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Examination of the etiopatomechanism of the otitis media with effusion in children

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1. INTRODUCTION

Otitis media is one of the most frequently observed inflammatory diseases in the early childhood. Ninety-three percent of the children at least once and 74% tree or more times have otitis media during the first decade of their life. Two types of the inflammatory process in the middle ear cleft (MEC) with intact tympanic membrane exist: acute (*otitis media acuta serosa*, *otitis media acuta suppurativa* - AOM) and chronic (*otitis media chronica catarrhalis serosa* - OME) type. Both the acute and chronic type of otitis media is characterized by fluid accumulation in the MEC, but distinguished by the presence or absence of acute inflammatory sings, such as fever, pain, hyperaemic and bulging ear drum. There is a strong connection between AOM and OME. It is widely accepted, that they more likely represent two stages of a disease than two different diseases, where OME is a result of a previous and completely unresolved acute inflammation. Forty percent of children 30 days after AOM and 10% of children 3 months after AOM still have persisting middle ear effusion (MEE).

1.1. Clinical features of OME

The prevalence of OME in children aged between 1 and 3 years is 10-30%, and the cumulative incidence of OME at 4 years of age is as high as 80%. There is no gender dominance.

The main feature of the disease is fluid accumulation and persistence in the MEC without any clinical signs or symptoms of an acute inflammation. The increased impedance of the sound transporting system caused by the MEE result a conductive hearing

loss. This mild hearing loss could be the first and only sign of the disease, and it is often diagnosed only with screening hearing tests. The hearing loss is reversible with the elimination of the MEE and the reestablishment of the normal aeration of the MEC.

OME is diagnosed by otologic and audiologic examination. Otomicroscopic examination shows a retracted, rarely normal tympanic membrane and fluid filled middle ear cavity behind it. Pure tone threshold audiometry shows a mild hearing loss and tympanometry a flat (B type) tympanogram.

OME could be treated by conservative and surgical way. Antibiotics show no effectiveness. Application of intranasal vasoconstrictors and/or antihisztamin compared to placebo do not result significantly faster recovery. Air inflation into the MEC (catheterization of the Eustachian tube, using of the Politzer balloon or autoinflation) gives only temporary improvement. The highest effectiveness is proven with paracentesis, ventilation tube insertion, adenoidectomy or the combination of these methods.

1.2. Etiologic factors in OME

The most important contributing factors in the development and persistence of OME are dysfunction of the Eustachian tube, hypertrophy of the adenoid tissue, cleft of the soft palate, upper respiratory airway pathogen viruses and allergy.

The most frequently detected pathogen viruses in the MEE in OME children are rhinoviruses, respiratory syncytial virus and human coronavirus. The role of viral infection in the pathogenesis of OME is confirmed by the presence of these viruses in the effusion,

but detectability alone does not necessarily mean a causal relationship between the viral infection and the fluid persistence. The most often studied respiratory viruses have been rhinoviruses, which are also the most important common colt viruses. The detection rates vary widely between 0 and 40 % for rhinoviruses. A substantial proportion of respiratory infections have also been shown to be caused by enteroviruses. The newly discovered human bocavirus (HBoV) in most of the cases is not a sole infective agent, but it shows a high coincidence with other viruses. The putative role of the HBoV in OME has not yet been established.

Previous studies have come to conflicting conclusions about the etiopathogenetic relationship between allergy and OME; some authors confirm, and others debate the causal relationship.

1.3. The patomechanism of the development of OME

Two main form of OME exist: 1) persistent MEE after an acute infection and 2) secretory OME.

Ad 1) The persistence of MEE after an acute infection is caused by the ineffective middle ear clearance. In case of normal clearance the effusion is continuously transported from the MEC through the Eustachian tube to the nasopharynx by the ciliated epithelium around the tubal orifice. Causes of blocked clearance are a) ciliar dysfunction, b) mucosal oedema and hyperplasia, c) higher viscosity of the effusion and d) decreased middle ear pressure. This form is characterized by the tubal dysfunction, the decreased middle ear pressure and retracted tympanic membrane. Insertion of

ventilation tube helps to normalize the negative middle ear pressure and results a spontaneous healing.

