOR count of 1.61 for benign breast neoplasms of breast. Contradictory to previous studies, Reddy GS et al.[53] reported a higher mAgNOR count of 7.45. Dhakwa et al[32] concluded the study in Cytology of breast neoplasms with mean AgNOR count of 2.63 +/- 1.36 while SAPA score of 6.26 +/- 1.19 in benign lesions. In malignant lesions, the mean AgNOR count and SAPA score were 8.42 +/- 2.53 and 10.05 +/- 2.22 respectively. Nepal N et al[48] reported the FNAC of breast neoplasms had mean AgNOR count and SAPA score of 1.736 +/- 0.2908 and 4.687 +/- 0.403 respectively in benign neoplasms. In malignant neoplasms of breast, the mean AgNOR count was 4.508 +/- 0.981 and SAPA score of 7.625 +/- 1.060.

AgNOR AND SAPA SCORE – PREMALIGNANT LESIONS:

Proliferative Breast disease with atypia is considered as the premalignant condition in FNAC. In our study, the mean AgNOR count in this lesion is 3.4 +/- 2.12 and the SAPA score is 5.416 +/- 1.41.

AgNOR AND SAPA SCORE – MALIGNANT LESIONS:

In FNAC, mean AgNOR count of malignant breast lesion was 6.7872 +/- 2.106 while SAPA score was 12.42 +/- 2.430. In Histopathologically confirmed malignant neoplasms, the mean AgNOR count in its cytology smear was 6.2526 +/- 1.763 and SAPA score was 11.33 +/- 2.253. The P value is statistically significant.

Simba M et al[51] had a lower mean AgNOR count of 3.5 in case of malignant breast lesions compared to the present study. Whereas Dasgupta A et al[52] reported the mean AgNOR value of 12.10 for malignant breast lesions, similar to that of

Reddy GS et al[53] reported a value of 12.72 for breast malignancies.

Proliferative Breast disease with atypia is a challenging one for the surgeon to decide whether it is benign or malignant and thus the patient can be proceeded to Excision biopsy or Mastectomy. This study helps the Surgeon to reduce this dilemma as mean AgNOR count and SAPA score assist in differentiating malignant from benign neoplasms. The mean AgNORcount in Proliferative Breast disease with atypia correlated with histopathology among

benign cases 3.4 +/- 2.12 and malignant were 5.416 +/- 1.41. The SAPA score in Proliferative Breast disease with atypia correlated with histopathology among Benign cases were 9.0 +/- 4.24 and 10.33 +/- 1.36 malignant.

Few of the cases appears contradictory like one case of Proliferative breast disease with atypia with mean AgNOR count of 4.9 and SAPA score of 12 proved to be Fibroadenoma. Here, the SAPA score is contradictory. Another case of Proliferative Breast disease with atypia with mean AgNOR count of 2.8 and SAPA score of 8 confirmed to be Invasive ductal carcinoma in histopathology. In this case, mean AgNOR count is contradictory. This contradiction may be due to reasons such as interobserver variation.

MALIGNANT VS BENIGN LESIONS:

In our study, the AgNOR staining done in cytology smears correlated with histopathologic diagnosis of benign and malignant cases. The mean AgNOR count of 3.4206+/- 0.905 in benign neoplasms while in malignant neoplasms was 6.252+/- 1.763. P value of AgNOR was statistically significant with value of 0.001 (<0.05).

SAPA Score was enumerated in the cytology smears correlating with histopathology diagnosis. In benign neoplasms, SAPA score was 6.82 +/- 1.585. Malignant neoplasms has a SAPA score of 11.33+/-2.253. P value of SAPA score was statistically significant with value of 0.001 (<0.05).

