AMJ
ISSN: 0005-2523

ISSN: 0005-2523
Volume 63, Issue 01, January 2023

IMMUNOHISTOCHEMICAL DEMONSTRATION OF CALRETININ IN THE BREAST CANCER

[1] **Noor Bassem Majed , [2] Ali Mahdi Mutlag , [3] Zainab Abbas Hassooni**

[1] Department of Biology College of Science / Wasit University [2]Assist.Prof Department of Pathological Analyses/ College of Science / Wasit Univercity

[3]Assist.Prof. Department of pathology and forensic medicine /college of medicine / wasit university

Abstract

Breast carcinoma is the second most common cancer following lung cancer, The invasive ductal carcinoma is the most common type of breast cancer. About 8 in 10 invasive breast cancers are invasive (or infiltrating) ductal carcinomas (IDC). IDC begins in the breast milk duct's lining cells. The malignancy then spreads to the adjacent breast tissues after penetrating the duct's wall. At this point, it may be able to spread (metastasize) to other parts of the body through the lymph system and bloodstream.Sixty samples were taken from Al Karama Teaching Hospital and private Laboratory in Kut /wasit during the period from (October 1 ,2021 to January 1,2022) totally (60) samples ,(40)samples cancer and (20) samples normal. For histopathological and immunohistochemistry analysis. The total patients were divided according to their age in to five groups (20-29),(30-39),(40-49),(50-59) and above 60.Reslts of present study revealed that a highest incidence of breast cancer (55%) occur in the age above 50 years ,there is a significant difference among patients groups ($p<0.001$). Histopathologically results revealed that 27 cases (67.50%) of them were classified as grade II ductal carcinoma while the remaining (7)cases (17.5%) and (5)15% were grade I and grade II in the same order .The results of immunohistochemical analysis showed that calretinin showed 0(0%) of normal were positive ,but 17(42.5%) of invasive ductal carcinoma were positive ,while 20(100%) of normal were negative and 23(57.5%) were negative.

Keyword: Breast cancer .Calretinin. Immunohistochemistry .PAS.

Introduction

Breast cancer is the most common cancer among women, comprising 18% of all female cancers, and worldwide, breast cancer is the fifth most common cause of cancer mortality (Bray *et al*., 2012). In Iraq it represents more than 24% of total cancer cases making it a great challenge to the health system in our country (Mjali *et al*., 2020).

The classification depending on two key determinations; whether the tumor is confines to the epithelial component of the origin (in situ carcinoma) or has invasive the stroma (invasive carcinoma), whether it arising from or involving a duct (ductal carcinoma) or arising from or involving the breast lobules (lobular carcinoma) (Ackerman′s , 2011).

Invasive ductal carcinoma IDC, often referred to as infiltrating ductal carcinoma, is a kind of cancer that started in a milk duct and has since spread to the fatty or fibrous tissue of the breast

outside of the duct. IDC accounts for 80% of all breast cancer diagnoses, making it the most prevalent kind of the disease (Sharma et al .,2010).

A lump or swelling, skin dimpling, nipple soreness, discharge, or retraction, and redness or scaliness of the nipple or breast skin are some of the early physical symptoms that might be associated with breast cancer (ACS, 2014).

According to Milne *et al*., (2011), epidemiological studies have identified several factors that are associated with an increased risk of developing breast cancer. These factors include age, family history, genetics, age of menarche, duration of lactation, parity, age of menopause, diet, and hormonal levels.

Immunohistochemistry has numerous important applications in diagnostic breast pathology, from helping to differentiate between benign epithelial hyperplasias and neoplastic proliferations to serving as predictive and prognostic biomarkers that aid in guiding patient treatment .This review covers a subset of novel uses, interpretation, and pitfalls of immunohistochemistry in breast pathology. Specifically, this review focuses on the use of immunohistochemistry in the following scenarios (Campagna *et al*., 2021).

Biomarker analysis in cancer not only provides additional information about classical clinical factors, but also enables patients with a more favourable benefit–risk balance to receive certain treatments (Polley *et al*.,2013) In breast cancer, biomarker analysis is routine practice. It originally began with testing for hormone receptor expression to guide tamoxifen therapy. The subsequent inclusion of targeted treatments against human epidermal growth factor receptor 2 (HER2) revolutionised the biomarker field (Palacios *et al* .,2009)

Calretinin is a calcium-binding protein that is intracellular and has a molecular weight of 29 kilodaltons. It is vitamin D-dependent and has some possible activities, including buffering calcium on the intracellular level and message targeting (Taliano *et al*.,2013) in most cases highly sensitive and specific for mesothelial origin,calretinin positivity has been reported in carcinomas arising in a myriad of other tissues including ovary,testis ,adenal cortex ,colon,breast,sinonasal tract ,thymus,skin and even soft tissue(Powell *et al*.,2011).

Materials & Methods:

Materials: Light microscope, Water Path, Camera (Digital), Rotary microtome, Refrigerator, Electric Oven, Glass slides, Cover Slide, Hot Plat stirrer, Slide Positively charged – Hydrophilic, Incubator, Micropipettes 5-50,0.5-10,100-1000 µl, Tips, Water Bath, Periodic acid – Schiff stain, Ethanol absolute, D.P.X (Disteren Plasticizer Xylene), Paraffin wax, Xylene, Calretinin kit.

Methods:

Samples collection: We collected (60) samples from patients when attended Al Karama Teaching Hospital and private Laboratory in Kut /wasit,(40)samples of invasive ductal carcinoma and (20) samples normal breast tissue.

Preparation of the paraffin Section

The sample was prepared for paraffin section ; according to (Suvarna *et al* .,2018),as follows: **Fixation:** All samples from patients with breast cancer instantly fixed in 10% buffered

neutral formalin as recommended, for general Histologic study and Immunohistochemistry (IHC) given that the tissues are not over-fixe in it . The fixation time for 24 hours at room temperature. **Dehydration**: Samples were dehydrated by a glass with a graded concentration of ethanol , with (70% , 80% ,90% , 100%) two hours for each concentration . **Clearing:** Tissue samples were cleared by but in xylene for 30 min .to ensure that ethanol was removed from the tissues. **Infiltration and Embedding**: Tissue samples were placed in liquid paraffin wax on (58-60 ˚C) for 2 hours changes (2 hours of each) embedded in paraffin wax in oven by melting point reach (57 ˚C) for 3 hours; then samples were blocks in paraffin wax . After 24 hours , the blocks were ready to cut. **Cutting:** Blocks were cut cross- section by microtome (Leice,Germany) ,figure (2-1) the thickness of section 6 micrometer , and all sections transported in a heated water bath at point (45 ˚C),then place on slide kept overnight in an incubator at 27 ˚C .

Periodic Acid – Schiff (PAS) Stain: This stain is used to identification of carbohydrate in normal and malignant lesions of mammary gland.

