World Journal of Internal Medicine and Surgery
ISSN(O): 3048-510X ISSN(P): 3051-2565

Research Article

2025, Volume 2, Issue 6, Page: 10-15. www.wjims.com

EXTRACTION OF CATHEPSIN S FROM BREAST CANCEROUS TISSUE (UNDERGO BREAST CONSERVING SURGERY ALONG WITH ADJUVANT RADIATION THERAPY.) AND ITS CHARACTERIZATION

[1]Muhammad Zubair. [2]Sadia Majeed, [3]Ruman Aamer, *[4]Rukhshan Khurshid, [1]Noor Ulain, [1]Amna Bakhtiar

[1]Department of Surgery, Watim Medical College Rawalpindi.
[2]Department of Pharmacology, Continental Medical College Lahore.
[3]Department of Biochemistry, M Islam Medical College Gujranwala.
[4]Akhtar Saeed Medical and Dental College, Lahore.
[4]Department of Biochemistry, Federal Post Graduate Medical Institute, Lahore.

Article Info

Article Received: 16 June 2025,
Article Revised: 09 July 2025,
Published on: 01 August 2025.

***Corresponding author:**

Rukhshan Khurshid
Akhtar Saeed Medical and Dental College, Lahore.
rakhshan99@yahoo.com
mzubairh@gmail.com
sadia.aimc@gmail.com
asmaamin31@gmail.com

ABSTRACT

Objective: Study was tried to extract/isolatecathepsin S from canceroust issue received breast conserving surgery along with radiation therapy. **Material and Methods:** Human tissue (normal/cancerous) was obtained from surgery department of Sir Ganga Ram Hospital Lahore. Tissue was characterized as malignant. Extraction of cathepsin S was carried out. Identification of cathepsin S was carried out by electrophoresis. Column chromatography was used to purify Cathepsin S. Level of cathepsin S was estimated using specific substrate and read photo metrically. Level of cathepsin was tried to associate with age (menarche / menopausal age) and clinic-pathological characteristics of patients. **Results:** Significantly high level of cathepsin S was observed in extract of breast cancerous tissue as compared to normal breast tissue. Level of cathepsin was high age at menarche <14years and at menopausal age. With increase tumor size (T3) and stage 111, the level of cathepsin was high as compared to tumor size T2 and stage 11. **Conclusion:** Increase level of cathepsin S in breast cancer tissue, marking it a biomarker and potential therapeutic target. Higher level of cathepsinS in breast cancer patients with small tumor size and late stage of breast cancer may help to investigate cathepsin mediated tissue modification and alteration is important for research science and for clinical purposes, such as biomarkers.

KEYWORDS: breast cancer tissue, cathepsin S; breast conserving surgery, radiation therapy.

INTRODUCTION

To date, breast cancer endures the most commonly diagnosed malignancy and the main cause of cancer-associated mortality in women globally, signifying about 25% of totally cancer issues and 15 % of total cancer deaths. Around 80 % of patients under gosurgery may be mastectomy / breast-conserving surgery (BCS). Majority of breast cancer patients treated with BCS in combination with adjuvant radiation remedy.[1]

Proliferation of epithelial tissue is the reason of breast malignancy that possibly invades nearby tissues or metastatic to different parts of body of women. Some of the risk factors in this cancer are age, family history, age at menarche, menopause, high BMI, sedentary life style etc.[2]

Cathepsins are group of proteolytic enzymes important in both cellular physiology and ailment. They are classified on the bases of amino acids present in active side of enzyme. The amino acid may be serine (cathepsin A & G), aspartic (cathepsin D & E), or cysteine (cathepsin B, C, F, K, L, S & others) proteolytic enzyme.[3]

The cysteine protease cathepsin S (CTSS) is member of cysteine cathepsin proteases, and has been found to be associated with a range of pathologies, including cancer. As compared to other cytsteine proteases, cathepsin S is normally reserved to lymphoid tissues and macrophage. This property of cathepsin S may relate with therapeutic ability in cancer. CTSS is implicated in tumor invasion and metastasis by the induction of tumor angiogenesis and the degradation of the tumor extracellular matrix. Besides,

CTSS may be elevated in patients with defects in DNA damage repair pathways, indicating it may be predictive of tumour sensitivity to DNA damaging agents.[4,5]

Amongst the family of cysteine cathepsin CTSS take special interest due to properties like restricted expression of profile normally, inducible up-regulation and action at a wide range of pH.[6] On the other hand abnormal expression of CTSS is observed in number of conditions and ailments, taking it as marker of disease and therapeutic goal. Level of cathepsins of cysteine family in breast cancer is estimated usually for its predictive value.[7] The use of cathepsin-targeted breast conserving surgery may help to find out the residual parts of tumor on the cavity surfaces and direct some additional excision, thereby lessening the need for re-resection, and recurrence.

