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# Clinical significance of vitamin D supplementation in women with mastopathy.

**L.I. Maltseva*, U.V. Garifullova, E.A. Gafarova, and D.M. Nikogosyan.**

SEI CPE MH RF Kazan Medical Academy, Department of Obstetrics and Gynecology (Head of Department, Prof. Maltseva L.I.), MSU KFU (Chief Physician, Cand. Sc. Med. Abashev A.R.).

**ABSTRACT**

Mastopathy is a benign breast disease extremely common among women of reproductive age. Mastopathy serves as a background for the development of breast cancer. According to available data, patients with breast cancer have an extremely low level of vitamin D in the blood, at the level of a high deficit. Strong evidence has been obtained of protective effect of vitamin D in patients with already developed cancer. Objective of the study is to determine the relationship between vitamin D levels in the blood, the clinical pattern of the disease and the characteristics of the hormonal status in women with various forms of mastopathy. To implement the objectives, 72 women aged 20 to 50 years were examined, among them - 42 with diffuse and nodular forms of mastopathy; the control group included 30 healthy women. The women underwent physical examination, ultrasound examination of the breasts, and mammography. Base levels of vitamin D in blood serum were determined by using ARCHITECT 25-OH Vitamin D system by Abbott Laborator, and the base levels of estradiol, progesterone, prolactin and the level of vitamin D binding protein - by ELISA. A reduction in the blood content of vitamin D was detected in 40.5% of patients with various forms of mastopathy, and its pronounced deficiency was found in 47.6%. The degree of decrease in vitamin D levels correlated with the severity of clinical symptoms, ultrasound-detected changes in the structure of the mammary glands, as well as higher prolactin levels in the patients as compared to healthy women.

**Keywords:** vitamin D, breast, breast fibroadenoma, fibrocystic mastopathy.

***Corresponding author***

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## INTRODUCTION

Mastopathy, according to WHO definition, is a fibrocystic disease with a wide spectrum of proliferative and regressive changes in the breast tissue and an abnormal ratio of epithelial and connective tissue components. The adopted Russian classification distinguishes diffuse (with a predominance of glandular, cystic, or mixed fibrous components) and nodular forms. Mastopathy is the most common breast pathology with no tendency to decrease, which refers to benign diseases and is not a precancer as such, but increases the risk of cancer pathology by 2 or more times. The etiology of the disease remains unclear. The possible causes include stress, the presence of breast trauma, metabolic and endocrine diseases, and gynecological pathology (adenomyosis, uterine fibroids, functional ovarian cysts, and anovulatory menstrual disorders) [1,2,3]. An established factor for the pathogenesis is a relative or absolute hyperestrogenia, resulting in the disruption of proliferative processes in the mammary gland. This condition is often accompanied by hyperprolactinemia and thyroid dysfunction. It is well known that the breast is the background for the development of breast cancer. For example, in case of diffuse mastopathy, the risk of malignancy increases by 4-37 times, and in case of nodular form - by 30-40 times [4]. However, the question of primary prevention of breast cancer in women with benign breast diseases remains still open. To date, the relationship between breast cancer and low vitamin D level has been established [5,6,7,8]. At the same time, there is no data on the relationship between vitamin D deficiency and development of benign breast disease. It is known that vitamin D is an important factor in the regulation of mammary carcinogenesis processes during modulation of the activity of genes involved in proliferation, apoptosis, cell differentiation, and the degree of invasion and angiogenesis [9]. The identification of high-risk groups and the correction of vitamin D deficiency may be an effective method of prevention and treatment of benign breast diseases.

Vitamin D deficiency, according to experts of the International Society of Endocrinology, is defined as the level of 25(OH)D in the blood serum of less than 20 ng/ml, values in the range of 20-30 ng/ml must be regarded as a “deficiency" of vitamin D, and the optimal level - more than 30 ng/ml [10,11]. Overdosing is possible at 150 ng/ml or more. It has been shown that the highest incidence of breast cancer is observed in patients with critical vitamin D deficiency - less than 13 ng/ml [12]. Vitamin D deficiency may result from the reduced sun exposure, possibly high concentration of vitamin D binding protein, reduced activity of enzymes transforming the vitamin D3 into the biologically active form - 1,25 dihydroxyvitamin D, a genetic polymorphism of receptors for vitamin D [12,13,14]. The effect of low levels of vitamin D on the development of benign breast pathology has not been studied, while the biological effects of vitamin D allow us to consider it as one of the possible protective factors in the prevention and treatment of benign breast diseases (BBD).

