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# Expression of metalloproteinases 2 and 9 and plasma zinc concentrations in women with fibroadenoma

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## SUMMARY

**OBJECTIVE:** This study aims to investigate the association between the immunohistochemical expression of matrix metalloproteinase-2 and matrix metalloproteinase-9 and plasma zinc in women with fibroadenoma.

**METHODS:** This cross-sectional study included 37 premenopausal women with fibroadenoma. Waist circumference and body mass index of the participants were measured. Plasma zinc concentrations were determined using atomic flame absorption spectrophotometry. Fragments of breast tissue were fixed and incubated with primary mouse monoclonal antibodies (monoclonal antibodies matrix metalloproteinase -2 -507 and monoclonal antibodies matrix metalloproteinase -9-439). Semi-quantitative analysis of matrix metalloproteinase-2 and matrix metalloproteinase-9 immunoreactivity was performed. Spearman's test and Friedman's test were used for statistical analyses. The $p<0.05$ were considered statistically significant.

**RESULTS:** The average age of the participants was 32.81±9.51 years. The body mass index and waist circumference values were within the normal range. The mean plasma zinc concentration was 42.73±13.84 µg/dL, with 94.6% inadequacy. A statistically significant difference was found between the positive expression of matrix metalloproteinase-2 and matrix metalloproteinase-9 ($p=0.0184$). There was no significant correlation between the matrix metalloproteinase expression and the plasma zinc levels.

**CONCLUSIONS:** Women with fibroadenoma had hypozincemia and positive expression of metalloproteinases.

KEYWORDS: Matrix Metalloproteinases. Fibroadenoma. Zinc.

## INTRODUCTION

Breast fibroadenomas are benign lesions in the breast tissue characterized by hyperplasia and polyclonal proliferation of the epithelial tissue and stroma. In this type of lesions, the presence of benign nodular masses is common. Its etiology is mainly due to the hormonal influences of estrogen, progesterone, and prolactin[1].

Studies have shown that fibroadenomas rarely cause breast cancer. However, invasive cancer can develop in fibroadenoma[2]. Breast cancer has high metastatic rates and often invades the liver, the brain, and the lungs. Moreover, biomarkers for determining invasiveness and tumor aggressiveness have been studied, especially the analysis of matrix metalloproteinases (MMPs), which are evaluated both in breast carcinoma and in benign tumors such as fibroadenoma[3].

MMPs are involved in the initiation and progression of breast cancer through interactions with tumor suppressor genes involved in the early stages of carcinogenesis. In addition, a

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positive correlation has been demonstrated between increased levels of MMP-2 and MMP-9 and an increased risk of metastasis and reduced survival in cancer patients[4].

MMPs are a family of zinc-dependent endopeptidases that are involved in regulating various physiological events such as cell growth, inflammation, invasion, and angiogenesis. When overexpressed, these enzymes can damage the integrity of the basement membrane and extracellular matrix by proteolysis, which are important barriers against cell invasion[5]. In particular, MMP-2 and MMP-9 favor the metastasis of tumor cells[6].

Zinc participates as a cofactor for MMPs and is essential for regulating their expression and activity. Zinc deficiency can decrease the activity and expression of MMPs; however, an increase in the amount of zinc in the cellular compartments favors the physiological activity of these proteins and their catalytic effects[7].

Considering the importance of zinc in regulating the expression and activity of MMPs, as well as the scarcity of literature on immunohistochemical expression of these proteins in fibroadenoma, this study aimed to investigate the association between the immunohistochemical expression of MMP-2 and MMP-9 and plasma zinc in women with fibroadenoma.

## MATERIALS AND METHODS

This was a cross-sectional study involving 37 premenopausal women, aged 20–59 years, with benign fibroadenoma tumors. Moreover, this study is an excerpt from a macro project entitled "Molecular Biomarkers in Women with Mammary Neoplasia" approved by the Ethics Committee of the Federal University of Piauí with opinion 1.022.962. All patients signed an informed consent form prior to the study initiation.