Study was therefore designed to extract/isolate cathepsin S from canceorous tissue received breast conserving surgery along with radiation therapy.

MATERIALS AND METHODS

Human tissue was obtained from surgery department of Sir Ganga Ram Hospital Lahore. Tissue was characterized as malignant. Extraction and autolysis was carried by modification of the methods.[8] The crude extract was purified employing column chromatography.[9] Cathepsin S estimation was carried out using the technique.[7]

Extraction and autolysis

Breast tissue collected from 25 breast cancer women in liquid ammonia and stored at -80^{0}c. Human breast tissue (normal/cancerous) was thawed at 4^{0}c. it was rinsed with 0.15M sodium chloride (pH 3.8) containing 1mM EDTA and homogenized (homogenizer Yamato Model K41) in 0.15M sodium chloride buffer containing 1mM EDTA and 0.05% triton x-100 to produce a fine suspension of the tissue. The pH of the homogenate was adjusted to 3.8 with 1M HCL and incubated overnight at 4^{0}c to allow autolysis to occur. The homogenate was centrifuged at 3000rpm for 15 min. The insoluble material was removed and supernatant was mixed with ether in 3:1 rationand centrifuged at 20,000 g for 30 min. the upper layer was concentrated in vacuo and protein was precipitated at 4^{0}c with 2M ammonium sulfate, store at -20^{0}c.[10]

Identification of cathepsin S using SDS PAGE electrophoresis. Gel used was 12.5 % polyacrylamide. Samples collected from normal and breast cancer tissue was loaded in wells formed by specific comb. Standard molecular marker (ladder) with range of weight 100-12 Kda was also run. Cathepsin s with molecular weight 37 Kda was identified after completing the electrophoresis.

Isolation of cathepsin S using HPLC gel permeation (Column: TSK G 2000 SW). Buffer used was 0.02 M sodium acetate buffer, pH 4.8 containing 1 mM EDTA and 0.02% NaN_3. The column was eluted stepwise by increase in pH. Fraction of Cathepsin S eluted at pH 6.2 with 0.4 M NaCl. Fraction (peak 3) containing cathepsin S activity was collected and estimation of cathepsin S of peak 3 /fraction was done using the specific assay of cathepsin S (Fig 1,2,3)

Assay of cathepsin S.

Cathepsin S is a cysteine proteinase was extracted from cancerous breast tissue of women. Its most susceptible substrates, such as Z-Val-Val-Arg-NHMec (pH 7.5), the specific determination of cathepsin S is possible. The substrate Z-Val-Val-Arg-NHMec is hydrolyzed to liberate 7-amino-4-methylcoumarin, which is quantified continuously by stopped assays after the termination of the enzymatic reaction by monochloroacetate. The level of the free aminomethylcoumarin is determined by 460 nm[7].

Statistical analysis

Data were analyzed for statistical significance using either one way analysis of variance (ANOVA) or Student's *t*-test.

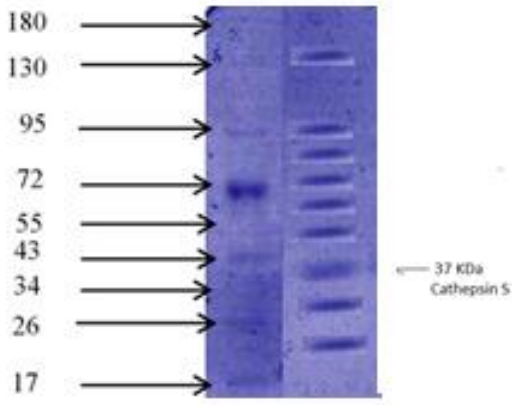

Fig 1: 12.5 % polyacrylamide gel electrophoresis with 37 Kdacathepsin s (right side). Ladder with standard protein from 17-180 Kda (left side).