Simba M et al[39] studied the cytology of about 200 cases of breast lesions which includes 140 malignancies, 55 benign lesions and remaining 5 normal breasts. They reported that the AgNOR counts are higher in malignant neoplasm compared to the benign ones. Another study by Dasgupta A et al[52] analysed AgNOR counts are not of more significant in differentiating fibroadenoma and fibrocystic disease that are the subtypes of benign lesion of the breast. He also reported that higher AgNOR values are noted in malignant lesions compared to the benign lesions. Roller E et al[35] conducted the study which had similar findings with higher AgNOR counts for malignant neoplasm of breast compared to benign neoplasm. Reddy GS et al[36] conducted the study on 10 benign and malignant epithelial lesions of breast and reported higher AgNOR values for malignant lesions compared to benign lesions. In a study conducted by Hasnan J et al[18] on 31 benign lesions and 25 malignant lesions of breast and observed that the AgNOR value in benign lesions were in the range 2.55 to 5.0. In contrast, the malignant lesions 5.8 to 17.2. Meehan et al[31] observed the mean AgNOR value of 4.44 for benign breast lesions and 9.52 for malignant breast lesions. They concluded that the median AgNOR score is 7 for benign lesions and 13 for malignant lesions. They also added the diagnostic accuracy of 90% for AgNORs in differentiating benign from malignant lesions. Khanna et al[33] analysed 27 benign and 46 malignant breast lesions. They took two parameters like Subjective AgNOR Pattern Assessment (SAPA) score and mean AgNOR count. They stated that the mAgNOR score and SAPA were quite similar in differentiating malignant from benign breast lesions. The mean

AgNOR score of benign lesion was 2.75 compared to malignant lesion which showed 6.94. These findings are almost similar to our study. This study also reported the SAPA score of 5.87 for benign lesions and 9.02 for malignant lesions. Kumar A et al[40] had higher AgNOR values for malignant neoplasms compared to benign neoplasms of breast. Karmakar T, Radhika S, Gupta SK[37] found a higher mean AgNOR count of 16.63 for malignant lesion and mean AgNOR value of 6.39 for benign lesions of breast. The overall AgNOR values are higher compared to the present study. They concluded by putting the cut-off value of 11 for differentiating benign from malignant lesions. Mehrotra A, Chandra T[38] assessed the cytological smears of 64 malignant and 31 benign neoplasm of breast and stated that the cut off value of 4 can be used in differentiating benign from malignant breast lesions with regard to mean AgNOR counts.

Ruschoff J, Plate K, Contractor H, Neumann K, Thomas C[29] analysed that there is a considerable overlap of mean AgNOR score between malignant and benign lesions. They reported the mAgNOR count for benign lesions were in the range of 1.2 to 3.8 and the mAgNOR count for malignant lesions in the range of 1.5 to 16.2. Giri DD, Dundas SA, Lawry J, Nottingham JF, Underwood JC[28] also noted overlapping of AgNOR counts in 25 to 30% of carcinomas with epithelial hyperplastic lesions in the range of 2 to 3 AgNOR dots per nuclear profile. There was a considerable variation in the value of mean AgNOR counts by different studies. These are attributed to the fact that different authors count AgNOR dots differently, some authors had counted

clustered dots as a single dot when individual NORs could not be easily discerned, while others leave off such cells where NORs could not be easily discerned. This explains for the variation in the values of mean AgNOR count. Crocker et al[30] recommends the counting of 100 cells as a standardised approach to count the AgNOR dot.

In the year 2014, Nepal N et al[48] proposed a study on AgNOR count and SAPA score of breast lesion in both benign and malignant neoplasms in cytology smears. It was a prospective study of 40 cases among the age group of 17 to 90 years. Out of 40 cases 29 were malignant and 11 were benign. Benign cases were fibroadenoma constituting 73%, fibrocystic disease of 20% and acute mastitis of 7%. Among the malignant cases, 20 cases were followed up out of which, 18 cases were Invasive carcinomas, no special type, 1 case of mucinous carcinoma and 1 case was of medullary carcinoma. The AgNOR count were 1.736 +/- 0.2908 and 4.508 +/- 0.981 in cytology of benign and malignant breast neoplasms respectively. While the SAPA score were 4.687 +/- 0.403 and 7.625 +/- 1.060 respectively. The P value was statistically significant <0.05 for both benign and malignant cases in FNAC[48].

