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GENERAL GENETICS

Buccal Micronucleus Cytome Assay for the Evaluation of Cytogenetic Status of Healthcare Professionals Contacting with Anti-Cancer Drugs

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Abstract—Healthcare professionals of chemotherapy departments are in a regular contact with anticancer drugs that have genotoxic properties. The analysis of the cytogenetic status of healthcare professionals (54 individuals) was for the first time carried out using a new promising noninvasive method, buccal micronucleus cytome assay with the calculation of integral indices. The effect was estimated with different duration of exposure to chemotherapy drugs (after an employee's vacation, one month after vacation, and before vacation). Statistically significant increases in almost all studied indices of cytogenetic damages, proliferation, and apoptosis as compared with the control group of office staff (47 individuals) were detected. Among healthcare professionals, an increase in the portion of individuals with an increased level of cytogenetic abnormalities with the maximum exposure duration from 24 to 38% (11% in the control) was established. Among cancer patients (9 individuals), the portion of individuals with a high level of cytogenetic abnormalities was 56% before the next course of chemotherapy and 87% after it.

Keywords: buccal micronucleus cytome assay, anticancer drugs, cytogenetic damages, healthcare professionals of chemotherapy departments, patients with cancer

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INTRODUCTION

The problem of risk of developing cancer in oncology healthcare professionals was raised in 1970s after conducting the first epidemiological studies. Since the development of oncogenic processes under the action of different factors is mainly associated with DNA damage, the experimental studies were started and genotoxic properties of a number of drugs used in chemotherapy of cancer patients were identified. At present, according to the classification of the International Agency for Research on Cancer (IARC), 14 anticancer chemotherapy drugs, exhibiting, among other things, a genotoxic effect, are classified as human carcinogens [1]. Cyclophosphamide, azathioprine, busulfan, chlorambucil, etoposide, and melphalan are proven carcinogens (group 1); azacytidine, adriamycin, cisplatin, thiotepa, treosulfan, and semustine are probable carcinogens (group 2A); chlorozotocin, bleomycin, dacarbazine, daunomycin, and mitoxantrol are possible carcinogens (group 2B). These drugs are used not only in the treatment of cancer but also in rheumatology, transplantology, gynecology, urology, surgery, and ophthalmology. Pharmacists, as well as employees working in the field of carcinogenesis and experimental chemotherapy, have direct contact with anticancer drugs. Despite compliance with the recommended safety measures, it is not possible to avoid the contamination of the production environment with these drugs on the premises of medical institutions. An increase in the extent of use and expansion of the spectrum of anticancer drugs and their combinations lead to an increase in the oncological risk in healthcare professionals, which was demonstrated in a number of foreign studies [2–4] and single studies in Russia [5, 6]. Another negative effect of anticancer drugs (their effect on the reproductive function) should also be taken into account. An increase in the risk of congen-

ital malformations in a child by 3.3 times with a professional exposure of the mother to cytostatics before the conception was demonstrated [7]. A statistically significant increase in the risk of spontaneous abortions was demonstrated in the study [8].

A search for the methods for early diagnosis of cytogenetic abnormalities (which are considered to be predictors of carcinogenesis) is an urgent problem of genotoxicology. And such diagnosis is of paramount importance when examining an individual. The methods based on the detection of chromosomal aberrations, micronuclei, and DNA damage in peripheral blood lymphocytes are used to estimate the carcinogenic hazard under the action of genotoxic agents on people. However, these methods require blood sampling from a vein (that is, they are invasive), as well as the cultivation of lymphocytes using the expensive reagents. In addition, many chemical compounds affecting a person have cytostatic or cytotoxic effects, which complicates the cultivation process. As an alternative, a noninvasive (without blood sampling) micronucleus method on epithelial cells of the buccal mucosa (which does not require cultivation) is increasingly used to estimate the chromosomal damages. The epithelium is of a special interest, since it is the first barrier on the way of influence of the factors coming with water, food, and air, while its active proliferation is provided by an intense blood supply. At the same time, blood-borne genotoxic compounds or their metabolites also affect epitheliocytes.