A. preparation of stain: periodic Acid -Schiff (PAS) was purchased commercially ready to use.

B .Staining procedure: Deparaffinizing and rehydration of slides to distilled water. Immersing slides for five minutes at room temperature (18-26 ˚C) in periodic Acid solution. Washing slide in distilled water. Immersing slides for fifteen mintes at room temperature (18-26 ˚C) in Schiffs Reagent for 15 min Rinsing slides in running tap water and incubate in D.W. for five minutes. Counterstaining slides in Hematoxline Solution, for 1 min differentiation with alcohol. Rinse slides in running tap water. Dehydrating, clearing, and mounting sections in toluene or xylene –based mounting media. Sections were imagined the light microscope and photos were taken with a canon digital apparatus.

Immunohistochemistry of calretinin:

These steps include preparation of slides for staining steps this can be accomplished by the following steps,the staining protocol was according to manufacturing instructions (Dako).

A. **Slide preparation:** Paraffin embedded sections were cut into 5µm thickness, then the sections were carried by adhesive positively charged slides, sections were left to dry to facilitate adhesion between the section and the charged glass surface.
B. **Deparaffinization and rehydration:** this step involve: Dewaxing of paraffin embedded sections were placed inside hot air oven at 65°C for 30 minutes. Deparaffinization was done by immersing the slides in xylene for 3 minutes then in fresh xylene for 5 minutes. Rehydration of tissue section accomplished through immersing of slides in sequential dilutions of ethanol as the following order: Absolute ethanol for 5 minutes. 95% ethanol for 5 minutes. 90% ethanol for 5 minutes. 70% ethanol for 5 minutes.50% ethanol for 5 minutes.Distilled water for 5 minutes
C. **Antigen Retrieval: According to your marker (30 minutes 90** ˚C) in Chamber Boiling method.
D. **Peroxidase block:** Slide encircled with Pap pen. Hydrogen peroxide was applied to cover the tissue and incubated for 20 minutes. Then the slides were rinsed with distilled water, drained and blotted gently.

E. Protein blocking of Non-specific binding of primary antibody:

Before adding the primary antibodies, slides were ready for blocking step, to block endogenous Fc receptor and high affinity proteins, 100 µl of Reagent 1 was added and incubate sections for 20 min to prevent any unspecific binding of primary antibody (FC region) with tissue section, this with prevent false positive results. then slides were drained and blotted without washing.

Immunostaining steps: these multi steps specific interaction between primary antibodies reacts with target antigen and ended by colored designation of target:

1) One hundred µl of primary antibody was placed onto the tissue section and incubated for 1 hour at 37˚C in humid chamber. After incubation, the slides were drained and blotted gently. Then Slides were placed in washing buffer bath for 5 minutes twice, drained and blotted gently.

2) One hundred µl of streptavidin HRP complex was placed onto the tissue section and incubated for 60 minutes at 37˚C in humid chamber, then slides were placed in washing buffer bath for 5 minutes, drained and blotted gently.

3) Fifty µl of the DAB-substrate chromogen (20 microliter of DAB mixed with 1ml of substrate diluent) was placed onto the tissue section and incubated for 5 minutes at 37˚C in humid chamber, and then slides were rinsed in distilled water, drained and blotted gently.

4) Counter-stain: the slides immersed in a bath of Mayer's Hematoxylin for 1 minute. Slides were washed three times in distilled water, 1 minute each; then drained and blotted gently.

Post staining steps: include preparation of stained slides for microscopic examination: 1: Slides were dehydrated by placing them in Ethanol and Xylene containing jars in the following order: 50% ethanol for 5 minute.70% ethanol for 5 minute.90% ethanol for 5 minute.Absolute ethanol for 5 minutes.Xylene for 5 minutes.Fresh Xylene for 5 minutes.2: A drop of mounting media placed onto section and the tissue section was quickly covered with cover slip and slides were left to dry. Negative control was included for each run of immunohistochemistry. The slide were not allowed to dry in any step of the immunostaining.

2.4.3.3 Evaluation of the Immunostaining:

Evaluation of IHC results for performed by light microscope (Genex 20, America) at 40X objective lens. The typical results of immunochemical staining counted in. All results counted as a relative percentage of positive cells stained with dark brown color out of total count of positive and negative cells.

Statistical analysis:

The collected data was entered, coded, and analyzed by the software program Statistical Package for Social Science (SPSS) version 26. Continuous (numerical) variables were presented by means and standard deviations while the qualitative variables (categorical) were presented by frequencies and percentages. The Chi-square test was used to assess the association between categorical variables while Fischer's Exact Test was used instead when more than 20% of the cells have expected values less than 5. The *P*-value equal to or less than 0.05 was considered significant.

Results & Discussions:

Histochemical results

The normal mammary gland when stained with Periodic Acid -Schiff (PAS) showed the basement membrane of the duct and intraluminal secretion by magenta(purple), confirming the presence of PAS positive substances like carbohydrate and neutral mucins in figure (1)and figure (2) .While invasive ductal carcinoma breast, when stained with PAS gave negative results because the absence of basement membrane that show in figure (3) and figure(4).

Periodic acid–Schiff PAS is a tissue staining procedure that may detect mucosubstances such as glycoproteins, glycolipids, and mucins in addition to polysaccharides such as glycogen. PAS can also detect glycogen. Periodic acid oxidizes the vicinal diols in these sugars, which breaks the bond between two adjacent carbons that are not involved in the glycosidic linkage or ring closure in the ring of the monosaccharide units that comprise the long polysaccharides and forms a pair of aldehydes at the two free tips of each broken monosaccharide ring. Additionally, periodic acid creates a pair of aldehydes at the two free tips It is essential that the oxidation state be appropriately maintained in order to forestall any further oxidation of the aldehydes. A magenta-purple color is produced as a result of the reaction between these aldehydes and the Schiff reagent. In most cases, a suitable basic stain is applied as a contrast stain (Abdelrouf and Idris 2022).

Current data were agreed with other studies showed that histochemical observations between cases have been inconsistent, as periodic acid-schiff (PAS) staining negative for glycogen in all cases (Sarma et al. 2011; Forman and Ferringer 2007). In addition another study concluded that breast cancer is mostly occurred in older age, age group 46-60 years is most frequent age group and invasive ductal carcinoma is most frequent type, and diastase resistant periodic acid schiff (DPAS) can be used in differentiated types of breast cancer even the subtypes of the same type (invasive from in-situ) (Abdelrouf and Idris 2022).

