World Journal of Medical Sciences 11 (2): 171-174, 2014
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2014.11.2.8486

Extracellular Nucleic Acids and Purine Bases in Blood of Patient with Locally Invasive Breast Carcinoma

[1]Valentina Bronislavovna Sirota, [1]Larisa Yevgenyevna Muravlyova, [1]Ryszhan Yemelyevna Bakirova, [1]Neila Amerbekovna Kabildina, [1]Indira Milatovna Omarova, [1]Olga Anatolyevna Ponamaryeva, [1]Dmitriy Anatolyevitch Klyuyev, [1]Sholpan Sarkenovna Zhakupova and [2]Ardak Nyssanovna Kuanyshkaliyeva

[1]Karaganda State Medical University Karaganda, Kazakhstan
[2]Astana Medical University, Astana, Kazakhstan

Abstract: The main purpose of our research was to study the extracellular purine bases and extracellular nucleic acids (DNA, RNA, acid soluble predecessors of nucleic acids) in patient's blood with locally invasive breast carcinoma (LIBC). 48 patients diagnosed for the first time with nodular form of breast carcinoma were included in the 1-st group. The control group consisted of 15 healthy women of same age group. The insignificant alteration of extracellular purine bases (Excepting uric acid) was fixed in blood plasma of patients with LIBC of the 2-3 stages. At the same time the significant reduction of extracellular DNA, RNA and ASP both in plasma and in blood erythrocytes was observed.

Key words: Extracellular Nucleic Acids · Purine Bases · Blood · Breast Carcinoma

INTRODUCTION

The breast carcinoma takes the first place in the structure of women's morbidity with malignant tumors. The patients' specific weight with II and III stages of breast carcinoma among all revealed for first time achieves 40%. These patients belong to patients with locally invasive form of disease, which demands complex therapy. At present the treatment of locally invasive breast carcinoma (LIBC) begins from neoadjuvant general influence on organism that is chemotherapy, which considerably widens possibilities of oncological patient's treatment [1-3].

At present the role of adenosine and another purines catabolism intermediate in mechanisms of development and progression of disease and pathological states is actively studied. The adenosine action mechanism is joined with cells function modulating involved inflammatory process: of neutrophils, eosinophil, lymphocytes and macrophages. There is an opinion that adenosine takes part in the genesis of chronic inflammation through release of pro-inflammatory cytokines and chemokine [4].

The analysis of literature data showed that the systematic investigations of purine bases and products of their catabolism in LIBC were not carried out. Another actual direction is the extracellular nucleic role investigation. The extracellular circulating nucleic acids are presented both DNA and RNA fragments. The extracellular circulating nucleic acids are present in blood of healthy ones. There is no clear understanding of the mechanism of their releasing in circulation of healthy ones [5]. The concentration of extracellular nucleic acids has been studied in various acute and chronic disorders. In pathology the nucleic acids are released into the circulation from apoptotic and necrotic cells; but it is assumed an existence of other ways; the exact mechanism is unclear. It was supposed that extracellular RNA molecules act as endocrine signals to alter the phenotypes of target cells [6].

Extracellular nucleic acids have ambivalent functions. It appears to act as host alarm signals that serve to amplify the defence response, but they also participate in thrombus formation [7, 8]. The role of extracellular circulating nucleic acids in mechanisms of disease development and progression has not clarified yet.

Corresponding Author: Larissa Muravlyova, Karaganda State Medical University, Karaganda, Kazakhstan.

Some investigations fulfilled in different scientific centers showed that the definition of DNA level may be interesting for diagnosis of different forms of tumorous process.

The analysis of literature data showed that the systematic investigations of extracellular circulating nucleic acids in LIBC were not carried out.

The main purpose of our research was to study the extracellular purine bases and the extracellular nucleic acids in patient's blood with LIBC.

MATERIALS AND METHODS

48 patients diagnosed for the first time with nodular form of breast carcinoma (T_2 N_1-$_2M_0$, T_3 N_{0-2} M_0) with histologic and immunohistochemical verification participated in clinical investigation.

The patient's age was from 18 to 75 years. From them 25 women had the II and 23 women had the III stage of cancer practically healthy women of analogous age group took part in the control group.

