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A note about the authors:
Abramov Alexei Fyodorovich – doctor of biological Sciences, Professor. Yakut Scientific Research Institute of Agriculture after M. G. Safronov (677001, Russia, Yakutsk, Bestuzheva-marlinskogo, D. 23/1, ANEESH, tel: 8(4112)214574, e-mail: yniicx@mail.ru.
Sleptsova Tatyana Vasilevna – researcher of the laboratory of agricultural products processing and biochemical analyses. Yakut Scientific Research Institute of Agriculture after M. G. Safronov (677001, Russia, Yakutsk, Bestuzheva-marlinskogo, D. 23/1, ANEESH, tel: 8(4112)214574, e-mail: yniicx@mail.ru).
Efimova Aleksandra Arkadyevna, candidate of agricultural Sciences, leading researcher of the laboratory of agricultural products processing and biochemical analyses. Yakut Scientific Research Institute of Agriculture after M. G. Safronov (677001, Russia, Yakutsk, Bestuzheva-marlinskogo, D. 23/1, ANEESH, tel: 8(4112)214574, e-mail: alekefim@mail.ru).
Vasilyeva Valentina Tikhonovna – candidate of biological Sciences, senior researcher of the laboratory of agricultural products processing and biochemical analyses. Yakut Scientific Research Institute of Agriculture after M. G. Safronov (677001, Russia, Yakutsk, Bestuzheva-marlinskogo, D. 23/1, ANEESH, tel: 8(4112)214574, e-mail: vasvalt@mail.ru.

M.M. Vinokurov, V.V. Savelyev, T.V. Yalynskaya

THE USE OF «DNA-COMET» METHOD FOR THE DETECTION AND ASSESSMENT OF DAMAGE TO THE BLOOD MONONUCLEAR CELLS INDUCED BY ENDOGENOUS INTOXICATION AT THE ACUTE DESTRUCTIVE PANCREATITIS

ABSTRACT
The method of analysis of DNA damage («DNA comet», DNA-comet assay, method of gel electrophoresis of individual cells DNA) found its application in clinical practice as a method of monitoring the effectiveness of treatment and the severity of genetoxicologic effect in the development of endogenous intoxication. The literature review data suggest a possible clinical application of the method in determining the level of endogenous intoxication in patients with acute destructive pancreatitis.
Keywords: DNA damage, mutations, repair, apoptosis, genotoxicity.

Most researchers dealing with human adaptation in the Far North have noted that for the majority of the inhabitants of the Republic Sakha (Yakutia) the reduction or distortion of most of biochemical processes and the violation of homeostasis of the body are indicative, which are expressed by changes in carbohydrate, protein and lipid metabolism, immunological reactivity [1, 2, 3, 4, 5], balance of prooxidant and antioxidant systems, the activity of enzymes involved in detoxification and protective processes of the body [1,3, 4, 7].

There is no doubt that all these pathological changes affect the homeostasis for any disease and require accounting and analysis for decision-making of their correction in a comprehensive program of treatment [2, 5, 10, 11]. It should be noted that these body systems are the most important in the pathogenesis of inflammation and also play a significant role in the development and progression of various complications [5,10]. For this reason, research aimed at studying the pathogenesis of significant violations homeostasis, their dynamics in order to control the efficiency and timely correction of the complex therapeutic measures have significant value.

The impact of adverse factors on any biological system (including the human body) is accompanied by the accumulation of DNA damage and repair systems activity change that may cause mutations, lesions and body cells. The review assessed the effectiveness of the method of «comet assay» to detect DNA damage caused by endogenous intoxication (including that causes , and acute destructive pancreatitis),which was the reason for choosing this method.

The method has a sensitivity required for registration of DNA damage at the level of individual cells, and can be used to evaluate the integrity of the integrated genome. Application method «comet assay»: any biological system [9].

