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Karyopathological Traits of Thyrocytes and Exposure to Radioiodines in Belarusian Children and Adolescents following the Accident at the Chernobyl Nuclear Power Plant

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Abstract

The Belarus-American (BelAm) Thyroid Study cohort consists of persons 0–18 years of age at the time of exposure to radioiodines from the 1986 Chernobyl nuclear power plant accident who have undergone serial thyroid screenings with referral for fine-needle aspiration biopsy (FNAB) using standardized criteria. We investigated thyrocyte nuclear abnormalities in cytological samples from FNABs in 50 BelAm subjects with thyroid nodules and 43 control patients from Leningrad, Russia, unexposed to Chernobyl fallout. Nuclear abnormalities such as internuclear chromosome bridges and derivative nuclei with broken bridges (i.e., “tailed” nuclei), formed from dicentric and ring chromosomes, may be cellular markers of radiation exposure. In the exposed BelAm cohort, thyrocytes with bridges were present in 80% of subjects with single-nodular goiters compared with 27% of unexposed controls. The average frequency of thyrocytes with bridges was also significantly higher in the BelAm subjects than in controls as was the mean frequency of thyrocytes with tailed nuclei. In the case of multi-nodular goiters, thyrocytes with bridges were present in 75.0% of exposed BelAm patients compared with 16.7% of unexposed controls. Thyrocytes with tailed nuclei were observed in all of the BelAm subjects but in only 35% of controls, and the mean frequency of tailed nuclei was significantly higher. Unusually long bridges were detected in 29% of BelAm patients with single-nodular goiters and 35% of cases with multi-nodular goiters, while no such abnormalities were observed in the follicular thyroid epithelium of patients from the Leningrad region. Further study is needed to understand whether these phenomena represent irradiation consequences in the human organism.

Keywords

thyroid gland; thyrocytes; nuclear abnormalities; Chernobyl accident

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Introduction

In the countries most affected by the Chernobyl nuclear accident in April of 1986, the elevated risk for thyroid cancer in exposed children and adolescents is the best documented and most significant health consequence observed to date (Bennett et al. 2006; Rozhko et al. 2008; Tronko et al. 1999). In 1996, the Belarus-American Thyroid Study (BelAm) was established to screen for a range of thyroid pathologies in subjects throughout the country who were 0–18 years old at the time of exposure and had thyroid radioactivity measurements taken within two months after the accident (Stezhko et al. 2004).

Fallout from Chernobyl was primarily to iodine radioisotopes, with Iodine-131 (I-131) being the most common (Likhtarev et al. 2006). Because the thyroid gland is an organ with rich blood flow, and thyroid cells have iodine pumps that transport iodine ions from blood into thyroid tissue, there is a possibility that radioiodines accumulated following the accident may have induced pathologic changes in thyrocytes. Internuclear chromosome bridges and “tailed” nuclei - broken bridge fragments – are considered likely cytopathological effects of radiation exposure (Kravtsov et al. 2000; Kuzovatov et al. 2000) as these abnormalities are formed from dicentric and centric ring chromosomes, which are established markers of radiation exposure (IAEA 2011). The proposed mechanism for development of internuclear bridges from dicentric and ring chromosomes involves pathologic mitoses in which, due to a failure of cell division, nuclear membranes are formed and persist throughout interphase. Tailed nuclei, considered to be derived from nuclei with broken bridges, are defined as morphological abnormalities that occur when thyrocyte nuclei extend long, thin protrusions into cytoplasm that never reach another nucleus. Given the low mitotic index of thyrocytes, it seems reasonable to postulate that cells with these karyopathologies induced by radioactive iodine from Chernobyl fallout could survive and be detectable a decade later.

To test this possibility, we carried out cytological studies in A-cells of thyroid follicular epithelium from biopsy materials of uni-nodular and mult-inodular goiters among subjects in the BelAm cohort and a comparison group of patients unexposed to Chernobyl fallout.

