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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 (5427 subjects of thyroid cancer, 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 was studied. 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** ***BRAF^{V600E}*****-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** ***BRAF^{V600E}*** **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** ***BRAF^{V600E}*****-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 *BRAF^{V600E}* 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.

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Abbreviations used: EC – extracellular domain; FTC – follicular carcinomas; PTC – papillary carcinomas; TK – tyrosine kinase

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*^{V600E} 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 $2^{-\Delta Ct}$, where $\Delta Ct = C_{t\ of\ target\ gene} - C_{t\ of\ reference\ gene}$ [7]. The ratio TK/EC was calculated for all studied samples. The value of TK/EC values 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 were 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 three last 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 before 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

Table 1. Number of thyroid cancer cases and incidence per 100,000 children population in 1986* by year, sex and region

	Year																						
	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Number (F)	4	9	7	18	24	32	61	54	80	92	100	106	135	162	149	231	211	188	236	239	306	333	370
Number (M)	4	2	4	8	17	15	27	30	29	33	43	31	38	59	38	55	52	54	42	60	65	71	72
Total number	8	11	11	26	41	47	88	84	109	125	143	137	173	221	187	286	263	242	278	299	371	404	442
Total incidence	0.07	0.10	0.10	0.23	0.37	0.42	0.79	0.75	0.97	1.12	1.28	1.22	1.54	1.97	1.67	2.55	2.35	2.16	2.48	2.68	3.31	3.61	3.95
6 regions incidence	0.14	0.00	0.14	0.38	0.76	1.10	2.38	2.10	2.62	2.90	3.33	2.76	3.81	5.14	4.86	6.81	6.19	5.05	5.33	5.76	7.24	7.86	7.95
21 regions incidence	0.05	0.12	0.09	0.20	0.27	0.27	0.42	0.44	0.59	0.70	0.80	0.87	1.02	1.24	0.92	1.57	1.45	1.49	1.82	1.96	2.41	2.63	3.02

Note: *Those aged 0–14 years at the time of the Chernobyl accident

Table 2. Number of thyroid cancer cases and incidence per 100,000 adolescents population in 1986* by year, sex and region

	Year																						
	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Number (F)	9	9	10	9	19	19	26	42	37	60	46	51	70	61	56	74	71	74	76	96	85	96	102
Number (M)	2	6	2	4	2	4	9	7	9	8	10	11	11	9	14	15	22	13	7	12	18	21	17
Total number	11	15	12	13	21	23	35	49	46	68	56	62	81	70	70	89	93	87	83	108	103	117	119
Total incidence	0.42	0.57	0.45	0.49	0.79	0.87	1.32	1.85	1.74	2.57	2.11	2.34	3.06	2.64	2.64	3.36	3.51	3.28	3.13	4.08	3.89	4.42	4.49
6 regions incidence	0.19	1.15	0.00	0.58	1.15	1.54	1.92	1.92	2.12	4.23	2.69	3.65	5.38	3.27	5.00	6.15	6.73	7.12	5.38	7.05	8.85	9.04	8.65
21 regions incidence	0.47	0.42	0.56	0.47	0.70	0.70	1.17	1.83	1.64	2.16	1.97	2.02	2.49	2.49	2.07	2.68	2.72	2.35	2.58	3.24	2.68	3.29	3.47