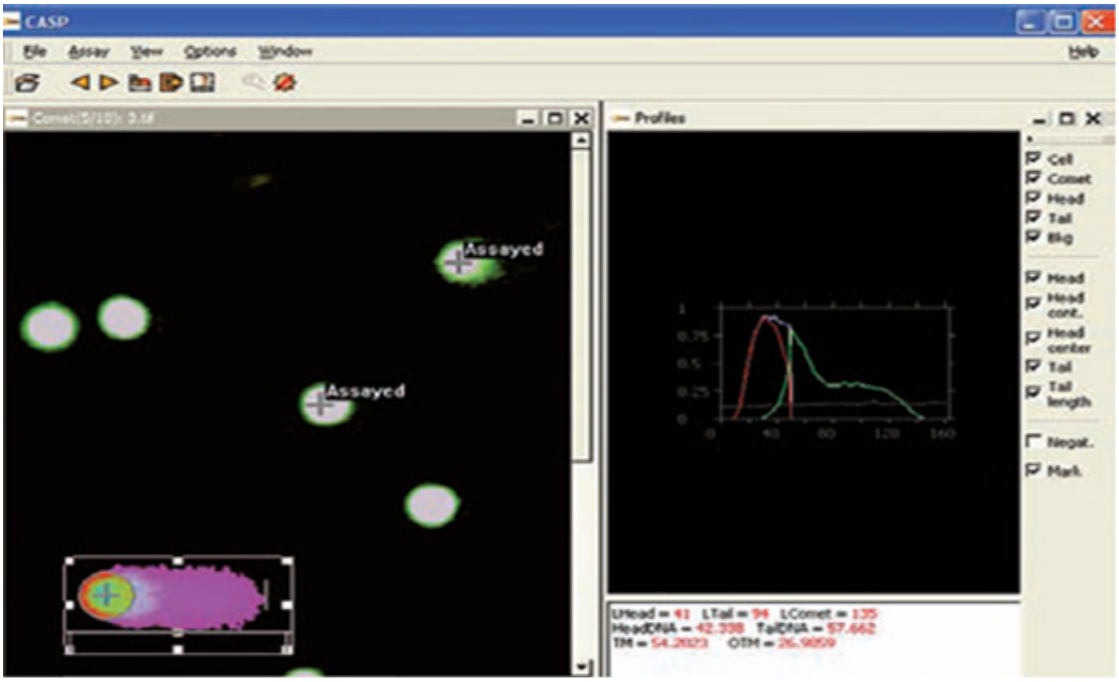


Figure 1. Analysis of DNA comets digital images in a software environment CASP 2.2.1. (CaspLab, USA).

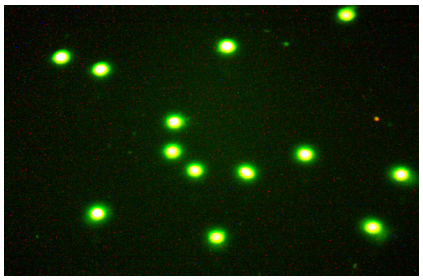
The principle of the method - based on the registration of varying speed of movement in a constant electric field is intact and fragmented DNA isolated cells enclosed in an agarose gel. Isolated DNA molecules of the entire compact cells moving in the electric field. In the presence of impaired DNA structure, the speed of advance in the electric field will be different from the speed of unmodified DNA molecule. Structure «comet tail DNA» due to the natural processes of DNA replication and transcription associated with cell division and protein synthesis processes [8,12].

Proceedings determination: Blood samples (2 ml) was mixed with an equal volume of RPMI- 1640 medium containing 10 % dimethyl sulfoxide and frozen and stored until analysis at -20° C. Then, for analysis of blood samples in a volume of 50 mkL were added to tubes containing 500 mkL of 1 % agarose solution , resuspended and loaded onto agarose precoated slides . After hardening of the agarose slides are lysed with cold buffer(10 mMTrisHCl [pH 10], 2,5 MNaCl, 100 mMEDTA-Na2,1% Triton X-100, 10% DMSO) at least 1 hour. After the end the slides were incubated in lysis buffer for electrophoresis (300 mM NaOH, 1 mM EDTA-Na2, pH>13) for 20 minutes for the implementation of an alkaline alkali-labile sites and denaturation of DNA. Electrophoresis was performed for 20 minutes at a field strength 1/V and a current intensity of from to - 300 mA. After electrophoresis, the slides were fixed in 70 % ethanol solution, dried and stored until analysis at room temperature [5, 8].

