ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 4, Issue 1, July 2014

The Use of Cytogenetic Technology in The Occupational Selection of Labour Resources for the Oil Fields of Siberia

Nicolai N. Ilyinskikh, Ekaterina N.Ilyinskikh, Anna E. Yankovskaya
Department of Ecology National Research Tomsk State University, Russia.
Department of Biology and Genetics Siberian State Medical University, Russia

We examined 225 males employed in the oil industry of Tomsk and Tyumen Regions of Siberia, Russian Federation. The frequency of micro nucleated urinary epithelial cells and concentrations of urino-excreted benz[a]pyrene in the workers were significantly higher than in the control group. The highest levels of the indices, including increased frequency of the cells with a large micronucleus (greater than 3 μm^2 in diameter), were determined in individuals with mutant alleles of GSTM1 gene. There was a good correlation between the frequency of micro nucleated cells and the concentrations of benz[a]pyrene in urine. The activation of the mutation process in the oil industry workers is presumed to be a result of combined effects of various factors on the genome including not only strong mutagens, but also co-mutagens. It should become a basis for the implementation of new, scientifically based criteria for hiring personnel for oil industry work in Siberia.

Keywords: **Cytogenetics, micronuclead cells, benz[a]pyrene in urine, GSTM1 gene, oil industry workers, northern Siberia (Russia)**

I. INTRODUCTION

Our studies [1] have shown that some oil workers employed in shift work have an increased level of cytogenetic damage (shown by a micronucleus test), and it was supposed that the oil has a genotoxic effect. Additionally, there is a view that the occurrence of such changes in the oil workers' bodies are caused, generally, by the extreme conditions of labor in northern Siberia [2]. Studies conducted by several scientists [3, 4] made it possible to prove that oil and its derivatives have a pronounced mutagenic and carcinogenic effect. Oil content affects the nucleic acid composition and content of free nucleotides. Individuals in contact with petroleum products have changes in the primary structure of DNA of the oral epithelium, bronchial tubes and lungs. Also, a genetic polymorphism set of people's sensitivity to carcinogenic and mutagenic effects of some oil components was found [3, 4, 5]. In this regard, particular attention is paid to the xenobiotic biotransformation of the enzymes gene *GSTM1*. The *GSTM1* locus is not a functional (mutant) null allele [3]. A considerable excess frequency of homozygote for GSTMI gene deletion in chronic respiratory diseases was found, along with an association between GSTM1 null genotype and gene predisposition to cancer of the oral cavity and lungs [3, 6]. Among the works devoted to the study of *GSTM1* genes, rather great attention is given to studies of workers exposed to the effects of different doses of industrial chemical agents. A number of studies found a clear association between *GSTM1* null gene variants and the level of potential mutagens excreted in urine, and it was also found that *GSTM1* enzyme deficiency may be a risk factor because of the increased sensitivity to chemical agents of some oil production workers [5]. Water-soluble components are the most dangerous among the migratory forms of oil [7,8]. This group is represented by Benz (o) pyrene. Obviously, the increased sensitivity to genotoxic mutagenic action of oil for oil workers is a poor prognostic sign of their health and it should naturally be taken into account in the course of occupational selection of shift workers for shift work in the oil sphere.

II. MATERIALS AND METHODS

We examined 118 males directly involved in the process of oil production in oil fields in the north of Tomsk and Tyumen regions. In this paper we examined only those persons who gave free and informed consent regarding the detection of cytogenetically abnormal cells in their epithelial cells (micronucleus test), the level of benz(o)pyrene in their urine and mutant forms of *GSTM1* genes. In all cases, the examined workers were engaged in modern mechanized physical types of shift work in oil fields in northwest Siberia. 107 employees of the oil fields' administrative units who were not directly in contact with the oil production process served as a control group. The age of the oil workers was 38.6 ± 5.4 years, administrative and management personnel – 39.0 ± 4.2. According to the questionnaire data and medical records during the year the examined persons did not undergo X-ray procedures and had not suffered from viral infections. All urine samples were obtained at the end of the work shift. The level of benz(o)pyrene in the urine was determined by a fluorescent photometry analyzer FLUORAT-2M-92 [9]. Moreover, epithelial cell swab samples were taken from the buccal mouth epithelial. After the Carnoy fixation method, the swab samples were stained with Romanovsky-Giemsa dyes and analyzed for the presence of cells with micronuclei according to the procedure and criteria described previously [10]. Simultaneously, from the cell pellet DNA was derived in which we investigated the presence of mutations in GSTM1 gene. Primers were added to the sample amplification. Separation of GSTM1 gene amplification products was