During a six-month investigation of 315 aspirates, each of which contained sufficient cellular material for PAS staining to be performed on a full slide, PAS staining was performed over a period of six months. Semi-quantitatively, PAS staining was reported as negative, ambiguous, or positive (+, ++, or +++). There was a correlation between the cytology results and any later histology conducted on these patients. PAS-positive substances were detected both intracellularly and extracellularly. It required cautious interpretation. In both benign and malignant instances, there were sporadic cells with apparent intracytoplasmic positivity (+). Malignancy was most strongly linked with intracellular DPAS positivity (++, +++) that was frequent or particularly strong. On the basis of intracytoplasmic DPAS staining, two patients were confidently upgraded from initial reports suspicious of malignancy to final reports diagnostic of malignancy. (Johnson and Wadehra 2001).

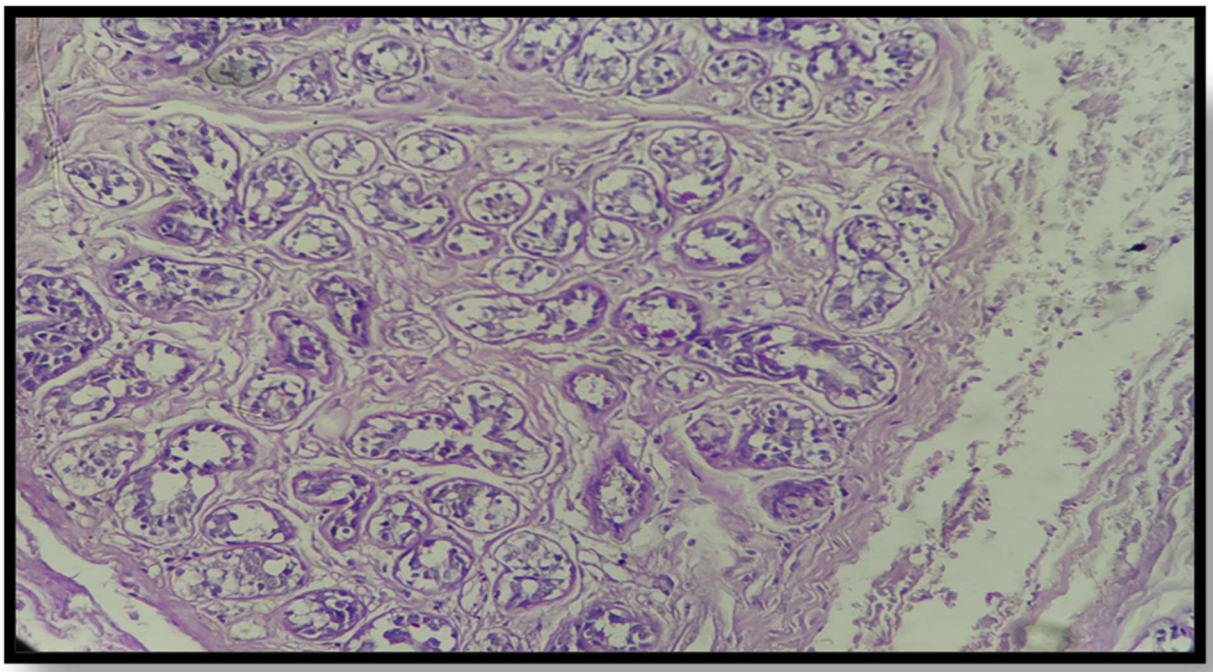
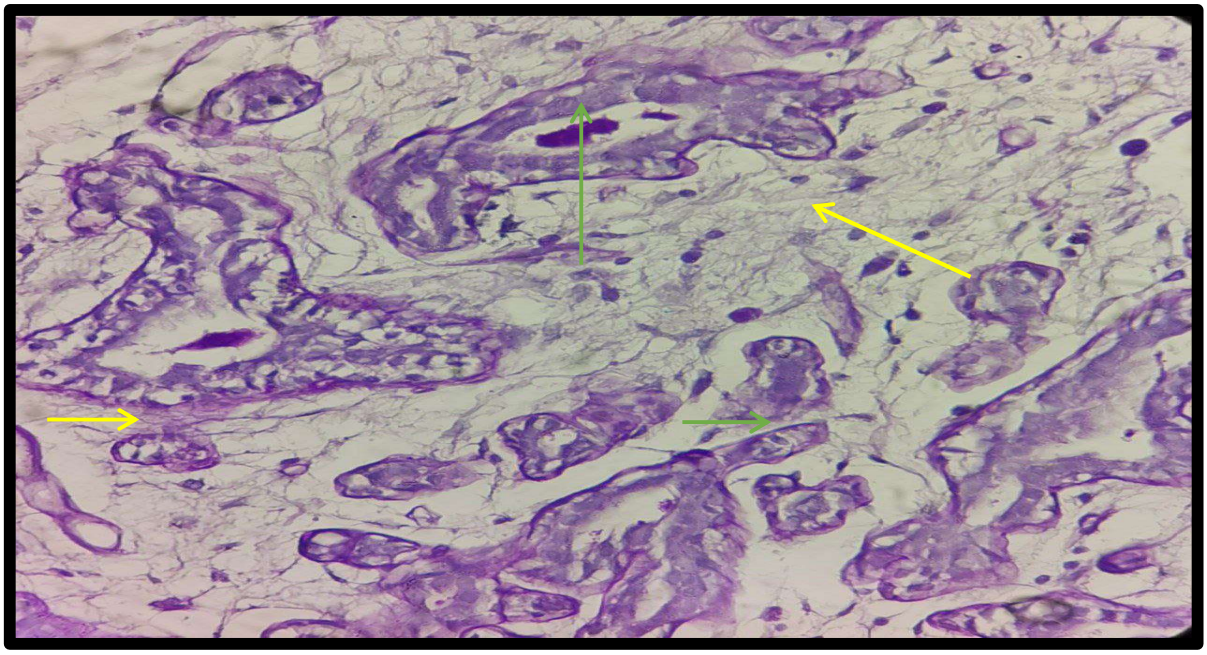


Figure (1) Histological Section of normal breast tissue the PAS stain stained the basement membrane of the ductales and intraluminal secretion.