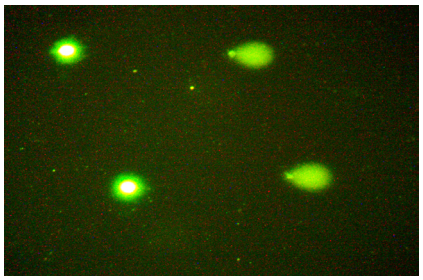
Immediately prior to microscopy slides were stained with appropriate fluorescent dye SYBR Green I (1: 10000) for 30 minutes in TE- buffer). The analysis was performed on the epi-fluorescence microscope Mikmed 2-12T («LOMO», Russia), combined with a high-resolution digital camera (the VEC - 335,»EMU», Russia), with an increase of x400. The resulting image from micropreparations «comet assay» was analyzed using CASP 2.2.1 software. (CaspLab, USA) (Picture 1).

Hardware-software complex consists of a coincident with a microscope a highly sensitive CCD- camera and specialized software that enables digital recording and processing parameters of «DNA - comets «, characterizing the integrity of the structure of DNA: the length of the «comet assay», tail length, head diameter, DNA content percentage in the head or the tail (% DNA) etc. (Figure 2).

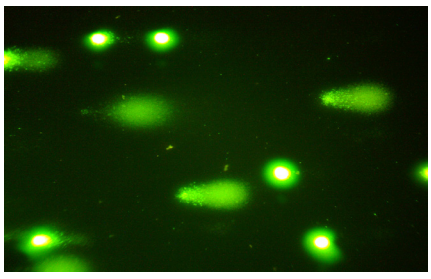
As an indicator of the damaged DNA is most often used of the tail length , percentage of DNA content in the tail or their work - so-called « tail moment » (tail moment) [13,14].Apoptotic DNA



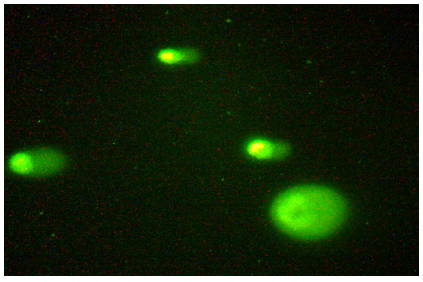
a. Mononuclear cells from a normal donor



b. DNA-comets ghost cells



c. DNA-comets apoptotic cells



d. DNA-comets necrotic cells

Figure 2. DNA-comets cells with varying degrees of damaged DNA.

comet was identified as specific DNA «DNA-comets» diffuse «tail» and is practically absent «head» as well as the extensive necrotic - diffuse «DNA-comets» of irregular shape (Picture 2 c, d). The identification of such abnormal DNA comets can be seen as an indirect indicator, respectively, apoptotic or necrotic cell death.The DNA comet micropreparations often show atypical (cytotoxic) DNA comet, with absent or virtually absent head and broad diffuse tail, known as ghost cells or hedgehogs [9], they singled out and treated separately. Since such DNA comet tail is represented in the form of short discrete fragments (Picture 2b), it is assumed that these DNA comets can form apoptotic cells are on chromatin fragmentation stage [9].

«Comet assay» method has a number of significant advantages over other methods of assessing the damaged DNA. This - high sensitivity, possibility of detecting DNA damage in the cells of any tissue in vivo, a minimum required amount of experimental material, relatively low cost, high «ductility», allowing for minor modifications of the method used for selective recording various categories of DNA damage and related events.It attracts speed of the experiments and the relative simplicity of the laboratory protocol. Today there is a consensus on the need to include the method of «comet assay» as a tracer test in expert assessment of genotoxicity in vitro and in vivo. In Russia, this method became a part of a series of guidelines and instructions [9.12].

Further, it should point to the prospect of applying the method of «comet assay» as a tracer test in epidemiological, different kind of experimental and clinical studies, the study etiopathogenic role of primary DNA damage, as well as to assess the «quality of life» of biological systems in different environmental conditions habitat.

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Authors:
Vinokurov Mikhail Mikhajlovich, doctor of medical sciences, professor of department of faculty surgery of medical institute of NEFU. 677000, Street of Dzerjinskogo 26 flat 41. Office telephone (43-20-91), home telephone (45-25-30), Yakutsk, Russia. E - Mail: mmv mi@rambler.ru;
Savelev Vyacheslav Vasilievich, candidate of medical sciences, associate professor of department of faculty surgery of medical institute of NEFU. 677000 street of Krupskoj 37 flat 58. Mobile telephone 89241706330. Yakutsk, Russia. E - Mail: vvsaveliev@mail.ru;
Yalinskaya Tatyana Vadimovna, student of the 5th course of medical institute of NEFU. 677009 street of Dzerjinskogo 45/2 flat 70. Mobile telephone 89143033999. Yakutsk, Russia. barbuzyaka@mail.ru.

