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The Reactive Carbonyl Derivatives of Proteins, Methylglyoxal, and Malondialdehyde in Blood of Women with Breast Cancer

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Abstract

BACKGROUND: Every year 1.5 million women in the world are diagnosed with breast cancer (BC). In 2018, more than 260,000 new cases of cancer and more than 40,000 deaths due to this disease were detected. At the same time, in Kazakhstan, an intensive indicator of the incidences of BC in 2018 amounted to 25.3% per population of 100 thousand people (2017–24.5%) with a growth rate of 3.1%, which in absolute numbers are 4,648 new cases per year. In terms of mortality, BC ranks third after lung and stomach cancer (6.8%).

AIM: This necessitates a detailed study of the molecular mechanisms that underlie the development and progression of BC. One of the mechanisms of carcinogenesis is oxidative stress (OS). An increase in malondialdehyde (MDA) levels was detected in the early stages of cancer. It was suggested that MDA, due to its high cytotoxicity, acts as a promoter of the tumor and cocarcinogen agent.

METHODS: Therefore, violation of the parameters of OS in BC is in no doubt. However, according to the literature data analysis, these results are ambiguous and contradictory. There are no studies on a comprehensive assessment of the oxidative destruction of lipids, proteins, and nucleic acids in BC.

CONCLUSION: The nature and direction of changes in various components of OS in patients with BC have not been adequately studied, which is necessary for a correct assessment of the involvement of OS in the mechanism of the pathological process and determination of a sensitive marker of the risk of BC or its progression.

Introduction

Every year 1.5 million women in the world are diagnosed with breast cancer (BC). In 2018, more than 260,000 new cases of cancer and more than 40,000 deaths due to this disease were detected [1].

At the same time, in Kazakhstan, an intensive indicator of the incidences of BC in 2018 amounted to 25.3% per population of 100 thousand people (2017–24.5%) with a growth rate of 3.1%, which in absolute numbers are 4,648 new cases per year. In terms of mortality, BC ranks third after lung and stomach cancer (6.8%) [2]. This necessitates a detailed study of the molecular mechanisms that underlie the development and progression of BC. One of the mechanisms of carcinogenesis is oxidative stress (OS).

OS is a complex multicomponent process of the formation of free radicals (FR), their neutralization and utilization [3]. Excessive generation of FR leads to damage of the structure and function of lipids lipid peroxidation (LP) [4], proteins (oxidative modification of proteins) [5], carbohydrates (oxidative degradation of carbohydrates), and nucleic acids [6].

In a few studies on LP and antioxidant system in the blood and tumor tissue in BC, conflicting results have been identified. Thus, the level of malondialdehyde (MDA) in the blood of women with BC in comparison with the healthy women was increased [7], the same [8], or reduced [9].

An increase in MDA levels was detected in the early stages of cancer. It was suggested that MDA, due to its high cytotoxicity, acts as a promoter of the tumor and cocarcinogen agent [10].

There are few studies on oxidized modified proteins in BC. Research by Rossner *et al*. [11] showed that the level of carbonyl derivatives (CD) of proteins increased in the blood of women with BC. According to the authors, an increase in the level of CD in blood plasma is a marker of the risk of BC. In this case, three proteins with a higher sensitivity to carbonylation were found. According to the authors of the study, identification of carbonylation-sensitive proteins in tumor tissue and understanding of their role in tumor progression may contribute to the development of targeted approaches to the treatment of BC [12].

In another study, growth of protein carbonyls was found in aspirated fluid from the nipple not only

in women with BC but also in women with precancer compared to that in healthy individuals. According to the authors of the study, the results confirm the hypothesis of development of OS in the microenvironment of a BC [13].

In 2017, the results of an epidemiological study on the role of biomarkers of OS in the risk of development and prognosis of BC were published. Two groups of women were examined. The first group included women in the postmenstrual period, the second one - women in the premenstrual period. In women in the postmenstrual period, the risk of development of BC is associated with a marker of oxidative damage to DNA (8-oxodG). At the same time, the survival of women in Group 2 after the diagnosis of BC correlated with the direction of changes in some parameters of LP [14].

Recently, the role of dicarbonyl stress (DS) has been actively studied. DS develops with the excessive formation of various dicarbonyl metabolites (DM), which form adducts with proteins and DNA. One of the DM is methylglyoxal (MG) [15].