Ad 2) There is no clinically evident acute otitis media in the preceding period in the secretory form of OME. This for is especially observed in older children, although most children have a history of AOM in earlier childhood. Fluid accumulation is the result of the prolonged antigenic stimulation on the sensitized middle ear mucosa. Transtubal infections by pathogens (viruses) or metabolically active bacteria in the biofilm on the surface of the mucosa can lead to chronic stimulation. In the secretory form the normal tubal function could be intact, therefore normal middle ear pressure and normal position of the ear drum can be observed.

There are two mechanisms leading to MEE development: a passive transudation caused by the increased microvascular permeability in the mucosa and an active exudation. Albumin, which is exclusively produced by the parenchymal cells of the liver, could easily penetrate through the capillary wall. The presence of albumin in the effusion indicates the underlying passive transudation. The presence of immunoglobulin-G in the effusion is not a result of the transudation because of its big molecular size (Mr 150.000 protein). Immunoglobulin-G is produced locally by the activated B-cells; therefore its presence is a marker of the active exudation.

2. OBJECTIVES

The aim of our study was to examine the contributing factors and the pathological mechanisms leads to development and maintenance of OME.

Ad 1) The two mechanisms of fluid development, such as the passive transudation caused by the increased vascular permeability and the active exudation caused by chronic stimulation of the mucosa, could be characterized by the content of albumin and immunoglobulin-G of the effusion. Our aim was to find out the possibility of selecting MEE samples into groups defined by different patomechanisms based on the ratio of the concentrations of albumin and immunoglobulin-G (A/G), and to characterize these groups with the contents of cytokines [proinflammatory (TNF-α and IFN-γ) and inflammatory (IL-4 and IL-8)] in the effusion.

Ad 2) Our aim was to describe the prevalence and asses the possible etiologic role of the newly discovered HBoV infection in conjunction with other common upper respiratory viruses (rhino and enteroviruses).

Ad 3) Because of the conflicting data in the literature about the relationship of OME and allergy, we aimed to assess the epidemiologic connection between these two diseases.

3. PATIENTS, MATERIALS AND METHODS

3.1. Patients and materials

The study group consisted of children with OME between age 1 and 13. OME was diagnosed with otologic and audiological examination. Otomicroscopic examination showed intact, transparent, retracted tympanic membrane without any sing of acute inflammation. Pure-tone threshold audiometry diagnosed a mild conductive hearing loss, and tympanometry showed a flat (B-type) tympanogram. Middle ear effusion showed unresponsiveness to

conservative treatment using nasal decongestants, mucolytics or antihistamines. Children with persistent MEE for at least six weeks were enrolled into the study.

Adenoidectomy, tonsilloadenoidectomy and paracentesis and/or ventilation tube insertion were performed. Samples were collected with gentle suction into a trap pipette. According to theirs viscosity, samples were characterized as mucous (thick, viscous, glue-like) or serous (thin, water clean). The amount of the MEE was measured precisely and then it was washed with 1 ml sterile PBS into an Eppendorf tube. Samples for virus detection were stored at -70 C temperatures. For immune protein measurements samples were centrifuged, and supernatants were frozen and stored for later testing.

We have examined two separate patient groups. The first group consisted of 56 children (23 boys, mean age 4.9 years, range 0.9 and 12.6 years) whose 88 MEE were used for measurement of the concentrations of immune proteins. The second group is consisted of 54 children (32 boys, mean age 5.4 years, range 2.8 to 9.8 years), where 75 samples were used for virus detection. Allergy tests were performed on 52 of the total 54 children of the second patient group (31 boys, mean age 5.1 years, range 2.8 and 9.8 years), and two children were lost to follow-up.

3.2. Measurements of inflammatory proteins from the MEE

3.2.1. Measurement of the concentrations of albumin, immunoglobulins (IgG, IgM, IgA), complement factors (C3, C4) and acute phase protein (CRP) by nephelometry

The concentrations of albumin, C3 and C4 were measured by the automated BN II. Dade-Behring Nephelometer (Dade Behring Marburg, Germany). Test light was 840 nm. Normal ranges were as follows: albumin, 33-52 g/l; C3, 0.9-1.8 g/l and C4 0.1-0.4 g/l.

The concentrations of IgG, IgA, IgM and CRP were measured by Cobas Mira Plus Analyzer (Dako AS, Glostrup, Denmark). Normal ranges were 2.7-16 g/l, 0.07-4.0 g/l, 0.12-2.3 g/l and 0.0-5.0 mg/l for IgG, IgA, IgM and CRP, respectively.