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 4, Issue 1, July 2014

performed in 3% horizontal agarose gel, prepared in a single tris-borate buffer with the addition of ethidium bromide and visualization of the transmitted UV light. The electric field strength in the separation of DNA fragments was 1-8 V/cm. Normal alleles of genes were characterized by the presence of PCR products of *GSTM1*gene : homozygote *GSTM1* +/+ heterozygote and *GSTM1* +/0. Deletion ("zero") homozygous variants (*GSTM1* 0/0) were identified by the absence of *GSTM1* gene fragments. Genotype 0/0 means absence of the fragment on the electro phoregram, respectively; this individual is also homozygous for the deletion. The "+" sign indicates the presence of the fragment and a particular donor is either heterozygous or homozygous by the absence of deletions in genes. Preparations of buccal smears were analyzed by light microscopy ("Reichert GIM-358", Austria) equipped with binoculars and a digital camera "Casio QV-100" (Japan). The resulting images were transmitting to a QV-Link program supplied with the camera. Analysis of the size of micronuclei in buccal epithelium cells was conducted with the use of the ImageJ program (Wayne Rasband, USA). At least 10,000 interphase cells were viewed for each surveyed person, according to the criteria presented previously [10]. Statistical processing was conducted with the use of the statistical software package STATISTICA v.6.0, BIOSYS- 2, Microsoft Access, BIOSTAT (Primer of Biostatistic version 4.03). Linked loci haplotype frequencies for the *GSTM1* gene was calculated with the use of the EH software program, Rockefeller University, NY. All quantitative studies were processed with the use of a Student t-test for independent samples, as far as testing the distribution principle with the use of the Kolmogorov-Smirnov test showed no deviations from the norm. Analysis of statistical differences of qualitative characteristics was conducted with the use of the $\chi 2$ test with Yates' correction for continuity [11]. Differences of the compared results (X±m, where X is the arithmetic mean of the sample, and m is the arithmetic mean error) were considered significant at the obtained level of significance of $p < 0.05$.

III. RESULTS AND DISCUSSIONS

As evidenced by our data (see Table I), the number of buccal epithelial cells of the oral cavity of control group workers (administrative staff at oil fields) was practically the same in patients with mutant and "normal" *GSTM1* gene genotypes (1.6 ± 0.2 and 2.3 ± 0.6% correspondingly; $p > 0.05$).

At the same time, the oil workers' samples had more significant differences ($p < 0.01$). Besides, for the workers with the GSTM1gene genotype 0/ 0 the number of epithelial cells with micronuclei exceeded the reference level by almost 300% ($p < 0.01$). Significant differences are marked with respect to the "normal" genotype ($p < 0.01$).

Table I. The number of buccal epithelial cells of the oral cavity with micronuclei of those workers who have different allelic variants of GSTM1 gene and work in the oil fields of northern Siberia

Note: True significant differences from the control group are marked with an asterisk, and the differences between the results of individuals with a homozygous null genotype and individuals with

Surveyed group	The number of the subject's epithelial cells (in %) depending on the genotype of the GSTM1 gene	
	0/0	+/+ and 0/+
Administrative staff (control group)	2.3±0.6	1.6±0.2
Oil workers	6.8±0.8*	3.6±0.3*

genotypes are marked with (+ / +) and (0 / +) - dash, one dash at $P < 0.01$, two dashes at $P < 0.05$.

Analysis of the recorded micronuclei size showed (Fig.) that large micronuclei were predominantly observed in the samples of oil workers with the mutant genotype, while the small number of micronuclei was sharply reduced.

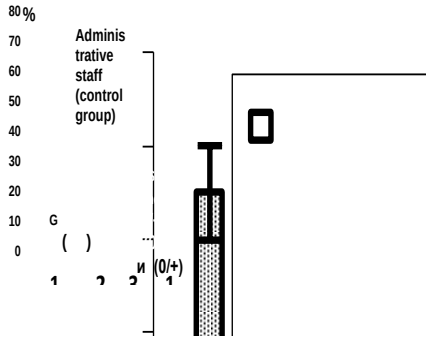


Fig 1. The number of individuals' epithelial cells with different sizes of micronuclei in the buccal epithelium of the oral cavity with different allelic variants of the GSTM1 gene, who work in the oil fields of northern Siberia (Note: Correlation coefficient values are marked at P <0.05.)