Studies on cell cultures of lines MDA MB 231 and MCF 7 showed that MG has a synergistic enhancement of the cytotoxic effects of doxorubicin and cisplatin [16].

A view has been proposed that MG has a pronounced antitumor effect due to a decrease in adenosine triphosphate (ATP) synthesis and limiting in the formation of FR. ATP synthesis decreases due to inhibition of glyceroaldehyde-3-phosphate dehydrogenase activity in tumor cells, as well as tissue respiration [17].

Nokin *et al*. – it has been shown in cell cultures that MG has the ability to enhance tumor growth and metastasis. The authors suggested that MG has a dual effect: In small doses it stimulates tumor growth, while in large doses it has the ability to inhibit tumor growth [18].

Increased expression of glyoxalase 1 confers multidrug resistance to cancer chemotherapy and is relatively high in liver, lung, and BC [19], [20].

Moreover, OS can be the main cause of damage to brain tissue, as well as in chronic inflammatory, vascular, and neurodegenerative diseases of the central nervous system, diabetes [5], [21], [22], [23].

Therefore, violation of the parameters of OS in BC is in no doubt. However, according to the literature data analysis, these results are ambiguous and contradictory. There are no studies on a comprehensive assessment of the oxidative destruction of lipids, proteins, and nucleic acids in BC. The nature and direction of changes in various components of OS in patients with BC have not been adequately studied, which is necessary for a correct assessment of the involvement of OS in the mechanism of the pathological process and determination of a sensitive marker of the risk of BC or its progression.

Study design

The aim of this study was to research LP, oxidative protein modification, and DS in the blood of women with BC of various stages. This study was conducted as a part of the research work on randomized post-registration multicenter clinical trials of the original drug Arglabin in the treatment of BC in an increased dose in 2018–2020. (Identification code AR 01/1).

The present study involved 76 BC patients with the first diagnosed nodular form of this disease with I-III stage and histological and immunohistochemical verification, who were admitted for treatment to the regional oncological clinic of the city of Karaganda. The age of patients ranged from 30 to 80 years. In the age group of women under 40 there were seven patients (9.2%); 41–55 years old – 30 patients (39.5%); at the age of 56–70 – 33 patients (43.4%); and aged 70 and older – six people (7.9%). With Stage I cancer (T1N0M0) 14 patients were observed, with the second (T2N0M0; T1N1M0; and T2N1M0) and third (T1N2-3M0 and T2N2-3M0) stages – 48 and 14 patients, respectively. Half of the patients were of reproductive age; the second half was in menopause. All patients were examined before any therapeutic measures after obtaining informed agreement. The blood of ten practically healthy women of the corresponding age was as control.

Blood was collected from the cubital vein (5 ml/sample) and was drawn into vacutainer tubes containing heparin. Erythrocytes were separated from plasma by centrifugation and washed for three times with physiological saline. Investigations of blood samples were done within 1–2 h after its collection.

Methods

Research methods included determination of the toxic LP metabolite – MDA, the level of reactive CD of proteins (RCDP) and advanced oxidation protein products (AOPP), as well as the content MG, MDA, RCDP, and MG were determined in red blood cells (RBC) and blood plasma.

The concentration of RCDP was measured following the protocol of Levine *et al*. [24]. To triplicate aliquots of plasma (0.8 mL), 0.2 mL of 10% trichloroacetic acid solution was added. The samples centrifuged. Then, either 1 mL of 2M HCl liquid solution or 10 mL of 2.4–dinitrophenylhydrazine (DNPH) in 2M HCl solution was added to the precipitates, and further this mixture was incubated at 37°C for 90 min. Next the samples were centrifuged again (8,000 rpm, 10 min) and the DNPH excess was removed with ethanol-ethyl acetate 1:1 (v/v). Finally, the samples were re-suspended in 6M of guanidine hydrochloride. Quantification was

performed using a spectrophotometer PD - 303 UV APEL (Japan) at a 366 nm absorbance. Concentration of CD was calculated using the molar absorption coefficient of 22,000 $mol^{-1}cm^{-1}$, RCDP values were given in nmol/ml.