Participants were recruited from the mastology clinic of a public hospital in Teresina-Piauí, according to the following eligibility criteria: premenopausal women with follicle-stimulating hormone (FSH) levels <30 mUI/mL; presence of fibroadenoma; nonsmoker; absence of chronic diseases, liver disease, anemia, or clinical inflammatory processes; and absence of vitamin–mineral supplementation. The participants underwent histological evaluation of the tumors.

The body mass index (BMI) and waist circumference (WC) were calculated and measured[8,9]. Ten milliliters of blood was collected in the morning after a 12-h fast and was transferred to the tubes containing 30% sodium citrate for zinc analysis. Plasma was obtained by centrifuging whole blood at 1,831 g for 15 min at 4°C (CIENTEC® 4K15) and stored in microtubes at -20°C for further analysis. Plasma zinc analysis was performed using atomic flame absorption spectrophotometry[10]. The reference value used was 75–110 µg/dL[11].

Semiquantitative analysis of MMP-2 and MMP-9 immunoreactivity was performed[12], and the following were considered: intensity of cell staining (I) and fraction of stained neoplastic cells (F). The intensity was classified as 0 (negative), 1 (weakly colored), 2 (moderately colored), or 3 (strongly colored). The fraction of stained cells was classified as: I (0–25%), II (25–75%), or III (75–100%). The final result was achieved by a combination of two parameters (I and F) ranging from 0 to 6. Cases with a final score ≥3 were classified as positive for MMP-2 and MMP-9. In all cases, brown cytoplasmic staining was used as the standard of positivity.

Data were analyzed using IBM SPSS statistical software for Windows, version 22 (Armonk, NY: IBM Corp.). Descriptive analyses were used for frequencies, percentages, means, and deviations. The Spearman's test was used to analyze the correlation between the data. For comparisons between the studied variables, the Friedman's test was performed. The $p<0.05$ were considered statistically significant.

## RESULTS

The mean age of the participants was 32.81±9.51 years. The average values of the anthropometric parameters used to assess the nutritional status of women with fibroadenomas are shown in Table 1. The average plasma zinc concentration (42.73±13.84 µg/dL) was found below the reference values for plasma zinc, as well as a high percentage of inadequate plasma zinc concentrations in women with fibroadenoma (94.6%) (Figure 1).

Analysis of the immunohistochemical expression of MMP-2 and MMP-9 revealed that a greater number of women showed a negative expression of MMP-2 (n=22) and a positive expression of MMP-9 (n=25). A statistically significant difference was found ($p<0.0184$) between the number of women with positive immunohistochemical expression of MMP-2 and MMP-9 (final score ≥3) (Figure 2). The correlation between the immunohistochemical expression of MMP-2 and MMP-9

Table 1. Mean values and standard deviations of age, weight, height, body mass index and waist circumference of women with fibroadenoma.

| Parameters | Women with fibroadenoma (n=37) |
|---|---|
| | Mean±SD |
| Age (years) | 32.81±9.51 |
| Weight (kg) | 57.49±11.33 |
| Height (m) | 1.57±0.07 |
| BMI (kg/m²) | 23.92±4.76 |
| WC (cm) | 79.70±12.13 |

SD: standard deviation.

with plasma zinc was not statistically significant (r=–0.1252, p=0.4602; r=0.1693, p=0.3164, respectively).

## DISCUSSION

In this study, reduced levels of plasma zinc concentrations were found in women with fibroadenoma. Although there were still no data on plasma zinc concentrations in women with fibroadenoma, the reduction in plasma zinc concentrations in women with breast cancer has already been observed[13].

It is noteworthy that hypozincemia present in breast cancer may be the result of changes in the compartmentalization of the mineral. It is likely that zinc is transported from the plasma compartment into the tumor cells and is used to stimulate the catalytic domain of MMPs, which might alter the activity of this enzyme, thereby contributing to the tumor development and progression[14]. Holanda et al. found that reduced concentrations of zinc in plasma and erythrocytes were positively related to the increased plasma concentrations of MMP-2 in patients with breast cancer[13].