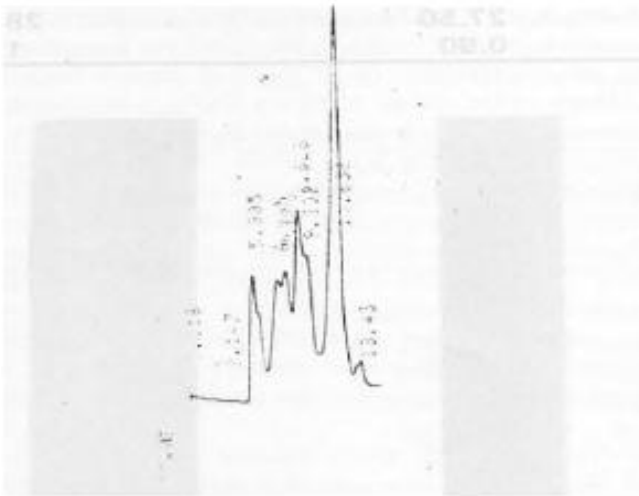

Fig. 2: HPLC gel permeation (Col: TSK G 2000 SW) of fraction (peak 3) containing cathepsin S activity after TSK G 3000 SW column.

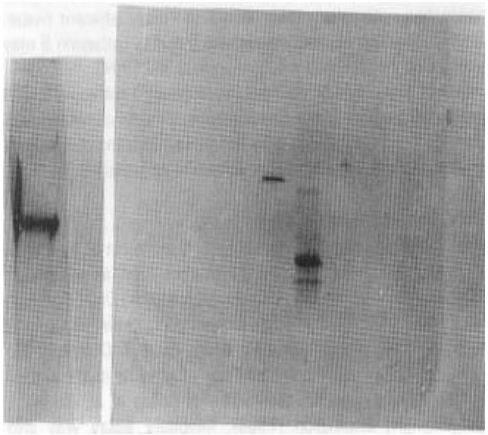

Fig 3: Fraction of TSK G 3000 SW containing cathepsin S activity was analyzed by 10 percent 12polyacrylamide gel.

Table 1: Demographic /Clinical Characteristics of Breast Cancer Patients (n=25).

Parameters	Breast cancer women
Age	50.80±10.6
Level of education	Graduate
Awareness of disease	Aware
Family history	Positive (50%)
Socioeconomic status	Middle (75 %)
Profession	Professional (40%)
Life style	Active (35%) Sedentary (65%)
Age at menarche	14 year (60 %)
Age at menopause	55 year (70%)
Staging	II & 111
Histologically diagnosed	In situ carcinoma (Ductal/Lobular) Invasive carcinoma Ductal (80%) Lobular (20%)
Tumor size (mean ±SD) Length width	 6.51 cm ± 3.89 cm 6.48 cm ± 3.91 cm
Site of breast involved	Right ---55% Left ---45%
Nodal status	77% positive
Mode of treatment	Radiotherapy (35%) Radiotherapy and Chemotherapy (65%)

Table 2: Mean, Standard Error of mean Cathepsins (Pixel) Between Normal and Tumor Breast Tissue Specimens (n = 25).

	Cathepsin S Mean±SD	P-value
Normal	25.22±5.5	>0.001
Patients	75.83±11.58	

Table 3: Activities of Cathepsininbreast cancer women age with menarche and menopause.

	Cathepsin S Mean±SD	P-value
Age in early menarche (< 14 years)	70.89±11.92	
Age in early menarche (≥14 years)	45.89±11.89	<0.05
Present age ≥50 years	65.88±9.80	
Present age ≤50 years	77.89±10.91	>0.05

