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## Squamous cell carcinoma of the head and neck in young adults – a preliminary assessment of genetic factor

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### Summary

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| <b>Aim</b>               | Patients with squamous cell carcinoma of the head and neck (HNSCC) aged less than 45 years are classed as young adults and in opinion of many authors compared to older (typical) patients appear more serious forms of the disease and often lack the classical risk factors associated with the illness. There is a need of an exact clinical analysis and a search for additional causative factors. The purpose of this study was to estimate the role of genetic factors in the aetiology of HNSCC in young adults.   |
| <b>Materials/Methods</b> | Studies carried out on 44 patients estimated: 1) the degree of chromosomal instability (bleomycin test), 2) the degree of spontaneous and induced DNA damage and potential of DNA repair (comet assay) and 3) polymorphisms of selected genes of carcinogens metabolism and DNA repair (genotyping).   |
| <b>Results</b>           | The degree of chromosomal instability was a little lower in young adults group than in typical patients group, but the differences were not statistically significant. The level of spontaneous and induced DNA damage and its removal by DNA repair were comparable in the groups of young adults and typical patients. Concerning genotyping we showed in the group of young adults a statistically significant more often co-occurrence of <i>GSTM1</i> (-) and <i>NAT2</i> *4/6A genotypes ( $p < 0.05$ ) and statistically significant lower frequency of allele <i>CYP1A1</i> *4 ( $p < 0.02$ ). Differences between the other risk genotypes and alleles ( <i>CYP1A1</i> *4/*4, <i>NAT2</i> *4/*6A, <i>XPD35931AA</i> , <i>NAT2</i> *4) were not shown to be statistically significant. |
| <b>Conclusions</b>       | The studies parameter revealed only a weak prevalence of genetic predisposition to HNSCC in young adults.  |
| <b>Key words</b>         | <b>squamous cell carcinoma of the head and neck • young adults • genetic factor</b>  |

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## BACKGROUND

Squamous cell carcinoma of the head and neck (HNSCC) is a malignant tumour arising most frequently in non-keratinised epithelial tissue of the upper part of the respiratory or gastrointestinal tracts. The disease accounts for 6% of all cancers and about 90% of all malignant tumours of the head and neck. In this category the most common tumours are of the larynx (about 70%) and the tongue and palatine tonsil (about 25%). These tumours are closely associated with well known causative factors such as tobacco smoking and alcohol abuse and develop most commonly in men in the sixth or seventh decade of life. However, it is possible to define three groups of patients among whom these cancers develop significantly rarer - they are: people who neither drink nor smoke, women and young adults.

Young adults are patients aged younger than 45 (or 40) years (this age limit is not exactly fixed, but the majority of authors set the limit at 40 or 45 years). This idea is used widely in medicine, not only in cancers of the head and neck but also in other neoplastic diseases (e.g. lung cancer) and in non-neoplastic diseases (e.g. heart disease). In head and neck cancer patients this small group (about 5–10%) has attracted the attention of researchers a long time ago [1,2], by the reason of observed differences in causes and results of treatment of these neoplasms in comparison with typical (older) patients. Many authors have noted worse results of treatment in young adults group (worse 3-years and 5-years survivals, more quickly spreading to the lymph nodes and more often local recurrences) [3–6], which may be the effects of more aggressive character of the tumour [4] or more advanced stage at the time of diagnosis (later appearance at doctor and later beginning the treatment) [5]. However some papers provided quite different findings including similar or even better outcome of treatment of HNSCC in young adults than in typical patients [7–13]. A number of studies have also shown a lack of typical cancer causing factors (tobacco smoking and alcohol abuse) in young adults [10,12,14–17], that resulted in search for additional causative factors. The later findings turned an attention for an increased genetic predisposition [18–25], weakened immune system [26] and narcotic abuse (marijuana and hashish) [27]. Some authors, however, link earlier incidence of HNSCC with very high exposure to cigarettes and alcohol [4].

## AIM

This study was aiming for the genetic conditions associated with squamous cell carcinoma of the head and neck in young adults. Using various tests, the study compares the level of chromosomal instability, the level of DNA damage (either spontaneous or induced), DNA repair capacity and a distribution of risk genotypes and alleles associated with carcinogens metabolism and DNA repair in groups of young and typical patients.

## MATERIALS AND METHODS

The study group consisted of 44 patients aged below 45 years treated in the Department of Otolaryngology and Laryngological Oncology of Karol Marcinkowski University of Medical Sciences in Poznań during the period 2001–2003.

The study material were leukocytes isolated from blood taken from patients before surgery. Patients were qualified for testing if they had not previously received chemotherapy or radiotherapy. Three analytical techniques were used.