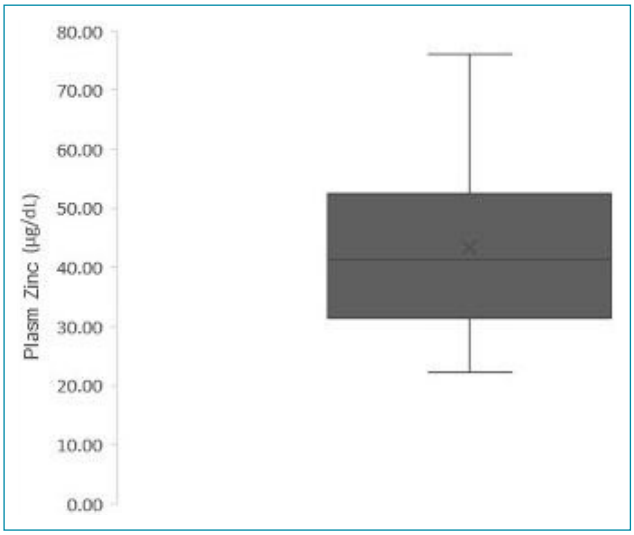
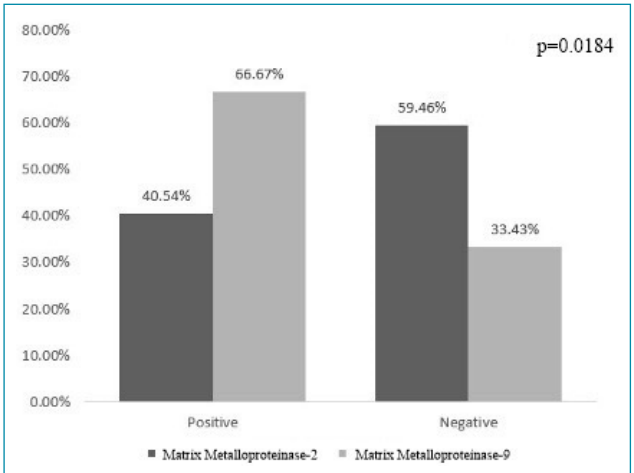



Figure 1. Mean values of plasma zinc concentrations in women with fibroadenoma.




*Statistically significant difference (p<0.05) for Friedman's test comparing the number of cases (%) with cells positively stained for matrix metalloproteinase-2 and matrix metalloproteinase-9.

Figure 2. Percentage of cases with cells expressing matrix metalloproteinase-2 and matrix metalloproteinase-9 in breast fibroadenoma.

Zinc is essential for the cell survival, growth, proliferation, and metabolism. Changes in zinc levels in the body contribute to cellular and metabolic dysfunction with pathophysiologic repercussions[15].

In this study, we evaluated the positive immunostaining of MMP-2 and MMP-9, which showed 40.54 and 67.57% of expression, respectively. Jinga et al. observed that both enzymes were expressed in both malignant and benign tumors, enzymes being more expressed in malignant than in benign tumors[16]. Vasaturo et al. observed that the plasma concentration of MMP-2 was significantly higher in patients with breast carcinoma than in patients with fibroadenoma[17].

MMP-9 participates in the progression of breast cancer; its high expression is associated with a higher incidence of metastasis and a worse prognosis[18]. Although the risk of developing breast cancer is low in fibroadenomas, some authors report the appearance of cancerous lesions within fibroadenomas[19,20]. Thus, investigation of the expression pattern of MMPs may contribute to the early identification of changes that favor carcinogenesis in fibroadenomas.

The change in the dynamics of MMP functioning is a critical factor in the process of tissue integrity; that is, the imbalance in the activity of these enzymes may favor excessive degradation or accumulation of tissues in the extracellular layer. Positive immunostaining for MMP-2 has been proposed as a marker of aggressiveness in breast carcinomas. It has been shown that blocking the secretion and activation of this enzyme can reduce the risk of breast cancer[21].

Although most studies have shown greater expression of these enzymes in breast cancer, the results of some studies have already shown higher MMP expression in women with fibroadenoma[22]. According to Sangma et al., patients with proliferative lesions had risk factors for developing invasive carcinoma[23]. As there is no consensus on the morphological risk markers, molecular and nutritional biomarkers can, therefore, help in risk stratification and improve clinical management.

