THE DETECTION OF PHAGOCYTES INVOLVED IN EXECUTIONAL PHASE OF APOPTOSIS IN HUMAN METANEPHROS

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Introduction
Apoptosis is the most frequent type of programmed cell death with a genetic basis and intra- and extra- cellular stimulation. Apoptosis has a large significance during morphogenesis as it is essential for conservation of homeostasis during intrauterine development (IUD). We distinguish two types of apoptosis – fast (critical) and slow (trickle-like) types. The slow type of apoptosis is specific for the development of the metanephros.

We focused on the degradation phase of apoptosis in human metanephros and we studied the role of professional and non-professional phagocytes in clearing apoptotic bodies. Macrophages have a main role for clearing apoptotic bodies. We focused on the time during IUD when they appear in the metanephros. The apoptotic cell exposes molecules of phosphatidylserine on an external part of the cytoplasmic membrane and this is detected by specific macrophage receptor – this is the initial step in phagocytosis.

Materials and methods
We used 18 histologically normal kidneys (collected from embryos and foetuses ranging from the 8th to 28th week of IUD). The tissues were processed by routine paraffin technique. Macrophages were detected by a standard indirect three-step immunohistochemical method. For the detection of macrophages, we used mouse monoclonal macrophage antibody Ab-1 (Macrophage Marker, Oncogene), goat polyclonal antibody CD64 (N-19, Santa Cruz Biotechnology, Inc.) and mouse monoclonal antibody MMP9 – Matrix Metalloproteinase 9 (NCL-MMP9-439, Novocastra Lab.). For the detection of apoptotic cells, TUNEL (TdT-mediated dUTP nick end-labeling method) was applied.

We used a double staining method to identify phagocytosis of apoptotic bodies. This is based on detection of macrophages and apoptotic bodies (cells) in the same section. This method enables us to determine collocation of macrophages and apoptotic bodies.

Results
MMP9+ macrophages were present from the 8th week of IUD but not in all observed ages. CD64 expressing cells were present from the 12th week, Ab-1 cells occurred from the 14th week of IUD. Positive cells were found sporadically or in small groups.

The number of Ab-1 positive cells slightly increases and in the 28th week of IUD, there were groups of positive cells. The number of CD64 positive cells fluctuated, but it was more prominent than Ab-1 positive cells. MMP9 positive cells appear also sporadically. Collocation of apoptotic bodies and macrophage markers was demonstrable in all three cases (TUNEL/Ab-1, TUNEL/CD64 and TUNEL/MMP9).

Conclusions and discussion
We showed the presence of macrophages in the anlage of definitive human kidney even in the early stage of IUD (the 8th week of IUD) and their participation in the clearance of apoptotic bodies was confirmed. Ab-1 and CD64 antigens are expressed at different stages of IUD. The presence of macrophages did not exclude non-professional phagocytes and their role in the clearing of apoptotic bodies. We do not consider MMP9 a reliable marker of macrophages because it can be synthetized by mesenchymal cells as well.

Acknowledgement
This work was supported by grants FRVŠ 27/G3/2003 and GACR 304/01/0485.

EVALUATION OF SPEECH DISORDERS AMONG CHILDREN WITH ORTHODONTIC ANOMALY

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Key words: Orthodontics / Sibilants

Introduction
The relationship between speech disorders and orthodontic anomaly is not invariable. Most patients learn correct pronunciation even with malocclusion. Severe orthodontic anomalies (open bite, severe protrusion, anodontia of incisors) may cause speech disorders. Short time pronunciation problems may appear at the beginning of treatment with fixed appliances and again after their removal. From the logopaedic point of view the key role plays, beside jaw and teeth position, also the quality of orofacial motor functions. The aim of this study was to evaluate speech disorders among children treated at the Department of Orthodontics, 2nd Clinical Dentistry, Faculty of Medicine, Palacky University, Olomouc, Czech Republic. The study focused on sibilant pronunciation problems (sigmatism) among patients before the beginning of active therapy. The study was further focused on finding a reliable method for speech evaluation in orthodontics.

Methods
Sigmatism was evaluated in a group of 24 randomly selected patients. The age mean of the group was 12,0 years. The control group constituted 19 children not treated by an orthodontist. The age mean of this group was 12,3 years. A czech logopaedic poem dealing with sigmatism was recorded: “Byl jeden les, v lese byl pes, v lese byl kos a ten byl bos”. We made use of minidisk MZ-R3 and the mixing box set Sennheiser HD250 linearII. All recordings were made in the standard surgery room of the clinic, thereby providing standard acoustic conditions. Sigmatism severity was evaluated categorically: not found, mild, severe. The evaluation was conducted by one evaluator several times, and in selected cases by FFT (spectral analysis) by the means of SoundForge 5.0 software.