3.2.2. ELISA method to measure the concentrations of cytokines

The concentrations of the two proinflammatory (tumour necrosis faktor-α, TNF-α; interferon-γ, IFN-γ) and the two inflammatory (interleukin-4, IL-4; interleukin-10, IL-10) cytokines were measured by sandwich ELISA (enzyme linked immunosorbent assay) method, OptEIA human cytokine Set (Pahrmingen, San Diego, California, USA). Values were given in pg/ml.

3.3. Virus detection in the MEE

3.3.1. Detection of Picornaviruses (rhino and enteroviruses) – reverse transcription, polymerase chain reaction and hybridization method (RT-PCR-H)

Extraction of viral RNA from MEE samples was performed with a commercial RNA isolation procedure (QIAamp, QIAGEN GmbH, Hilden, Germany). Reverse transcription PCR was carried out. The primers were targeted to highly conserved 5'NCR sequences shared by rhino- and enteroviruses. The nucleotide sequences of the primers were 5'-GAAACACGGACACCCAAAGTA-3' and 5'-TCCTCCGGCCCCTGAATG-3', and sequences of the probe was

5’-AGGGTTAAGGTTAGCC. Human rhinovirus-2, Coxsackie virus-A16 and -B5 and echovírus-23 were used as positive controls. Several negative controls were used in every step of the procedure.

The differentiation of rhinoviruses from enteroviruses was carried out by a liquid-phase hybridisation assay with Europium-labelled oligonucleotide probes (Wallac Oy, Turku, Finland, one for rhinovirus and the other for enterovirus). Quantification of lanthanide fluorescence was performed in a time-resolved manner. The cut-off value of positive samples for both europium-labelled probes was the mean of the negative controls plus 5 times the SD of the mean.

3.3.2. Quantitative PCR for HBoV detection

DNA was purified from 200 µl fluid of serous or 20 mg glue of mucoid MEE sample with the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) and eluted in 200µl of distilled water, of which 5µl was used in a total reaction volume of 25µl. The TaqMan universal PCR master mix (PE Applied Biosystems) was used and the PCR, amplifying a part of the NP1 gene was performed using the Stratagene Mx3005P thermal cycler. A positive quantifiable result was obtained with down to 10 copies/µl of the HBoV ST2-containing plasmid. To avoid contamination, samples and PCR mixtures were prepared under laminar flow hoods in separate rooms. Water was used as negative control.

3.4. Allergy tests

3.4.1. Case history

Case history of previous allergic diseases (such as atopic dermatitis, allergic rhinitis and asthma, food allergy) and symptoms possibly related to allergy (such as seasonal rhinorrhoea and sneezing, enteral problems after certain food intake, drug hypersensitivity) were recorded.

3.4.2. Prick- test

Intracutaneous Prick-tests were performed by commercial allergen extracts (HAL Priktest, Haarlems Allergenen Laboratorium B.V., Haarlem, Netherlands) suitable for detection of hypersensitivity against 20 common allergens. Visual positivity scale were used (one to four plus) to give the degree of the reaction on the skin.

3.4.3. Total IgE concentration

ADALTIS Personal Lab Allergy ELISA Automat was used to measure the serum total IgE concentration. Total IgE concentration over 120 kU/l was considered as elevated.

3.4.4. Concentration of allergen specific IgE in the sera

Allergen specific IgE concentrations for 40 inhalative and 40 nutritive allergens were measured with sandwich ELISA method using the commercial CLA Allergen-specific IgE Assay (Hitachi Chemical Diagnostics, Mountain View, California, USA). Samples were divided into six positivity classes (0-4) (Table 1), where 0, 1/0 and 1 values mean no or slight reactions against the specific allergen (allergy negative), 2 or a higher value means hypersensitivity (allergy).

3.4.5. Control group to compare the occurrence of allergen specific IgEs

The occurrence rate of allergen specific IgEs was analyzed by comparing the results of the patient and the control group. The control group consists of 818 children (age range: 1-6 years) selected randomly from outpatients examined at general and allergic paediatric clinics with enteral or respiratory problems.

3.5. Statistical analysis

The statistical analysis was performed with the computer program SAS for Windows 8.2. (SAS Institute Inc., Cary, NC. 27513 USA). Immunological parameters were reported by descriptive statistics (mean, standard deviation, median and quartiles). The differences of logarithmically transformed values of the two groups were analyzed by the Student's unpaired t-test.

The prevalence of viruses in the two MEE group based on viscosity was compared with the Fisher exact test. Differences between the patient and the control groups were calculated by the chi square statistical test, where the level of significance was $p \leq 0.05$.