	AgNOR Count		**SAPA Score**	
	Mean +/- SD	**Range**	**Mean +/- SD**	**Range**
Benign	1.736 +/- 0.2908	1.2-2.16	4.687+/- 0.403	4-8
Malignant	4.508 +/- 0.981	3.3-6.04	7.625+/- 1.060	6-11

Table 23: AgNOR count and SAPA score in benign and malignant breast lesion in FNAC by Nepal N et al[48]

The difference in the value of mean AgNOR count and SAPA score in various study is due to the inter-observer variations, different methods of counting and different incubation periods in silver nitrate solution. Only clearly discernible AgNOR dots were counted while the vague ill-defined dots were excluded in counting[32].

AgNOR staining technique in FNA smears has more advantage in comparison with histological sections. In FNA smears, fresh tissues can be obtained; increased thickness of sections in histology is difficult to interpret the AgNOR dots. FNA smears has a monolayered, single cells and they are easily discernible. So, evaluating AgNOR dots in FNA smears is quite easy and reliable when compared to the histology sections.

In1993, Roller E et al[35] conducted the study on 20 cases of benign and 56 cases of malignant neoplasm. The malignant lesions shows significantly higher AgNOR counts than the benign counterparts .

Reddy GS, Sesikeran B, Bhaskaran CS[36] also conducted a study among ten benign and neoplasms of breast. They concluded that quantitative analysis of AgNORs enables us to differentiate benign from malignant lesions.

In 1995, Karmakar T, Radhika S, Gupta SK[37] conducted a prospective study on the cytological smears of both benign and malignant breast lesions including fibroadenoma, proliferative breast lesions, fibrocystic changes and ductal carcinoma of breast. They stated that the mean AgNOR count is 16.63 in malignant breast lesions which was higher and statistically significant when

compared with 6.39 of mean AgNOR count in benign breast lesions. They also added that cut-off AgNOR value of 11 can reliably be used in differentiating benign from malignant neoplasm.

Whereas a study proposed on assessing the number of AgNOR dots in 64 malignant and 31 benign breast neoplasm on cytological smears by Mehrotra A, Chandra T[38] concluded by providing a cut off point of 4 to be the reliable indicator to differentiate malignant from benign neoplasm of the breast.

In 1996, Simha et al[39] conducted a study about the prognostic value of AgNORs in breast neoplasm, which showed that the AgNOR counts correlates with mitosis, desmoplasia and size of the tumor. Higher Ag NOR counts were noted in ER/PR negative breast neoplasms.

In the year 1997, Kumar et al[40] conducted a study by assessing the AgNOR count of malignant breast neoplasm in the cytology smears of 56 cases and concluded that the AgNOR counts correlates well with stage of the cancer, tumor size, lymph node status and recurrence rate of tumor.

Hasnan J, Jayaram G[18] conducted the prospective study on the cytology smears of 31 cases of benign and 25 cases of malignant breast neoplasm with histological correlation in about 26 cases, found that mean AgNOR count ranged from 2.55 to 5.0 in benign breast neoplasm and 5.8 to 17.2 range in malignant neoplasm. The difference in mean AgNOR count among the benign and malignant lesions was statistically significant. None of the cases showed overlap of mean AgNOR values in the cytological smears of breast neoplasms.

Khanna AK, Kumar M, Ansari MA, Khanna A[41] studied both Histology and cytology of 73 breast lesions that included 27 benign and 46 malignant neoplasm. The study correlates the cytology and histology using Subjective AgNOR Pattern Assessment (SAPA) score and mean AgNOR dot counts. They concluded that both SAPA score and mAgNOR counts were useful in differentiating malignant from benign neoplasm in both histology specimens and the cytology smears and both gave similar results. Mean AgNOR count of benign neoplasm was 2.75 while in malignant neoplasm it was 6.94 in Fine Needle Aspiration Cytology. SAPA score of benign neoplasm was 5.87 and 9.02 in malignant neoplasm. They concluded that Subjective AgNOR Pattern Assessment score is rapid, reproducible and convenient method of AgNOR assessment[41].