To date, we improved a micronucleus test on buccal epithelium and supplemented it with the analysis of a wide range of the state of the nucleus of exfoliative cells by cytogenetic indices and proliferation and apoptosis indices [9, 10]. Simultaneously, this extended version of the method was accepted by the international community. In 2007, the HUMNxL project was launched to harmonize studies using the micronucleus test. At present, this approach is generally accepted and was called "buccal micronucleus cytome assay" (BMCA) [11]. To evaluate BMCA, we for the first time included integral indices of cytogenetic damages, proliferation, and apoptosis and determination of the level of cytogenetic stress depending on the excess of the approximate normative values and complex index of accumulation of cytogenetic damage, which combines the whole spectrum of indices of the cell nucleus structure [12–14].

In this regard, the aim of this study was to estimate the cytogenetic status of healthcare professionals under conditions of professional exposure of different duration to anticancer drugs using a new promising method (buccal micronucleus cytome assay, BMCA) in an extended version.

MATERIALS AND METHODS

The study was carried out in accordance with WHO Guidelines [15] according to our published methodological recommendations [13].

The smears of buccal epithelium of the employees of the Blokhin National Medical Research Center of Oncology (Ministry of Health of the Russian Federation) who have regular contact with anticancer drugs were studied. Physicians and nurses, a total of 54 employees (49 women and 5 men) aged from 19 to 63, participated in the study. A comparison of the exposure group with negative and positive controls and a conjugate sample approach, which means the analysis of changes in the index in the same group of individuals before and after the effect of any factor, were used (Table 1). The biological material was taken at three time points in each subject from the group of healthcare professionals: at a minimal exposure, on the first working day after vacation (group 2); one month of active work after vacation (group 3); at the point of maximal exposure to anticancer drugs (a day before vacation) (group 4). The vacation of employees involved in the study was 14–28 calendar days. The employees of Moscow office, 47 women aged up to 25–40 years having no contact with genotoxic compounds, conditionally healthy at the time of examination, were included in the comparison group (group 1, hereinafter negative control). The evaluation of the cytogenetic status of nine oncological patients (women aged from 40 to 64 suffering from breast cancer, ovarian cancer, or uterine body cancer) before and one week after the treatment with cytostatics (cyclophosphamide and cisplatin) was also conducted (groups 5 and 6, respectively). These groups served as a positive control. Informed consent was obtained from each subject for this noninvasive examination and the corresponding questionnaire was completed.

Microscopic preparations of buccal mucosa cells were prepared according to [13]: scraping on both sides from inside of the cheek was done with a sterile wooden spatula, applied to glass slides, and dried. The preparations were fixed in ethanol–acetic acid (3 : 1); DNA was stained with acetoorcein; cytoplasm was stained light green. Microscopic analysis was performed on encrypted preparations; 1000 cells from each individual were calculated at magnification $\times 1000$; the indices of cytogenetic and cytotoxic effect were estimated. Previously, the biological significance, classification, and criteria for determining these indices were described [9]. Each of the analyzed cells was assigned to the following categories: cells are normal; cells with cytogenetic damages (micronuclei, protrusions, atypical nuclei); cells with two nuclei (isolated, double) that are indices of impaired proliferation; cells at an early stage of nuclear destruction (with perinuclear vacuole, with nuclear membrane damage, chromatin condensation, early karyolysis) and at a late stage of nuclear destruction (with karyorrhexis,

Table 1. BMCA indices in the groups of healthcare professionals in contact with anticancer drugs and controls, *X*av. ± *m* (95% CI)