Objective of the study is to determine the relationship between vitamin D levels in the blood, the clinical pattern of the disease and the characteristics of the hormonal status in women with various forms of mastopathy in order to justify the possibility for using vitamin D for the prevention and treatment.

## MATERIALS AND METHODS

The study included 72 women aged 20 to 50 years, 42 of them - women being managed for benign breast diseases - represented the main group, and 30 healthy women - the control group. All women underwent a comprehensive examination: physical examination, ultrasound breast examination, and mammography, if necessary. The base vitamin D level was evaluated by using ARCHITECT 25-OH Vitamin D system by Abbott Laboratories. This method is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of 25-hydroxyvitamin D (25-OH vitamin D) in the serum and plasma of human blood. ELISA was applied to study the hormonal status, which was assessed based on the blood content of estradiol, progesterone, and prolactin, and the level of vitamin D binding protein.

The pathology of the mammary glands was represented by diffuse fibrocystic mastopathy (DFM) in 26 patients, and by fibroadenoma in 16 women. The vast majority of women with DFM (21) were diagnosed with cystic form, 5 women had glandular component prevailing. Six women with fibroadenoma had primary multiple fibroadenomas, 2 patients had a combined form of cystic mastopathy and fibroadenoma. The diagnosis was confirmed by biopsy and the results of histological examination of tissue obtained through sectoral breast resection.

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Statistical analysis of the results was performed by using IBM SPSS Statistics 20. The indicators measured in nominal scale were presented as percentages. Their comparison was performed with the use of Pearson test $\chi^2$. To describe the quantitative indicators, the values of arithmetic means (M) and standard error (m) were calculated. Previously, each indicator was assessed for compliance with the normal distribution, by Shapiro-Wilk test. Quantitative indicators were compared by using Student's t-test, and the differences in indicators in the related aggregates were evaluated with the use of one-way ANOVA method with repeated observations. To identify the relationship between the quantitative indicators the parametric correlation analysis was used, which implied the calculation of values of the Pearson correlation coefficient and determination coefficient $R^2$. Differences in the indicators, as well as the correlations between them were considered significant at $p<0.05$.

## RESULTS AND DISCUSSION

During the assessment of blood levels of vitamin D and sex hormones in the structure of the main group, two additional subgroups were identified according to the nosological BBD. First subgroup included 26 women with DFM (61.9%), the second - 16 patients being managed for breast fibroadenoma (38.1%).

Analysis of the initial content of vitamin D in the blood showed that the values in the main group varied from 8.9 to 50.4 ng/ml and averaged 21.4+1.3 ng/ml (Table 1), which was significantly lower than in healthy women.

**Table 1 – Initial blood level of vitamin D in women with different forms of mastopathy.**

| Study category | Vitamin D blood content, ng/ml | | |
|---|---|---|---|
| | min - max | M±m | 95% CI |
| Healthy women | 12.0 – 55.4 | 30.5±2.2 | 26.0-35.0 |
| Patients with BBD, including: | 8.9 – 50.4 | 21.4±1.3* | 18.8-24.1 |
| - with fibrocystic mastopathy | 8.9 – 50.4 | 20.8±1.7* | 17.3-24.3 |
| - with breast fibroadenoma | 9.7 – 39.7 | 22.5±2.1* | 18.1-26.9 |

*significance of differences between the indicators of healthy and sick patients $p<0.05$

The differences in the levels of vitamin D in healthy women and patients with DFM and breast FA were also statistically significant ($p=0.002$ and $p=0.034$, respectively) and reflected a significant decrease in vitamin D content. Comparison of vitamin D content in patients with different forms of mastopathy revealed no statistical differences, which indicates the absence of the relationship between the specific forms of the disease and the blood levels of vitamin D.