The full clinical examination including collection of anamnesis and survey, clinical and biochemical blood analyses for 1-2 days before the beginning of treatment; mammography and USI of lactiferous glands, USI of regional lymph nodes, organs of peritoneal cavity; radiography of lungs, ECG and examination by specialized doctors were made to patients. The control group consisted of 25 healthy women of same age group.

Ethics: The medical ethics committee of the Medical University (Karaganda) approved the study. All patients and healthy subjects have received the full information on probable inconveniences and complications at the blood sampling before giving their consent to participate.

Blood collected from the cubital vein (3 ml/sample) 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 plasma and red blood cells were done within 1-2 hours after its collection.

In erythrocytes and blood plasma the contents of extracellular DNA, RNA and acid-soluble precursors (ASP) of nucleic acids were defined by following the protocol of Markusheva *et al.* [9]. Units of measure are ng/ml.

The purine bases concentration in blood plasma: guanine (G) hypoxanthine (HX), adenine (A), xanthine (X) and uric acid (UA) were detected by following the protocol of Oreshnikov *et al.* [10]. The concentration of purine bases was expressed in units of extinction (U.ext), UA was expressed in mkmol /l.

For definition of different links work activity of xanthine oxidase, catalyzing oxidation of hypoxanthine into xanthine and xanthine into UA the concentration correlation indexes for all three products of the reaction were calculated. The relation X/HX reflexes activity of the first phase of xanthine oxidase work, relation UA/X reflexes activity of the second phase and relation UA/HX reflexes general activity of enzyme.

Data were presented as mean ± SD. Comparisons or results between patients and controls were performed using non-parametric Mann-Whitney U-test (For independent variables).

RESULTS

The results of investigation are presented in Tables 1-3.

From the data of the table 1 it follows that in patients' blood plasma the unreliable changes of purine metabolism indexes were fixed. It is documented by reduction of contents for all intermediates of purine metabolism excluding guanine, whose level was higher than the control by 9%.

The significant reduction of uric acid concentration in 1.94 times in patients' blood with breast carcinoma in comparison with control attracts attention.

It is revealed that the level of HX in patients' blood plasma with breast carcinoma decreased by 17,5%, the adenine contents decreased by 18,7%, the xanthine contents decreased by 12% in comparison with control ones.

The speed of reaction hypoxanthine± xanthine was reduced by 9%, the speed reaction xanthine ± urine acid didn't change and general activity of xanthine oxidase was reduced by 19% in patients with LIBC of the 2-3 stages in comparison with control ones.

Table 1: The purine bases in blood plasma of LIBC patients (Mean ± SD)

Index	Control (n=25)	Patients with LIBC (n=48)
Guanine	146,85± 8,4	160,2 ± 45,4
Hypoxanthine	164,71 ± 27,06	135,33 ± 43,4
Adenine	122,86±19,33	103,5 ± 37,96
Xanthine	142,93±23,39	125,5 ± 18,52
UA	286,57±28,35	147,67 ± 16,83*
X/HX	0,89±0,15	0,81 ± 0,13
UA/HX	1,47 ± 0,2	1,19 ± 0,41
UA/X	1,22± 0,13	1,19 ± 0,18

Note: *-in comparison with control ones ($p<0,05$)

Table 2: Acid-soluble predecessors (ASP), extracellular DNA and RNA in LIBC patients (Mean ± SD)

Groups of patients	ASP, ng/ml	RNA, ng/ml	DNA, ng/ml
Control, n=25	1,58 ± 0.236	0.122 ± 0.0313	0.045 ± 0.0028
Patients, n=48	0.017 ± 0.009*	0.0028 ± 0.0015*	0.0046 ± 0.0015*

Note: *-in comparison with control ones (p<0,05)

Table 3: Acid-soluble precursors, extracellular DNA and RNA linked with erythrocytes of LIBC patients (Mean ± SD)

Groups	ASP, ng/ml	RNA, ng/ml	DNA, ng/ml
Control, n=32	1,58 ± 0,236	0,122 ± 0,0313	0,045 ± 0,028
Patients, n=48	0,025 ± 0,0015*	0,067 ± 0,0019*	0,028 ± 0,0011*

Note: *-in comparison with control ones (p<0,05)

The reduction of xanthine oxidase activity testify about reduction of active acid forms generation that may be connected with disorder of immune system cells activity, for example neutrophils.