Results
In the group of 24 patients sigmatism was found severe 2 times, mild 6 times, not found 16 times. In the control group of 19 children severe sigmatism 0 times, mild 5 times, not found 14 times. Sibilant pronunciation disorders were found in patients with anomalies in frontal segment of the dental arch (severe protrusions, tremata). Number of children in presented groups is not sufficient to conduct significant
statistical analysis. The method presented may be used for evaluation of speech disorders in larger groups of children.

Discussion
Sibilants are produced in the sibilant slot between the tongue apex and the palatal surface of upper incisors. Sigmatism was observed, as one might expect, with different orthodontic anomalies, when there were severe protrusions or tremata present. In these cases the function of the tongue is impaired and consequently sigmatism occurs. The results of the presented study might be negatively influenced by difficult communication with children during recording in medical environment.

Conclusion
The study confirmed to some extent the role of the frontal segment of dental arch in sibilants pronunciation. Sibilant speech disorders might occur in patients with severe orthodontic anomaly of the frontal segment. In these cases one might recommend cooperation between an orthodontist and a logopaedist. In this study was presented the evaluation of speech disorders by the means of recording and FFT analysis.

References

Objective
The aim of our study was to analyse quantitative characteristics of perfusion and function of left ventricle new program 4D-MSPECT using.

Methodology
8-frame gating was used to collect data from stress/rest Tc-99m MIBI or Tl-201 on 2-detecting camera Siemens e.cam. To date there are 205 coronaryogic tested patients registered where software 4D-MSPECT was used for evaluation: 66 women and 139 men average age 61 years (39–83), 61 patients after myocardial infarction and 44 after previous revascularisation. Quantification of perfusion defects: automatic determination of summing stress score (SSS) using 20 segmental model of left ventricle and 5 point scale, where 0 = normal perfusion (2 SD) and 4 = absence of radioisotope trapping (6 SD). Data recorded by ECG-gated SPECT were automatically processed by programme 4D-MSPECT to determine the volume of left ventricle in end-diastole and systole and to quantify ejection fraction (EF).

Results
Coronarography was negative at 57 examined patients, restriction of arterial lumen more than 50 % was verified in 148 patients (disease of 1 artery at 51 patients, disease of 2 or 3 arteries at 49 or 48 patients). Visual evaluation of imagery showed the sensitivity and specificity of SPECT for detection of ischemic heart disease 95 % and 79 % and was not statistically different to the subset of patients who did not suffer myocardial infarction and at men and women (Tab. 1).

Quantitative analysis of the perfusion showed SSS values according to the number of afflicted arteries: SSS 8 at disease of 1 artery, SSS 14 and 16 for 2 or 3 arteries. In patients with negative coronaryogic stress ejection fraction was 62 % and did not vary from rest EF. In patients with 1 artery disease average stress EF was 55.2 % and stress 57.3 % (variation about 2.1 %), at 2 or 3 artery disease stress EF was 50.6 % or 46.7 % at rest 55.3 % or 51.8 % (variation 4.7 % or 5.1 %).

Conclusion
Quantitative analysis of perfusion showed increasing SSS with cumulative count of afflicted arteries. Gated SPECT data evaluated by the programme 4D-MSPECT permitted identification of patients with stress aggravation of ejection fraction (postischemic stupor of left ventricle), and multivessel disease can be expected in these patients.

Table 1. Characteristics of coronaryogic tested patients.

<table>
<thead>
<tr>
<th></th>
<th>Whole group</th>
<th>Patients without myocardial infarction</th>
<th>Men</th>
<th>Women</th>
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<tr>
<td></td>
<td>205</td>
<td>144</td>
<td>139</td>
<td>66</td>
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<tr>
<td>Sensitivity</td>
<td>95 % (141/148)</td>
<td>92 % (82/89)</td>
<td>95 % (105/110)</td>
<td>95 % (36/38)</td>
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<tr>
<td>Specificity</td>
<td>79 % (45/57)</td>
<td>80 % (44/55)</td>
<td>76 % (22/29)</td>
<td>82 % (23/28)</td>
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