Meehan SM, Carney DN, Magee H, Dervan PA[31] evaluated the cytological preparations obtained from surgical specimens for AgNOR count, shape, size and clustering. The malignant lesion revealed a mean AgNOR count of 9.52 while benign lesion was 4.44.They concluded that the diagnostic accuracy was 90% in combined pattern assessment and counting of NORs in distinguishing benign and malignant neoplasm. The median score for benign lesions was 7 and for malignant lesions was 13.

SUMMARY

Fine Needle Aspiration Cytology aims at differentiating malignant from benign neoplasms and this investigation is done as a preoperative diagnosis. As a pathologists, we undergo dilemmas in some of the cases to conclude as a benign or malignant which throws the light for the surgeons to decide the type of surgery.

In such situations, AgNOR which is a proliferative marker helps in differentiating malignant from benign neoplasms of the breast. AgNOR staining as a simple method of silver staining technique which can be reliably and effectively used to differentiate benign from malignant neoplasms. Not only the breast cases, all malignant neoplasms consistently reveals a higher AgNOR values comparing with their benign counterparts.

Several studies showed the discrepancies between the absolute value of mean AgNOR count and the cut off values for differentiating malignant from benign neoplasms of the breast. This variation is due to the lack of standardisation in tedious counting of AgNOR dots and interobserver variability.

In our study, the mean AgNOR count in cytology correlated with histopathology reveals 3.4 +/- 0.905 and SAPA score was 6.82 +/- 1.585 among benign lesions of breast. In malignant lesions, the mean AgNOR count and SAPA score was 6.252+/- 1.763 and 11.33 +/- 2.253 respectively.

The present study has taken measures to prove that AgNORs can be used reliably to differentiate malignant from benign neoplasms of the breast and also proliferative breast disease with atypia from without atypia.

CONCLUSION

AgNOR count and SAPA score reflects the proliferative activity of the cell in which AgNOR dots are quite increased in malignant neoplasms compared to the benign. Mean AgNOR count and SAPA score together provides better accuracy for distinguishing malignant from benign neoplasms.

AgNOR counts are constantly higher in malignant neoplasms when compared to benign neoplasms of the breast cytology smears. SAPA score is found to be superior than AgNOR count as it considers different variables for analysis.

AgNORs helps in distinguishing Proliferative Breast disease with atypia from proliferative breast disease without atypia. Here, the mean AgNOR count and SAPA score is comparatively higher in cases of proliferative breast disease with atypia than witout atypia. In Proliferative breast disease with atypia, Surgeon has the confusion to put into the diagnosis of benign or malignant. This study helps them to proceed with excision biopsy or lumpectomy in cases of low AgNOR count and SAPA score whereas mastectomy in case of high AgNOR count and SAPA score.

Robinson's Cytological grading were analysed in the FNA smears of breast malignant neoplasms such as Ductal carcinoma of breast and were graded cytologically. The grade was correlated with AgNOR count and SAPA score which revealed that as the cytological grade increases, both AgNOR count and SAPA score also get increased.

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ANNEXURE I

ஒப்புதல் படிவம்

நோயாளியின் பெயர்:

பாலினம் :

வயது :

பெற்றோர் பெயர் :

முகவரி :

கோவை மருத்துவக்கல்லூரி, பரிசோதனை நடத்த சம்மதம் அளிக்கிறேன். இந்த ஆய்வின் செய்முறை மற்றும் இது தொடர்பான அனைத்து விளக்கங்களையும் கேட்டுக் கொண்டு, எனது சந்தேகங்களையும் தெளிவுபடுத்திக் கொண்டேன் என்பதையும் தெரிவித்துக் கொள்கிறேன். இந்த ஆய்வில் என் விவரங்கள் பாதுகாக்கப்படுவதுடன் இதன் முடிவுகள் ஆய்விதழில் வெளியிடப்படுவதில் ஆட்சேபனை இல்லை என்பதையும் தெரிவித்துக் கொள்கிறேன்.