the

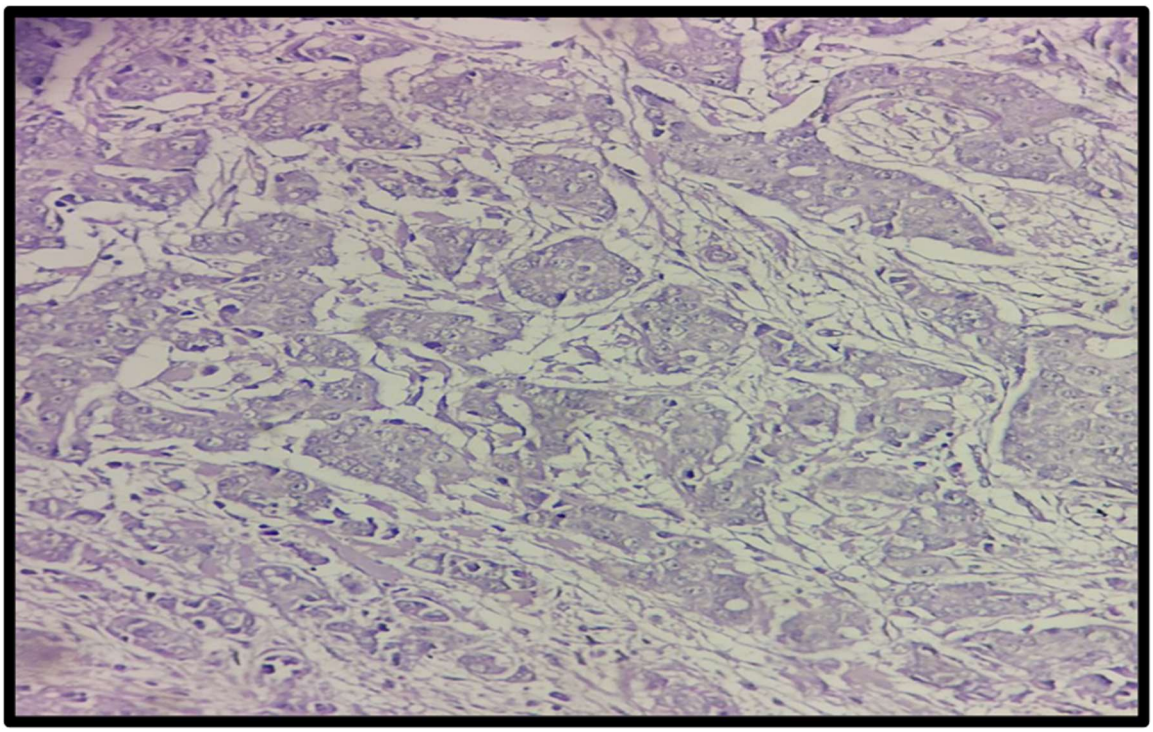
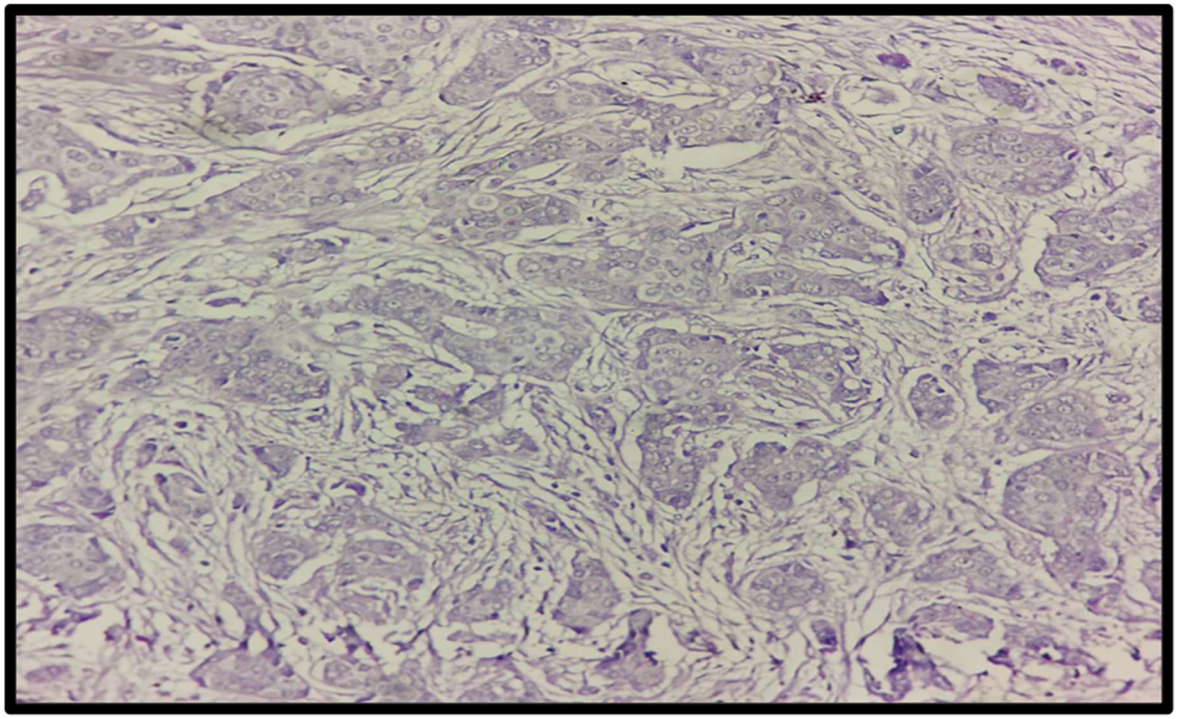


Figure (3) Histological section of invasive ductal carcinoma NST with negative PAS stain.



Figure(4) Histological section of invasive ductal carcinoma NST with negative PAS.

Immunohistochemistry Staining

Positive calretinin was found in about 17 (42.5%) of the study sample with invasive ductal carcinoma that gave positive results (brownish color) in figure(5),(6),(7),(8),normal people didn't

have positive calretinin not stained as figure (9),(10).Negative calretinin was found in about 23(57.5%) of the study sample with invasive ductal carcinoma that gave negative results in figure (11),(12). So, there was a significant association (*P*-value <0.001) between the detection of calretinin and the case diagnosis in this study as appeared in table (1).

Table 1:Association between calretinin and the diagnosis.

Calretinin	Diagnosis		Total	*P*-value (Chi-square test)
	Normal	Invasive ductal carcinoma		
Positive	0(0%)	17(42.5%)	17(28.3%)	<0.001
Negative	20(100%)	23(57.5%)	43(71.7%)	

Calretinin is a 29-kD, intracellular, vitamin D-dependent calcium binding protein that likely has multiple functional roles including intracellular calcium buffering and message targeting, first identified in the central nervous system, it has also been identified in a wide variety of non-neural cells, both neoplastic and non-neoplastic. In the oncologic arena calretinin is most commonly used as part of a panel in the separation of pleural mesothelioma from poorly differentiated pulmonary adenocarcinomas, while in most cases highly sensitive and specific for mesothelial origin, calretinin positivity has been reported in carcinomas arising in a myriad of other tissues including ovary, testis, adrenal cortex, colon, breast, sinonasal tract, thymus, skin and even soft tissue (Dezfoulian *et al*., 2020).