MG was determined by reaction with DNPH according to a modified Racker method [25]. 25 μl of blood sample with 350 μl of DNPH (0.1% DNPH in 2N HCl) and then added 2.125 mL of distilled water to each tube. Finally, samples incubated for 15 min at 37°C and after the incubation added 1.5 mL of 10% NaOH. Absorbance for spectrophotometer was set at 576 nm; MG levels were expressed in percent absorbance of MG.

MDA in RBCs was determined according to the protocol of Goncharenko and Latinova [26]. The washed RBC was hemolyzed with distilled water with a 1:5 ratio. 0.3 mL of hemolysate was added 0.3 mL of 10% phosphotungstic acid and 10 min later centrifuged the samples at 3000 rpm for 10 min. The resulting precipitate was washed with 1 mL of distilled water and centrifuged once again. Then, 3 mL of water and 1 mL of thiobarbituric acid (TBA) (80 mg of TBA in a mixture of 5 mL of water and 5 mL of acetic acid) were added to the precipitate and incubated for 60 min in a boiling water bath. Finally, the solution was centrifuged and tested for absorbance at spectrophotometer set at 532 nm against water. In the calculations, we used the molar extinction coefficient, which is $1.56 \times 10^5 M^{-1}cm^{-1}$, expressed in μmol/mL.

MDA in blood plasma was determined according to the protocol of Korobeinikova [27] 0.5 mL of blood plasma was added 5.0 mL of 20% phosphotungstic acid and 15 min later centrifuged the samples at 2500 rpm for 15 min. The resulting precipitate was added 2 mL of distilled water and centrifuged once again. Then, 2 mL of water and 1 mL of 0.8% TBA were added to the precipitate and incubated for 60 min in a boiling water bath. Finally, the solution was centrifuged at 6000 rpm and tested for absorbance at spectrophotometer set at 532 nm and 580 nm against water to avoid the effect of non-lipid compounds reacting with TBA. In the calculations, we used the formula C = 0.21+26.5 Δ D (D_{535}-$_{580}$). C – the concentration of MDA (nmol/ml).

For statistical processing, we used the procedures of mathematical statistics implemented in the application programs STATISTICA 10 and EXCEL. The significance of differences between groups of patients was determined by the Chi-square method.

Results

When studying the concentration of MDA in blood plasma, a gradual increase in its concentration from Stage I to Stage III of BC was established, and reached its maximum values in the group with Stage III of BC, so MDA plasma Me (Q25; 75) was 1.99 (1.61; 2.09) and all three groups significantly exceeded the values of the control group (p = 0.003).

Plasma MG concentration was statistically significantly higher than the control group values in groups with Stages I and II of BC and amounted to 34.95% (24.8; 37.5) and 33.65% (28.2; 43.3; p = 0.000), respectively. While in erythrocytes, there was a decrease in MG concentration with a minimum value in the group with Stage II of BC, and amounted to 18.1 (13.3; 24.6; p = 0.000), compared with the control group.

When studying the concentration of RCDP in plasma, a predominance of concentration was revealed in groups with Stages I and III of BC, so the median RCDP in patients with Stage I of BC was 2.46 (0.94; 8.21; p = 0.000), and with Stage III of BC - 2.38 (1.56; 3.61; p = 0.000). A similar trend, as in the case of MG, continued for RCDP, as its concentration decreased in RBC and reached its minimum in the group of patients with Stage III of BC and amounted to 6.0 (2.19; 6.67; p = 0.000) compared with a control group (Table 1). MDA in erythrocytes and AOPP did not show significant differences in Table 1.

Discussion

Obtained data showed that in the RBC of women with BC, regardless of the stage of the disease, there is a simultaneous increase in MDA (toxic product of LP), RCDP (which indicates an increase in the processes of oxidative modification of proteins), and MG (one of the metabolites of DS), with the highest level of MG, as well as AOPP, is observed in patients with Stage III, which indicates the prevalence of the tumor process. In the RBC of women with BC, regardless of the stage of the disease, there was a significant decrease in RCDP with a significant increase in MG. There were no significant differences in the level of MDA in the RBC of women with BC of varying severity.

Excessive reactive oxygen species (ROS) in BC is due to a number of factors associated with dysregulation of enzymes such as NADPH-oxidase, thymidine phosphorylase and lactoperoxidase, and macrophage tumor infiltration. Macrophages produce not only ROS but also tumor necrosis factor α, which induces intracellular OS [28], [29]. MG is also able to induce OS. ROS generation not only causes direct damage to biopolymers but also promotes the activation of intracellular signaling pathways, which also lead to overproduction of ROS [30].

