Clinicopathological significance of Fractional Allelic Loss (FAL) in prognosis of follicular cell-derived thyroid tumors

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Background:

Thyroid cancer is a serious epidemiological problem of endocrine diseases. Thyroid carcinoma develops as a result of malignant transformation of nodular goiter (NG), which at an early stage can lead to the development of follicular adenoma (FA). The progression of FA can be associated with the transformation of this benign neoplastic lesion into: papillary thyroid carcinoma (PTC), characterized by slow growth and mild outcome, and thyroid follicular cancer (FTC), the more aggressive form of cancer. The final differentiation of thyroid lesions (FA, PTC, FTC) is usually carried out post-operatively (lobectomy, total thyroidectomy). Therefore, it appears advisable to look for markers enabling the proper preoperative diagnosis.

The microsatellite alterations represent molecular disorders acquired by the cell during neoplastic transformation. The genetic instability, i.e., loss of heterozygosity (LOH) and microsatellite instability (MSI) are frequent molecular events in thyroid tumor etiopathogenesis. They were found in several chromosomal critical areas, including 3p12-p21.2, 3p24.2-p25.3, 7q21.1-q31.2, 10q22-24, 15q11-q13, with *loci* of oncogenes and tumor suppressor genes. Since the results seem to be controversial, in this study we decided to focus on the following chromosomal regions: 1p31.2, 3p21.3, 3p24.2, 9p21.3, 11p15.5 and 16q22.1, thus we evaluated the frequency of genetic instability as well as the association of these DNA alterations with clinicopathological variables.

Aim of the study:

Evaluation of usefulness of LOH/MSI as diagnostic/prognostic biomarker in follicular cell-derived thyroid tumors.

Material:

- Thyroid tissue specimens obtained from 93 patients operated for PTC or *„neoplasma folliculare".*
- Histopathological classification: NG, n=43; FA, n=11; PTC, n=31; FTC, n=8.
- Macroscopically unchanged thyroid tissue samples (control).

Methods:

- DNA isolation (QIAamp DNA Mini kit (QIAGEN®).
- Qualitative and quantitative spectrophotometric analysis of DNA (BioPhotometer, Eppendorf).
- PCRs with microsatellitare primers specific for 10 markers (D1S2137, D1S368, D3S3615, D3S1583, D9S974, D9S1604, D11S4088, D11S1318, D16S496, D16S3025).
- Allelotyping analysis (3130 xl Genetic Analyzer, Applied Biosystems).
- FAL values assessment (LOH/MSI coincidence in various chromosomal regions).
- Statistical analysis (Statistica for Windows 10.0).

Results:

Percentage of LOH/MSI at particular microsatellite marker and for each chromosomal region

- LOH/MSI changes were observed in all (100%) microsatellite *loci* and in 27 out of 93 patients (29%).
- LOH/MSI frequency was the highest in 11p15.5 (14.29%), followed by 1p31.2, 3p21.3 and 9p21.3 (~12%).
- The lowest LOH/MSI frequency (~10%) was observed in 3p24.2 and 16q22.1 region.
- No significant differences in LOH/MSI frequency between particular marker, or between studied chromosomal regions (p>0.05; χ^2 test).

Marker	D1S2137	D1S368	D3S3615	D3S1583	D9S974	D9S1604	D11S4088	D11S1318	D16S496	D16S3025
LOH/MSI-positive lesions	3.80 %	11.11 %	12.28 %	10.42%	6.67%	9.43%	7.32%	8.75%	8.96%	5.45%

Table 1. LOH/MSI-positive cases for each microsatellite marker.