Indices	Group 1, negative control, 47 individuals	Group 2, healthcare professionals after vacation, 54 individuals	Group 3, healthcare professionals one month after vacation, 49 individuals	Group 4, healthcare professionals before vacation	Group 5, positive control, cancer patients before treatment, 9 individuals	Group 6, positive control, cancer patients after treatment, 8 individuals
Integral index of cytogenetic abnormalities, ‰ (Icyt)[0–5]	0.53 ± 0.14 (0.25–0.82)	1.41 ± 0.19 (1.02–1.80)	1.45 ± 0.19 (1.06–1.83)	2.02 ± 0.28 (1.46–2.58)	5.78 ± 1.89* (1.41–10.14)	16.4 ± 5.69* (2.93–29.8)
Integral index of proliferation (Ipr), ‰ [0–8]	1.95 ± 0.25 (1.45–2.46)	7.0 ± 0.53 (5.93–8.07)	7.31 ± 0.67 (5.96–8.65)	7.78 ± 0.66 (6.45–9.10)	11.9 ± 3.33 (4.23–19.6)	67.6 ± 33.1* (–10.7–146.0)
Apoptotic index (Iapop), ‰ [0–600]	167.1 ± 11.0 (145.0–189.3)	373.3 ± 12.7 (347.9–398.7)	375.7 ± 11.8 (352.0–11.8)	402.4 ± 12.7 (376.9–427.8)	369.9 ± 55.9 (240.9–498.9)	293.6 ± 31.87* (218.3–369.0)
Index of accumulation of cytogenetic damages (Iac)	1.90 ± 0.31 (1.28–2.53)	3.48 ± 0.47 (2.55–4.43)	3.90 ± 0.83 (2.34–5.56)	5.10 ± 0.95 (3.18–7.02)	28.6 ± 13.8* (–3.19–60.3)	1101 ± 821.4* (–841.1–3043)
Level of cytogenetic stress in points	1.13 ± 0.05 (1.03–1.23)	1.70 ± 0.07 (1.57–1.84)	1.55 ± 0.10* (1.36–1.75)	1.86 ± 0.08 (1.70–2.02)	2.33 ± 0.24* (1.79–2.88)	2.75 ± 0.16* (2.36–3.14)

All studied indices in the negative control group differ statistically significantly from the appropriate indices in other groups according to Mann–Whitney *U* criterion at $p < 0.05$. When comparing with group 4, * $p < 0.05$.

pyknosis, complete karyolysis), which are indices of physiological cell death. The integral index of cytogenetic abnormalities (Icyt) (cells with micronuclei and protrusions total), integral index of proliferation (Ipr) (cells with two nuclei and double nuclei total), and apoptotic index (Iapop), which includes the cells at early and late stage of nucleus destruction, except for the cells with perinuclear vacuole and nuclear membrane damage, were also determined. A complex evaluation of the cytogenetic status was carried out according to two indices: level of cytogenetic stress and index of accumulation of cytogenetic damage. The level of cytogenetic stress was determined for each individual in points depending on the excess of approximate normative values (ANV) [13]. In the absence of excess of ANV, the index is 1 (low level); when any or several indices of proliferation or destruction of the cell nucleus are exceeded, the index is 2 (permissible level); if there is an excess in cytogenetic indices (the most dangerous), the index is 3 (high level). According to the average group values, the following levels of cytogenetic stress can be allocated: 1, low level (no excess of ANV in all individuals in the group); ≤2, permissible level (there is an excess of ANV in some of the subjects in the group); >2, high level (there is an excess of ANV in the majority of the subjects in the group, predominantly by cytogenetic indices). The previously proposed index of accumulation of cytogenetic damage (Iac) [12] was calculated according to the formula $Iac = (Icyt \times Ipr/Iapop) \times 100$, where the integral index of cytogenetic abnormalities (Icyt) and integral index of proliferation (Ipr) are in the numerator and the apoptotic index (Iapop) is in the denominator. This index makes it possible to characterize human cytogenetic status and, unlike the index "frequency of cells with micronuclei," takes into account the ratio between the frequency of cytogenetic abnormalities and "intensity" of cell kinetics. Determination of the index of accumulation of cytogenetic damage makes it possible to distinguish three risk groups: low ($Iac \leq 2$), moderate ($2 < Iac < 4$), and high ($Iac \geq 4$).

The statistical analysis of data was performed using the computer programs Excel and Statistica 10.0 for Windows. The comparison of data by the groups was performed using the Mann–Whitney criterion. Differences were considered significant at $p < 0.05$.