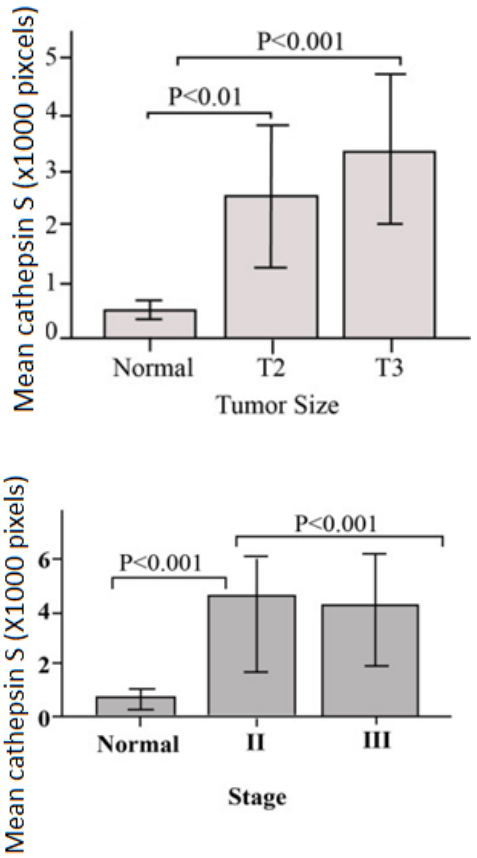

Fig 4: Tumor size / stage specific difference of cathepsin S activity in breast cancer women. Activity of cathepsin was quantify by band densitometry. Values were expresses as mean±SEM.

RESULTS

Pre-fractionation of crude tissue extract on gel filtration (Sephadex G-75) resulted in 3 peaks (Fig. 1). Fractions of these peaks were pooled and concentrated in vacuo. The concentrated fraction of peak 2 containing cathepsin S (active fraction) was observed by 8-25% polyacrylamide gradient gel on phast system which give a single band of approximately 25,000 dalton (Fig. 2,3). Level of cathepsin was estimated by using the substrate Z-Val-Val-Arg-NHMec.

Socio-Demographic characteristics of the Patients

Mean age of patients was 50 years. Majority of women were educated, having awareness of disease, middle class. About 50 % breast cancer women had family history of

breast cancer. 40% were professional. Majority had sedentary life style. Age of menarche was > 14 years in 60 % of women; age at menopause was 55 years in 70 % women. Majority of women had intraductal carcinoma (right breast) with tumor size (length –6.51cm and width 6.48 cm). None of the patients had the habit of alcohol use and smoking. However, about 32.6% patients used oral contraceptive pills (table 1).

Clinical /Pathological Outcomes

Of the total 25 breast cancer women, 77% were node-positive. The histopathologicalreports showed that 80 % had infiltrative ductal carcinoma (table 1). Level of cathepsin S was significantly increased ($p<0.001$) in patients compared to normal subjects (table 2).

Level of cathepsin S was non-significantly high in age group less than 50 years as compared to age group greater than 50 years. On the other hand increase level of cathepsin S was significantly high in women with their age < 14 years (menarche age) as compared to age > 14 years (Table 3).

Figure 4 showed tumor size / stage specific difference of cathepsin S activity in breast cancer women. It is observed that level of cathepsin S was significantly high with tumor size > 6.51 (T3) cm compared to size < 6.51 cm (T2). Level of cathepsin S was significantly high in patients with stage 111 as compared to in patients with stage 11.

DISCUSSION

The technique used in the current study help to identify the cathepsin S and isolate from the tissue. Estimation of quantity of cathepsin was done by using specific substrate. However techniques used for identification and isolation of cathepsin from breast tissue was also carried out by a group of workers proposed the method of zymography. This technique is based on polyacrylamide gel staining showed visually the separation protein's cathepsin. The quantity of cathepsin was found out via band densitometry. This method also grading of tissue.[11, 12]

We observed that mean age of cancer patients was 50 years. Majority of women were educated, having awareness of disease, middle class. Majority had sedentary life style. Age of menarche was > 14 years in 60 % of women, age at menopause was 55 years in 70 % women. Majority of women had intraductal carcinoma (right breast) with tumor size (length –6.51cm and width 6.48 cm).