In current study our data were compatible to Taliano *et al*., (2013) aimed to test calretinin expression in non-neoplastic and malignant breast tissue. High-level calretinin expression was identified in five (38.5%) of these tumors while negative in (57.4%). With 53% of basal-like tumors showing high-level expression of calretinin, our findings of the association of calretinin expression with basal-like tumors are in also in agreement with two recent, but much smaller studies by (Powell, Roche, and Roche 2011). Using a binary cutoff of 1%, Duhig *et al* observed calretinin expression in 28 of 53 (53%) cases of grade 2 and 3 breast carcinoma (Duhig *et al*., 2011). Micello *et al*., (2017) showed that among the 22 cases of IDC 14 (64.6%) cases showed negative calretinin expression, 4 (36.4) cases showed weak positivity, 1 moderate positivity and 3 cases showed high positivity which includes one case of IDC with necrosis. Among the 4 metaplastic carcinoma 2 cases showed low calretinin expression. The cystic papillary carcinoma, mucinous carcinoma and the mixed ductal and lobular carcinoma showed negative calretinin expression. Statistical analysis found out the association was not significant with P value (0.354).

While one study have addressed the expression of calretinin in breast carcinoma. In a comprehensive immunohistochemical tissue microarray study involving more than five thousand tissue samples from 128 different tumor types, Lugli, *et al* identified calretinin expression in less than 10% of 158 breast carcinomas (Lugli *et al*., 2003). calretinin expression was found in 1.9% of invasive ductal carcinoma and 4.4% of ductal carcinoma in-situ (DCIS). No calretinin immunoreactivity was detected in invasive lobular, cribriform or tubular carcinomas. Although the

expression of calretinin was not analyzed in relationship to molecular subtype or hormone receptor status, the highest expression of calretinin was observed in medullary and apocrine breast carcinoma; tumor subtypes that frequently exhibit a basal-like phenotype (Jacquemier *et al*., 2005).

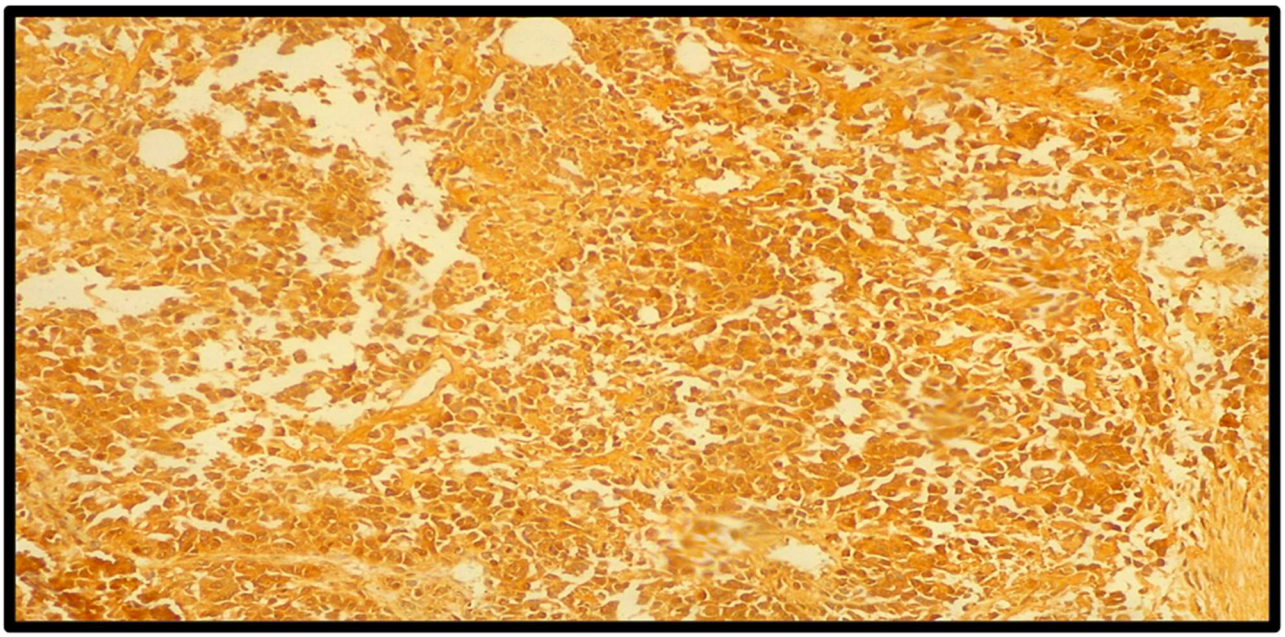


Figure (5) Histological section of invasive ductal carcinoma immuno-histochemical stained with calretinin appear positive (brownish) stained (10x).

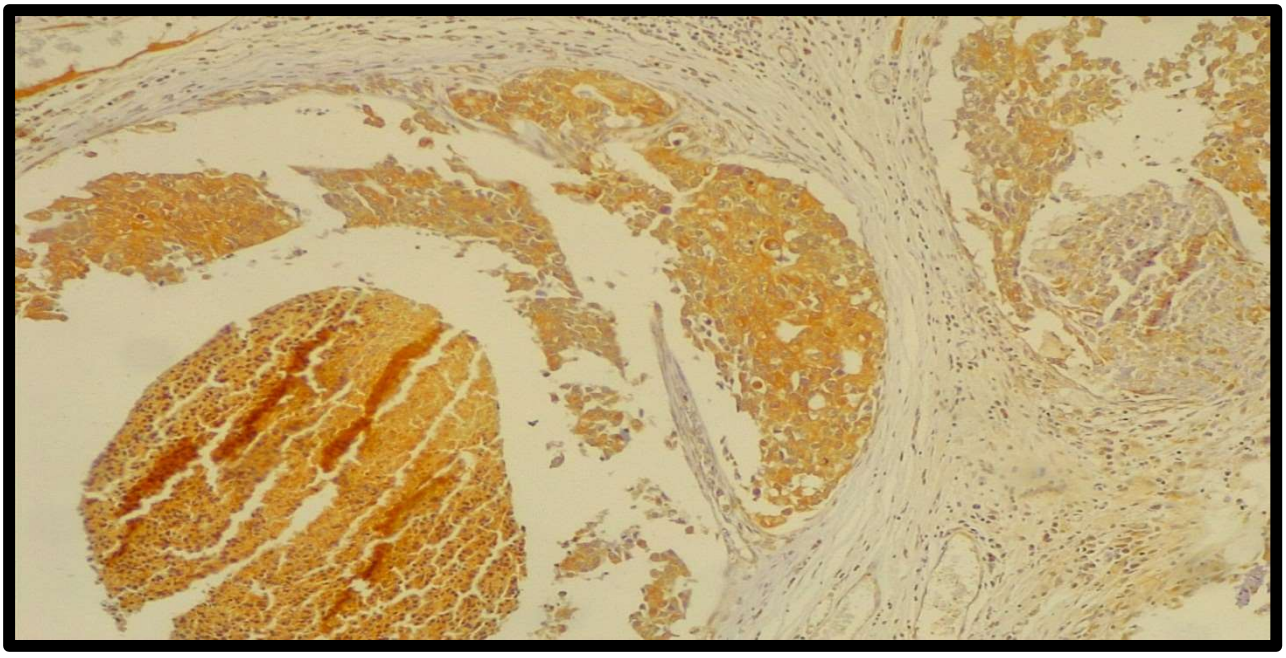


Figure (6) Histological section of invasive ductal carcinoma immunohistochemical stained with calretinin appear positive grade II (10x).