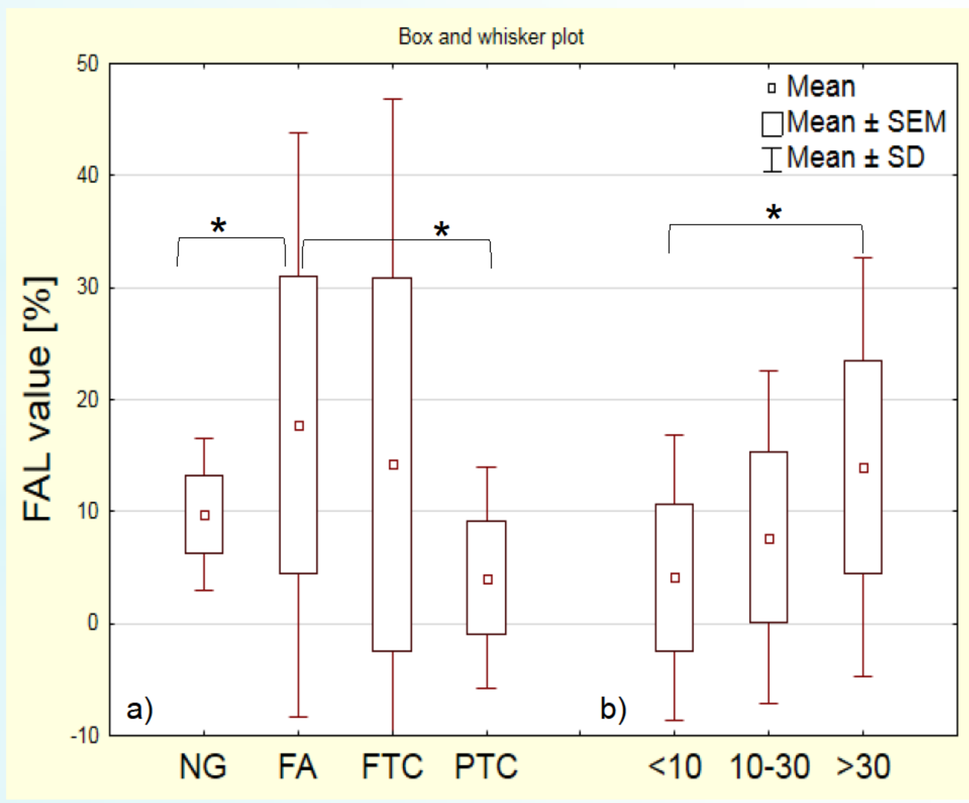

Figure 1.
Box and whisker plot showing differences between mean FAL values:
a) in all histopathological types of thyroid tumors (NG *vs* FA *vs* FTC *vs* PTC).
b) primary tumor diameter (<10 mm, 10-30 mm, >30 mm).

LOH/MSI frequency in correlation with clinicopathological parameters

- Statistical analysis of regional LOH/MSI score revealed significantly increased frequency of studied alteration for group:
  - FA in the region 3p24.2 (p=0.001; χ^2 test),
  - FTC in 1p31.2 and 3p21.3 (p=0.017, 0.001; χ^2 test),
  - PTC in 3p21.3 (p=0.001; χ^2 test).
- Significantly increased LOH/MSI was found in:
  - 3p21.3 for pT1 tumors, AJCC stage I, and tumors with diameter <10 mm,
  - 1p31.2 for pT2-T4, stage II-IV, and tumors with diameter 10-30 mm,
  - 11p15.5 for pT2-T4, stage II-IV, and tumors with diameter >30 mm (p<0.05; χ^2 test).

Correlation of FAL value with clinicopathological parameters

FAL values were significantly higher:

- in men *vs* women (mean 22.66 % *vs* 5.21%, p=0.002; Mann-Whitney U-test),
- in younger patients: <40yrs *vs* 40-60yrs (mean 10.52% *vs* 4.32%, p=0.016; Mann-Whitney U-test),
- in tumor size pT2-T4 *vs* pT1 according to pTNM classification (mean 15.69% vs 2.95%, p=0.021; Mann-Whitney U-test).
- FA and FTC compared with NG and PTC (p=0.033; Kruskal-Wallis test) *(see Fig. 1a)*,
- FA *vs* NG (mean 17.73% *vs* 8.82% p=0,026) and FA *vs* PTC (mean 17.73% *vs* 4.06%, p=0.003; Neuman-Keuls' multiple comparison test).
- in group with increasing diameter of a tumor: >30 mm *vs* 10-30 mm *vs* <10 mm (p=0.044; Kruskal-Wallis test),
- tumor diameter group >30 mm *vs* <10 mm (mean 13.97% *vs* 4.10%, p = 0.027; Neuman-Keuls' multiple comparison test) *(see Fig. 1b)*.

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Conclusions:

Our findings confirmed LOH/MSI occurrence in 3p21.3 at early stage of tumorigenesis while in 1p31.2 and 11p15.5, not recognized as critical areas, seems to be characteristic for advanced stage of thyroid tumors. FAL defined as LOH/MSI coincidence in various chromosomal regions may be useful biomarker in prediction of tumor progression. The increased FAL values in FA/FTC can be regarded as promising distinguishing biomarker from PTC and NG.

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