We agreed with a study carried out in United Kingdom found that 80% of breast cancers were diagnosed in age greater than 50 years with sedentary life style.[12] Another study was carried out in Karachi-Pakistan including about 1000 breast cancer women. Study found higher incidence rate of breast cancer in women aged 40 to 44 years & 65 to 69 years.[13] It is proposed that risk of breast cancer in age > 40 years is related with imbalance of hormone.[12] A cross-sectional study was carried out on 135 breast cancer women to find out relationship between obesity, sedentary lifestyle, and risk of breast cancer. It is noted that high BMI, physical inactivity, and lower WC were associated with the lower breast cancer risk.[14]

We observed significantly high value of cathepsin S in tumor of breast as compared to normal breast tissue. Studies also observed high values of cysteine proteases along with cathepsin S in breast cancer women. A study reported that cathepsin S help in the progression of cancer via proteolyticbreakdpwm of the BRCA1, causing a suppression in repair of break of double strand of DNA.[15] Another study found that increased proteolytic activity and abnormal localization of number of cysteine cathepsinsmotivate the progression of tumor, proliferation, process of invasion, & metastasis.[16]

Tumor size / stage specific difference of cathepsin S activity was also observed in breast cancer women. It is observed that level of cathepsin S was significantly high with tumor size > 6.51 (T3) cm compared to size < 6.51 cm (T2). Level of cathepsin S was significantly high in patients with stage 111 as compared to in patients with stage 11. A study also observed the increase level of cathepsin S and it may be related to tumor size and stage of cancer.[17] A study also found the role of cathepsin S motivating the development / progression of the cancer. Study also found increased expression of cathepsin S related with increased volume of tumor, higher stage of tumor and increased the metastasis results (involvement of lymph node) in poor prognosis of patients.[18] It is proposed that interactions between cells of cancer and the tumour micro-environment happen as chains of mechanical edges and signaling episodes leads to progression of tumor.[19] Of the total 25 breast cancer women, 77% were node-positive. It is found that inflammatory cancer cell lines of breast show increased levels of cathepsin and it may be positively related with metastases of lymph node.[20]

The histo-pathological reports showed that 80 % had infiltrative ductal carcinoma. A study observed high percentage of ductal carcinoma. However it is found that various molecular sub-types of breast cancer and appears to rise during the switch the ductal carcinoma to invasive breast carcinoma, signifying this cysteine protease gives to the tumor's invasiveness.[21, 17]

Study observed level of cathepsin S was non-significantly high in premenopausal age as compared to postmenopausal age group. A study also found in postmenopausal women with high BMI, a 50% high risk of development of breast cancer. This may be due to increasedadipokines, which may cause progression of cancer.[22] Findings of a group of workers found that usage of hormonal treatment in menopausal older ages and unawareness about breast screening in women of Pakistani may be the reason of developing breast cancer.[23] Furthermore, illiteracy, pitiable socioeconomic

position, and lack of healthcare facility may be the reason of increase cases of breast cancer in postmenopausal women.[24] It is therefore proposed that up-regulation of cancerous tissue, and hypoxia resulting the speedygrowth in tissue, causes increase level of cathepsin.[25]

On the other hand increase level of cathepsin S was significantly high in women with their age < 14 years (menarche age) as compared to age > 14 years. Number of studies agreed with our study and reported that age at menarche and childbearing arrays may affect the development of mammary gland and create breast cancer discrepancy. The timing of menarche is affected by biology of female, genetic and environmental features, as well as nutritional factors.[26] Up-regulation of cysteine protease cathepsinin breast cancer women, with time of menarche was at <14 years of age. As at this period the breast cells are very sensitive, therefore pathogenesis of the tissue of breast results an increase expression of cysteine protease cathepsin. It is therefore proposed cells of tumor and the stromal cells present in surroundings shows high values cathepsin for metastases of tumor.[27] As increase proteolyticactivity of cathepsin in the tumor micro-environment can breakdown the ECM of cell that may help in metastasis.[28]

CONCLUSION

Increase level of cathepsin S in breast cancer tissue, marking it a biomarker and potential therapeutic target. Higher level of cathepsinS in breast cancer patients with small tumor size and late stage of breast cancer may help to investigate cathepsin mediated tissue modification and alteration is important for research science and for clinical purposes, such as biomarkers.

External factors, such as socioeconomic factors demonstrate a link in breast cancer risk such as low-income, poor diet, lack of exercise, and lifestyle choices including smoking and drinking reveal an environmental connection to breast cancer risk and cancer disparity.Furthermore, a deeper appreciation of the biology underlying this disease will help guide treatment regimens and possible application of cathepsin S inhibitors in the future.