Distribution of women according to the levels of vitamin D, based on its blood content (Table 2) showed the difference between healthy and sick patients.

**Table 2. Distribution of women according to the levels of vitamin D based on the form of mastopathy.**

| Vitamin D content, ng/ml | Main group | | | | | | Control group n=30 | |
|---|---|---|---|---|---|---|---|---|
| | Overall for group n=42 | | DFM n=21 | | FA n=16 | | | |
| | Abs. | % | Abs. | % | Abs. | % | Abs. | % |
| Normal (30 and more) | 5 | 11.9 | 3 | 11.5 | 2 | 12.5 | 11 | 36.7 |
| Insufficiency (20-30) | 17 | 40.5 | 9 | 34.6 | 8 | 50.0 | 15 | 50.0 |
| Deficiency (13-20) | 15 | 35.7 | 11 | 42.3 | 4 | 25.0 | 3 | 10.0 |
| Severe deficiency (less than 13) | 5 | 11.9 | 3 | 11.5 | 2 | 12.5 | 1 | 3.3 |
| TOTAL: | 42 | 100 | 26 | 100.0 | 16 | 100.0 | 30 | 100.0 |

As follows from the table, the healthy women had the highest incidence of normal vitamin D level - 36.7%, at the same time, its insufficiency was observed in 15-50%, and its deficiency - in 13.3%. The proportion of women with normal vitamin D levels in the study group was only 11.9%, and 47.6% had its deficiency and a pronounced deficiency, which is 3.6 times higher than in the control group. Differences between healthy women and patients in the vitamin D levels, evaluated by using Pearson $\chi^2$ test, were statistically significant (p=0.01), while the content of vitamin D in women with fibrocystic mastopathy and its nodular forms was reduced to the same degree. It is noteworthy that 50% of healthy women have also a decreased rate of vitamin D in their blood, despite the lack of breast pathology. Moreover, the analysis of the somatic status showed an increased body mass index (25-30) in 5 patients of the control group (16.6%), which may be an independent factor for low vitamin D.

No differences were revealed in the content of vitamin D binding protein in the surveyed patients. The average blood level in the main group was 305.9±18.6 g/ml, in the control group - 345.0±25.1 (p=0.2), in patients with fibrocystic mastopathy - 293.6±21.4 μg/ml, with mammary fibroadenoma - 281.5±29.3 μg/ml, which was statistically insignificant. It can be assumed that the vitamin D binding protein does not play a significant role in reducing the vitamin D content in patients with various forms of mastopathy.
Results of the analysis of blood levels of sex hormones in sick women are presented in Table 3.

**Table 3 – Blood levels of sex hormones in women with different forms of mastopathy.**

| Hormone | | Main group | | | Control group | p |
|---|---|---|---|---|---|---|
| | | Total | DFM | FA | | |
| Prolactin (μIU/ml) | M±m | 324.5±13.0 | 315.3±17.0 | 339.5±20.2 | 256.4±12.7 | 0.001** |
| | 95% CI | 298.2-350.8 | 280.2-350.3 | 296.4-382.7 | 230.1-282.7 | |
| Progesterone (ng/ml) | M±m | 29.9±2.6 | 32.1±4.1 | 26.9±2.8 | 35.9±2.2 | 0.105 |
| | 95% CI | 24.5-35.4 | 23.3-40.8 | 20.6-33.2 | 31.4-40.5 | |
| Estradiol (pg/ml) | M±m | 41.7±1.5 | 39.6±1.8 | 45.0±2.4 | 44.1±1.8 | 0.31 |
| | 95% CI | 38.7-44.7 | 35.9-43.3 | 39.9-50.1 | 40.4-47.8 | |

** - differences in the indicators are statistically significant (p<0.05)

According to the findings, the average content of prolactin in two groups had statistically significant differences as compared to healthy women both overall for the group and in some forms of mastopathy, where prolactin values were significantly higher than in healthy women. Estradiol and progesterone levels were not statistically different from normal. We can note the tendency to reduction in the progesterone levels and to increase in estradiol in patients with nodular forms as compared with fibrocystic mastopathy.

