ORIGINAL ARTICLE

CORRELATION BETWEEN THE NUMBER OF BREAST DUCT CELLS EXPRESSING ANDROGEN, ESTROGEN AND PROGESTERONE RECEPTORS AND THE SIZE OF THE CHANGED TISSUE IN PATIENTS WITH GYNECOMASTIA

KORELACIJA IZMEĐU BROJA ĆELIJA DUKTUSA DOJKE KOJE EKSPRIMIRAJU ANDROGENSKE, ESTROGENSKE I PROGESTERONSKE RECEPTORE I VELIČINE PROMENE KOD OBOLELIH OD GINEKOMASTIJE

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Abstract

Introduction: Gynecomastia is an abnormal growth of the male breast due to excessive proliferation of ductal epithelial cells. It usually occurs during puberty, so the influence of hormones on the stimulation of ductal cells is indicative. Androgen, estrogen and progesterone are steroid hormones that exert their influence by binding to intracellular receptors and thus activating transcriptional mechanisms, i.e. act as trophic factors.

Material and methods: A retrospective study for the period 01.01.2021 - 31.12.2021 was performed and 6 patients were found, of which in two cases left and right breast gynecomastia surgeries were performed. Hematoxylin-eosin (HE) stained slides and corresponding paraffin blocks were taken from the archive, and a tissue microarray was constructed, with 4 tissue cylinders for each tissue sample. Immunohistochemical staining was performed for androgen, estrogen, and progesterone receptors. The obtained slides were scanned on a Leica Biosystems Aperio AT2 slide scanner, and image analysis was performed in the Aperio Image Scope program. Statistical analysis of the data was performed by the EZR R Commander program.

Results: Of the 8 samples, none showed significant androgen receptor expression. Statistical analysis of the number of cells expressing estrogen receptors ($p_s = 0.03676$; $rho = 0.7619048$) progesterone ($p_s = 0.002439$; $rho = 0.8982197$) and the volume of tissue removed showed a statistically significant positive correlation.

Conclusion: The obtained results confirm the hypothesis that the volume of removed breast tissue in patients with gynecomastia correlates with the expression of estrogen and progesterone receptors, but further research on a larger sample is necessary for more reliable conclusions.

Keywords:
gynecomastia,
androgen,
progesterone,
estrogen

Sažetak

Uvod: Ginekomastija predstavlja abnormalan porast muške dojke usled prekomerne proliferacije epitelnih ćelija duktusa. Obično se javlja u periodu puberteta, pa je uticaj hormona na stimulaciju ćelija duktusa indikativan. Androgen, estrogen i progesteron su hormoni steroidne građe koji svoj uticaj ostvaruju vezivanjem za intracelularne receptore i na taj način aktiviraju transkriptorne mehanizme, tj. deluju kao trofički faktori.

Materijal i metode: Urađena je retrospektivna studija za period 1.1.2021 - 31.12.2021. i pronađeno je 6 pacijenata, od kojih su u dva slučaja rađene operacije ginekomastije obe dojke. Iz arhive su preuzete hematoksilin-eozin obojene pločice i odgovarajući parafinski kalupi, te je konstruisan tkivni mikroniz, sa po 4 tkivna cilindra za svaki tkivni uzorak. Imunohistohemijska bojenja su urađena za receptore za androgen, estrogen i progesteron. Dobijeni preparati su skenirani na skeneru pločica *Leica Biosystems Aperio* AT2, a analiza slika je izvršena u programu *Aperio Image Scope*. Statistička analiza podataka vršena je EZR *R Commander* programom.

Rezultati: Od 8 uzoraka nijedan nije pokazao značajnu ekspresiju receptora za androgen. Statistička analiza broja ćelija koje eksprimiraju receptore za estrogen ($p_s = 0,03676$; rho $= 0,7619048$) progesteron ($p_s = 0,002439$; rho $= 0,8982197$) i zapremine odstranjenog tkiva pokazala je statistički značajnu pozitivnu korelaciju.

Zaključak: Dobijeni rezultati potvrđuju postavljenu hipotezu da zapremina odstranjenog tkiva dojke kod pacijenata sa ginekomastijom korelira sa ekspresijom receptora za estrogen i progesteron, ali je za pouzdanije zaključke neophodno dalje istraživanje na većem uzorku.