RESULTS

Comparison of Cytogenetic Status of Healthcare Professionals in Groups with Different Exposure to Anticancer Drugs

The average values of the frequency of cells with micronuclei were 0.64, 0.71, and 0.9‰ in groups 2–4. According to international HUMNxL project, the background frequency of buccal cells with micronuclei is 1.1‰ [11]; that is, the frequency of this index in healthcare professionals does not exceed the background level. The cells with nuclear protrusions were 0.76, 0.73, and 1.12‰; Icyt was 1.41, 1.45, and 2.02‰ (Table 1). It is obvious that the average values increase with increasing load; however, differences between the groups are statistically insignificant.

The average values of Ipr were 7.0, 7.31, and 7.78‰ in the group after vacation, one month after vacation, and before vacation, respectively. They did not differ statistically significantly and did not exceed ANV.

Close values of the indices of early and late stages of cell nucleus destruction were determined at three exposure times that slightly increased by the last time; however, the differences were not statistically significant. On average, these values did not exceed ANV, except for apoptosis (to a very small extent). The apoptotic index was increased by 7% (statistically insignificant) with the largest exposure duration.

The level of cytogenetic stress was slightly increased at a maximum duration of exposure and corresponded to 1.70, 1.55, and 1.86 in groups 2–4 ($p < 0.05$). It was determined as permissible (not low). The most pronounced changes were noted by the index of accumulation of cytogenetic damage, which was 3.48, 3.90, and 5.10. An excess of the effect at the last exposure time was 46%. Moreover, considering this index, one can characterize healthcare professionals examined immediately and one month after vacation as a group of moderate risk of the effect on cytogenetic status, while the same group examined before vacation is a high-risk group [13].

Comparison of Cytogenetic Status of Healthcare Professionals and Negative Control Group

The average values of the whole spectrum of cytogenetic status indices in group 1 (negative control) differ statistically significantly from the appropriate indices in all other groups according to the Mann–Whitney *U*-criterion at $p < 0.05$. The average values of the frequency of cells with micronuclei in this group were 0.3‰; with nuclear protrusions, 0.23‰; Icyt, 0.53‰. The average values of these indices in groups 2–4 of healthcare professionals were significantly higher ($p < 0.05$).

Ipr corresponded to 1.95‰ in the control and 7.0, 7.31, and 7.78‰ in groups 2–4 ($p < 0.05$); at the same time, no excess of ANV was noted. The level of indices of cell nucleus destruction in group 1 was 2–3 times lower than in group 2 of healthcare professionals as compared with the negative control group (minimal level of exposure). This also refers to the apoptotic index in general, which characterizes faster elimination of the cells with cytogenetic abnormalities in the group of healthcare professionals ($p < 0.001$).

A low level of cytogenetic stress was noted both in the control and in group 2 (1.13 and 1.70), but the portion of individuals who had an increase in the fre-

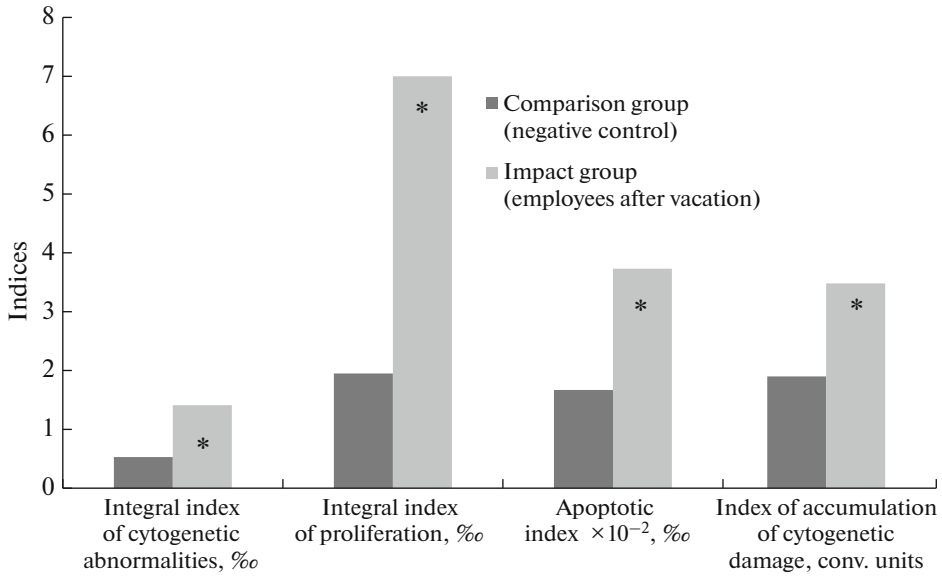

Fig. 1. Average values of integral indices of cytogenetic status. Asterisk, $p < 0.05$.