Correlation analysis showed a negative relationship between the blood level of vitamin D and prolactin (r=-0.345, p=0.02), i.e. a low vitamin D values corresponded to higher levels of prolactin. Comparison of the levels of vitamin D and the content of other hormones in the blood revealed no statistically significant relationship.

Additionally, the ratio of chances was calculated for the quantitative description of the relationship of the factor (level of vitamin D less than 20 ng/mL, and the presence of mastopathy). In the main group, deficiency of vitamin D was observed in 20 patients, which amounted to 47.6%. In the control group, the number of patients with vitamin D deficiency was 4, or 13.3%. The ratio of the chances that characterizes the relationship of vitamin D deficiency with the presence of the BBD, was 5.91 with 95% confidence interval ranging 1.75 to 19.9, indicating a statistically significant direct relationship between the factor and an outcome.

All women (56) with the detected deficiency or insufficiency of vitamin D (less than 30 ng/ ml) were prescribed substitution therapy at a dose of 4000 IU/day for one month with further evaluation of vitamin D level and transition to a maintenance dose of 2000 IU/day. These doses allowed for the achievement and maintenance of blood level of vitamin D at 40 ng/ml. No other methods of treatment were used to evaluate

the effects of vitamin D on clinical symptoms. The total follow-up period was 6 months. The clinical pattern was evaluated on month 1, 3 and 6 of therapy, and the ultrasound was conducted in 3 and 6 months from the beginning of the treatment.

The analysis of the clinical pattern showed that patients with severe deficiency of vitamin D (13-20 ng/ml) had a clear clinical manifestations of the disease: a strong pain and more pronounced changes according to the ultrasound pattern. For example, the women with fibrocystic mastopathy had an ultrasound pattern characterized by the presence of multiple cysts of up to 20 mm on the background of fibrotic changes, and the history of the repeated punctures and cyst sclerotherapy, and inefficiency of conservative therapy. In patients with fibroadenoma, the pronounced vitamin D deficiency was accompanied by relapses of nodular formations and repeated sectoral resection of the breast. Women with vitamin D insufficiency (20-28 ng/ml) had moderately severe pain, and also more longer duration and incomplete effect of conservative therapy. The correlations have shown a direct relationship of tension and pain in the breast with a deficiency of vitamin D in the blood ($r= 0.46$, $p<0.05$). Patients with fibroadenoma had a nonspecific clinical picture with vitamin D insufficiency and deficiency: a sense of discomfort and indurated formations detected in the mammary gland.
In the course of observations in 1, 3 and 6 months, a progressive reduction of pain and tension in the mammary glands was detected. According to the ultrasound pattern, no significant dynamics was registered in 3 months, but after 6 months a decrease was detected in adenosis foci and in the number and size of small and medium-sized cysts (1-1.5 cm) by 50%. Large cysts up to 2 cm did not change during 6 months of observation, despite the pain relief. Over the entire study period, neither the progression of the disease in the form of increased pain, increased number of cysts and size of adenosis foci, the emergence of new fibroadenomas, or growth of the "old" small formations was recorded.

## SUMMARY

- Thus, patients with BBD have a decreased blood content of vitamin D of 88.5%, almost every second woman (47.6%) has a deficiency of vitamin D established. Clinical and ultrasound data directly depend on the deficiency level of vitamin D. At the same time, a critical vitamin D deficiency is combined with severe pain and changes in the structure of the breast based on ultrasound data, and the lack of effect of conservative therapy. The analysis of hormonal status showed higher prolactin levels in patients with BBD as compared with healthy women, and revealed no differences in the levels of estradiol and progesterone. It is important that low vitamin D levels negatively correlate with prolactin indicators.
- Hormone therapy at a dose of 4000 IU/day for the first month and 2000 IU/day for 5 months was highly effective: by the end of the first month of therapy no single case of persistent vitamin D deficiency has been reported, and a maintenance dose of vitamin D prescribed for 5 months has maintained normal levels of vitamin D.

