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The Content of Extracellular Nucleic Acids in the Blood and Ejaculate of Men of Reproductive Age Living in the Ecologically Unfavourable Regions of the Aral Sea

Svetlana Jangildinova, Yelena Tatina, Gulzhan Kaliyeva*, Kuttykyz Kuvatbaeva, Nursaya Beygam

Department of Biology, Karaganda Medical University, Karaganda, Kazakhstan

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Keywords: Extracellular DNA; Men of reproductive age; Ejaculate; Plasma acid soluble fractions (ASF); Plasma RNA; Plasma DNA

***Correspondence:** Gulzhan Kaliyeva. Department of Biology, Karaganda Medical University, Karaganda, Kazakhstan. E-mail: G.Kalieva@kgmu.kz

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Abstract

AIM: We aimed to study the influence of adverse environmental factors on molecular-cellular processes in the population living in the Aral Sea region.

METHODS: Extracellular RNA (ecRNA) and ecDNA were determined in blood. We obtained the ejaculate of the studied men after 4-5 days of abstinence. The ejaculate was placed in a warm tube with a ground stopper. The examination of the ejaculate was started in 20-30 minutes after receiving, as during this time it is subjected to liquefaction. Spectrophotometry of ASF, RNA and DNA hydrolysates was performed on an SF 26 at a wavelength of 290 nm against H_2O.

RESULTS: In the ejaculate of the studied groups of men, significant deviations in the content of extracellular nucleic acid fractions from the indicators of the comparison group were also detected. Statistically significant differences in the content of extracellular RNA were observed in men of the younger age group living in the territory of all study regions. A significant increase in the content of extracellular DNA was detected in two regions, but not in all age groups.

CONCLUSION: The study revealed a significant increase in the content of extracellular nucleic acids in the biological fluids of men of reproductive age living in the Aral Sea region. The most significant are the changes in the level of extracellular RNA in the blood plasma and ejaculate in men of the younger age group and the increase in ASF content in the ejaculate in men of all age groups.

Introduction

The Aral problem as the largest ecological catastrophe of the planet poses a direct threat to the sustainable development of the region, the health and future of the people living in it. Environmental pollution from exposure to various chemical and physical factors leads to the development of environmentally dependent pathologies, which manifest as clinical, pathophysiological, immunological and biochemical changes, which leads to several relevant diseases [1].

It has been revealed that effect of dust-salt aerosols and ecotoxicants (hydrazine, heavy metals and increased radiation background) in the Aral Sea regions can induce disturbances at the molecular-cellular and subcellular levels, leading to disruption of intercellular signaling, membrane transport and the genetic apparatus of the cell, which are starting mechanism of development of pathophysiological processes in the body [2].

Thus, it seems relevant to study the biochemical markers, indicating violations of molecular cell processes, to assess the impact of negative environmental factors on the body of the population in ecologically unfavourable regions [3]. We have investigated the content of various fractions of extracellular circulating nucleic acids in the blood and ejaculate in men of reproductive age living in the Aral Sea region.

We aimed to study the influence of adverse environmental factors on molecular-cellular processes in the population living in the Aral Sea region. For this purpose, the content of extracellular circulating nucleic acids was studied: the acid-soluble fraction (ASF) of nucleic acid precursors, extracellular RNA and DNA in the blood and ejaculate of men of reproductive age.

Material and Methods

The object of the study was the blood and ejaculate of men of reproductive age living in the Aral Sea regions. Depending on the age the examined persons were divided into three groups. As a comparison, we used the indicators of healthy men of reproductive age living in the territory of the Karaganda region, not exposed to dust-salt aerosols.

Blood sampling was carried out in the morning on an empty stomach; the ingestion of food and liquids was excluded 8-10 hours before the study. The volume of blood obtained from patients was 5.0 ml with the addition of 0.5 ml of heparin. The storage time of the biomaterial from the moment of collection to the research was no more than 1 hour. The biomaterial was stored in a special container at a temperature of +2 to +6°C.

Extracellular RNA (ecRNA) and ecDNA were determined in blood using the method of L. I. Markusheva and M. I. Savina [4]. The principle of ecRNA and ecDNA quantitative determination consists of the extraction of nucleic acids after hydrolysis at different temperatures in a water bath with perchloric acid of a certain concentration. The units of optical density/1.0 ml are used when calculating.

We obtained the ejaculate of the studied men after 4-5 days of abstinence. The ejaculate was placed in a warm tube with a ground stopper. The examination of the ejaculate was started in 20-30 minutes after receiving, as during this time it is subjected to liquefaction.

Spectrophotometry of ASF, RNA and DNA hydrolysates was performed on an SF 26 at a wavelength of 290 nm against H_2O.

Statistical Analysis

Statistical processing of research results was carried out by methods of variation statistics, non-parametric data processing methods. Statistical analysis was performed using SPSS 7.0, Windows Statistica 8.0 [5], [6].

Results

According to the results of a study of blood plasma, most often significant changes in the content of extracellular RNA were observed in men living in ecologically unfavourable regions of Kazakhstan. At the same time, the largest deviations from the corresponding indicators of the comparison group were observed in men of the younger and middle age groups in all regions of the study (Table 1).