Materials and methods

Methodological details of the BelAm Thyroid Screening Study have been described previously (Stezhko et al. 2004). In brief, the screening protocol included, among other procedures, thyroid palpation and ultrasonographic examination of the thyroid gland. Referral for fine-needle aspiration biopsy (FNAB) was made for all nodules 10 mm detected either by palpation or sonography as well as for nodules 5–10 mm that were partially solid and suspicious for malignancy.

For the current study, cytological samples were obtained from 50 BelAm cohort members drawn from the Gomel region with uni-nodular or multinodular goiters diagnosed at screening. For purposes of comparison, we also obtained cytological samples from FNABs performed for nodular goiters by the Nikiforov Russian Centre of Emergency and Radiation Medicine in the Leningrad region of Russia, an area unaffected by the Chernobyl nuclear accident. Thyroid radioactivity measurements performed on the examined BelAm cohort members allowed estimation of their individual thyroid doses from intake of I-131 (Likhtarev et al. 2006). The patients from the comparison group had no known history of exposure to environmental radiation.

Cytopathological analyses for both groups were carried out in 2008–2009 at the Republican Research Centre for Radiation Medicine and Human Ecology in Gomel, Belarus, the site of the BelAm project. The analyses were conducted on samples from a total of 47 subjects with

uni-nodular goiter (25 from BelAm, 22 from the Leningrad region) as well as 43 subjects with multinodular goiter (25 from BelAm, 18 from the Leningrad region).

Cytological smears from thyroid follicular cell material obtained after FNABs were prepared using a standard method (Kost 1975), with two to five smears from every subject. In the case of multi-nodular goiters, we focused on nodules with a pathologic sonographic structure. The smears were air dried, fixed with 96% ethanol, and stained with azure 2-eosin by Romanovsky. To establish that the bridges identified were actually composed of DNA, the smears were stained with ethidium bromide in order to rule out artifacts that might resemble internuclear chromosome bridges. Microscopic examination was carried out under immersion (1000x) and only in smears with whole cells. For microscopic photography, we used fluorochrome ethidium bromide. Thyrocytes were arranged either as a group or individually. Cell nuclei of follicular epithelium were stained equally and had ideal round contours. In a few cases we detected proliferative changes, but we observed no cells with signs of atypia. All cytological summaries corresponded to colloid nodules. We excluded from analysis any case with concomitant thyroiditis since nuclear abnormalities have been observed in enlarged glands from such patients.

Frequencies of thyrocytes with nuclear abnormalities were determined per 500–1000 scored A-cells of follicular epithelium. We calculated the percentage of patients in each group who had thyrocyte bridges present. For each group, we also calculated the mean frequency of thyrocytes with bridges as well as the mean frequency of cells with tailed nuclei. Statistical testing was carried out using the Mann-Whitney non-parametric U-test.

Results and discussion

Descriptive data on study groups

Table 1 provides data on basic demographic characteristics by study group. In each goiter category, there were between-group differences in age, with the BelAm subjects being on average about 20 years younger than the control patients from Leningrad. The possible implications of this age difference are discussed later. Data on estimated I-131 doses for BelAm subjects show similar means and ranges for those with uni-nodular and multinodular goiters.

Results of cytopathological studies

We observed a variety of morphological types of bridges (Fig. 1): cone-shaped bridges, bridges having the same thickness along the whole bridge and bridges with enlargements in the form of micronuclei. In most cases, we found cells to have one bridge; no thyrocytes with more than two bridges were observed. In some cases, we detected bridges of abnormal length (30–50 μm) (Fig. 2).

Thyrocytes with bridges were detected in 80% of uni-nodular cases from the BelAm cohort as compared with only 27% of the control group. In the BelAm cohort, the mean frequency of thyrocytes with bridges was $5.16 \pm 0.69 \times 10^{-3}$, substantially higher than the frequency of $0.73 \pm 0.28 \times 10^{-3}$ in cells from Leningrad region control patients ($z=4.64$, $p<0.001$). It should be noted that 29% of subjects from the BelAm cohort compared to 0% of controls had bridges of abnormal length (see Fig. 2).