கையொப்பம்

தேதி:

ANNEXURE II

"ANALYSIS OF AGNOR COUNT AND SAPA SCORE IN FNAC OF BREAST NEOPLASMS"

PROFORMA

FNAC NO: _____ . IP/OP. NO. : _____ .

PATIENT NAME:

_____.

AGE: _____ . SEX: M / F UNIT/WARD: _____.

ADDRESS: _____ .

CLINICAL DIAGNOSIS: _____.

1) PRESENTING COMPLAINTS: _____ .

2) PERSONAL HISTORY: _____.

3) FAMILY HSITORY: _____ .

4) GENERAL EXAMINATION:_____ .

5) LOCAL EXAMINATION: _____

6) FNAC DIAGNOSIS: _____.

7) ROBINSON'S CYTOLOGICAL GRADE: _____.

8) HPE DIAGNOSIS: _____ .

9) mAgNOR VALUE: _____.

10) SAPA SCORE: _____.

11) AgNOR SIZE VARIATION: _____.

12) AgNOR CLUSTER DISTRIBUTION: _____.

ABBREVIATIONS

AgNOR	:	Argyrophilic Nucleolar Organizer Region
DNA	:	Deoxy ribonucleic acid
DPX	:	Di-N-Butyle Phthalate in Xylene
FNAC	:	Fine Needle Aspiration Cytology
mAgNOR	:	Mean Argyrophilic Nucleolar Organizer Region
NCI	:	National Cancer Institute
NOR	:	Nucleolar Organizer Region
NORAP	:	Nucleolar Organizer Region Associated Protein
NOS	:	Not Otherwise Specified
NST	:	No Special Type
rDNA	:	Ribosomal Deoxyribonucleic acid
RNA	:	Ribonucleic acid
rRNA	:	Ribosomal Ribonucleic acid
SAPA	:	Subjective Argyrophilic Nucleolar Organizer Region Pattern Assessment
TDLU	:	Terminal Duct Lobular Unit

KEY FOR MASTER CHART

1. R- Right
2. L- Left
3. UO - Upper Outer
4. UI - Upper Inner
5. LO - Lower Outer
6. LI - Lower Inner
7. B - Benign
8. M - Malignant

