Determination of the Level of Extracellular DNA in the Blood of People During the Reproductive Period under Actions of Ascaridiasis Invasions

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Abstract: The numbers of extracellular DNA can growth under influence of disease, which is likely to be taken into consideration as an early sign of pathological process. Experiment was carried out on state of extracellular DNA under actions of ascaridiasis in the blood of men and women during the reproduction period. Those persons who are under influence of ascaridiasis have changes in extracellular DNA. The number of extracellular DNA decreases in the erythrocytes of the effected people by helminthic invasion. The increase of concentration of precursors DNA, acid soluble fractions (ASF) is watched that is one of the reliable signals of the elimination DNA from a cell.

Key words: Extracellular DNA · Helminthic process · Reproduction health · Ascaridiasis

INTRODUCTION

For a few years past the learning of pathogenesis has been explored actively. The disorders of endocrine system and reproductive system are known to have been occurred under influence of helminthes invasions.

According to the some information of WHO (World Health Organization) parasitic and infection disease are the main reason for more than 16 million of died people. The helminthes influence on our body negatively: invaded kids have some patomorphological changes in organs and tissues, enzymatic, hormone and reproduction functions are violated, micro flora of intestine is affected as well, also immunodeficiency develops in their body [1, 2].

There was an established mutagenic influence on genetic system under actions of helminthes. Nuclear proteins and especially proteins included to chromatin, substantially determine the level of the functional activity of a cell.

Other explores showed the availability of the close relation between quantitative content of the proteins’ fraction of chromatin, their quantitative fluctuation towards each other may be used as a point to estimate synthetic and proliferative level. Some numbers of DNA are detected out of the cells, especially in plasma of the blood, existing in blood circulation as a result of death of the cellular elements enriched with nucleus and birth of erythrocytes and thrombocytes and also as a result of the nucleic acid’s secretion to the extracellular capacity [3, 4].

The interest to extracellular DNA increased after the established moment, which says that the number of DNA may growth significantly especially while having some diseases, that is possible to consider it as a sign of coming pathological process. That provided with a huge practical meaning for the further learning of nucleic acids, which is related with the diagnostic and practical significance of this index having ray radiation, oncological and autoimmune diseases and neurological disorders [5, 6].

It is established that the level of extracellular nucleic acids increase in blood under having such pathological diseases, like malignant growth and also some explores were carried out at the persons having a pathology of pregnancy. It is determined that the content of extracellular nucleic acids increase under influence of ray radiation that shows their participation in factors of stress signaling.
Obviously that extracellular DNA of the blood can be used as an option for diagnostic of some parasite invasions. There is no information about DNA, RNA with Helminthes invasions, which determined as a goal of our exploration in some literary sources.

The goal of this examination is to determine extracellular DNA, RNA and predecessors of the nucleic acids at the persons in reproductive period with ascaridiasis invasions.

**MATERIALS AND METHODS**

About 68 people, living in Kazakhstan were examined in this study. All of the examined people are man and women at the age of (18-45 years) diagnosed like "Ascaridiasis". All of the patients were determined with availability the eggs in feces by using method of a native smear.

On defatted subject glass slide was put drop of 50% of glycerin or water and then with the spatula or stick was taken small amount of feces from different sample sites and been added to this drop and then accurately have been mixed before obtaining equable emulsion.

Particulate matter was removed from the droplets; drop was crushed with glass coverslip and examined under the microscope at low magnification. Investigated no less than 10 smears of each sample.

Healthy people have been considered as a control group without having ascaridiasis. We learnt circulating DNA with ascaridiasis invasions at the persons in reproductive period. Extracellular DNA was determined with methods of Markushreva [7]. As an example was taken amount of the blood of examined people: To 0.5 ml of the blood add 2.55 ml of 0.5 n HClO₄ mix together and centrifuge for 30 minutes under 3000 revolutions per minute. Pour out supernatant (it is soluble fraction of acids, which contains free nucleotides, oligonucleotides and other substances of nucleotide nature) in test tube. After that add to supernatant 5 ml 10% HClO₄ mix and leave in thermostat for 10 minutes under temperature of 37°C, centrifuge for 30 minutes under 3000 revolutions per minute. Pour out supernatant (it is hydrolyzed of RNA) in test tube. Add to supernatant 3 ml of 10% HClO₄ mix and leave in water bath for 7 minutes under temperature of 70°C, centrifuge for 30 minutes under 3000 revolutions per minute. Pour out supernatant (it is hydrolyzed of DNA) in test tube. Spectrophotometry of soluble fraction of acids, DNA and RNA hydrolyzed realize with spectrophotometer 26 under wavelength 290 nm [7].

Statistical handling of getting data had been provided by parametrical methods with estimation of the difference by t-Student criteria [8].

| Table 1: Nucleic acids and their predecessors in the plasma of healthy people and the ones who are ill with ascaridiasis. |
|---|---|---|
| Indications | Sick with ascaridiasis | The control |
| ASF of plasma, Conv.units/ml | 19.31±0.92* | 10.719±0.055 |
| RNA of plasma, Conv.units/ml | 0.55±0.29* | 0.16±0.13 |
| DNA of plasma, Conv.units/ml | 0.44±0.046* | 0.11±0.07 |

Note: * significant in comparison with the control, p <0.01; p < 0.05

| Table 2: The nucleic acid and their precursors in the erythrocytes of healthy people and sick ones before treatment. |
|---|---|---|
| Indications | Sick with ascaridiasis | The control |
| ASF erythrocytes Conv.units/ml | 54.8±4.51* | 70.2±6.5 |
| RNA erythrocytes Conv.units/ml | 3.15±0.22* | 3.6±0.31 |
| DNA erythrocytes Conv.units/ml | 0.52±0.015* | 0.625±0.052 |

Note: * significant in comparison with the control, p <0.01; p < 0.05

**RESULTS AND DISCUSSION**

The results of extracellular DNA in the plasma of affected people are demonstrated in Table 1.

According to the results of examinations which say that the content of ASF increases in the plasma of sick people in comparison with previous indexes of nearly healthy people.

The indication of extracellular DNA and RNA in the plasma also increases in comparisons with the control indexes.

Obtained information demonstrates that the sick people have deficiency of extracellular DNA while they have ascaridiasis invasion for the erythrocytes are died and their DNA involved the plasma and ripped into fragments in Table 2.

Also there was some marked situation showing increase of acidity soluble fractions of nucleic acids for example precursors of DNA, that is authentic indication of the strong illumination of DNA.

Thus people who are affected by ascaridiasis invasion may have an increase of concentration of extracellular DNA in plasma of their blood.

Conducted researches allow us to conclude that the change in the concentration of extracellular DNA in the blood of individuals with ascaridiasis invasion may be due to endogenous intoxication caused by metabolic products of acarida, which is consistent with the published data [4, 5, 9].

Contact DNA fragments in the blood may be caused since a large number of apoptosis in cells, which leads to disruption of the processes of elimination of apoptotic cells, as the endogenously minutes under intoxication rate of cell death exceeds the ability of phagocytes to absorb and destroy DNA, which explains the increase in its blood level [9].
CONCLUSION

Ascaridiasis invasion provokes the dying of the human cells, this is why erythrocytes have less extracellular DNA than before and obtained knowledge show prove the availability of oxidative stress called toxic products of metabolism.

REFERENCES