In other cases, the incidence of micronuclei with an area of more than 3 MK2 , 3 to 1 and less than 1 MK2 MK2 did not differ ($p > 0.05$). Determining the level of benz(a)pyrene in the oil workers' urine (see Table II) showed that this index exceeded the same index compared to the control group workers 6-8 times. In particular, this figure was raised in the sample of oil workers with the mutant genotype. A correlation analysis (see Table.III) revealed a reliable direct relationship between the excreted benz(o)pyrene and the number of cells with micronuclei ($p < 0.01$). In other cases, this pattern was not observed.

Table II. Level of benz(o)pyrene in the urine (ng / L) of patients with different allelic variants of GSTM1 gene who work in the oil fields of northern Siberia

Surveyed group	The level of benz(o)pyrene in urine depending on the subject's GSTM1 gene genotype.	
	0/0	+/+ and 0/+
Administrati	1.8±0.3	1.1±0.2

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 4, Issue 1, July 2014

ve staff (control group)		
Oil workers	11.3±0.7*	8.6±0.5*

Note: True significant differences from the control group are marked with an asterisk, and the differences between the results of individuals with a homozygous null genotype and individuals with genotypes are marked with (+ / +) and (0 / +) - dash, one dash at P <0.01, two dashes at P <0.05.

The obtained results suggest that the workers of oil fields, the bulk of whom were drillers working in direct contact with oil, had significantly increased levels of cells with micronuclei in the buccal epithelium of the oral cavity, which was not observed in the control group's samples. Analysis of micronuclei values held by a computer program IMAGE- 6 revealed a definite pattern. Most workers' micronuclei had an area of more than 3 MK2 , while in the control group, the cells with such micronuclei were significantly less observed (p <0.01).

Table III. Correlation analysis by comparing the level of benz(o)pyrene in urine and the number of epithelial cells with micronuclei of individuals with different allelic variants of GSTM1 gene, working in the oil fields of northern Siberia

Surveyed group	GSTM1 gene genotype	Correlation coefficient with micronucleus test indicators and the level of benz(o)pyrene in urine
Admini-strative staff (control group)	0/0	0.08
	+/+ and 0/+	0.03
Oil workers	0/0	0.21
	+/+ and 0/+	0.26**

Note: Correlation coefficient values are marked at P <0.05.

It is known that large micronuclei are formed mainly from lagging whole chromosomes [10]. Usually, this is the result of destruction of achromatin unit cell division threads. A rise in the number of aneuploid cells is observed under the influence of certain toxic hydrocarbons included in the oil and petroleum products [3], which is associated with depolymerisation of achromatin spindle cells filaments [9,12]. Apparently, our findings also indicate it with regard to the level of benz(o)pyrene excreted in urine that has significant clastogenic activity [3,6]. The presence of the mutant form of GSTM1 gene xenobiotic biotransformation in the oil workers' samples significantly increases the number of epithelial cells with micronuclei, at the same time the level of benz(o)pyrene excreted in urine was also increased . Correlation analysis leads to the conclusion that there is a significant direct correlation between these parameters. It would seem that the rise in the number of epithelial cells with micronuclei is a consequence of the high content of benz(o)pyrene entering the body in the conditions of oil fields of northern Siberia. In the scientific literature there is also evidence that shows that the null genotypes of glutathione -S-transferring enzyme (GSTM1 gene) are associated with higher levels of chromosomal aberrations [13]. Our data confirm this finding with respect to the null GSTM1 gene genotype. The workers with a GSTM1 null gene genotype were especially sensitive to the genotoxic factors of oil production conditions. An analysis of ethnicity of shift oil workers suggests that most of them were representatives of the Finno-Ugric people, or Finno-Ugric and slavic mestizo (mainly Russian) groups. According to the published data, the number of Russian people with a zero (mutant) genotype for this gene is 42.6 - 46.2%, whereas the same index among the Udmurt, Mordovians and Chuvash (Finno-Ugric group) is represented by more than 38% of shift oil workers in this region, and the frequency of such workers reaches 61.3% [14]. The number of oil workers in the oil fields of Western Siberia with a zero GSTM1 gene genotype, according to our data, was 23.8% , while in the control group, the same number was significantly higher - 55.8%. Since the zero genotype for this gene significantly increases the likelihood of serious diseases [13, 14], an "occupational selection" of people with this genotype in oil fields cannot be discounted. So, the analysis of working experience in the oil fields of Siberia showed that the number of oil workers with a GSTM1 gene null genotype in cases of 1-3 years professional experience is particularly high (46.2 %) and this figure falls almost 3 times (15.3%) in a group of persons who had worked for more than 10 years in oil production. There are studies showing the genotoxicity of smoking [15]. It was found that especially significant cytogenetic changes are observed in smokers with this zero GSTM1 gene genotype [16]. In our studies, an analysis of smokers' and non-smokers' numbers suggests that their frequency is almost the same in the examined groups of donors, ranging from 38.2 - 40.4%. At the same time, indeed, the level of the cytogenetic abnormalities of smokers with a zero GSTM gene genotype was significantly higher (p > 0.05) than the same level of non-smokers. The rise in the number of cytogenetic abnormalities of the oil workers may also be due to the high radioactive background in the field of oil production. In their work, Berezin and Gorbachev [19] emphasized that in the process of oil extraction, along with the reservoir water there also extracted oil sludge with a high content of radionuclide of the uranium and thorium series. In addition, in order to monitor the integrity of pipelines in the oil-gas enterprises complex X-ray and radionuclide methods are widely used, which can cause the personnel to encounter radiation doses exceeding the maximum permissible levels. It should also be noted that the northern Tyumen and Tomsk regions were repeatedly covered with fallout resulting from nuclear tests at the Novaya Zemlya test site and there was evidence of radioactivity of lichen and reindeer meat [20],