Thyrocytes with bridges were detected in 75% of multinodular cases from the BelAm cohort as compared with only 16.7% of the control group. The average frequency of thyrocytes with bridges in multinodular goiter patients from BelAm was almost 10 times higher than that in the Leningrad region control group ($4.30 \pm 0.67 \times 10^{-3}$ vs. $0.39 \pm 0.20 \times 10^{-3}$; $z=4.2$,

$p < 0.001$). Giant bridges were found in 35% of cases from BelAm while, as in the uni-nodular goiter group, no such abnormalities were detected among controls.

The frequency distributions of tailed nuclei in patients from the two study groups with uni-nodular and multi-nodular goiters are shown in Fig. 4(c) and (d), respectively. Among subjects with single-nodular goiters, the frequency of thyrocytes with tailed nuclei varied from 0.0 to 18.9×10^{-3} in the BelAm cohort and from 0.0 to 8.0×10^{-3} in the control group from the Leningrad region (Fig. 4(c)). In the BelAm group, the mean value was $7.82 \pm 0.77 \times 10^{-3}$, significantly higher ($z = 4.54$, $p < 0.001$) than the average frequency in the control group ($2.27 \pm 0.56 \times 10^{-3}$). In patients with multi-nodular goiters (Fig. 4(d)), all subjects from the BelAm cohort had thyrocytes with tailed nuclei. The value of the parameter varied from 2.0 to 14.0×10^{-3} , and the mean frequency was $6.33 \pm 0.64 \times 10^{-3}$. In patients with multi-nodular goiters from the control group, thyrocytes with tailed nuclei were detected in only 35% of cases. The maximum frequency was 4.0×10^{-3} , with a mean of $1.09 \pm 0.30 \times 10^{-3}$, significantly lower than the mean in the BelAm cohort group ($z = 5.59$, $p < 0.001$).

The principal finding from our study of thyrocyte cell populations obtained from FNA biopsies of single- and multi-nodular goiters is the significantly higher frequency of internuclear bridges and bridge fragments (tailed nuclei) in Chernobyl-exposed patients compared with those from an area unaffected by Chernobyl fallout. These nuclear abnormalities are formed as a result of pathologic mitoses with chromosomal bridges by dicentric and centric ring chromosomes, a mechanism shown in a series of studies using fluorescence in situ hybridization (FISH) by a wide spectrum of probes on whole chromosomes and their loci (Gisselsson 2000; Gisselsson et al. 2000). Since dicentric chromosomes and centric rings are markers of radiation exposure (IAEA 2001), bridges and tailed nuclei in thyrocytes would seem to represent cellular effects of radiation exposure to the thyroid gland. If, as has been proposed, internuclear bridges reflect breakage-fusion-bridge cycles leading to increased genomic instability (Gisselsson 2000) and karyotypic heterogeneity in cell populations (Gisselsson et al. 2000), they may in turn be associated with an increased risk of malignant transformation. Our findings are consistent with the results from a study of chromosomal painting of chromosomes 1, 4 and 12 of cultured thyroid cells from 40 Belarusian children with papillary carcinoma of the thyroid, which showed an increased frequency of stable translocations in tumor as well as non-tumor tissues, especially among the cases from the Gomel region, compared with reference thyroid cancer cases without radiation history (Lehmann et al. 1996).

Another notable finding from our studies of thyrocytes in patients exposed to fallout from Chernobyl is the detection of a special form of karyopathology: namely, internuclear bridges of abnormally great length (20–50 mkm) detected in the follicular epithelium. As a rule, giant bridges were observed in clusters consisting of 7–20 cells, where they might begin from the thyrocyte nucleus at one side of the cluster and end in the nucleus of a thyrocyte in another area of the cluster, although in a number of cases they were separately situated in mononucleated cells. These giant bridges were thinner than those of typical length (Fig. 2). In the clusters of cells that retained volume structure in cytological smears (so-called cell blocks), it was obvious that a huge bridge could be situated above or below the neighboring cells and the cells themselves could be located quite far from each other. This means that, most likely, the growth of thyroid follicles in follicular epithelium and the accumulation of colloid in them at low mitotic activity promoted formation of bridges stretched slowly and long. If so, one might infer that giant bridges are witnesses of irradiation – including iodine radioisotopes – that occurred in the past.