## CONCLUSION

Recovery of normal levels of vitamin D leads to pain control, tension relief in the mammary glands, improvement of the ultrasound pattern of the disease. Relapses of the disease were observed during the study. It is already clear at this stage of research that the determination of the initial level of vitamin D in women with BBD and the subsequent correction of the deficiency should be included in the program of comprehensive examination and treatment of patients. However, the issues arise relating to the optimal blood level of vitamin D in these patients. Perhaps it should be higher, as well as vitamin D drug doses. It is not clear yet whether this is a life-long therapy. However, the first results encourage and force to move on.

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## REFERENCES

[1] Kravchenko E.N., Ozherelieva M.A. Condition of mammary glands in gynecological diseases (literature review). Mother and child in the Kuzbass. 2014, No.2, Pp. 19-23.

[2] Tagieva T.T. Risk factors for breast diseases. Bulletin of N.N. Blokhin RCRC 2007, Volume 18, No.2, Pp. 68-73.
[3] Trubnikova L.I., TikhonovaN.Iu., Savinova N.A. A comprehensive examination and treatment of women with diseases of the mammary glands and genitals. Ulyanovsk Medical and Biological Journal 2013, No.3, Pp. 65-75.
[4] Practical mammology. Ed. M.I. Davydov, V.P. Letiagin. Publishing house "Practical Medicine", Moscow. 2007
[5] Garland CF, Gorham ED, Mohr SB, Grant WB, Giovannucci EL, Lipkin M, et al. Vitamin D and prevention of breast cancer: Pooled analysis. J Steroid Biochem Mol Biol. 2007;103:708–711.
[6] Reduced pre-diagnostic 25-hydroxyvitamin D levels in women with breast cancer L. Rejnmarka, A. Tietzeb, P. Vestergaarda, L. Buhlc, M. Lehbrinkb, L. Heickendorffd, L. Mosekildea Abstracts / Bone 44 (2009) S162–S167
[7] Garland CF, Gorham ED, Mohr SB, Grant WB, Giovannucci EL, Lipkin M, et al. Vitamin D and prevention of breast cancer: Pooled analysis. J Steroid Biochem Mol Biol. 2007;103:708–711.
[8] Vitamin D and breast cancer risk Kay W. Colston Best Practice & Research Clinical Endocrinology & Metabolism Vol. 22, No. 4, pp. 587–599, 2008
[9] Lopes et al. BMC Cancer 2010, 10:483 http://www.biomedcentral.com/1471-2407/10/483 Alterations in Vitamin D signalling and metabolic pathways in breast cancer progression: a study of VDR, CYP27B1 and CYP24A1 expression in benign and malignant breast lesions Vitamin D pathways unbalanced in breast lesions Nair Lopes, Bárbara Sousa, Diana Martins, Madalena Gomes, Daniella Vieira, Luiz A Veronese, Fernanda Milanezi, Joana Paredes, José L Costa, Fernando Schmitt
[10] Bischoff-Ferrari, H.A., P. Burckhardt, K. Quack-Loetscher, B. Gerber, D. I'Allemand, J. Laimbacher, M. Bachmann, and R. Rizzoli, Vitamin D deficiency: Evidence, safety, and recommendations for the Swiss population. Report written by a group of experts on behalf of the Federal Commission for Nutrition (FCN) 2012. http://www.iccidd.org/p142000804.html. 3.
[11] Dawson-Hughes B, Mithal A, Bonjour JP, et al.: IOF position statement:
[12] vitamin D recommendations for older adults. Osteoporos Int 2010; 21(7): 1151-4.
[13] Garland F, Garland C, Gorham E, Young J Jr. Geographic variation in breast cancer mortality in the United States: a hypothesis involving exposure to solar radiation. Prev Med. 1990;19:614–622.
[14] Raimondi S., H. Johansson, P. Maisonneuve, S. Gandini, Review and metaanalysis on vitamin D receptor polymorphisms and cancer risk, Carcinogenesis 30 (2009) 1170–1180.
[15] Tang C., N. Chen, M. Wu, H. Yuan, Y. Du, FokI polymorphism of vitamin D receptor gene contributes to breast cancer susceptibility: a meta-analysis, Breast Cancer Res. Treat. 117 (2009) 391–399.