quency of cytotoxic effect indices is still higher among healthcare professionals than in the control (Fig. 1).

Iac was 1.9 in group 1, while it increased in healthcare professionals from 3.48 to 3.90 (permissible level of risk) and 5.1 (high level of risk) with an increase in exposure ($p < 0.01$).

Comparison of Cytogenetic Status of Cancer Patients before and after Taking Anticancer Drugs

As a positive control, the cytogenetic status was determined in nine cancer patients before and one week after a course of treatment with cytostatics. The average values of the frequency of cells with micronuclei in cancer patients before and after the treatment were 5.22 and 9.37‰ ($p > 0.05$); with protrusions, 0.56 and 7.0‰ ($p < 0.05$); Icyt, 5.78 and 16.4‰ ($p < 0.05$), respectively. It is obvious that the average values significantly increase after the treatment of patients with cytostatics, and this is logical, since both drugs they received (cyclophosphamide and cisplatin) are characterized by a strong mutagenic effect.

The frequency of cells with two nuclei was 8.0 and 16.6‰ (higher than ANV); cells with three, four, five nuclei or even groups of 7–8 small nuclei of approximately the same size were noted. The frequency of cells with double nuclei was even higher (3.9 and 51‰). Some cells have multiple nuclear abnormalities (separate and double lying nuclei, protrusions, and micronuclei). Such pictures are typical of the processes of significant disturbances of nuclear fission and damage to the spindle and DNA. Ipr was 11.9 in patients before the treatment and 67.6 after the treatment (which significantly exceeds ANV). This phenomenon is typical of the drugs characterized by a cytostatic effect.

A decrease in almost all apoptosis indices was noted in patients after the treatment: the frequency of cells with chromatin condensation (from 207.3 to 184.2‰), with the beginning of karyolysis (from 53.3 to 15.0‰), with apoptotic bodies (from 16.3 to 5.5‰), with pycnosis (from 94.7 to 74.0‰), Iapop in general (from 369.9 to 293.6‰). The level of cytogenetic stress is high and is 2.33 and 2.75 in patients before and after the treatment ($p > 0.05$). Iac in cancer patients after the treatment was 1101, which is 40 times higher than the Iac value in the same patients before the therapy (28.6, $p = 0.075$). In general, a significant deterioration of cytogenetic status was noted in cancer patients one week after the treatment.

Comparison of Cytogenetic Status of Healthcare Professionals and Positive Control Groups

Group 4 (healthcare professionals with the longest exposure) was compared with the groups 5 and 6 (cancer patients before and after the treatment). The average values of the frequency of cells with micronuclei in the group of healthcare professionals and cancer patients were 0.90, 5.22, 9.37‰ ($p < 0.05$); the average values of the frequency of cells with nuclear protrusions were 1.12, 0.56, and 7.0‰; the values of Icyt were 2.02, 5.78, and 16.4‰, respectively (Table 1). It is obvious that indices in the groups of cancer patients are significantly higher, especially in the group of patients after the treatment ($p < 0.001$). Ipr was 7.8, 11.89, and 67.6‰, respectively. It significantly