Sl.No	FNAC No.	AGE	SIDE	QUAD RANT	SIZE (cm)	FNAC DIAGNOSIS	BENIGN/ MALIGNANT	ROBINSON'S GRADE (malignancy)	Mean AGNOR Count	SAPA SCORE	HISTOPATHOLOGY DIAGNOSIS	BENIGN/ MALIGNANT
1	F2595/17	45	R	UO	4	Ductal carcinoma of Breast	Malignant	II	9	12	Invasive ductal carcinoma	Malignant
2	F2588/17	57	L	UO	2	Suspicious of malignancy	Malignant	I	6	13	Invasive Carcinoma	Malignant
3	F2614/17	35	L	UI	1.5	Fibrocystic disease of Breast	Benign		4	7	Fibroadenoma	Benign
4	F2685/17	16	L	UI	3	Fibroadenoma	Benign		3.2	5	Fibroadenoma	Benign
5	F2689/17	48	R	UI	4	Ductal carcinoma of Breast	Malignant	II	4.4	10	Lost to follow up	
6	F54/18	47	L	UO	4	Ductal carcinoma of Breast	Malignant	II	7.2	13	Invasive ductal carcinoma	Malignant
7	F1217/18	23	L	UO	1.5	Fibrocystic disease of Breast	Benign		4.8	8	Lost to follow up	
8	F1218/18	19	L	UI	4	Proliferative breast disease without atypia	Benign		4	8	Fibroadenoma	Benign
9	F1225/18	65	L	UI	1.5	Ductal carcinoma of Breast	Malignant	I	6	11	Invasive ductal carcinoma	Malignant
10	F1243/18	40	R	UO	2	Fibrocystic disease of Breast	Benign		4	5	Fibroadenosis	Benign
11	F1245/18	68	L	UO	6	Ductal carcinoma of Breast	Malignant	III	6.2	13	Invasive ductal carcinoma	Malignant
12	F1288/18	46	R	UO	12	Ductal carcinoma of Breast	Malignant	II	10.2	15	Lost to follow up	
13	F1318/18	73	R	LO	3	Fibrocystic disease of Breast	Benign		3.7	7	Lost to follow up	
14	F1330/18	18	R	LO	3	Fibroadenoma	Benign		4	7	Lost to follow up	
15	F1331/18	48	R	LO	5	Fibrocystic disease of Breast	Benign		3	5	Fibroadenoma	Benign
16	F1340/18	30	L	LO	1	Proliferative breast disease without atypia	Benign		3.7	7	Fibroadenoma	Benign
17	F1344/18	48	R	UO	3	Ductal carcinoma of Breast	Malignant	II	7.9	13	Lost to follow up	
18	F1345/18	66	L	UO	5	Ductal carcinoma of Breast	Malignant	II	9.8	15	Invasive ductal carcinoma	Malignant
19	F1413/18	29	L	UO	1	Fibroadenoma	Benign		3.8	6	Fibroadenoma	Benign
20	F1423/18	45	R	UO	7	Ductal carcinoma of Breast	Malignant	III	10	15	Lost to follow up	
21	F1424/18	31	L	UI	1	Benign phyllodes Tumor	Benign		3	7	Fibroadenoma	Benign
22	F1426/18	34	R	UI	3	Benign phyllodes Tumor	Benign		5.8	9	Malignant Phyllodes tumor	Malignant
23	F1526/18	38	L	UO	6	Ductal carcinoma of Breast	Malignant	III	9	15	Invasive Carcinoma Breast	Malignant