The results of extracellular nucleic acids and ASP definition in patients with LIBC are presented in Tables 2 and 3.

From the data of table 2 it follows that in patients with breast carcinoma of the 2-3 stages the significant decreasing of extracellular nuclear acids and ASP concentration in blood plasma was fixed.

We defined significant reduction of ASP (In 2.3 times), extracellular RNA (In 43,6 times), DNA (In 9,8 times) in blood plasma of patients with LIBC in comparison with control ones.

In the table 3 the concentration of nucleic acids and ASP linked with erythrocytes of LIBC patients was presented.

The significant reduction of ASP and extracellular DNA and RNA linked with erythrocytes of LIBC patients was observed. In comparison with control ones the RNA concentration decreased in 1.8 times, DNA-in 1.6 times and ASP concentration decreased in 63.2 times.

Thus the significant reduction of extracellular nucleic acids and ASP was shown in blood plasma and erythrocytes of LIBC patients.

DISCUSSION

The insignificant alteration of extracellular purine bases (Excepting uric acid) was fixed in blood plasma of patients with LIBC of the 2-3 stages. At the same time the significant reduction of extracellular DNA, RNA and ASP both in plasma and in blood erythrocytes was observed. The decreasing of the extracellular DNA, RNA and ASP concentration may be connected with high activity of nucleases. Based on the results of Pinkel [6] and Fischer and Preissner [7], we assume that very low concentration of extracellular nucleic acids may contribute to suppression of the defence response of patients with locally invasive breast carcinoma. In any case the further challenge will be to expand analyzing of extracellular DNA and RNA in blood of patients with LIBC to estimate their impact in progression of breast carcinoma.

REFERENCES

1. Mohammed Amr Saad, Azza Abd-Alla Mohammed, Amal Mohammed Nour-Eldin, Ahmed Mostafa Ahmed and Mostafa Saif-Elnasr, 2013. Evaluation of Activated Leukocyte Cell Adhesion Molecule as a Biomarker for Breast Cancer in Egyptian Patients. Academic Journal of Cancer Research, 6(1): 29-37.
2. The indexes of oncological service of the Republic of Kazakhstan in 2011. (Statistical materials), 2012. Almaty, pp: 1-105.
3. Arzykulov, Zh. A., 2002. The oncological aid to population of the Republic of Kazakhstan. Oncology and radiology of Kazakhstan, 1: 5-9.
4. Mohsenin, A. and M.R. Blacburn, 2006. Adenosine signaling in asthma and chronic obstructive pulmonary disease. Curr Opin Pulm Med., 12(1): 54-59.
5. Muravlyova, L.Y., V.B. Molotov-Luchanskiy, D.A. Kluyev, N.U. Tankibayeva and V.V. Koiykov, 2010. Extracellular nucleic acids: origin and functions. Minisurvey. The modern problems of science and education, 2: 15-20.
6. Pinkel, D., 2011. New Funding opportunities in Extracellular RNA Communication! The Journal of Biological Chemistry, 286: 41949-41962.
7. Fischer, S. and K.T. Preissner, 2013. Extracellular nucleic acids as novel alarm signals in the vascular system. Mediators of defence and disease. Hamostaseologie, 33(1): 37-42.
8. Komissarov, A., G. Florova and S. Idell, 2011. Effects of Extracellular DNA on Plasminogen Activation and Fibrinolysis. Journal of Biological Chemistry, 9(286): 41949-41962.

9. Markusheva, L.I., M.I. Savina and V.M. Reshina, 2000. The nucleic chromatine proteins in the patients with psoriasis treatment efficiency estimation. Clinical laboratory diagnostics, 7: 18-20.

10. Oreshnikov, Y.V., A.G. Gunin, I.V. Madyanov and S.F. Oreshnikova, 2008. Purines of blood and cerebrospinal fluid in pregnancy. Problems of reproduction, 6: 74-80.