Significant differences with the comparison group in the content of ASF and extracellular DNA in the blood plasma were not observed in all regions of the study. Thus, the greatest deviations in the content of extracellular DNA in the blood plasma are characteristic of residents of the South Kazakhstan region, and increased content of ASF was detected in residents of the Aktobe region.

Table 1: Indicators of extracellular nucleic acids in blood plasma in men (M ± m)

Region	Age	ASF, (standard units per ml)	RNA, (standard units per ml)	DNA, (standard units per ml)
South Kazakhstan region				
Arys-town, n = 300	18-29 years, n = 100	0.41 ± 0.03	1.25 ± 0.27***	1.18 ± 0.16***
	30-39 years, n = 100	0.58 ± 0.21	1.51 ± 0.82***	1.26 ± 0.21**
	40-49 years, n = 100	0.65 ± 0.09	1.81 ± 0.63**	1.35 ± 0.21***
Aktobe region				
v. Argyz, n = 150	18-29 years, n = 50	0.49 ± 0.06**	1.46 ± 0.27***	1.38 ± 0.51
	30-39 years, n = 50	0.60 ± 0.02***	1.47 ± 0.22***	1.39 ± 0.67
	40-49 years, n = 50	0.71 ± 0.03	1.51 ± 0.02***	1.67 ± 0.94
Shalkar-town, n = 225	18-29 years, n = 75	0.49 ± 0.15	1.57 ± 0.28***	1.28 ± 0.62
	30-39 years, n = 75	0.61 ± 0.29	1.63 ± 0.21***	1.30 ± 0.16***
	40-49 years, n = 75	0.69 ± 0.04*	1.18 ± 0.74	1.41 ± 0.26**

Note: the reliability of differences between the study groups and analogous indicators of the comparison group: * p < 0.05; ** p < 0.01; *** p < 0.001.

In the ejaculate of the studied groups of men, significant deviations in the content of extracellular nucleic acid fractions from the indicators of the comparison group were also detected (Table 2).

The increased content of ASF in the ejaculate is typical for men living in all regions of the study. Moreover, for two of the three study areas, a significant increase in the ASF content was observed in all age groups.

Table 2: Indicators of extracellular nucleic acids in the ejaculate in men, (M ± m)

Region	Age	ASF, (standard units per ml)	RNA, (standard units per ml)	DNA, (standard units per ml)
South Kazakhstan region				
Arys-town, n = 300	18-29 years, n = 100	0.41 ± 0.03**	1.25 ± 0.27**	1.18 ± 0.16***
	30-39 years, n = 100	0.58 ± 0.02***	1.51 ± 0.32	1.26 ± 0.21***
	40-49 years, n = 100	0.65 ± 0.1***	1.81 ± 0.63	1.35 ± 0.21***
Aktobe region				
v. Argyz, n = 150	18-29 years, n = 50	0.49 ± 0.06**	1.46 ± 0.27***	1.38 ± 0.51
	30-39 years, n = 50	0.60 ± 0.01***	1.47 ± 0.62	1.39 ± 0.67
	40-49 years, n = 50	0.71 ± 0.24***	1.51 ± 3.02	1.67 ± 0.94
Shalkar-town, n=225	18-29 years, n = 75	0.49 ± 0.15	1.57 ± 0.28***	1.28 ± 0.62
	30-39 years, n = 75	0.61 ± 0.29	1.63 ± 0.21*	1.30 ± 0.16***
	40-49 years, n = 75	0.69 ± 0.04***	1.18 ± 0.74	1.41 ± 0.26**

Note: the reliability of differences between the study groups and analogous indicators of the comparison group: * -p < 0,05; ** -p < 0,01; *** -p < 0,001.

Statistically significant differences in the content of extracellular RNA were observed in men of the younger age group living in the territory of all study

regions. A significant increase in the content of extracellular DNA was detected in two regions, but not in all age groups.

Discussion

Extracellular nucleic acids are actively studied under various pathological conditions. Increasing the concentration of extracellular nucleic acids in the blood leads to various metabolic consequences. Now it has been established that the content of circulating nucleic acids varies at diabetes, myocardial infarction, diseases of the kidneys and lungs, hepatitis, oncopathology [7], [8], [9].

The results of the study of extracellular nucleic acids have allowed developing new methods for diagnosing, determining the stage and monitoring the treatment of certain types of cancer, identifying certain congenital malformations [10], [11].

In conclusion, the study revealed a significant increase in the content of extracellular nucleic acids in the biological fluids of men of reproductive age living in the Aral Sea region. The most significant are the changes in the level of extracellular RNA in the blood plasma and ejaculate in men of the younger age group and the increase in ASF content in the ejaculate in men of all age groups.

It is possible that the change in the values of ASF, extracellular RNA and extracellular DNA is associated with the death of nucleated cell elements, as well as secretion of nucleic acids into the extracellular space, activated by exposure to dusty salt aerosols and other negative environmental factors in the Aral Sea region.

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