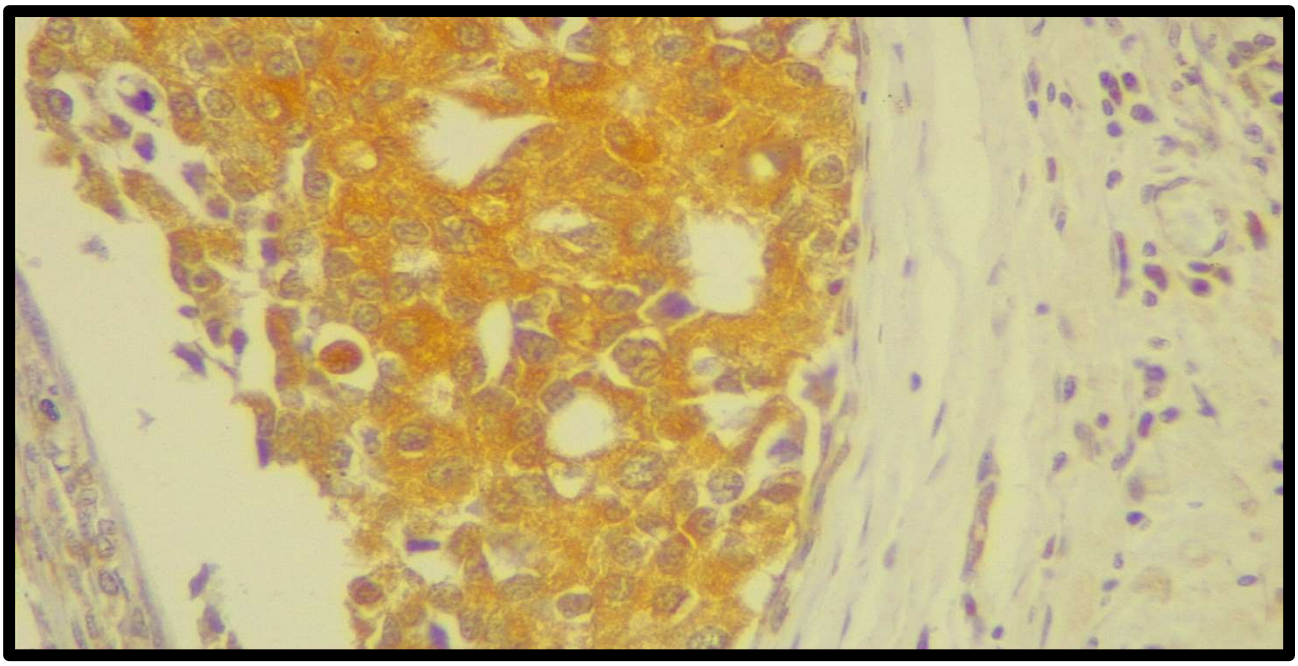


Figure (7) Histological section of invasive ductal carcinoma immunohistochemical stained with calretinin appear positive(brownish color) grade II (40x).

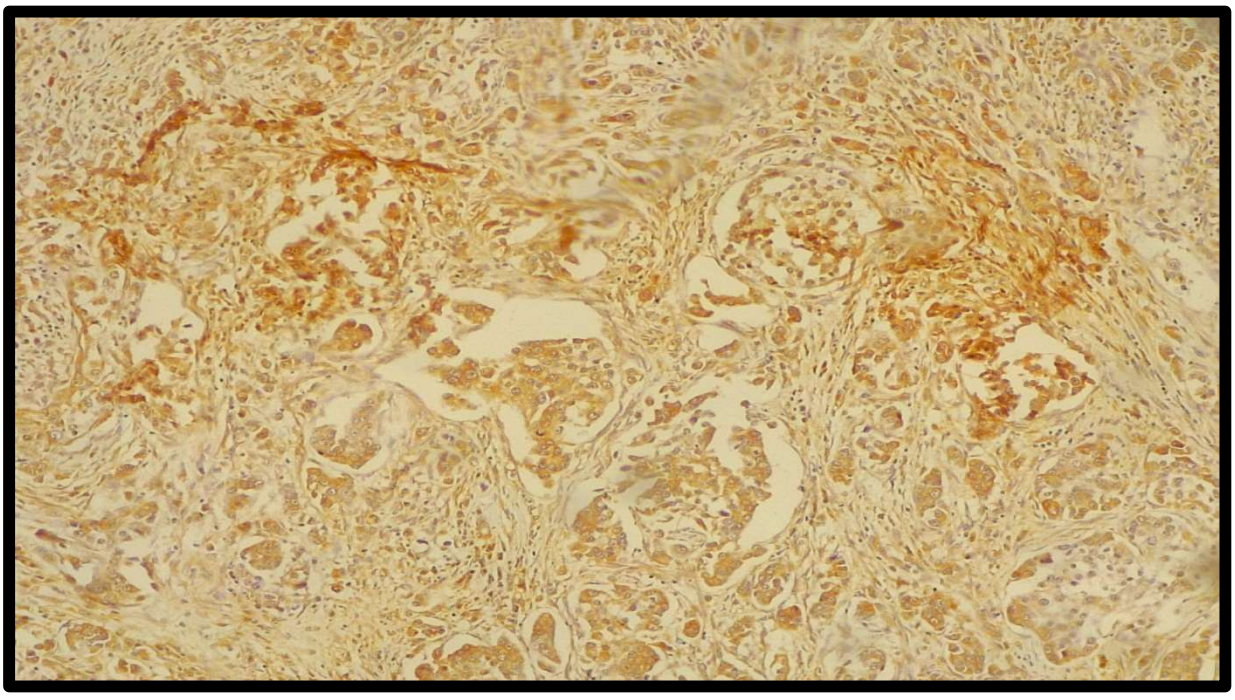


Figure (8) Histological section of invasive ductal carcinoma immunohistochemical stained with calretinin appear positive(brownish color) grade II (10x).

Figure (9) Histological section of normal breast tissue immuno-histochemical stained with calretinin appear negative stained (10x).