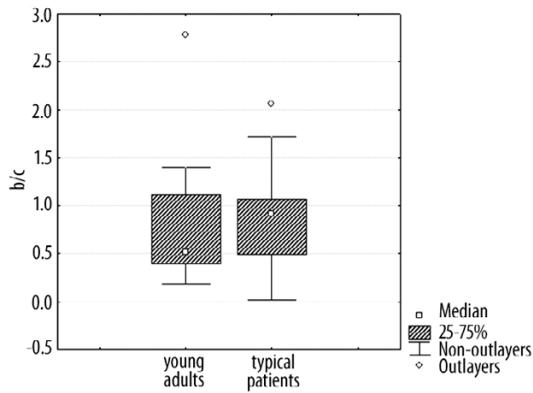
1. **Bleomycin test** was used to determine chromosomal instability by induction chromosomal aberrations by bleomycin in cells cultured *in vitro* [28]. For every patient 2 cultures were maintained (12 cases, 72 hours, 37°C). After 67 hours bleomycin (Bleocin – Nippon Kayaku Co, Ltd.; 30 mU/ml of culture) was applied and after 71 hours colchicine was added (to stop the cell cycle at metaphase). Cultures were harvested using typical cytogenetic methods; the material was collected on slides and stained in a 5% solution of Giemsa's stain and analysed by light microscope. For each patient 50 metaphases were estimated for two parameters: b/c ratio (breaks per cell – a number of breaks of chromosomes in a cell) and the percentage of damaged cells. The scale of values for b/c were described by Hsu and co-workers as follows: b/c value <0.8 represents stable chromosomes, b/c >0.8 and <1.0 shows unstable chromosomes and b/c >1.0 shows increased chromosomal instability.
2. The **comet assay** allows to determine the spontaneous and induced DNA damage and DNA repair capacity [29]. Lymphocytes were isolated from circulatory blood (5 ml from 20 donors) and from these: (A) part was used for determining spontaneous DNA damage, (B) part was exposed to the model mutagen bleomycin (induced damage) and (C) part was exposed to bleomycin and then incubated for 30 minutes at 37°C to determine DNA repair. The lymphocytes were then embedded in agarose gel, lysed, denaturated, electrophoresed, neutralised, stained (DAPI) and analysed by fluorescent microscopy. The length of 100 comets was determined for each sample.
3. **Genotyping**. We analysed the polymorphisms of selected genes involved in the metabolism of carcinogens and DNA repair (24 patients) [30]. Polymorphic variants were determined for the following genes: *CYP1A1*, *NAT2*, *GSTM1* and *XPD* and the frequency of these genotypes and alleles was calculated. We estimated the frequencies of these genotypes and alleles in young adults group, which had appeared significantly more often in pre-analysed large group of almost 300 typical patients with laryngeal cancer than in the healthy people group (risk genotypes and alleles). DNA was isolated from circulatory blood by the phenol method and fragments to be tested were amplified by the PCR technique. In the case of analysis of the polymorphism of genes connected with the presence of deletion variants (*GSTM1*) the PCR products were identified by agarose gel electrophoresis. In the case of analysis of the polymorphism of remaining genes PCR products were hydrolyzed by restriction enzymes (RFLP technique) followed by agarose gel electrophoresis.

The control group in all these tests consisted of typical patients treated in the same period.

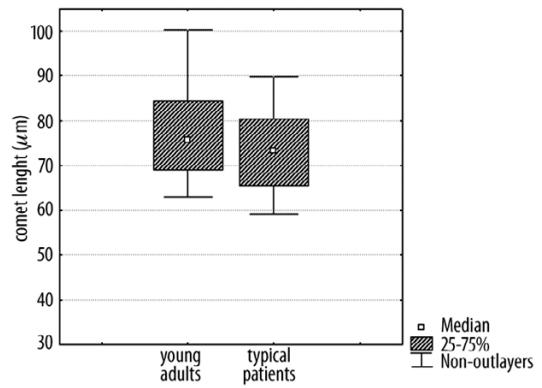
## RESULTS

The results are shown in Figures 1–6.