Thus, MMPs have been evaluated in breast carcinoma, normal breast tissue, and benign tumors, as these proteins are involved in tumor progression[24]. Somiari et al. suggested that the preoperative plasma concentration and the activity of MMP-2 and MMP-9 may allow the subclassification of patients with

breast diseases[25]. In that study, individuals without breast disease and with a low risk for developing breast cancer showed significantly different concentrations and activities of these proteins in the plasma, compared to individuals with high risks for benign disease and breast cancer.

This study had limitations due to the small number of participants; however, it was possible to verify a considerable percentage of cells stained for MMP-2 and MMP-9 and a reduced plasma zinc concentration in women with benign breast tumors.

## CONCLUSIONS

The data obtained in this study do not show a correlation between the MMPs evaluated and the plasma zinc concentrations in women with fibroadenoma, despite the low values found for this micronutrient. The study also showed positive expression of gelatinases, particularly MMP-9, which had a higher expression than MMP-2. Thus, it is recommended that further studies should be conducted to obtain more in-depth knowledge on the topic.

## AUTHORS' CONTRIBUTION

**LMM, ISB, ESF, AGSN, CSMED, VAO, ARSO, JBSM, DNM, BBS:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

## REFERENCES


1. Meng X, Yamanouchi K, Kuba S, Sakimura C, Morita M, Matsuguma K, et al. Giant fibroadenoma of the breast: a rare case in a mature woman. Int J Surg Case Rep. 2019;63:36-9. https://doi.org/10.1016/j.ijscr.2019.09.015
2. Shiino S, Yoshida M, Tokura M, Watase C, Murata T, Jimbo K, et al. Locally advanced triple negative breast cancer arising from fibroadenoma with complete response to neoadjuvant chemotherapy: a case report. Int J Surg Case Rep. 2020;68:234-8. https://doi.org/10.1016/j.ijscr.2020.02.059
3. Li H, Qiu Z, Li F, Wang C. The relationship between MMP-2 and MMP-9 expression levels with breast cancer incidence and prognosis. Oncol Lett. 2017;14:5865-70. https://doi.org/10.3892/ol.2017.6924
4. Skerenova, M, Mikulova V, Capoun O, Zima T, Tesarova P. Circulating tumor cells and serum levels of MMP-2, MMP-9 and VEGF as markers of the metastatic process in patients with high risk of metastatic progression. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2017;161(3):272-80. https://doi.org/10.5507/bp.2017.022
5. Holanda AON, Oliveira ARS, Cruz KJC, Severo JS, Morais JBS, Silva BB et al. Zinc and metalloproteinases 2 and 9: what is their relation with breast cancer? Rev Assoc Med Bra. 2017;63(1):78-84. https://doi.org/10.1590/1806-9282.63.01.78
6. Song Z, Wang J, Su Q, Luan M, Chen X, Xu X. The role of MMP-2 and MMP-9 in the metastasis and development of hypopharyngeal carcinoma. Braz J Otorhinolaryngol. 2019;85(5):30145-4. https://doi.org/10.1016/j.bjorl.2019.10.009
7. Kang M, Zhao L, Ren M, Deng M, Li C. Zinc mediated hepatic stellate cell collagen synthesis reduction through TGF-β signaling pathway inhibition. Int J Clin Exp Med. 2015;8(11):20463-71. PMID: 26884962
8. World Health Organization. WHO expert consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. The Lancet. 2004;363(9403):157-63. https://doi.org/10.1016/s0140-6736(03)15268-3
9. World Health Organization. Obesity: preventing and managing the global epidemic. Geneva: WHO; 2000. Technical report series, 894.
10. Rodriguez MP, Narizano A, Demczylo V, Cid A. A simple method for the determination of zinc human plasma levels by flame atomic absorption spectrophotometry. At Spectrosc. 1989;10(2):68-70.
11. Gibson RS. Assessment of chromium, copper and zinc status. In: GIBSON, R.S. Principles of nutritional assessment. 2nd ed. New York: Oxford University Press; 2005. p. 683-748.
12. Van Slooten HJ, Clahsen PC, Van Dierendonck JH, Duval C, Pallud C, Mandard AM, et al. Expression of Bcl-2 in node-negative breast cancer is associated with various prognostic factors, but does not predict response to one course of perioperative chemotherapy. Br J Cancer. 1996;74:78-85. https://doi.org/10.1038/bjc.1996.319
13. Holanda AON, Barros AQS, Araújo CG, Rocha CVS, Hipólito TLB, Silva BB, et al. Influência da zincemia sobre as concentrações das metaloproteinase 2 e 9 em mulheres com câncer de mama. REAS. 2017;9(1):1003-10.
14. Marcos NY, Zakhary NI.; Said MM.; Tadros MMM. Postoperative simple biochemical markers for prediction of bone metastases in Egyptian breast cancer patients. Ecancermedicalscience. 2013;7:305. https://doi.org/10.3332/ecancer.2013.305
15. Costelo LC, Franklin RB. Decreased zinc in the development and progression of malignancy: an important common relationship and potential for prevention and treatment of carcinomas. Expert Opin Ther Targets. 2017;21(1):51-66. https://doi.org/10.1080/14728222.2017.1265506
16. Jinga DC, Blidaru A, Condrea I, Ardeleanu C, Dragomir C, Szegli G, et al. MMP-9 and MMP-2 gelatinases and TIMP-1 and TIMP-2 inhibitors in breast cancer: correlations with prognostic factors. J Cell Mol Med. 2006;10(2):499-510. https://doi.org/10.1111/j.1582-4934.2006.tb00415.x
17. Vasaturo F, Solai F, Malacrino C, Nardo T, Vincenzi B, Modesti M, et al. Plasma levels of matrix metalloproteinases 2 and 9 correlate with histological grade in breast cancer patients. Oncol Lett. 2013;5(1):316-20. https://doi.org/10.3892/ol.2012.977

