

ERYTHROCYTE BLOOD GROUP ANTIGENS AND ALTERATIONS OF THE HORMONAL STATUS AMONG THE REPRODUCTIVE AGE WOMEN WITH BREAST TUMORS

Irina Nakashidze¹, Nanuli Kotrikadze², Anzor Diasamidze¹, Marina Nagervadze¹, Manana Alibegashvili²,
Liana Ramishvili², Manana Gordeziani²

¹ Department of Biology, Faculty of Education and Sciences, Batumi Shota Rustaveli State University, Batumi, Georgia

² Department of Biology, Faculty of Exact and Natural Sciences, Ivane Javakishvili Tbilisi State University, Tbilisi, Georgia

ABSTRACT

The significance of ABO blood system groups antigens in development of some malignant tumors is already established. The alteration of hormonal homeostasis must also be taken into account. Hence the aim of the investigation was to study ABO and Rh blood system antigens and hormonal status among reproductive age women with benign and malignant breast tumors. Methods: The determination of hormones was made by the enzymatic analysis method (ELAIZA), provided by proper ELAIZA kits. For the study of ABO and Rh-Hr system antigens, internationally recognized immunoserology methods were used. Results: High index of the breast gland tumors were revealed in patients with A(II) phenotypic group, according to the ABO system. The frequency distribution of O(I) phenotypic group was low among women with breast tumors. Among D, C, E, c and e antigens of the Rh system, the frequency of D and E antigens were increased in benign and malignant breast tumors patients. The study of hormonal balance revealed thyroid gland hypofunction and increased level of estradiol on the background of increased testosterone and decreased progesterone levels. Such hormonal imbalance and excess production of estradiol creates conditions for malignant tumor formation in reproductive age women. Conclusion: The highest frequency of breast cancer in reproductive age was revealed in A(II) group patients. The wide spectrum of hormonal disorders were revealed in breast tumor patients of the reproductive age, which was especially clear in cases of malignant tumor.

UDC CODE & KEYWORDS

■ UDC: 616-006 ■ Breast tumors ■ Blood group antigens ■ Rh system ■ Hormones

INTRODUCTION

Breast cancer is mostly wide spread oncologic disease among women (Kim et al., 2007). According to American Cancer Society 180 150 new cases of breast cancer were registered in 2007 in America. According to the same data, 2300 cases of breast cancer were revealed among men (American Cancer Society, 2007).

Among numerous risk-factors, initiating breast cancer are: ionizing radiation, chemical compounds, viruses (Donovan, 2007; Lawson and Heng, 2010), smoking (Marcus, 2000), the status of the reproductive system of a woman (early menarche, late menopause), delayed or late child-bearing (Schack-Nielsen, 2005), hormone therapy (Althuis, 2003; Ozmen, 2008) and environmental factors (Olivera, 2007).

Progression of mammary gland cancer is associated with many other factors, among which are erythrocyte blood group antigens, in particular the antigens of the ABO system play an important role in the immune system, and may stimulate some malignant growth (Stamatatos, 2009). The ABO blood group antigens are the major antigens, which present on the surface of erythrocytes and in different epithelial cells. As for cancers, majority of their formation and development begin from epithelial cells.

It is known that genetic changes of genes of the blood ABO system in patients with malignant tumor takes place (Hu, 2000). Moreover, it was demonstrated that expression of antigens of the ABO system in case of cancer, is associated with tumor dissemination (Nakagoe, 2001). In tumors, changes in glycosylation are found in ABO system genes (Le Pendu, 2001). However, several recent studies have shown that altered glycosylation plays a major role in most aspects of malignant phenotype, including signal transduction and apoptosis (Hakomori, 2002).

Besides ABO system antigens, the Rh-Hr system antigens are also significant (Connie, 2007). The latter take an active part in different cell processes and are responsible for membrane stability. Small amount of the Rh in cell changes its morphology and as a result, the life span of the cell diminishes (Denomme, 2004).

According to above mentioned, investigation of some erythrocyte blood group antigens among breast benign and malignant tumor patients is very important today.