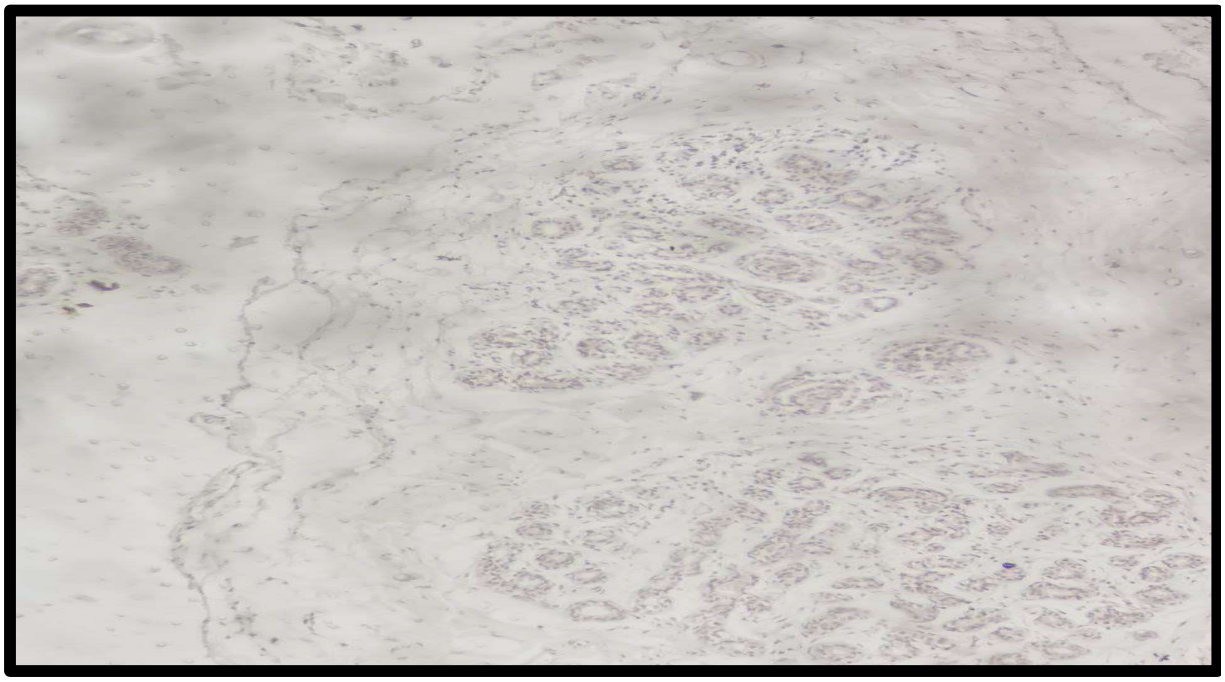


Figure (11) Histological section of invasive ductal carcinoma immunohistochemical stained with calretinin appear" negative not stained grade II (x10).

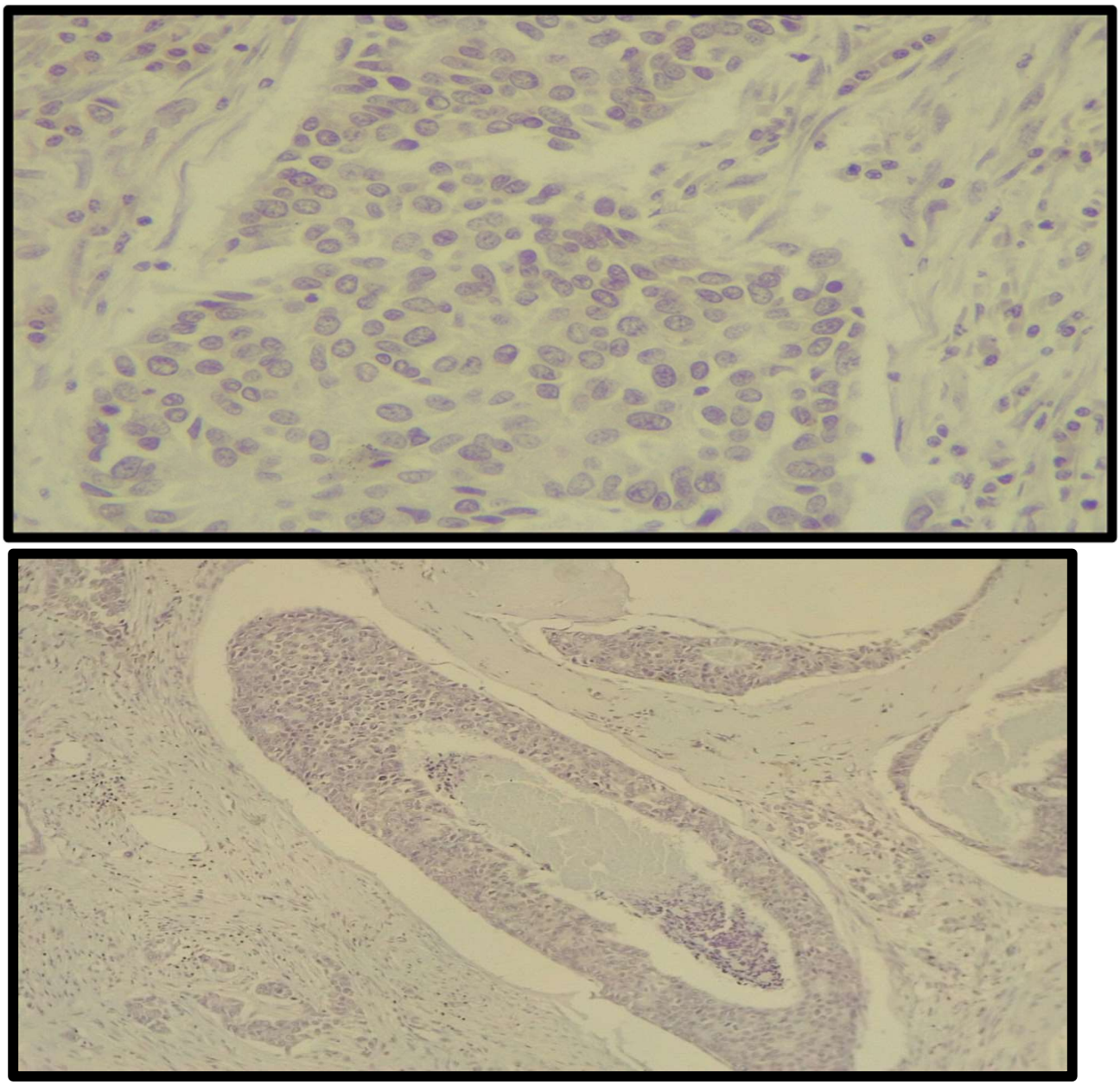


Figure (12) Histological section of invasive ductal carcinoma immunohistochemical stained with calretinin appear negative not stained grade II (x10).

Conclusion:

The normal mammary gland when stained with periodic Acid –Schiff showed the basement of the duct and intraluminal secretion by magenta (purple).The presence of PAS positive substances like carbohydrate and neutral mucins. while invasive ductal carcinoma breast ,when stained with PAS gave negative results because the absence of basement membrane.