Note: *Those aged 15–18 years at the time of the Chernobyl accident

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 χ^2-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 χ^2-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 χ^2-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 χ^2-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 detected among children compared to adolescents, and especially young adults (64.8; 38.9 and 24.1%, respectively). However, when we pooled together all age groups, a marked decrease in the percentage of tumors with extrathyroidal spreading was observed in those cases with a longer latency period varying from 61.2% in cases diagnosed in 1990–1995 to 16.3% in those diagnosed in 2006–2008 ($p < 0.001$ by χ^2-test). A similar change was observed when the prevalence of regional metastases to cervical lymph nodes was considered. Lymph node metastases in the neck were most often reported among children who had surgery at the age up to 15 years (68.6%), while the percentage of these metastases was decreasing with the increase of the latency period varying from 58.2% in 1990–1995 to 28.8% in 2006–2008 ($p < 0.01$ by χ^2-test). The analysis of cases with distant metastases to lungs has shown a similar tendency. Particular attention should be given to the fact that for the last period of follow-up (2006–2008) only 1.9% out of 584 PTC cases showed distant metastases to lungs. From our standpoint, this fact was likely favored both by the above change in the PTC structure, 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 χ^2-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 χ^2-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 χ^2-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 χ^2-test) was significant 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 χ^2-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 $BRAF^{V600E}$ 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 that in another case *RET/PTC3* was present in association with $BRAF^{V600E}$ 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

	PTC, % (n = 35)
RET/PTC rearrangements	51.4 (18/35)
RET/PTC1	11.4 (4/35)
RET/PTC3	17.1 (6/35)
RET/PTC1+ RET/PTC3	2.9 (1/35)
RET/PTC3 + BRAF	2.9 (1/35)
RET/PTCX	17.1 (6/35)
$BRAF^{V600E}$ mutation (including one case with *RET/PTC3* rearrangenemt)	24.0 (6/25)
Total	68.6 (24/35)

$BRAF^{V600E}$ point mutations have been discovered as the most common genetic alteration in sporadic adult papillary thyroid carcinomas [7, 19]. Our previous stud-

ies 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*^{V600E} 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*^{V600E} 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*^{V600E} 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.

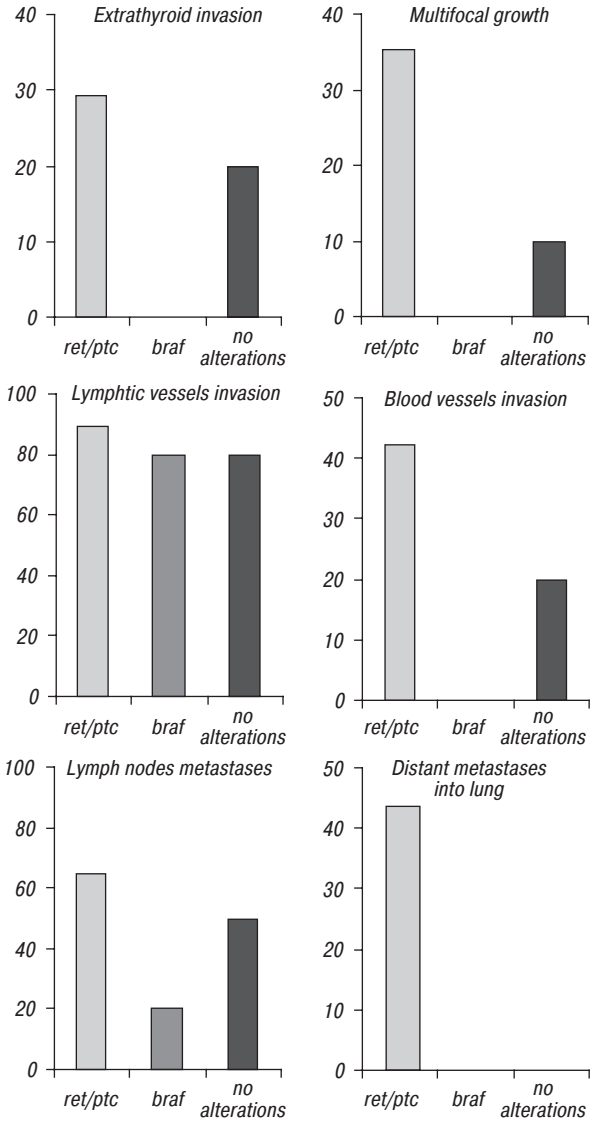

Figure. Invasive properties of papillary thyroid carcinomas with *RET/PTC* rearrangements, *BRAF*^{V600E} mutations, and without any alterations

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*^{V600E}-positive tumors or PTC without gene alterations.

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