The most of the mammary gland tumors are hormon-dependent. Accordingly, investigation of the hormonal status of breast tumor patients, together with above mentioned parameters, is of great significance. Recent tendency of rejuvenation of breast cancer points to the importance of hormonal status assessment in reproductive age females as well.

Data and methodology

ABO system assay:

Blood samples of 20 female patients with benign and malignant tumors of mammary glands were tested for investigation the ABO system antigens. The same number of blood samples of healthy women served as control.

Rh-Hr system antigens assay:

Sixty samples of the blood of benign breast tumor female patients and 130 samples of malignant ones were tested. The same number of blood samples of healthy women used for control group. The internationally acknowledged immunoserological methods were applied, while investigating the antigens of ABO and Rh-Hr systems (Judd, 1994). The test systems with specificity: anti - A, - B, - D, - C, - c, - E, - e were used to reveal ABO and Rh-Hr system antigens.

Hormones's assay:

The immunoenzyme method (ELAIZA) was used for hormone determination. The blood of reproductive age (19-45 years) female patients with benign (fibroadenoma) and malignant tumors of mammary glands were tested to study the hormone status. Statistical analysis of experimental data were processed using the variance method, by means of computer program (Graphpad prisma 6) $P < 0.05$ was regarded as statistically significant.

Results and discussion

Frequency distribution of ABO system phenotypic groups of the reproductive age women was studied both in female patients with benign (fibroadenoma) and malignant breast tumors.

All four phenotype groups were revealed in control variants according the ABO blood group system of women with benign (fibroadenoma) and malignant breast tumors. The distribution of phenotypic groups of the ABO system was following: O(I)→A(II)→B(III)→AB(IV).

High index of the mentioned disease was revealed in patients with A(II) phenotypic group, according to the ABO system. The frequency distribution of O(I) phenotypic group was low among women with breast tumors, compared to control group. It must be more resistant to the mentioned pathologies. The frequencies of the B(III) and AB(IV) phenotypic groups are low as in the breast gland tumors, as in the control group (Table 1). Our results are in accordance with the data of other authors (Layla, 2008; Amini, 2010).

On the next step of the investigation the frequency of Rh-Hr system antigen occurrence was studied both in control groups and in women with benign (fibroadenoma) and malignant breast tumors.

Investigations revealed that the frequency distribution of antigens, belonging to Rh-Hr system in all studied groups was following: e → c → D → C → E; Moreover, among the D, C, E, c and e antigens of the Rh system, frequency of D and E antigens is increased among breast tumor patients (both, benign and malignant), which is the indication to sensitivity of mentioned antigens towards breast tumors (Table 2).

Table 1: The distribution frequencies of ABO system phenotypic groups among reproductive age women with benign and malignant breast tumors

The ABO system phenotypic groups	Control group n=20; P<0,05	Women diseased by the breast benign tumor n=20; P<0.05	Women diseased by the breast cancer n=20; P<0.05
O (I)	60± 10.6%	20±8.9%	30±8.9%
A (II)	30±10.2%	65±10.6%	60±10.6%
B (III)	5±4.8%	10±6.7%	5±6.7%
AB (IV)	5±4.87%	5±4.8%	5±4.8%

Source: Authors

Table 2: The Rh-hr system antigens distribution frequencies in the blood of women with breast benign and malignant tumors

Rh-hr system antigens	Control group n=130	Women with breast benign tumor n=60	Women with breast malignant tumor n=130
D	83.07±3.2%	93.3±3.2%	94.6±1.98%
C	54.61±4.3%	48.3±6.4%	46.92± 4.3%
c	92.30±2.3%	95±2.8%	93.84±2.1%
E	18.46±3.4%	33.3±6.08	23.07±3.6%
e	99.23±0.7%	95±2.8	97.69±1.3%

Source: Authors

As it was mentioned above, both, benign and malignant breast tumors are mostly hormone-dependent (formation and functional activity of mammary glands is influenced by hormones of ovary, pituitary gland and adrenal gland as well). Accordingly, to establish the role of both, sex steroid and non-steroid hormones in formation of breast tumor pathologies in reproductive age women, following indices were investigated in the blood of breast cancer patients: variation of the content of gonadal steroids – estradiol, progesterone and testosterone, functional characteristics (in particular, hypofunction) of the pituitary gland – changes in the amount of thyroid hormone thyroxine (T₄) and thyrotrophic hormone of adenohypophysis (TSH), also the amount of adenohypophysis hormone – prolactin (PRL).