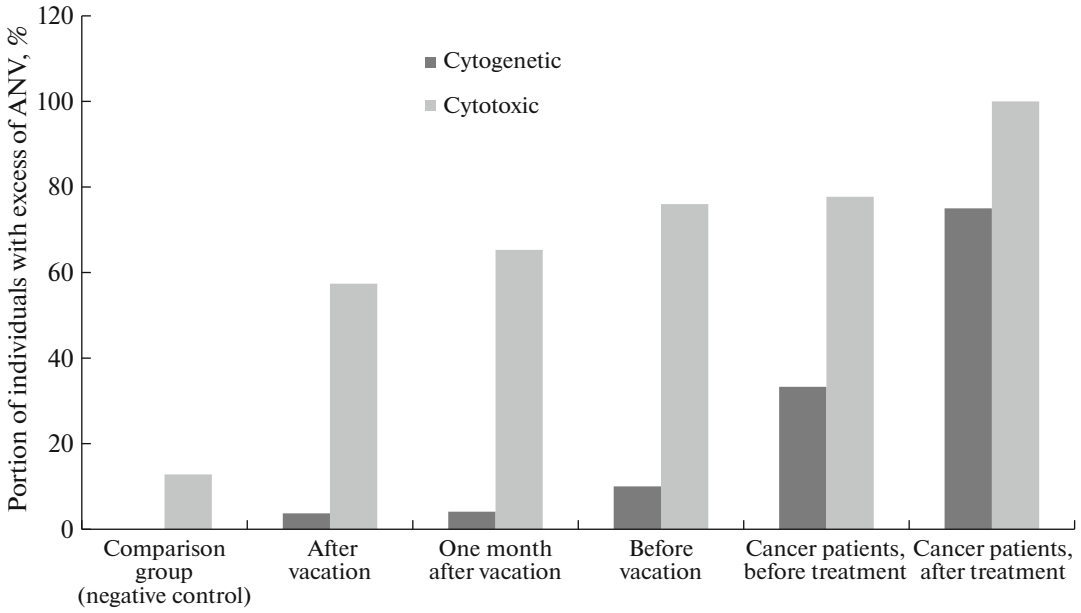

Fig. 2. Portion of individuals with an excess of ANV by cytogenetic and cytotoxic indices.

exceeded ANV in the group of cancer patients. The level of indices of cell nucleus destruction (including apoptotic index) was higher in the group of healthcare professionals.

The level of cytogenetic stress was 1.86, 2.33, and 2.75; that is, the portion of individuals who have an excess in the frequency of indices of cytotoxic effect is greater in the group of cancer patients. Iac in the group of cancer patients is 5.6 and 220 times higher than in healthcare professionals.

The results of comparing the groups by the portion of individuals with an excess of ANV by cytogenetic and cytotoxic indices are given in Fig. 2. An increase in the portion of individuals with an excess of ANV by cytogenetic and cytotoxic indices in the series of groups 1–6 was noted.

DISCUSSION

According to the aim of this study, it was necessary to characterize most completely the cytogenetic status of healthcare professionals who have contact with anticancer drugs. For this, we used three approaches: we compared the groups with and without contact with these drugs; we determined the cytogenetic status of healthcare professionals of the same group with different exposure duration; we determined the cytogenetic status of patients in the same department before and one week after taking anticancer drugs and performed a comparison with the indices of healthcare professionals.

The average values of the indices of cytogenetic stress, proliferation, and apoptosis disorders in groups 1–4 are in general in the range determined for a number of populations that we and other research groups examined [14–16]. However, higher levels of the indices ($p < 0.05$) were determined in the group of healthcare professionals as compared with those in the office staff even at a minimal duration of exposure.

When analyzing the exposure duration, the level of cytogenetic stress was statistically significantly increased in the group with a maximal duration of exposure. The portion of individuals with a high risk of cytogenetic abnormalities during the examination before vacation (maximal exposure duration) was 38%, which was 1.6 times higher as compared with the groups after vacation (Fig. 3). It should be taken into account that buccal epithelium is regenerated in 7–10 days; that is, the cells predominantly not exposed to the effect of negative production factors were collected in healthcare professionals after vacation. When working with anticancer drugs, such an effect is possible, despite the compliance with recommended security measures. According to the published data, anticancer drugs are detected in the air and on the surfaces of the premises in medical institutions [17, 18]. Meta-analysis demonstrated the presence of residual quantities of at least one drug on the premises of a hospital [19]. Trace amounts of drugs were detected in the places of preparation of solutions and on dishes and packages [20]. Moreover, secondary packaging of vials does not prevent contamination [21]. Studying the stability of some drugs (methotrexate, cyclophosphamide, 5-fluorouracil, paclitaxel) demonstrated that they remain active even 6 days after entering the environment [22]. Trace amounts of anticancer drugs were found in urine