An alternative explanation for the observation of an extra-long chromosome bridge may be a technical artifact, and this cannot be ruled out. However, considering the fact that the smears were prepared using the same technique in both the BelAm and comparison groups, it is noteworthy that we did not observe any extra-long chromosome bridges among thyrocytes from the control goiter patients. Also, from the figures presented it can be seen that bridges are arranged crosscut (transversely) to the longitudinal axis of a smear and are situated within the same cell cluster where cells bear against each other and the cluster is round in shape. In the case of a technical artifact, one would expect a cluster form to be extended parallel to the axis of a smear.

The average age at diagnosis of goiter in BelAm cohort patients was younger than that of subjects from the Leningrad region since screening results in detection of nodular disease earlier than clinically evident cases. However, as the frequency of cytogenetic changes also increases with age, it might have been expected that thyrocyte nuclear abnormalities would be more numerous in the control group than in the younger patients from BelAm. The excess of karyopathology observed in the Chernobyl-exposed group is therefore unlikely to be accounted for by patient age.

Finally, we emphasize again that giant bridges in follicular epithelium were observed only in smears from thyroid goiters in patients from the BelAm cohort, and not in any patient of the Leningrad group. Thyrocytes with giant internuclear bridges may therefore be candidates for the role of specific markers of radiation effects on the thyroid gland, the search for which continues (Tuttle and Becker 2000; Achille et al. 2009; Takeichi et al. 2006; Zotti-Martelli et al. 1999). Further study of the retrospectively detected cellular phenomena in cytological smears obtained after fine needle biopsies of the thyroid gland among people who were exposed in the first months following the Chernobyl accident is of importance to extend understanding of long-term irradiation consequences in the human organism.

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Fig. 1. Internuclear chromosome bridges between thyrocyte nuclei. Cytological smear of biopsy material of single-nodular goiter of thyroid gland, stained with azure 2- eosin by Romanovsky. 1000-power magnification.

Fig. 2. Giant (50 μ m long) internuclear chromosome bridges between thyrocyte nuclei. Cytological smear of bopisy material of single-bodular goiter, stained with azure-2eosin by Romanovsky (a) and Ethidium bromide (b). 1000-power magnification.

Fig. 3.
Tailed nuclei – broken bridge in binucleated thyrocyte. Cytological smear of biopsy material from single-nodular goiter, stained with azure 2-eosin by Romanovsky. 1000-power magnification.

Fig. 4.

(a) Distribution of patients with single-nodular goiter from the BelAm cohort and Leningrad region by the frequency of thyrocytes with internuclear bridges. (b) Frequency of goiter among patients with multi-nodular. (c) Distribution of patients with single-nodular goiters from the BelAm cohort and Leningrad region by the frequency of thyrocytes with tailed nuclei. (d) Frequency of thyrocytes with tailed nuclei among patients with multi-nodular goiter.

Table 1
Descriptive characteristics of the BelAm and Leningrad region study groups

	Number of patients (male:female)	Thyroid dose Mean (range), Gy	Mean age at accident, yr	Mean age at diagnosis, yr	Mean time from accident to diagnosis, yrs
Single-nodular goiter					
BelAm	25 (11:14)	1.2 (0.2 – 2.3)	8.9 ± 1.7	23.0 ± 2.0	13.8 ± 1.8
Leningrad	22 (7:15)	-	-	45 (21 – 63)	-
Multi-nodular goiter					
BelAm	25 (9:16)	1.5 (0.3 – 2.8)	9.1±2.1	21.0 ± 2.0	12.4 ± 2.3
Leningrad	18 (8:10)	-	-	42 (21 – 60)	-