Ključne reči:
ginekomastija,
androgen,
progesteron,
estrogen

Introduction

Gynecomastia is a disease of the male breast. It is characterized by abnormal proliferation of epithelial cells of the ductus and peridutal connective tissue. Macroscopically, a spherical tumor-like enlargement is observed, which is of solid consistency and vaguely limited by the environment. Microscopically, the proliferation of duct cells is observed. Ducts become expanded and surrounded by proliferated connective tissue. Cystic changes with flattened epithelium can occur, with several rows of cells often seen. It does not usually alter to malign altered tissue (1, 2).

Androgen, estrogen and progesterone are sex hormones whose levels change significantly during puberty. These are steroid hormones that achieve their activity by binding to receptors in the nucleus, thus activating transcription factors. The influence of sex hormones on the development of gynecomastia has been previously investigated with the results that the disease is stimulated by estrogen and inhibited by androgen.

In this paper, the focus is on the influence of androgen, estrogen and progesterone receptors on size itself, i.e. the volume of removed altered breast tissue. Hypothesis is that higher estrogen and progesterone receptor expression would result in greater volume of tissue removed while higher androgen expression would result in less volume.

Material and methods

A retrospective study for the period 01.01.2021 - 31.12.2021 was performed. By searching the laboratory information system (LIS) of the Institute of Pathology 6 patients were found, of which in two cases left and right breast gynecomastia mastectomy were performed, making a total of 8 tissue samples analyzed. All patients were clinically diagnosed with gynecomastia (N62 – *Hypertophia mammae*), and confirmed by pathohistological analysis. All subjects were in puberty at the time of diagnosis (2021).

Hematoxylin-eosin stained slides and corresponding paraffin blocks were taken from the archive, and a tissue microarray was constructed, with 4 tissue cylinders for each tissue sample. Tissue sections, 4 microns thick, were made, applied to superfrost plates that were stained histochemically for hematoxylin-eosin and immunohistochemically for androgen (abbr. AR; Lab Vision; 1: 200), Estrogen (abbr. ER; Lab Vision 1: 100), and progesterone (Novocastra, 1: 200) – the example can be seen in **figure 1**.

Methods for antigen detection, antibody incubation and visualization were performed according to the manufacturer's recommendations. The obtained samples were scanned on a Leica BiosystemsAperio AT2 plate scanner, and image analysis was performed in the Aperio Image Scope program, as can be seen in **figure 2**. Statistical analysis of the data was performed by the EZR Commander program.

Tissue volume was calculated according to the following formula for calculating the volume of the ellipsoid $V = \frac{3}{4} * l * h * w * \pi$ (3) - where l - half the length, h - half the height, w - half the width. Data on the length height and width of the tissue change were obtained from the macroscopic findings in the histopathological report.

