RADIATION INDUCED THYROID CANCER: FUNDAMENTAL AND APPLIED ASPECTS

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Aim: To describe the epidemiology and pathology of thyroid cancer in Ukraine, and to perform the molecular analysis of genetic alterations more frequently found to be associated to papillary carcinomas (PTC) in a selected group of PTC.

Materials and Methods: Relationship between the thyroid cancer incidence and gender, age, and place of residence of subjects aged 0–18 years at the time of the Chernobyl accident. Pathologically analyzed thyroid carcinomas were obtained from 640 patients (20–40 years old at the time of surgery and born before the Chernobyl accident), and from 90 patients (11–22 years old at the time of surgery and born after the accident). All patients were operated during 2006–2008. RET/PTC rearrangements and BRAFV600E-mutation were analyzed in 35 cases of PTC.

Results: A comparison between the thyroid cancer incidence rates in the 6 highest contaminated regions of Ukraine and in the other 21 regions shows the most significant difference between the rates for the last three years of follow-up, which confirms that a direct relationship is still present between the rise in thyroid cancer incidence and the post Chernobyl radiation exposure. Much lower incidence of thyroid cancer in subjects, who were born after the accident, additionally confirmed a direct relationship between the Chernobyl accident and thyroid cancer development at least in those who were aged up to 18 years at the time of the nuclear accident. Pathological results showed that with increasing latency the decrease has been noted in the percentage of PTC with solid structure, a decrease in invasive properties of tumors, as well as an increase in the percentage of PTC with papillary-follicular structure, encapsulated forms, and small carcinomas measuring up to 1 cm. Molecular–biological studies of PTC revealed more common RET/PTC1 and RET/PTC3 rearrangements (34.3% of cases), than BRAFV600E-mutation (24% cases).

Conclusion: After 22 years from the Chernobyl nuclear accident the number and incidence of thyroid cancer cases in Ukraine was steadily increased in the cohort of those who were children and adolescents at the time of the accident. Most common thyroid tumors (PTC) were characterized by significant changes in histological structure with increasing latency. PTC with any RET/PTC rearrangements had more aggressive behavior than BRAFV600E-positive tumors or PTC without gene alterations.

Key Words: Chernobyl nuclear accident, thyroid cancer, papillary carcinomas.

After 22 years from the Chernobyl accident the number of thyroid cancer cases in persons been children and adolescents at the time of this catastrophe was steadily increased in Ukraine, and 561 newly diagnosed cases have been registered in 2008. An estimation of Clinical-Morphological Register’s data by age at the time of the accident shows that for the post-Chernobyl period (1986–2008) 5427 cases of thyroid cancer have been registered in the above age group, among which 3996 (73.6%) were children aged 0 to 14 years, and 1431 (26.3%) were adolescents aged 15 to 18 years at the time of the accident. As well as in previous years, also in 2006–2008 the highest thyroid cancer incidence was registered in the 6 most contaminated northern regions of Ukraine. In the cohort of those born in 1968–1986 and operated on in 2006–2008, thyroid cancer was observed only in young adults aged 20–40 years, and 91.2% of them were represented by papillary carcinoma. These tumours were mainly with papillary, follicular or papillary-follicular structure (70.4% of cases) and presented with low levels of regional and distant metastases. Thyroid cancer incidence between children and adolescents born after the accident was much lower than in patients born before the accident. Nevertheless the pathological features of papillary carcinomas in both groups were similar. Molecular–biological studies showed that in papillary thyroid carcinomas RET/PTC1, RET/PTC3, RET/PTCX rearrangements and BRAFV600E mutations were detected. Papillary carcinomas with RET/PTC rearrangements were characterized by more prominent aggressiveness with respect to tumors with BRAF mutation or without any genetic alterations.

MATERIAL AND METHODS

Statistical data were obtained from clinical-morphological Register of the State Institution «Institute of Endocrinology and Metabolism of the Academy of Medical Sciences of Ukraine» [1]. Pathologically studied thyroid carcinomas were obtained from 640 patients who were 20–40 years old at the time of surgery and born before the Chernobyl accident, and from 90 patients who were 11–22 years old at the time of surgery and born after the accident. All patients were treated at the Hospital of the State Institution «Institute of Endocrinology and Metabolism of the Academy of Medical Sciences of Ukraine» during 2006–2008. All patients gave informed written consent. Studies were performed according to the rules of local Ethical Committee.