REFERENCES

1. Kedrzycki MS, Chon HTW, Leiloglou M, Chalau V, Leff DR, Elson DS. Fluorescence guided surgery imaging systems for breast cancer identification: a systematic review. J Biomed Opt. 2024 Mar; 29(3): 030901. Available from doi: 10.1117/1.JBO.29.3.030901
2. Sun T, Jiang D, Zhang L, Su Q, Mao W, Jiang C. Expression profile of cathepsins indicates the potential of cathepsins B and D as prognostic factors in breast cancer patients. OncolLett. 2016; 11(1):575–583. Available from doi: 10.3892/ol.2015.3960
3. Lalmanach G, Saidi A, Bigot P, Chazeirat T, Lecaille F, Wartenberg M. Regulation of the Proteolytic Activity of Cysteine Cathepsins by Oxidants. Int J Mol Sci. 2020; 21(6): 1944. Available from doi.org/10.3390/ijms21061944
4. Wilkinson RDA, Burden RE, McDowell SH, McArt DG, McQuaid S, Bingham V, Williams R, Cox ÓT, O'Connor R, McCabe N, Kennedy RD, Buckley NE, Scott CJ. A Novel Role for Cathepsin S as a Potential Biomarker in Triple Negative Breast Cancer JOncol. 2019 Jun 27; 2019: 3980273. Available from doi: 10.1155/2019/3980273.
5. Liang S, Dang B, Chen S, Mi H. Prognostic value and immunological role of cathepsin S gene in pan-cancer. OncolLett. 2023 Dec 4; 27(1):41. Available from doi: 10.3892/ol.2023.14175.
6. Yoo Y, Choi E, Kim Y, Cha Y, Um E, Kim Y, Kim Y, Lee YS.Therapeutic potential of targeting cathepsin S in pulmonary fibrosis. Biomed Pharmacother. 2022 Jan; 145:112245. Available from doi: 10.1016/j.biopha.2021.112245.
7. Smyth P, Sasiwachirangkul J, Williams R, Scott CJ. Cathepsin S (CTSS) activity in health and disease - A treasure trove of untapped clinical potential.Mol Aspects Med. 2022 Dec; 88: 101106. Available from doi: 10.1016/j.mam.2022.101106.
8. Menacho-Melgar R, Lynch MD. Simple Scalable Protein Expression and Extraction Using Two-stage Autoinducible Cell Autolysis and DNA/RNA Autohydrolysis in Escherichia coli.BioProtoc. 2022 Jan 20; 12(2):e4297. Available from doi: 10.21769/BioProtoc.4297.
9. Dou J, Heinonen J, Vuorinen T, Xu C, Sainio T. Chromatographic recovery and purification of natural phytochemicals from underappreciated willow bark water extracts. Separation and Purification Technology 2021; 261:118247 Available from doi.org/10.1016/j.seppur.2020.118247.
10. Singh S, Sharma S, Agarwal SK. A simple purification procedure of buffalo lung cathepsin H, its properties and influence of buffer constituents on the enzyme activity.BiochemBiophys Rep. 2020 Feb 9; 22: 100739. Available from doi: 10.1016/j.bbrep.2020.100739.
11. Rani V, Aggarwal K, Varshney S, Atale N. Zymographic Techniques. In: Chakraborti, S., Ray, B.K., Roychoudhury, S. (eds) Handbook of Oxidative Stress in Cancer: 2022; Mechanistic Aspects. Springer, Singapore. Available from doi.org/10.1007/978-981-15-9411-3_26.
12. Yu DMT, Dauth S, Margineanu MB, Snetkova V, Rehders M, Jordans S, Brix K. Characterization of Cysteine Cathepsin Expression in the Central Nervous System of Aged Wild-Type and Cathepsin-Deficient Mice. Applied Sciences. 2022; 12(5): 2608. Available fromdoi.org/10.3390/app12052608
13. Zaheer S, Yasmeen F. Historical trends in breast cancer presentation among women in Pakistan from join-point regression analysis. Pak J Med Sci. 2024 Jan-Feb; 40(1Part-I): 134-139. doi: 10.12669/pjms.40.1.7123
14. Solikhah S, Perwitasari D, Permatasari TAE, Safitri RA. Diet, Obesity, and Sedentary Lifestyle as Risk Factor of