The EZR Commander statistical analysis program found that the variable - volume of removed tissue does
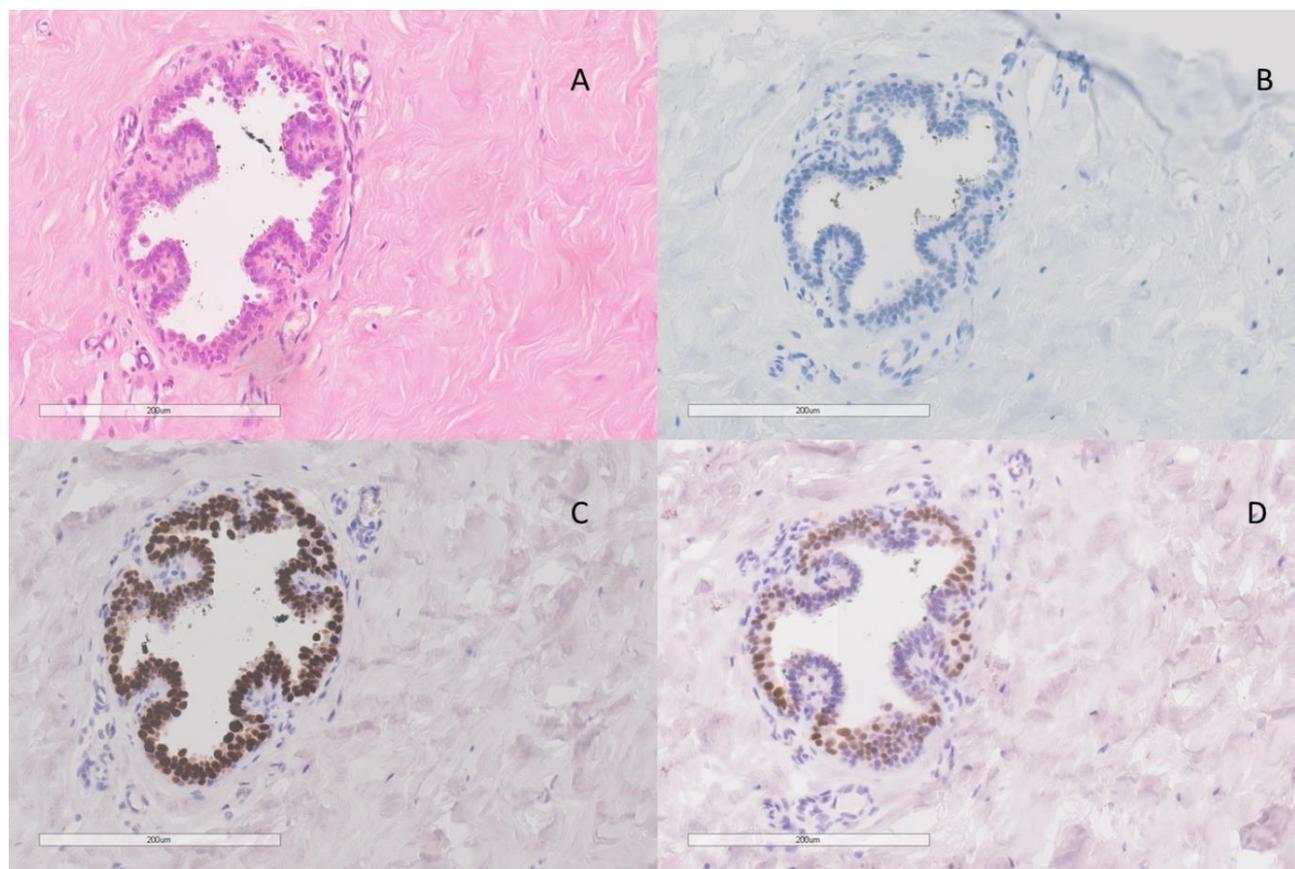
Figure 1. One duct of one of the sample stained in hematoxyline-eosin (A) and immunohistochemically stained for androgen (B), estrogen (C), progesterone (D) at magnification 20 times.
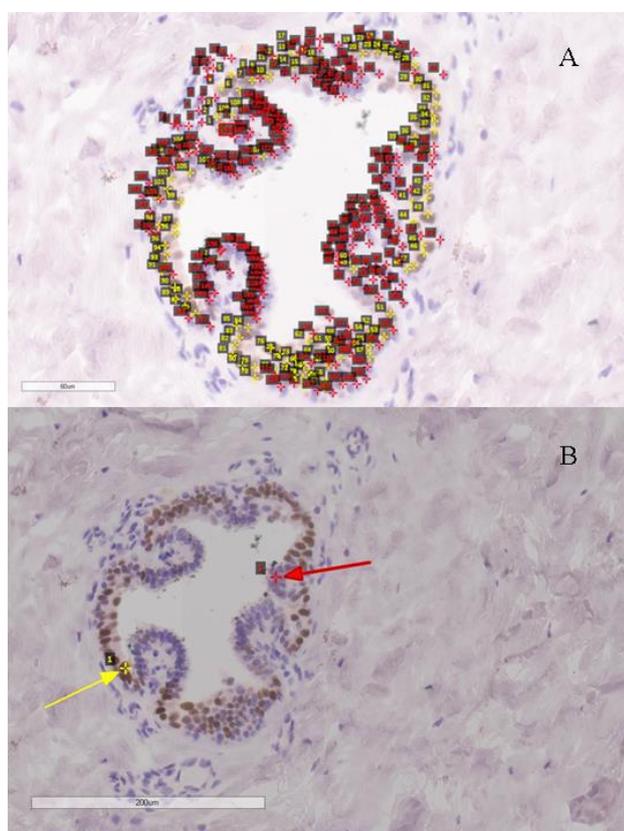

Figure 2. Counting cells of one of the ducts in the Aperio Image Scope program, all cells counted at magnification 40 times (A) and single cell type marked at magnification 10 times (B): red mark and red arrow - a cell that doesn't express the receptor; yellow mark and yellow arrow - a cell that expresses the receptor.

not show a normal distribution (CV > 30%), unlike the variable – number of estrogen receptor expressing cells and variables – number of progesterone receptor expressing cells showing normal distribution (CV < 30%), which means that Spearman's correlation test is suitable test for correlation analysis.

Results

After examination of tissue microarray, data on the number of cells expressing receptors in an approximation of 200 total cells were obtained and presented in **table 1**.