In particular, extracellular matrix remodeling and activation of MG of MEK/ERK/SMAD1 of the

Table 1: Biochemical parameters of the blood depending on the stage of BC

Parameters	I stage; Me (Q25;Q75)	II stage; Me (Q25;Q75)	III stage; Me (Q25;Q75)	Control group; Me (Q25;Q75)	p-level
MDA, plasma, μmol/mL	1.82 (1.72; 1.95)	1.97 (1.76; 2.34)	1.99 (1.61; 2.09)	1.05 (0.79; 1.13)	0.003
MDA, RBC, μmol/mL	7.5 (27.8;11.28)	9.35 (7.05; 11.73)	9.10 (8.97; 12.17)	9.35 (7.82; 10.89)	0.68
MG, plasma %	34.95 (24.8;37.5)	33.65 (28.2; 43.3)	29.4 (24.8; 43.5)	0.32 (0.29; 0.36)	0.000
MG, RBC%	21.7 (17.8; 26)	18.1 (13.3; 24.6)	23.98 (13.3; 34.4)	0.57 (0.54; 0.6)	0.000
RCDP, plasma, nmol/ml	2.46 (0.94; 8.21)	1.32 (0.86; 2.34)	2.38 (1.56; 3.61)	0.76 (0.49; 1.12)	0.000
RCDP, RBC, nmol/ml	7.47 (5.48; 8.21)	7.47 (5.48; 8.22)	6.0 (2.19; 6.67)	21.34 (12.71; 21.64)	0.000
AOPP	0.24 (0.21; 0.38)	0.28 (0.21; 0.41)	0.29 (0.22; 0.33)	0.23 (0.2; 0.32)	0.63

(*): Data are presented as Me (Q25; Q75). Differences in groups were evaluated by Chi-square, BC: Breast cancer, MDA: Malondialdehyde, RBC: Red blood cell, MG: Methylglyoxal, RCDP: Reactive carbonyl derivatives of proteins, AOPP: Advanced oxidation protein products.

migration signaling pathways are considered as one of the mechanisms of metastasis of BC cells. The increase in MG levels, according to the literature, is due to an increase in glycolysis, which is associated with malignant transformation and cancer progression [31]. MG can form not only adducts with proteins, lipids, and nucleic acids, forming advanced glycation end-products but also damage RBC, white blood cells, and platelets [32].

We have found that in Stage I of BC, the level of MDA is reduced comparatively to the control value; however, in Stages II and III it tends to increase. The level of RCDP and, especially MG, in plasma in BC significantly increases from Stage I. It is noteworthy that the content of MG in blood plasma in Stage III of BC increases by 90 times compared with the control value, which undoubtedly shows the prospect of its further study as a marker of the progression and effectiveness of the therapy.

The increase in the level of RCDP in RBC requires special attention. In our opinion, this can be explained by the accumulation of oxidized proteins, mainly hemoglobin, in red cells [33]. Intracellular metabolic changes in erythrocytes associated with OS can lead to increased suicidal death of RBC [34].

Thus, obtained data showed the promise of the further study of the OS characteristics in women with BC. In addition to understanding the mechanisms of the pathogenesis of this disease, research in this direction will provide an answer to the question of the advisability of modulating the OS as a strategy for antitumor therapy.

Conclusion

Thus, this study indicate a correlation between the stage of the tumor process and OS. Furthemore, this study demonstrated an increased production of ROS in the blood of breast cancer patients; in addition, there are high levels of MDA, MG and RCDP in the blood of these patients simultaneously. Based on the foregoing, markers MDA, MG and RCDP can be used as a diagnosis of the prevalence of the tumor process and assessment of OS.

Author Contributions

LM conceived of and designed the study, drafted the manuscript. SZ identified eligible patients. VS conceived of and designed the study. AN, XM, RB, and NK interpreted data. VML critically revised the manuscript for important intellectual content.

Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval and Consent to Participate

The clinical study is conducted in accordance with ethical principles based on the Helsinki Declaration and in accordance with the requirements of the GCP and applicable law. The topic was approved at a meeting of the Central Commission on Ethics under the Ministry of Health of the Republic of Kazakhstan dated 06.06.2018 under No. 6. All patients received informed consent to participate in a clinical trial.

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