Breast Cancer among Women at Yogyakarta Province in Indonesia. Open Access Maced J Med Sci. 10(E): 398-405.
15. Kim S, Jin H, Seo HR, Lee HJ, Lee YS. Regulating BRCA1 protein stability by cathepsin S-mediated ubiquitin degradation. Cell Death Differ. 2019; 26(5): 812–825. Available fromdoi: 10.1038/s41418-018-0153-0.
16. Linders DGJ, Bijlstra OD, Fallert LC, Hilling DE, Walker E, Straight B, March TL, Valentijn ARPM, Pool M, Burggraaf J, Basilion JP, Vahrmeijer AL, Kuppen PJK. Cysteine Cathepsins in Breast Cancer: Promising Targets for Fluorescence-Guided Surgery. Mol Imaging Biol. 2023 Feb; 25(1): 58-73. Available from doi: 10.1007/s11307-022-01768-4.
17. McDowell SH, Samantha A. Gallaher [a 1], Roberta E. Burden [b], Christopher J. Scott. Leading the invasion: The role of Cathepsin S in the tumour micro environment. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 2020; 1867(10): 118781.
18. Tan G, Liu Q, Tang X, Kang T, Li Y, Lu J, Zhao X, Tang F. Diagnostic values of serum cathepsin B and D in patients with nasopharyngeal carcinoma, BMC Cancer 2016; 16: 241.
19. Sara H. McDowell, Samantha A. Gallaher, Roberta E. Burden, Christopher J. Scott, Leading the invasion: The role of Cathepsin S in the tumour microenvironment, Biochimicaet Biophysica Acta (BBA) - Mol Cell Res 2020; 1867(10): 118781, 10.1016/j.bbamcr.2020.118781.
20. Ibrahim SA, El-Ghonaimy EA, Hassan H, Mahana N, Mahmoud MA, El-Mamlouk T, El-Shinawi M, Mohamed MM. Hormonal-receptor positive breast cancer: IL-6 augments invasion and lymph node metastasis via stimulating cathepsin B expression. J Adv Res. 2016; 7(5): 661–670. Available from doi:10.1016/j.jare.2016.06.007
21. Toss M, Miligy I, Gorringe K, Mittal K, Aneja R, Ellis I, Green A, Rakha E. Prognostic significance of cathepsin V (CTSV/CTSL2) in breast ductal carcinoma in situ. J ClinPathol. 2020; 73(2): 76–82. Available from doi: 10.1136/jclinpath-2019-205939.
22. Kim JW, Kim JH, Lee YJ. The Role of Adipokines in Tumor Progression and Its Association with Obesity. Biomedicines. 2024; 12(1):97. Available from doi.org/10.3390/biomedicines12010097
23. Gulzar F, Akhtar MS, Sadiq R, Bashir S, Jamil S, Baig SM. Identifying the reasons for delayed presentation of Pakistani breast cancer patients at a tertiary care hospital. Cancer Manag Res. 2019; 11: 1087. Available from doi:10.2147/CMAR.S180388.
24. Manzoor S, Anwer M, Soomro S, Kumar D. Presentation, diagnosis and management of locally advanced breast cancer:Is it different in low/middle income countries? Pak J Med Sci. 2019; 35(6): 1554–1557. Available from doi:10.12669/pjms.35.6.165
25. Karin E. de Visser, Johanna A. Joyce, The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth, Cancer Cell 2023; 41(3): 374-403. Available from doi.org/10.1016/j.ccell.2023.02.016.
26. Krisanits B, Randise JF, Burton CE, Findlay VJ, and Turner DP. Pubertal mammary development as a “susceptibility window” for breast cancer disparity.Adv Cancer Res. 2020; 146: 57–82.
27. Marques P, Madeira T, Gama A. Menstrual cycle among adolescents: girls' awareness and influence of age at menarche and overweight. Rev Paul Pediatr. 2022; 40:e2020494
28. Tsegaye S, Mehdi M, Labisso WL, Melka DS. Cysteine Cathepsins and Matrix Metalloproteases Among Breast Cancer Patients. Breast Cancer (Dove Med Press). 2021 May 7; 13: 271-283. Available from doi: 10.2147/BCTT.S305387.
29. Sung H, Siegel RL, Rosenberg PS, Jemal A. Emerging can certrendsamong young adultsin the USA: analysis of a population-based cancer registry. The Lancet Public Health. 2019 Mar 1; 4(3): e137-47.