Positive calretinin was found in about (42%) invasive ductal carcinoma that gave positive results (brownish color), normal people didn't have appositive calretinin not stained and (57%) of invasive ductal carcinoma that gave negative results (not color).

References:

- **Abdelrouf, Roaa Hassan, and Alkhair Abd Almahmoud Idris. 2022**. 'Role of diastase resistant periodic acid schiff (DPAS) in improve diagnosis of breast cancer with different grades and subtypes, Science Progress and Research (SPR), 2: 409-13.
- **Ackerman. (2011)**. Ackerman's surgical pathology. Tenth edition Am J Clin Pathol 127:103-113.
- **Bray , F., Ren, JS., Masuyer , E., Ferlay, J. (2012)**. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. Int. J. Cancer,
- **Campagna, R., Pozzi, V., Spinelli, G., Sartini, D., Milanese, G., Galosi, A. B., & Emanuelli, M. (2021)**. The Utility of Nicotinamide N-Methyltransferase as a Potential Biomarker to Predict the Oncological Outcomes for Urological Cancers: An Update. *Biomolecules*, *11*(8), 1214.
- **Campagna, R., Pozzi, V., Spinelli, G., Sartini, D., Milanese, G., Galosi, A. B., & Emanuelli, M. (2021)**. The Utility of Nicotinamide N-Methyltransferase as a Potential Biomarker to Predict the Oncological Outcomes for Urological Cancers: An Update. *Biomolecules*, *11*(8), 1214.
- **Cancemi, P.; Bivona, S.; Pucci-Minafra, I.; Buttacavoli, M.; Di Cara, G.; Albanese, N.N.; Feo, S**. A multiomics analysis of S100 protein family in breast cancer. Oncotarget **2018**, 9, 29064–29081.
- **Cancer Facts & Figures 2014 - American Cancer Society(ACS).**
- **Dezfoulian, Omid, Hesameddin Akbarein, Shahram Jamshidi, Hananeh Golshahi, Hamed M Lakooraj, and Leila Haghighi. 2020**. 'Calretinin expression as a risk biomarker for metastatic canine mammary carcinoma', Veterinarski arhiv, 90: 453-66.
- **Donato R**. S100: a multigenic family of calci¬um-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol 2001; 33: 637-668.
- **Duhig, Edwina E, Lydia Kalpakos, Ian A Yang, and Belinda E Clarke. 2011**. 'Mesothelial markers in high-grade breast carcinoma', Histopathology, 59: 957-64.
- **Forman, Seth B, and Tammie C Ferringer. 2007**. 'Clear-cell basal cell carcinoma: differentiation from other clear-cell tumors', The American journal of dermatopathology, 29: 208-09.
- **Jacquemier, Jocelyne, Laetitia Padovani, Laetitia Rabayrol, Sunil R Lakhani, Frédérique Penault-Llorca, Yves Denoux, Maryse Fiche, Paulo Figueiro, Véronique Maisongrosse, and Viviane Ledoussal. 2005**. 'Typical medullary breast carcinomas have a basal/myoepithelial phenotype', The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 207: 260-68.
- **Johnson, SJ, and V Wadehra. 2001**. 'The importance of intracytoplasmic DPAS positivity in fine needle aspirates of breast lesions', Journal of clinical pathology, 54: 146-51.

- **Lugli, Alessandro, Yvonne Forster, Philippe Haas, Antoinio Nocito, Christoph Bucher, Heidi Bissig, Martina Mirlacher, Martina Storz, Michael J Mihatsch, and Guido Sauter. 2003**. 'Calretinin expression in human normal and neoplastic tissues: a tissue microarray analysis on 5233 tissue samples', Human pathology, 34: 994-1000.
- **Micello, Donata, Alberto Bossi, Alessandro Marando, Emanuele Dainese, Fausto Sessa, and Carlo Capella. 2017**. 'Expression of calretinin in high-grade hormone receptor-negative invasive breast carcinomas: correlation with histological and molecular subtypes', Virchows Archiv, 471: 13-21.
- **Milne, RL. And Antoniou, AC. (2011)** . Genetic modifiers of cancer risk for BRCA1 and BRCA2 mutation carriers. Annals of oncology : official
- **Mjali A, Najeh Hasan Al Baroodi B**. Some Facts About Cancers in Karbala province of Iraq, 2012-2020. Asian Pacific Journal of Cancer Care. **2020** 06 07;5(2):67-69.
- **Palacios J, Andreu X, Calasanz MJ, Concha A, Corominas JM, García-Caballero T, et al**. Recomendación para la determinación de HER2 en cáncer de mama. Consenso nacional de la Sociedad Española de Anatomía Patológica (SEAP) y de la Sociedad Española de Oncología Médica (SEOM) Rev Esp Patol. 2009;42:3–16.
- **Polley MY, Freidlin B, Korn EL, Conley BA, Abrams JS, McShane LM. Statistical and practical considerations for clinical evaluation of predictive biomarkers. J Natl Cancer Inst. 2013**;105:1677–1683.
- **Powell G, Roche H, Roche WR**. Expression of calretinin by breast carcinoma and the potential for misdiagnosis of mesothelioma. Histopathology. **2011**; 59:950–6.
- **Powell G, Roche H, Roche WR**. Expression of calretinin by breast carcinoma and the potential for misdiagnosis of mesothelioma. Histopathology. **2011**; 59:950–6.
- **Sarma, Deba P, Daniel Olson, Jennifer Olivella, Tracey Harbert, Bo Wang, and Stephanie Ortman**. 2011. 'Clear cell basal cell carcinoma', Pathology Research International, 2011.
- **Sharma, G. N., Dave, R., Sanadya, J., Sharma, P., & Sharma, K. (2010).** Various types and management of breast cancer: an overview. *Journal of advanced pharmaceutical technology & research*, *1*(2), 109.
- **Taliano, R. J., Lu, S., Singh, K., Mangray, S., Tavares, R., Noble, L., & Yakirevich, E. (2013).** Calretinin expression in high-grade invasive ductal carcinoma of the breast is associated with basal-like subtype and unfavorable prognosis. Human pathology, 44(12), 2743-2750.
- **Taliano, R. J., Lu, S., Singh, K., Mangray, S., Tavares, R., Noble, L., & Yakirevich, E. (2013)**. Calretinin expression in high-grade invasive ductal carcinoma of the breast is associated with basal-like subtype and unfavorable prognosis. Human pathology, 44(12), 2743-2750.
- **Yen, M.C.; Huang, Y.C.; Kan, J.Y.; Kuo, P.L.; Hou, M.F.; Hsu, Y.L**. S100B expression in breast cancer as a predictive marker for cancer metastasis. Int. J. Oncol. **2018**, 52, 433–440.