These cases were analyzed together with 1342 cases of thyroid carcinomas of patients who were 4–36 years old at the time of surgery and born before the accident, and 118 cases of thyroid carcinomas...
of children and adolescents who were born after the accident and described in our previous study [2].

The pathological diagnosis was made according to the WHO Histological Classification [4]. Most of the cases were additionally reviewed by the International Pathology Panel, established in the framework of the Tissue Bank Project [5]. The diagnosis of thyroid carcinoma was confirmed in all cases.

The papillary carcinomas (PTC, n = 35) were also studied by molecular biology approach. In particular, total RNA was extracted from the frozen thyroid tumors and normal tissues, obtained from Chernobyl Tissue Bank (http://www.chernobyltissuebank.com). The mean age at surgery of this selected group of patients was 21 ± 5 years. The mean latency period was 14 ± 1 years.

For RET/PTC analysis RT-PCR and southern blot was used as previously described [6]. BRAF<sup>V600E</sup> mutations were studied by direct sequencing of exon 15 [7]. Real-time RT-PCR method was used to measure the expression levels of the tyrosine kinase domain (TK) and extracellular domain (EC) of the RET gene [8]. Target gene mRNA levels were expressed as 2^ΔΔCt, where ΔΔCt = Ct of target gene – Ct of reference gene [7]. The ratio TK/EC was calculated for all studied samples. The value of TK/EC higher than 2 suggested the presence of a RET/PTC rearrangement. Molecular biological investigations have been carried out in collaboration with the University of Pisa (Italy).

**RESULTS AND DISCUSSION**

**Epidemiology and statistics.** 1556 newly diagnosed thyroid cancer cases have been reported in Ukraine during the period of 2006–2008. An estimation of Register’s data by age at the time of the accident showed that for all post-Chernobyl period (1986–2008) 5427 cases of thyroid cancer have been registered in the above age group, among which 3996 (73.6%) were children aged 0 to 14 years at the time of the accident, and 1431 (26.3%) were adolescents aged 15 to 18 years (Tables 1, 2).

Undoubtedly, this steady increase in thyroid cancer cases may be to some extent associated with a gradual increase in the age of the cohort under study for the period 1986–2008. At the same time, a comparison between the thyroid cancer incidence rates in the six highest contaminated regions of Ukraine and in the other regions of Ukraine shows the most significant difference between the rates for the last three years of follow-up, which confirms that a direct relationship is still present between the rise in thyroid cancer incidence and the post Chernobyl radiation exposure (Tables 1, 2).

At the time of surgery total of 5732 patients who were 4–40 years at surgery (5427 born before, and 305 – after the Chernobyl accident) were included in the Register for the period of 1986 to 2008. All 1556 cases revealed during the last three years in patients who were born before the accident have been detected in young adults who had surgery at the age of 20–40 years. The incidence per 100,000 significantly increased during this period, especially in the most contaminated 6 north regions of Ukraine (7.87) in comparison with less contaminated 21 regions (2.87).

If we consider the incidence among children, adolescents and young adults born before and after the Chernobyl accident separately, it appears that in children born before the accident who were up to age of 15 years at the time of surgery, the incidence was highest in 2000 (5.21 per 100,000 in most contaminated regions). Beginning from 2001, these children have gone over to the category of adolescents. At the same time, the incidence in children born after the accident were and remained much lower (for example, in 2000 — 0.13; in 2008 — 0.21).

A similar tendency was also noted when comparing the incidence in adolescents and young adult patients. For example, in 2008 in young adults born born after the accident the incidence in most contaminated regions was 8.09, but in young adults born after the accident — 1.91. These data represent an additional evidence of a direct relationship between the Chernobyl accident and thyroid cancer development at least in those who were aged up to 18 years at the time of the nuclear accident.

**Pathology.** The histological examination of 640 thyroid carcinomas diagnosed between 2006 and 2008 showed that 91.2% of cases were represented by PTC. It is of interest that the percentage of follicular...
carcinomas (FTC) was increased from 3.0% in cases diagnosed in 1990–1995 to 6.4% in those diagnosed in 2006–2008. This finding suggests that FTC incidence might be related both to an older age at diagnosis and to a longer latency period when compared to PTC. However, the relatively small number of FTC does not allow any definitive conclusions on this matter.