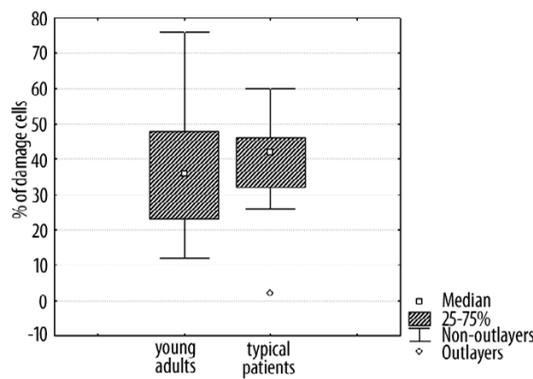
- A) **Bleomycin Test**. In young adults group the level of chromosome damage was lower than in the typical patients group (ratio b/c – median value 0.52 vs 0.96 and percentage of damaged cells – median value 36% vs 44%),



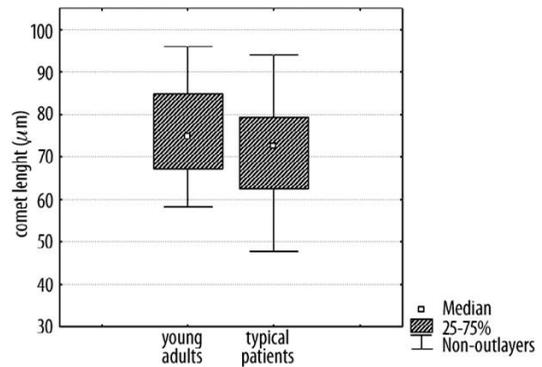
**Figure 1.** Bleomycin test. Induced chromosomal damage – b/c ratio.



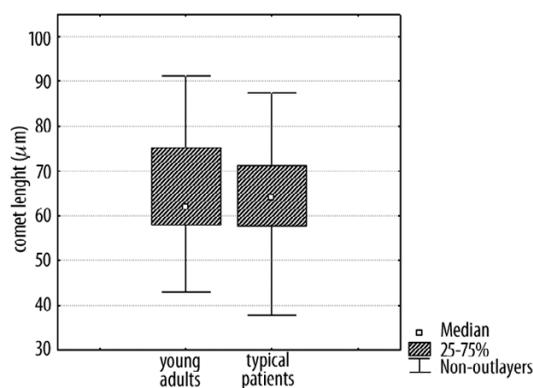
**Figure 4.** Comet Assay. Induced DNA damage (sample BLM).



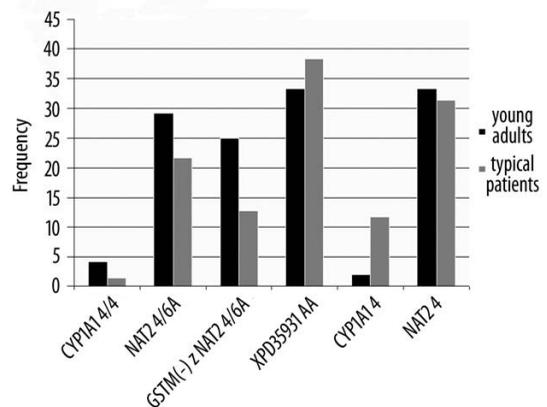
**Figure 2.** Bleomycin test. Induced chromosomal damage – percentage of damaged cells.



**Figure 5.** Comet Assay. Induced DNA damage followed by DNA repair (sample BLM-N).



**Figure 3.** Comet Assay. Spontaneous DNA damage (sample 0).



**Figure 6.** Genotyping. Frequency of risk genotypes and alleles.

but the differences were not statistically significant (U Mann Whitney test) (Figures 1 and 2).

B) Comet Assay. The levels of spontaneous and bleomycin-induced DNA damages and DNA repair potential were similar in both young and typical patients groups (Figures 3–5). Median values for spontaneous damages (sample 0) for young adults and typical patients

were 62.00 and 64.05, for bleomycin-induced damages (sample BLM) were 75.55 and 73.33, and the median values for differences between induced damages and induced damages with DNA repair (sample BLM – sample BLM-N) were 0.69 and 0.85. In no instance there was a statistically significant difference (U Mann Whitney test).

C) Genotyping. A distribution of risk genotypes and alleles among young adults was slightly different than those among typical patients (Figure 6). In young adults there was a statistically significant more often co-occurrence of *GSTM1(-)* and *NAT2\*4/6A* genotypes ( $p < 0.05$ ) and statistically significant lower frequency of allele *CYP1A1\*4* ( $p < 0.02$ ) than in typical patients. The differences between the remaining genotypes and alleles (*CYP1A1\*4/\*4*, *NAT2\*4/\*6A*, *XPD35931AA*, *NAT2\*4*) were not found to be statistically significant.

## CONCLUSIONS

Genetic factors may have a role in the etiopathogenesis of squamous cell carcinoma of the head and neck in young adults. Predisposition to suffering from this disease may be linked to risk genotypes and alleles involved in the metabolism of carcinogens.