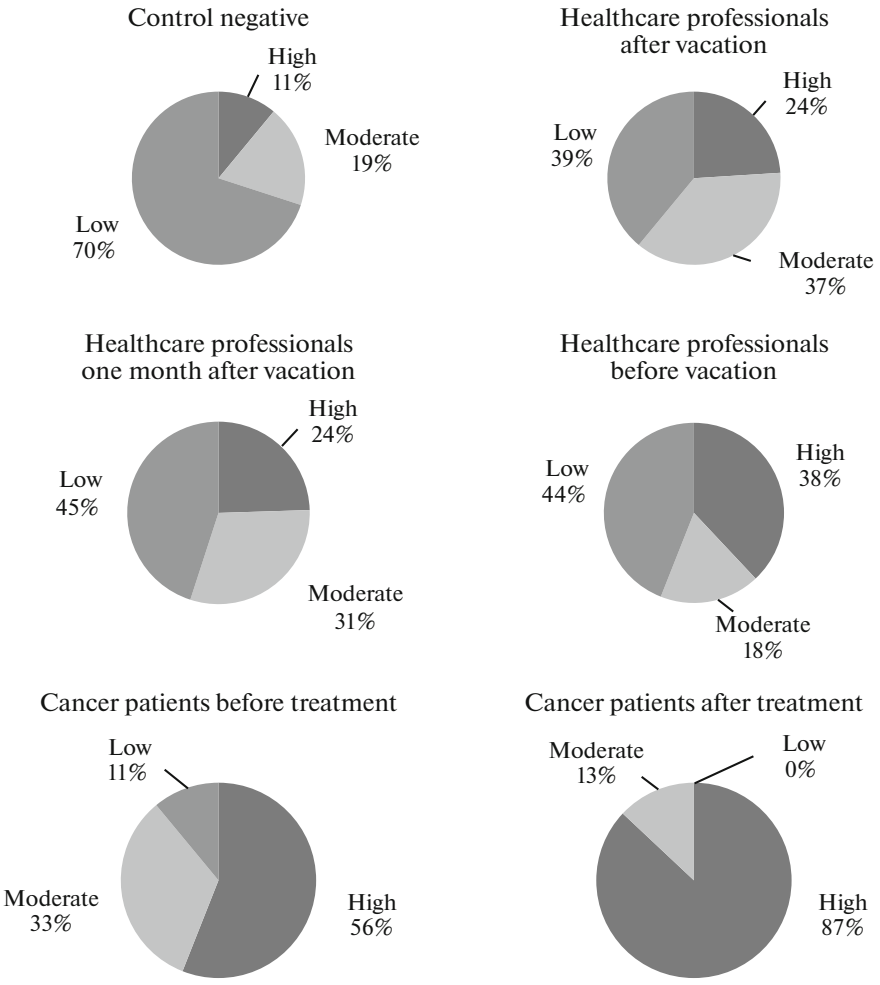

Fig. 3. Portion of individuals with low, moderate (lightest color), and high (darkest color) risk of cytogenetic damage in the examined groups according to the index of accumulation of cytogenetic damage.

tests of healthcare professionals [20]. But since the effective concentrations of drugs are extremely small, it is quite difficult to detect a genotoxic effect in healthcare professionals, as our study demonstrated.