18. Mehner C, Hockla A, Miller E, Ran S, Radisky DC, Radisky ES. Tumor cell-produced matrix metalloproteinase 9 (MMP-9) drives malignant progression and metastasis of basal-like triple negative breast cancer. Oncotarget. 2014;5(9): 2736-49. https://doi.org/10.18632/oncotarget.1932
19. Tiwari A, Singh BMK, Varshney S, Yadav ML. Incidental detection of carcinoma in-situ in fibroadenoma of the breast in a young woman: a rare Finding. Cureus. 2018;10(12):e3797. https://doi.org/10.7759/cureus.3797
20. Marumoto A, Steinemann S, Furumoto N, Woodruff S. An uncommon pairing of common tumors: case report of ductal carcinoma in situ within fibroadenoma. Hawaii J Med Public Health. 2019;78(2):39-43. PMID: 30766763
21. Araújo RVS, Silva FO, Melo-Júnior MR, Porto ALF. Metaloproteinases: aspectos fisiopatológicos sistêmicos e sua importância na cicatrização. Rev Ciênc Med Biol. 2011;10(1):82-8. https://doi.org/10.9771/cmbio.v10i1.5470
22. Zhang J, Yin L, Wu J, Zhang Y, Xu T, Ma R, et al. Detection of serum VEGF and MMP-9 levels by Luminex multiplexed assays in patients with breast infiltrative ductal carcinoma. Exp Ther Med. 2014;8(1):175-80. https://doi.org/10.3892/etm.2014.1685
23. Sangma MBM, Panda K, Dasiah S. A clinico-pathological study on benign breast diseases. J Clin Diagn Res. 2013;7(3):503-6. https://doi.org/10.7860/JCDR/2013/5355.2807
24. Davies B, Miles DW, Happerfield LC, Naylor MS, Bobrow LG, Rubens RD, et al. Activity of type IV collagenases in benign and malignant breast disease. Br. J. Cancer. 1993;67:1126-31. https://doi.org/10.1038/bjc.1993.207
25. Somiari SB, Shriver CD, Heckman C, Olsen C, Hu H, Jordan R, et al. Plasma concentration and activity of matrix metalloproteinase 2 and 9 in patients with breast disease, breast cancer and at risk of developing breast cancer. Cancer Lett. 2006;233(1):98-107. https://doi.org/10.1016/j.canlet.2005.03.003