24	F1544/18	41	R	UO	3	Fibrocystic disease of Breast	Benign		4.2	8	Fibroadenoma	Benign
25	F2052/18	45	L	UO	4	Ductal carcinoma of Breast	Malignant	II	8	15	Lost to follow up	
26	F2181/18	33	L	UO	3	Proliferative breast disease with atypia	Premalignant		6	11	Invasive ductal carcinoma	Malignant
27	F2189/18	53	L	UO	15	Benign Phyllodes tumor	Benign		3.6	10	Lost to follow up	
28	F2192/18	40	L	UI	7	Fibroadenoma	Benign		3	5	Lost to follow up	
29	F2222/18	57	L	UO	5	Ductal carcinoma of Breast	Malignant	I	1.9	6	Lost to follow up	
30	F2227/18	75	L	UO	15	Ductal carcinoma of Breast	Malignant	II	10	15	Lost to follow up	
31	F2234/18	23	L	UO	2	Fibroadenoma	Benign		3.8	8	Fibroadenoma	Benign
32	F2252/18	50	R	UI	2	Proliferative breast disease without atypia	Benign		4.2	11	Fibroadenosis	Benign
33	F2256/18	25	L	UI	1	Fibroadenoma	Benign		3.71	5	Lost to follow up	
34	F2272/18	50	L	UO	5	Ductal carcinoma of Breast	Malignant	II	6	11	Invasive ductal carcinoma	Malignant
35	F2298/18	38	R	UO	4	Fibroadenoma	Benign		3.5	6	Fibroadenoma	Benign
36	F2300/18	42	R	LO	5	Proliferative breast disease with atypia	Premalignant		5	12	Lost to follow up	
37	F2301/18	53	L	LO	5	Ductal carcinoma of Breast	Malignant	III	7.8	13	Lost to follow up	
38	F2312/18	70	L	UI	4	Ductal carcinoma of Breast	Malignant	III	8	15	Lost to follow up	
39	F2346/18	38	L	LO	1	Proliferative breast disease without atypia	Benign		2.4	7	Ductal carcinoma in situ	Malignant
40	F2354/18	48	R	UO	5	Proliferative breast disease with atypia	Premalignant		2.8	8	Invasive ductal carcinoma	Malignant
41	F2369/18	45	L	UI	1.5	Fibrocystic disease of Breast	Benign		1.7	7	Fibroadenoma	Benign
42	F2380/18	70	L	UO	3	Fibroadenoma	Benign		4.4	11	Lost to follow up	
43	F2415/18	27	L	UI	2	Fibroadenoma	Benign		4.2	8	Lost to follow up	
44	F2459/18	38	R	UI	1	Proliferative breast disease without atypia	Benign		5	7	Fibroadenoma	Benign
45	F2485/18	39	R	UI	2	Fibroadenoma	Benign		4	7	Fibroadenoma	Benign
46	F2491/18	21	L	UO	3	Fibroadenoma	Benign		3.5	7	Lost to follow up	
47	F2498/18	29	L	UO	2	Proliferative breast disease without atypia	Benign		4	7	Fibroadenoma	Benign
48	F2510/18	47	L	UI	2	Fibrocystic disease of Breast	Benign		3.5	8	Fibrocystic disease of Breast	Benign
49	F2524/18	36	L	UI	2	Proliferative breast disease with atypia	Premalignant		4.9	12	Fibroadenoma	Benign