Meta-analysis of studies examining the cytogenetic status of healthcare professionals having contact with anticancer drugs (presented in the publication [23]) demonstrated a significant increase in the level of chromosome aberrations in peripheral blood lymphocytes as compared with the control. The same conclusion follows from the publication [24], in which meta-analysis on the evaluation of the cytogenetic status of healthcare professionals using a micronucleus test on peripheral blood lymphocytes is presented. An increased frequency of lymphocytes with micronuclei was found in 15 out of 24 studies (1988–2015). In 16 studies, other indices of the cytogenetic effect of the drugs (chromosome aberrations, sister chromatid exchanges, DNA comets, micronuclei in buccal epitheliocytes) were additionally determined. A good agreement between the results of genotoxic tests was noted. Our studies coincide with the studies carried out in Italy [25–27]. The frequency of buccal epitheliocytes with micronuclei in the examined nurses in the oncology clinic was 0.89 and 0.94‰ (day nurses and department nurses), which differed significantly from the control (0.46‰). An increase in the frequency of buccal epitheliocytes with micronuclei was also detected in the studies [28–31]; however, this index was high even in the control (1.6, 2.7, 3.4‰). This can be associated with the staining preparations according to the Giemsa method, in which it is difficult to distinguish between micronuclei and bacterial cells almost always present in the oral cavity. In the study [32], no effect of anticancer drugs was detected: the frequency of cells with micronuclei in the group of exposed nurses was 0.89‰ (0.9‰ in our study); in the control, 0.84‰. At the same time, the control group was represented by hospital employees who could have come in contact with other genotoxic com-

pounds. Taking into account the indicated peculiarities of the studies, we can note that our data are in good agreement with the results of other study groups. At the same time, our approach has certain advantages. As compared with the chromosome aberrations, the analysis of micronuclei makes it possible to detect the cytogenetic effect of not only clastogens but also aneugens. The analysis of buccal epithelium has broad prospects owing to noninvasive sampling of material, no need to cultivate the cells, and the ability to analyze a wide range of states of the cell nucleus, including proliferative disorder and apoptosis. We for the first time applied such advanced cytogenetic analysis, confirming and expanding ideas about the effect of the conditions of working with anticancer drugs on the cytogenetic status of healthcare professionals.

An extremely high Iac was noted in the positive control group. At the same time, the portion of patients with a high risk of accumulation of cytogenetic abnormalities was 56 and 87%, respectively. An increased level of cytogenetic abnormalities in the buccal epithelium of cancer patients was noted in a number of publications [33], but in this study, we present a more complete picture with determination of integral indices in accordance with our approach. The analysis of this group before and after the treatment demonstrates that the cytogenetic status of cancer patients is significantly impaired; these impairments are determined not only in the tumors but also in other tissues of the body. In such patients, both exogenous genotoxicants (different drugs) and endogenous genotoxic metabolites are apparently present in the body.

In the process of evolution, a hierarchy of protective systems was formed in eukaryotic cells, including barriers to the entry of toxicants, their detoxification, repair of DNA damage, and other high molecular weight compounds. Moreover, each organism has its own peculiarities associated with polymorphism of genes. However, an increase in the frequency of oncopathology noted in the world, apparently, depends on the breakdown of adaptive mechanisms and disturbances in the stability of the cellular genome. BMCA, which makes it possible to carry out such monitoring noninvasively, has good prospects for identification of risk groups by the level of cytogenetic abnormalities and prediction of a carcinogenic effect, and most importantly for identification of individuals with a high index of accumulation of cytogenetic damage.

The study of the health status of healthcare professionals in contact with anticancer drugs was carried out by identifying early predictors of unfavorable changes, namely, indices of cytogenetic status using BMCA. This is especially relevant, since the drugs possessing genotoxicity and cytostatic and carcinogenic effects are the main drugs used by employees of chemotherapy departments. The group of healthcare professionals can be characterized as a group with a permissible level of cytogenetic stress and with a high level of risk according to the index of accumulation of cytogenetic damage, which significantly distinguishes it from the negative control group. This is also confirmed by determination of the portion of individuals with an excess of ANV (77% as compared with 13% in the control group), as well as by determination of the portion of individuals with a high index of accumulation of cytogenetic damage (38% against 11%). The results demonstrate that healthcare professionals are exposed to the effect of carcinogenic factors in a production environment and require the additional security measures reducing the risk of the adverse effect of anticancer drugs. At the same time, BMCA can be routinely used for cytogenetic monitoring of healthcare professionals to identify individuals with an increased risk of accumulation of cytogenetic damage.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in the study involving human participants are in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from each of the participants involved in the study.

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