The ratio of PTC subtypes in young adults for the 2006–2008 period confirmed our previous data concerning an inverse relationship between the latency period and the prevalence of more aggressive variants (i.e., solid variant) [2]. Also in the Ukrainian series we observed the decrease in the percentage of solid variant from 21.4% in 1990–1995 to 6.3% in 2006–2008 (p < 0.01 by χ²-test), and an increase in the percentage of typical papillary and mixed variants from 21.4% in 1990–1995 to 34.4% in 2006–2008, and from 21.4% in 1990–1995 to 43.0% in 2006–2008 respectively (p < 0.05 by χ²-test).

The structural combinations of mixed variant have also changed over time: the percentage of tumors with solid-follicular structure was substantially decreased (from 66.6% in 1990–1995 to 21.1% in 2006–2008, p < 0.01 by χ²-test), while the percentage of tumors with papillary-follicular structure was increased (from 16.7% in 1990–1995 to 46.2% in 2006–2008, p < 0.01 by χ²-test).

An analysis of invasive properties of PTC has revealed two main relationships: age and time dependences. Extrathyroid tumor spreading to soft tissues adjacent to the thyroid, which allowed to refer such a tumor to T3 category according to the 6th edition, was more often associated with the increase of both the patients’ age and latency period of tumor development.

Other possible reasons to explain this change of the biological behavior of PTC might be related to two new interesting findings: a) The significant increase in the percentage of completely encapsulated tumors found in the last years (30.5% in 2006–2008) with respect to that observed previously (7.4% in 1990–1995, p < 0.001 by χ²-test); b) The progressive increase of «small» tumors with the biggest diameter up to 1 cm (microPTC): from 4.1% in 1990–1995 to 26.0% in 2006–2008 (p < 0.001 by χ²-test). Such increase of the percentage of microPTCs is undoubtedly the result of an intensification of screening examinations [9] and improvement of the diagnostic facilities (i.e. modernization of ultrasound equipment and wide use of fine-needle aspiration biopsy) [10, 11].

A comparison of different histotypes of thyroid carcinomas in children and adolescents born before and after the Chernobyl accident, shows that in both groups the PTC was the prevalent histotype (93.0% and 84.1% respectively). However, it should be noted that in patients born after the accident (i.e. in 1987 and later) the percentage of FTC was notably higher (4.8% and 12.5%, respectively, p < 0.01 by χ²-test).

Invasive properties of PTC are similar in both groups either when considering the extrathyroid spreading (54.8% in children and adolescents together born before the accident, and 48.2% in those born after the accident) or the presence of regional lymph node metastases (60.0% and 54.8%, respectively). The percentage of distant metastases to lungs (22.7% and 9.5%, respectively, p < 0.001 by χ²-test) was significantly lower in patients born after the accident, but this finding might be due to an early diagnosis as well as to the higher percentage of microPTC in this group (6.3% and 13.7%, respectively, p < 0.05 by χ²-test).

Molecular-biological study. About 15 different isoforms of RET/PTC rearrangements were described in the literature till now, but the most common among them are RET/PTC1 and RET/PTC3 alterations [12, 13]. RET/PTC rearrangements (RET/PTC1, RET/PTC3 and unknown RET/PTCX) and BRAFV600E point mutations have been screened in 35 cases of post-Chernobyl Ukrainian papillary carcinomas. As shown in Table 3, the most common RET/PTC1 and/or RET/PTC3 rearrangements were detected in 12 of 35 (34.3%) PTC, one of which showed the simultaneous expression of both, RET/PTC1 and RET/PTC3. It should be noted in another case RET/PTC3 was present in association with BRAFV600E mutation. When the prevalence of the two types of rearranged RET oncogenes was analyzed, we found that RET/PTC3 was more frequent than RET/PTC1: 8/35 (22.9%) vs. 5/35 (14.3%), respectively. In general, in 18 from 35 PTC (51.4%) the presence of rearranged oncogenes RET/PTC was shown.