## DISCUSSION

The character of squamous cell carcinoma of the head and neck among young adults is a topic of some controversies. Typical and non-typical factors in etiopathogenesis and differences in clinical progress, as compared with those for typical (older) patients, are discussed in many articles [4,5,10,12,14,20–22,24,26,27]. The questions of whether to consider young adults as a distinct group and where to set the upper age limit (45, 40 or 35 years) remain open. Among authors who confirmed the worse results of treatment of HNSCC in young adults there is no agreement of the reasons of such situation. It may be the effect of more advanced stage of illness at the time of diagnosis or more aggressive character of the tumour connected with different etiological factors.

In order to clarify this problem genetic factors should be taken into account.

To date, a few papers has been published about the role of genetic factor in the etiopathogenesis of squamous cell carcinoma of the head and neck in young adults [18–25]. In 1989 Schantz et al. [22] used the bleomycin test in a group of young people (age <40 years) with HNSCC and showed an increased level of induced chromosomal damage connected with a high degree of chromosomal instability (b/c ratio 0.96). The control group of young healthy subjects showed a b/c ratio of 0.62 and young patients with nonsquamous cell carcinoma of the head and neck showed a b/c ratio of 0.50. Papworth et al. [20] confirmed the increased susceptibility of young adults to exogenous factors showing high levels of chromosomal aberrations and micronuclei formation caused by X-rays. Similarly, Llewellyn et al. [31] reviewed 46 publications and indicated that genetic instability may be one of the causes of squamous cell cancers of the head and neck in young adults. During the course of our study we did not find chromosomal instability to be a cause of HNSCC in young adults and found even a little lower level of chromosomal damage in group of young adults than in group of typical patients. The reason for differences between earlier and our results is not clear and is perhaps related to 3 facts. The most important is the difference in control group – Schantz compared young adults with HNSCC with young healthy individuals and with young pa-

tients with nonsquamous cell cancers of the head and neck, but we compared young adults with typical patients. The results showed by Schantz doesn't answer the question about the differences in etiology of the HNSCC in young adults and typical patients, because higher levels of chromosome instability in typical patients group than in healthy controls were shown in many papers. The another reason of these differences may be the small numbers included in the analysed groups (Schantz – study included 20 subjects, our group – 12 subjects) or the variation in age limits (Schantz – 40 years, our group – 45 years), but also in our group patients aged less than 40 years showed the level of chromosomal damage no higher than in the control group.

Comet assay and analysis of polymorphisms of selected genes of carcinogens metabolism and DNA repair have not been carried out before.

We found no differences between the results of comet assay in our tested groups suggesting that the sensitivity of DNA to carcinogens does not account for early occurrence of these neoplasms.

Several studies have tested the effects of genetic changes in genes involved in regulation of the cell cycle (*p53*, *p21*, *Rb*, *MDM2*) [18,19,21,23,25], but the results of these studies were not equivocal. Wang et al. [24] showed that young patients with HNSCC more commonly than typical patients display microsatellite instability but there is no difference in genes connected with DNA repair (*hMLH1* and *hMSH2*). Jin et al. [19] searched a loss of heterozygosity in the neoplastic cells of the oral cavity and healthy epithelium and showed similar results in young adults and typical patients.

Our analysis of the frequency of risk genotypes and alleles in young adults is very preliminary, because of the small number of genes analysed and the small largeness of groups. However analysed genes were selected such that they represent the processes of activation of carcinogens (*CYP1A1*), their detoxication (*GSTM1* and *NAT2*) and DNA repair (*XPD*). The results point to decrease of the correct process of detoxication connected with increase in so called risk genotypes.

The answer to the question of whether genetic factors play a role in the etiopathogenesis of HNSCC in young adults is not equivocal. The role of chromosomal instability remains unsolved. The level of spontaneous and induced DNA damage and DNA repair potential is comparable between groups of young adults and typical patients. The results obtained in our study show at the possible role of risk genotypes and alleles connected with the metabolism of carcinogens. The definition of the role of genetic factors in the etiopathogenesis of squamous cell cancer of the head and neck will require further studies on larger groups of patients. In this context our work is getting a character of pilot study. There remains the question about the role of exogenous factors in the etiopathogenesis of HNSCC in young adults. In the patients we studied, the mean number of cigarettes smoked daily was 20.91 while in typical patients it was 18.65 which suggests an important role of this factor. In the next stage of our work we plan to continue genetic testing in a larger group of patients and to analyse cases of squamous cell carcinoma of the head and neck in young

people retrospectively with the aim of identifying possible etiological factors.

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