50	F2524/18	36	L	UO	0.5	Fibroadenoma	Benign		4	7	Lost to follow up	
51	F2525/18	45	L	UO	3	Fibroadenoma	Benign		3.8	6	Fibroadenoma	Benign
52	F2526/18	45	L	UO	2	Fibroadenoma	Benign		3.7	5	Lost to follow up	
53	F2634/18	54	L	UO	4	Ductal carcinoma of Breast	Malignant	III	9	13	Lost to follow up	
54	F2649/18	45	R	UO	0.5	Fibroadenoma	Benign		3	7	Lost to follow up	
55	F2652/18	23	R	LO	2	Fibroadenoma	Benign		2.5	5	Fibroadenoma	Benign
56	F2660/18	65	L	UO	3	Ductal carcinoma of Breast	Malignant	I	6	11	Invasive ductal carcinoma	Malignant
57	F2688/18	42	R	UO	3	Proliferative breast disease with atypia	Premalignant		3.8	9	Lost to follow up	
58	F2692/18	42	R	UO	2.5	Fibrocystic disease of Breast	Benign		2.2	5	Fibroadenosis	Benign
59	F247/19	60	R	LO	9	Ductal carcinoma of Breast	Malignant	II	7.9	15	Lost to follow up	
60	F729/19	55	L	LO	3	Proliferative breast disease with atypia	Premalignant		1.9	6	Fibroadenoma	Benign
61	F730/19	47	R	UI	2.5	Proliferative breast disease without atypia	Benign		1.8	7	Fibroadenoma	Benign
62	F743/19	16	L	LO	1	Fibroadenoma	Benign		3.1	9	Lost to follow up	
63	F769/19	65	L	UI	4	Ductal carcinoma of Breast	Malignant	I	6	9	Lost to follow up	
64	F782/19	60	R	UO	6	Ductal carcinoma of Breast	Malignant	II	3.4	8	Lost to follow up	
65	F785/19	57	R	UO	3	Ductal carcinoma of Breast	Malignant	I	7.2	12	Invasive ductal carcinoma	Malignant
66	F814/19	57	L	UO	10	Ductal carcinoma of Breast	Malignant	II	6	12	Lost to follow up	
67	F821/19	52	R	UI	4	Proliferative breast disease without atypia	Benign		1.9	6	Fibroadenosis	Benign
68	F824/19	39	L	UO	2	Fibroadenoma	Benign		2.6	7	Lost to follow up	
69	F828/19	41	L	UO	6	Ductal carcinoma of Breast	Malignant	I	5	10	Ductal carcinoma in situ	Malignant
70	F987/19	74	L	UI	6	Ductal carcinoma of Breast	Malignant	I	4	10	Lost to follow up	
71	F1013/19	61	L	UO	4	Ductal carcinoma of Breast	Malignant	II	4.12	13	Lost to follow up	
72	F1014/19	75	R	UO	3	Malignant Phyllodes tumor	Malignant		3.32	9	Malignant Phyllodes tumor	Malignant
73	F1016/19	49	L	UI	5	Fibroadenoma	Benign		4.3	7	Fibroadenoma	Benign
74	F664/19	53	L	UI	1.5	Fibrocystic disease of Breast	Benign		3.7	6	Lost to follow up	
75	F666/19	50	L	LO	6	Ductal carcinoma of Breast	Malignant	II	7	13	Invasive ductal carcinoma	Malignant
76	F702/19	45	L	UO	3	Ductal carcinoma of Breast	Malignant	II	6.9	12	Invasive ductal carcinoma	Malignant
77	F706/19	30	R	UO	3	Fibroadenoma	Benign		4	7	Fibroadenoma	Benign
78	F711/19	23	R	LO	3	Fibroadenoma	Benign		3.7	6	Lost to follow up	
79	F717/19	55	R	UO	7	Ductal carcinoma of Breast	Malignant	II	5	14	Invasive ductal carcinoma	Malignant
80	F721/19	54	R	UO	4	Benign Phyllodes tumor	Benign		6	8	Malignant Phyllodes tumor	Malignant
81	F836/19	36	L	UO	5	Proliferative breast disease with atypia	Premalignant		6.2	10	Invasive ductal carcinoma	Malignant
82	F841/19	55	R	UO	4	Ductal carcinoma of Breast	Malignant	III	7.5	15	Invasive Carcinoma	Malignant
83	F896/19	75	L	UO	10	Ductal carcinoma of Breast	Malignant	II	8.8	15	Lost to follow up	
84	F923/19	70	R	UO	10	Ductal carcinoma of Breast	Malignant	II	4.8	15	Lost to follow up	
85	F954/19	40	L	UI	4	Proliferative breast disease with atypia	Premalignant		5.7	11	Ductal carcinoma in situ	Malignant
86	F955/19	32	R	UO	3	Fibroadenoma	Benign		5	9	Lost to follow up	
87	F970/19	55	R	UO	5	Ductal carcinoma of Breast	Malignant	I	6	8	Ductal carcinoma in situ	Malignant
88	F1033/19	50	L	UI	3	Proliferative breast disease without atypia	Benign		4.1	7	Fibroadenoma	Benign
89	F1181/19	39	R	UO	4	Proliferative breast disease with atypia	Premalignant		6	12	Lost to follow up	
90	F1234/19	53	L	UO	3	Proliferative breast disease with atypia	Premalignant		6.8	12	Invasive ductal carcinoma	Malignant
91	F1272/19	32	R	UO	2	Proliferative breast disease without atypia	Benign		2.4	5	Fibroadenoma	Benign
92	F1296/19	29	R	LO	4	Fibroadenoma	Benign		2.4	5	Fibroadenoma	Benign
93	F1310/19	42	R	UO	2	Proliferative breast disease without atypia	Benign		3	7	Fibroadenoma	Benign
94	F1324/19	55	R	UO	7	Proliferative breast disease without atypia	Benign		4.6	8	Fibroadenoma	Benign
95	F1363/19	40	R	LO	4	Proliferative breast disease without atypia	Benign		4.6	5	Lost to follow up	
96	F1587/19	70	R	UI	2	Proliferative breast disease with atypia	Premalignant		5	10	Ductal carcinoma in situ	Malignant
97	F1726/19	35	L	UO	2	Proliferative breast disease without atypia	Benign		3	7	Fibroadenosis	Benign
98	F1750/19	48	L	UI	1	Fibrocystic disease of Breast	Benign		2.9	5	Fibroadenoma	Benign
99	F1764/19	60	L	UO	10	Ductal carcinoma of Breast	Malignant	II	9	13	Invasive Ductal carcinoma	Malignant
100	F1765/1	26	R	UI	1	Fibroadenoma	Benign		4	8	Fibroadenoma	Benign