Table 3. Gene alterations in post-Chernobyl thyroid papillary carcinomas

<table>
<thead>
<tr>
<th>RET/PTC rearrangements</th>
<th>PTC, % (n = 35)</th>
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<tbody>
<tr>
<td>RET/PTC1</td>
<td>11.4 (4/35)</td>
</tr>
<tr>
<td>RET/PTC3</td>
<td>17.1 (6/35)</td>
</tr>
<tr>
<td>RET/PTC1 + RET/PTC3</td>
<td>2.9 (1/35)</td>
</tr>
<tr>
<td>RET/PTC3 + BRAF</td>
<td>2.9 (1/35)</td>
</tr>
<tr>
<td>RET/PTC3</td>
<td>17.1 (6/35)</td>
</tr>
<tr>
<td>BRAFV600E mutation</td>
<td>24.0 (6/25)</td>
</tr>
<tr>
<td>rearrangement</td>
<td></td>
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<tr>
<td>Total</td>
<td>68.6 (24/35)</td>
</tr>
</tbody>
</table>

BRAFV600E point mutations have been discovered as the most common genetic alteration in sporadic adult papillary thyroid carcinomas [7, 19]. Our previous stud-
ties of post-Chernobyl PTC in children and adolescents of Ukraine revealed a low frequency of such alterations [2, 15, 20]. Present study showed, that BRAF<sup>V600E</sup> point mutations in patients with mean age 21 years, and mean latency 14 years were found in 6 PTC out of 25 (24%). This is higher than in children and adolescents, and confirmed previous conclusion, that frequency of BRAF mutations are increasing with age of patients [2, 18, 21]. Among them coexistence of RET/PTC3 and BRAF alterations, as mentioned above, was detected in one of the tumors. Any gene alterations in normal surrounding tissues have not been detected.

The correlation between specific genetic alterations and histological structure of PTC was observed. In tumors with RET/PTC1 rearrangements the majority of cases (4/5, 80.0%) were with typical papillary structure. In contrary, solid variant of PTC was most specific for carcinomas with RET/PTC3 alterations (6/8, 75.0%). Among them tumor presented coexistence of both RET/PTC1 and RET/PTC3 alterations had papillary structure. This study confirmed our previous results [2, 14, 15] and the results of other studies [13, 16–18] showing the association of RET/PTC1 with typical papillary variant of PTC, and RET/PTC3 with solid one. It should be noted that the correlation between unknown RET/PTCX rearrangements and histological features was not revealed. All main histological variants (follicular, papillary, solid and mixed) were represented in this group of PTC.

The majority of BRAF positive tumors (4/6) were of typical papillary variant, one case was FTC, and one (with both BRAF mutation and RET/PTC3 rearrangement) was papillary-solid. So, the majority of PTC with BRAF mutation had typical papillary structure, what completely agree with our previous results [2, 15, 20, 21].

One PTC in our study had both RET/PTC3 and BRAF<sup>V600E</sup> mutations. This tumor had mixed papillary-solid histological structure. It should be noted that such two genetic events in the same tumor was not detected in our previous studies of Ukrainian post-Chernobyl PTC [15, 20, 21], but were described by other authors as very rare event in PTCs [22].

The half of tumors without gene alterations were characterized by mixed histological papillary-follicular structure.

It was shown, that signs of extrathyroid invasion, multifocal growth, blood vessels invasion, regional and distant metastases were present mainly in papillary carcinomas with RET/PTC rearrangements, whereas they were practically absent in tumors with BRAF<sup>V600E</sup> mutations (Figure). The invasion of tumor cells to lymphatic vessels was revealed in the majority of PTCs, and this characteristic was not related to the presence of gene alterations.

According to some publications [13, 16] BRAF-positive papillary carcinomas had higher incidence of extrathyroid invasion and lymph node metastases, higher tumor stage, and patients with such carcinomas had less favorable prognosis. However, other observations carried out on post-Chernobyl PTCs did not reveal such association [15, 20, 21]. Maybe, it could be due to the difference of the age of the patients and to the fact that aggressiveness of BRAF mutants increases with the age of patients. Our results indicate that in young adults (mean age 21 years) prognosis for patients with BRAF-positive papillary carcinomas is more favorable with respect to other PTCs, both with RET/PTC rearrangements and without them.

In summary, on the bases of these observations, it appears that papillary carcinomas with any RET/PTC rearrangements had a more aggressive behavior than BRAF<sup>V600E</sup>-positive tumors or PTC without gene alterations.

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