Experimental results demonstrate that amount of estradiol is increased (~1.2 times) in the blood of reproductive age breast benign tumor (fibroadenoma) patients, and sharply gained (~ 2 times) in case of breast cancer, compared with the control group. As for antiestrogenic hormone – progesterone, its amount was decreased among benign tumor patients and significantly decreased in case of breast cancer women, compared with the control. The studies of the testosterone levels has shown that in case of breast benign tumor its amount was increased for ~ 1.3 times and more sharply elevated (~ 2.7 times) in patients with breast cancer (Table 3).

Table 3: The sex steroid hormones quantitative changes in the blood of the reproductive age women with breast gland tumors

The object of the study (blood)	Sex steroid hormones		
	Estradiol (E2) pg/ml	Progesterone (P) ng/ml	Testosterone (T) ng/ml
Control group	40.4±0.4	0.90±0.06	0.67±0.009
Benign tumor (Fibroadenoma)	50.33±0.2 P<0.0001	0.60± 0.01 P<0.0001	0.89±0.003 P=0.0007
Malignant tumor (Cancer)	81.87±0.9 P<0.0001	0.57±0.0001 P<0.001	1.81±0.07 P<0.0001
n=15 (the amount of the patients in each group the average age of the patients 19-45; P<0.05)			

Source: Authors

It is known that formation and functional activity of mammary glands is influenced not only by sex steroids, but by non-steroid hormones too. Thus, quantitative changes of thyroid gland hormone – free thyroxin (fT4) and thyrotrophic hormone of adenohypophysis (TSH) has been investigated among reproductive age patients with fibroadenoma and breast cancer.

Experimental results demonstrate that in the blood of reproductive age women with fibroadenoma amount of free thyroxin decreased on the background of rising of thyrotrophic hormone level. While in malignant tumor patients level of thyroxin decreased ~ 1.2 times, and secretion of thyrotrophic hormone increased (~ 2.6 times) (Table 4).

Secretion of thyrotrophic hormone and prolactin are regulated by hypothalamus. Accordingly on the further step of our investigation the quantitative changes of adenohypophysis hormone – prolactin in the blood of breast gland tumors patients were studied. Testing revealed insignificant increase of prolactin level in case of benign tumor, while significant gain of the hormone was mentioned in the blood of breast glands malignant tumor patients (Table 4).

Table 4. The non-sex steroid hormones quantitative changes in the blood of the reproductive age women diseased by the breast tumors

The object of the study (blood)	Non-sex hormones		
	Free thyroxin (fT4) ng/ml	Thyrotrophic hormone P(TSH) ml/ml	Prolactin (P) ng/ml
Control group	1.7±0.02	2.43±0.05	11.54±0.4
Benign tumor (Fibroadenoma)	1.46±0.20.04 P<0.0001	2.98± 0.03 P<0.04	14.98±0.02 P=0.0001
Malignant tumor (Cancer)	1.36±0.006 P<0.0001	6.36±0.009 P<0.024	38.93±0.5 P<0.0001
n=15 (the amount of the patients in each group the average age of the patients 19-45; P<0.05)			

Source: Authors

Thus, breast benign and malignant tumors are associated with A(II) phenotype group, while in case of O(I) phenotype group the risk of evolving of the breast tumors (benign, malignant) is less.

It must be taken into account that lowering of the expression of A antigen represents probable marker for cancer progression. Especially in patients with A group. In malignant tumor patients cases of early mortality is especially high in women with A(II) group, and is comparatively low in B(II) groups (Stamatakis et al., 2009). Moreover, changes taking place in glycosyl transferases of the epithelial cells may affect the expression of ABO blood group antigens, which from its side, may be responsible for cancer formation (Wolpin et al., 2009).