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 4, Issue 1, July 2014

which may also lead, in human cells, to the appearance of chromosomal aberrations that was evidenced by the results of cytogenetic examination of the indigenous people of northern Siberia [21]. Undoubtedly, in the extreme conditions of oil production in northwest Siberia there are many factors that can have both a mutagenic and co-mutagenic effect on the human genetic apparatus. In addition to the anthropogenic and natural factors, there are: low temperatures, the strong geomagnetic field of the auroral zone, geomagnetic anomalies, in particular the light mode (polar night and day) and a deficit of some essential trace elements [7].

IV. CONCLUSION

1. It was found that in the presence of the deleted (null) allele forms of the *GSTM1* gene genotype in a person working shifts in the north of Siberia, there is a rise in the number of cells with micronuclei in the buccal epithelium of the oral cavity, resulting from abnormalities in chromosome segregation during cell division, while in the urine of these people there is a high level of benz(o)pyrene, which has a distinctive mutagenic effect.

2. Increased levels of cytogenetic damage, identified in the survey of oil workers are, it would seem the result of the cumulative influence of various factors on the human genetic apparatus. In this case, it is not only the impact of oil mutagenic components, but also factors of the diverse nature of both natural and anthropogenic origin, characteristic of northern Siberia.

3. The results of the study also generate a conclusion about the urgent need for new science-based criteria for the occupational selection of labour resources in the oil fields of Western Siberia, in which the genotyping data for genes of xenobiotic biotransformation should have a significant role, for the GSTM1gene, in particular.

The article presents the results of research supported by a grant from the Russian Foundation for Humanities no. 13-06-00094 and 13-06-00709

REFERENCES

[1] N. Ilyinskikh, M. Medvedev, P. Potapov, L. Perepechaev, A. Urazaev, D. Kudryavtsev, Comprehensive study of various parameters of cytogenetic and physiological health status of workers in the oil industry, Gig . and Sanitation, vol. 12, pp. 18-21, 1989.

[2] N. Ilyinskikh, L. Perepechaev, D. Urazaev, D. Kudryavtsev, «Complex physiological and cytogenetic examination of workers - Petroleum and dispensary problem» . In the book " Professional pathology in the eastern regions of the country, questions and medical examination of working, Novokuznetsk University Press, pp. 134 – 136, 1988.

[3] S.De Ftona, K.E.Wetterhahn, Mechanisms of benzol metabolism and genotoxicity, Life Chem. Rep. vol. 7, no. 1, pp. 169-244, 1989.

[4] Y.J.Kim, J.Y.Choi, D.Paek, Association of the NQO1, MPO, and XRCC1 polymorphisms and chromosome damage among workers at a petroleum refinery, J. Toxicol. Environ. Health, vol. 71, no. 5, pp. 333-341, 2008.

[5] A.M. Khalil, Chromosome aberrations in blood lymphocytes from petroleum refinery workers, Arch. Environ. Contam. Toxicol. vol. 28, no. 2, pp. 236-239, 1995.

[6] P.Wu, C.Loh, L.Hsieh, T.Liu, C.Chen, S. Liou, Clastogenic effect for cigarette smoking but not areca quid chewing as measured by micronuclei in exfoliated buccal mucosal cells, Mut. Res. Gen. Toxic. and Environ. Mutagenesis, vol. 562, no.1, pp. 27-38, 2004.