CLINICAL CASE

A.A. Tappakhov, T.Ya. Nikolaeva, A.D. Alexeeva, L.T. Okoneshnikova, L.A. Klarov, T.K.Davydova

DIFFERENTIAL DIAGNOSIS OF MULTIPLE SYSTEM ATROPHY AND ESSENTIAL TREMOR WITH PARKINSON'S DISEASE (CLINICAL CASES)

ABSTRACT
Differential diagnosis of Parkinson's disease is a difficult task especially at the level of primary medical care. It is one of the reasons for late diagnosis of some neurodegenerative diseases.
The article attracted the attention of doctors to clinical features of diseases similar with Parkinson's disease for early diagnosis and adequate treatment. We provide our own clinical cases of patients with neurodegenerative diseases (multiple system atrophy, essential tremor) who in the debut were diagnosed with Parkinson's disease.
Thus, there is hyperdiagnostics of Parkinson's disease, not only under diagnostics. Some neurodegenerative diseases such as multiple system atrophy, are accompanied by the development of parkinsonism, but they have a number of clinical features that contribute to choosing the right tactics and timely diagnosis. In the differential diagnosis of multiple system atrophy apart from typical clinical picture magnetic resonance imaging is important. Differential diagnosis of tremor form Parkinson's disease and essential tremor is often difficult, especially in the early stages of the disease, when there is no clinically severe rigidity. Timely clinical diagnosis involves the use of optimal methods of treatment based on evidence-based medicine, the identification of reliable epidemiological indicators and, consequently, appropriate use of health care resources.
Keywords: diagnostics, multiple system atrophy, essential tremor, parkinsonism, Parkinson's disease, MRI.

INTRODUCTION

Parkinson's disease (PD) is the most common cause of parkinsonism. Parkinsonism is a clinical syndrome characterized by hypokinesia with rest tremor, muscular rigidity and/or postural instability [1, 4, 5]. If at a later stage of PD patients have stereotypes clinical picture, then at an early stage even skilled experts have difficulty in diagnosis [1]. Therefore, PD should be differentiated with essential and dystonic tremor and other disorders that are accompanied by the development of Parkinsonism. For example, symptomatic parkinsonism may develop as a consequence of stroke or chronic vascular diseases of the brain, and traumatic brain injuries. Parkinsonism may accompany neurodegenerative diseases such as multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and dementia with Lewy bodies (DLB) [1, 10].

Differential diagnosis of Parkinson's disease is a difficult task especially at the level of primary medical care. This is evidenced, for example, by the existing shortage of primary diagnosis of PD, which is associated with both underdiagnosed Parkinsonism and reducing the available symptoms to the natural aging, and insufficient information and late negotiability of the population for health care [3].

Each nosology accompanied by the development of parkinsonism has several distinctive clinical features. Vascular parkinsonism is characterized by a temporary connection with cerebrovascular disease, lesions mainly the lower half of the body, early onset of gait disturbances, symmetrically symptoms and the low efficiency of levodopa [2]. The diagnosis of PSP is considered in cases of early postural instability with falls, early cognitive dysfunction, slowing of vertical saccades, and supranuclear vertical gaze palsy [7]. Various combination of progressive autonomic failure, parkinsonism with the low efficiency of levodopa, cerebellar ataxia, urinary urgency and pyramidal syndrome most often occurs in MSA [8].

Essential tremor (ET) is characterized by slowly progressive isolated tremor without muscular rigidity and hypokinesia, in the most cases patients have family history of disease and the positive effect of alcohol [6, 9].

Aim of study: To focus doctors on clinical features of diseases similar with Parkinson's disease for early diagnosis and adequate treatment. In this article, we present our own clinical cases of patients with neurodegenerative diseases (multiple system atrophy, essential tremor), who in the debut were diagnosed with Parkinson's disease.

RESULTS OF STUDY

Clinical case 1. A 66-year-old man admitted to the neurological department of the Republican Hospital №2 – The Center emergency medical care (Yakutsk city) in August 2015 with