We suppose that sharp increase of the frequency of D antigen in breast cancer patients points to lack of deletions in locus of the mentioned antigen (Flegel and Wanger, 2002). As for the C antigen, it decreased slightly in benign and malignant tumor patients, compared with the control group (Table 2). Presumably carriers of C antigen were less sensitive to cancer development.

In reproductive age the biosynthesis of estrogens mainly takes place in follicular cells of the ovary (Bender et al., 2011). At the same time, estrogens are formed from androgens of the adrenal gland. According to obtained data it may be supposed that estradiol, formed in ovary (on the background of sharp decrease of progesterone) together with androgens of the adrenal gland (androstendione as precursor of estrogens), is responsible for extra production of estradiol in case of breast cancer.

Moreover, the supposition exists that androgens (namely testosterone, which is significantly high in content) may directly stimulate division of mammary gland cells and malignant tumor formation (Key et al., 2002).

As for the comparatively low levels of progesterone in blood of breast cancer patients, it diminishes together with increase of estradiol amount in blood (Table 3).

It is well known that biosynthesis of androstendione raises in the blood of breast cancer patients. As progesterone is precursor of androstendione (Берштейн, 2000), on the background of intensive synthesis of the latter, active metabolism of progesterone must take place, with its consequence decrease. This was especially clear in case of breast malignant tumor.

Thus, in our observations imbalance between estradiol and progesterone was depicted. We suppose that increased amount of estrogens and reduction of its antagonist – progesterone may become a target for tissue growth and reason for disorder in differentiation in case of breast malignant tumor of reproductive age women (Bender, 2011).

As for testosterone, its high level may be associated with increased secretion of estrogen in reproductive age women with malignant breast tumor (Secreto, 1996). We suppose that high level of testosterone in breast cancer patients may directly stimulate division of mammary glands' cells and evolution of malignant tumor (Key, 2002).

Thus, gaining of the amount of steroid hormone estradiol and testosterone and decline of progesterone was revealed in reproductive age women. This imbalance is responsible for extra proliferative stimulation of estradiol on mammary glands' cells and creates conditions for malignant tumor development among reproductive age women (as in this age gonadal steroids are synthesized also in ovary and adrenal glands).

Sharp increase of prolactin in reproductive age women with breast cancer may be stimulated by expressed hypofunction of the pituitary gland, since the increased secretion of thyrotropin – releasing hormone of the hypothalamus by the feedback mechanism stipulates enhancement of both thyrotrophic hormone and prolactin secretion on the one hand, and reduction of thyroxin production may be responsible for decrease of dopamine synthesis and correspondingly intensive synthesis of prolactin, on the other hand (Ben-Joanathan and Hnasko, 2000).

The hypofunction of the pituitary gland in breast cancer women may be one of the risk-factors for development of these type cancers.

Conclusion

Thus, the wide spectrum of hormonal disorders were revealed in breast tumor patients of the reproductive age, which was especially clear in cases of malignant tumor. Mentioned disorders comprise quantitative changes of both gonadal steroids and non-steroid hormones. The latter are linked with inhibition of regulatory mechanisms and cell immunity and may promote evolution of cancer pathologies and deepening of the complex changes in an organism.

The highest frequency of breast cancer in reproductive age was revealed in A(II) group patients. The frequency distribution of O(I) phenotypic group was lower among women with breast tumors, compared to control group. It was revealed that among D, C, E, c and e antigens of the Rh system the frequencies distribution of D and E antigens enhanced among benign and malignant breast tumor patients. This demonstrates sensitivity of the mentioned antigens towards breast tumors.

Sharp increase of the gonadal steroid estradiol on the background of decreased progesterone and increased testosterone was revealed in reproductive age women with breast malignant tumor. Clearly expressed hypofunction of the pituitary gland was revealed among the reproductive age women with breast cancer, which was proved by sharp increase of prolactin level and reduction of free thyroxine level in blood.

Acknowledgements

My appreciations goes to the laboratory doctor of the clinical-biochemistry and Immunologic laboratory of Oncology Centre of Adjara - Nona Kharabadze and head of the laboratory of Maternity home's named after Yoseb Charkviani - Tsiuri Kakhidze.

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