[7] A. Sobakin, The efficiency of the personnel of the gas fields in the extreme conditions of the North, Novosibirsk University Press, 2004.

[8] G. Kharlampovich, V. Churkin, Phenols, Chemicals Publishing House, 1974.

[9] A. Daly, Molecular basis of polymorphic drug metabolism, J.Med.Genet. vol.73, no. 1, pp 539-553, 1974.

[10] N. Ilyinskikh, V. Novitsky , I. Ilyinskikh, Micronucleus assay and cytogenetic instability. Publishing House of Tomsk University, 1992.

[11] V. Borovikov, I. Borovikov, Statistical analysis and data processing for Windows. Moscow: Publishing House " Filin ", 1997.

[12] N. Ilyinskikh, I. Ilyinskikh, E. Bocharov, Cytogenetic homeostasis and immunity. Novosibirsk: Publishing House “Nauka”, 1986.

[13] B. Baranov, T. Ivashchenko, N. Swede, M.Yarmolinskaya, S. Sel'kov, S. Gorbushin, G. Sawicki, P. Malet ,M.Canis, M. Bruhat, E. Baranova, Genetic predisposing factors and therapy of endometriosis, Genetics, vol. 35, no.2, pp. 243- 248, 1999.

[14] M. Larramendy, S. Knuutila, Increased frequency of micronuclei in B and T8 lymphocytes from smokers, Mutat Res. vol. 259, no.2, pp.189-195, 1991.

[15] R.Scarpato, A.Hirvonen, L.Migliore, Influence of GSTM1 and GSTT1 polymorphisms on the frequency of chromosome aberrations in lymphocytes of smokers and pesticide-exposed greenhouse workers, Mutat Res. vol.389, no. 2, pp.227- 235, 1997.

[16] A.A.Edwards, D.C. Lloid, J.S.Prosser, Chromosome aberrations in human lymphocytes a radiobiological review, London: Academic Press, 2000, 432 p.

[17] N. Ilyinskikh, V. Bulatov, A. Adam, B. Smirnov, N. Plotnikov, I. Ivanchuk. Radiation eco-genetics of Russia. Tomsk University Press, 1998.

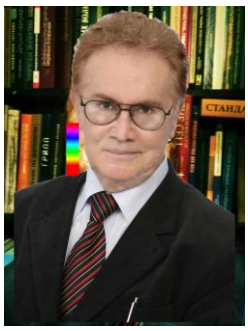
[18] I. Berezin , D. Gorbachev, Radiation- hygienic aspects of labor on oil and gas companies, Basic Science and Practice, vol.1, no.3, pp.12-15, 2010.

[19] B. Shcherbov, V. Stakovenko, L. Osipova, Cesium-137 in fur and reindeer moss as a radioactive pollution in Western Siberia, Scientific conference " Actual problems of geology and geography of Siberia." Tomsk University Press, vol. 3, 302-304.

[20] N. Ilyinskikh, L. Ogorodova, L. Bezrukov. Epidemiological genetic toxicology of heavy metals and human health, Siberian Medical University Press, 2003.

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 4, Issue 1, July 2014

AUTHOR BIOGRAPHY



Prof. Nicolai N. Ilyinskikh obtained his DSc in Genetucs. Head of Biology and Genetics Department at Siberian State Medical University (Tomsk, Russian Federation) and the Professor of Environmental Health Department of Tomsk State University. He is the author of 16 monographs and more than 600 articles in the field of cytogenetics and environmental epidemiology.
E-mail: nauka-tomsk@yandex.ru

Prof. (Mrs) Ekaterina N. Ilyinskikh has obtained her Ph.D. degree and D.Sc in Pathological Physiology and Infectious Diseases from Siberian Medical State University (Tomsk, Russian Federation). She is working as a Professor of Infectious Diseases and Epidemiology Department at Siberian State Medical University. She is author of 5 books and more than 54 research papers including 15 articles in the international journals in the field of cellular biology, environmental epidemiology and infectious diseases.
E-mail: infconf2009@mail.ru

Prof. **Anna Yankovskaya** obtained her DSc in Computer Science from the Tomsk State University in Russia. She is currently a head of the Intelligent Systems Laboratory and a professor of the Applied Mathematics Department at Tomsk State University of Architecture and Building and professor of the Computer Science Department at Tomsk State University and a professor of Tomsk State University of Control Systems and Radio electronics. She is the author of more than 600 publications and 6 monographs. Her scientific interests include mathematical foundations for test pattern recognition and theory of digital devices; artificial intellect; logical tests, cognitive graphics; advanced technology in education. E-mail: ayyankov@gmail.com