As can be seen in **table 1**, the results are such that none of the samples after immunohistochemical staining express androgen receptors in significant numbers.

On the other hand, a large number of cells express the estrogen receptors. Effect on the volume of removed tissue was measured in relation to the level of correlation with number of cells that express estrogen receptors. The results of Spearman's correlation test are such that a statistically significant correlation is shown ($p_s = 0.03676$; $p_s < 0.05$), as well as a strong correlation in the positive direction (rho = 0.7619048), between the number of cells which express the estrogen receptor and the volume of tissue removed. This correlation can be graphically represented in **figure 3**.

Also, progesterone follows the same rules as estrogen. The results of the Spearman test are such that there is a statistically significant correlation ($p_s = 0.002439$;

Table 1. Results after tissue microarray analysis.

patients	patients age	sample number	number of cells that express androgen receptors	number of cells that express estrogen receptors	number of cells that express progesterone receptors	volume of tissue in cm^3
I	18	1	0	120	73	34,02
		2	0	127	76	35,34
II	17	3	0	97	55	12,03
III	18	4	0	80	46	6,48
IV	15	5	0	119	76	75,40
V	17	6	0	138	82	44,24
		7	0	147	86	54,12
VI	18	8	0	110	63	18,85

$p_s < 0.05$), and a strong correlation in the positive direction (rho = 0.8982197) between the number of cells expressing the progesterone receptor and volume of tissue removed.
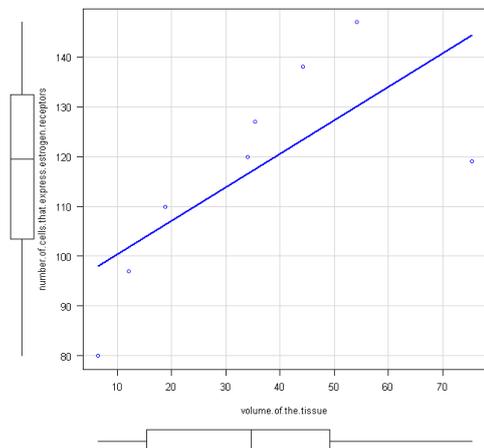

Figure 3. Graphical presentation of the correlation between the number of cells expressing the receptor for estrogen and the volume of the tissue removed.

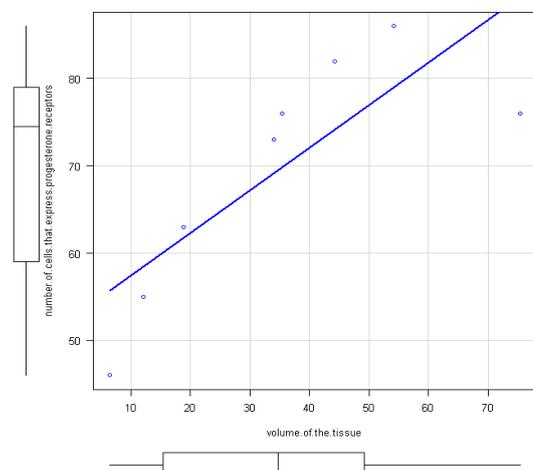This dependence can also be shown graphically in **figure 4**.

Figure 4. Graphical presentation of the correlation between the number of cells expressing the receptor for progesterone and the volume of the tissue removed.

Discussion

Based on the obtained results, the androgenic effect on the volume of changed tissue in gynecomastia is not considered significant. This is not in accordance with previous papers, where the expression of androgen receptors in ductal epithelial cells was observed in a certain number of subjects (4-7).

On the other hand, the presence of a large number of cells positive for estrogen and progesterone receptors is in accordance with existing researchs (4-7). It can also be noticed that although a larger number of cells express the estrogen receptor compared to the progesterone, Spearman correlation test gives a stronger correlation with the presence of progesterone (rho = 0.8982197) than estrogen (rho = 0.7619048) receptor, and even statistically significant correlation (estrogen – p_s = 0.03676; progesterone – p_s = 0.002439).

Conclusion

To conclude, the influence of number of cells with androgen receptors expression has a minor value on the size of the removed tissue volume. Contrary, if number of cells which express estrogen receptors and progesterone receptors were compared with the volume of removed tissue then it can be said there is a positive correlation between those two. Moreover, there is even greater correlation of removed tissue volume with number of cells that express progesterone receptors.

The obtained results confirm the hypothesis that the volume of removed breast tissue in patients with gynecomastia correlates with the expression of estrogen and progesterone receptors, however, further research on a larger sample is necessary for more reliable conclusions.

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