4. RESULTS

4.1. Examination of the inflammatory proteins in the MEE

4.1.1. Distribution of MEE samples on the basis of the ratio of albumin and IgG concentration

A/G ratios in the plasma of the control group of 31 children showed a normal distribution, mean±SD = 4.12±1.13. The 88 effusions of 56 patients could be separated into two groups on the basis of albumin and IgG concentration ratios (A/G) in the effusions.

The cut-off ratio between these two groups was defined by subtracting three times the SD (1.13) from the mean value (4.12) of the A/G ratio in the plasma of the control children group. It equals 0.73. Group A: A/G<0.7 (16 MEE samples of 13 children, 18.2 %) and group B: A/G>0.7 (72 samples of 43 children, 81.8 %).

4.1.2. *Levels of IgM, IgA, C3, C4 and CRP*

Whereas albumin and IgG were measurable by laser nephelometry in all MEE samples, IgA, IgM, C3 and C4 levels were found to be measurable only in 98.8%, 68.1%, 68.1% and 38.6% of the samples, respectively. CRP level was higher than that of the detectability threshold in only one sample; subsequently it was excluded from further analysis. There was no significant difference in the levels of IgA, IgM, C3 or C4 in these groups.

4.1.3. *Comparison of cytokine levels in the two MEE group*

In group A significantly elevated levels of TNF-α and IFN-γ characterized the effusions (TNF-α: A=2.75 versus B=0.0001, p<.001; IFN-γ: A=7.80 versus B=0.0001, p<.001), whereas IL-4 and IL-10 were the significantly dominating cytokines in group B (IL-4: B=6.54 versus A=0.73, p<.001; IL-10: B=13.26 versus A=0.84, p=.019). There were strong correlations between cytokine levels and the A/G ratio; positive correlation for IL-4 (r=.58) and IL-10 (r=.36) and negative for IFN-γ (r=-.62) and TNF-α (r=-.66).

4.1.4. *Comparison of the clinical background of the patients in the two groups*

There were no statistically significant differences in the protein contents between the two MEE groups according to previous

adenoidectomies and/or ventilation tube insertions in the history of OME.

4.2. Virus detection from the MEEs

4.2.1. Virus positive middle ear effusion samples

Altogether, 26 (34.7%) of the total 75 MEE samples were positive for viral nucleic acid, 22 (29.3%) for enterovirus, 10 (13.3%) for rhinovirus and 2 (2.7%) for bocavirus. The two HBoV positive samples had 4.4 HBoV-genome copies/mg in mucoid effusion and 596 copies/ml in serous effusion, respectively. A sole virus species was detected in 19 of the 26 virus-positive samples; enterovirus in 15 (78.9%), rhinovirus in 3 (15.8%) and bocavirus in 1 (5.3%) sample. There were 6 cases of dual (rhino and enterovirus in each) and one case of triple viral infection detected.

4.2.2. Middle ear effusion types and virus positivity

According to the type of the effusion, the samples were divided into two groups: mucoid and serous effusions. In the mucoid MEE group, the proportion of virus positive samples (except for bocavirus) was higher than in the serous group, but there was no statistically significant difference between these two groups.

4.2.3. Bilateral otitis media cases

Twenty-one (39%) children had a bilateral disease while 33 (61%) children had MEE only in one ear. Virus detection showed identical results for both ears in 12 (57%) of the 21 children who had a bilateral disease; 8 of them were negative for any of the three viruses, 3 were positive for enterovirus and 1 for rhino- and enteroviruses. There were no children with bilateral OME with a

different virus detected in the other ear. Nine (43%) children had a virus negative MEE sample on one side and positive sample from the contra-lateral ear, 1 being positive for rhinovirus, 5 for enterovirus, 2 for rhino- and enterovirus and 1 for all the three viruses.

4.3. OME and allergy tests

4.3.1. Case history

Fifteen of the 52 children (28.8%) were previously established to be allergic; allergic rhinitis and asthma together (n=3), allergic rhinitis alone (n=3), allergic asthma alone (n=2), hypersensitivity against milk (n=3) and against eggs (n=2) and atopic dermatitis (n=2), respectively. A further 3 children were suspected to be atopic based on their symptoms. The remaining 36 children (69.2%) had no allergy.

4.3.2. Prick-test

Hypersensitivity against 2.6 (between 1 and 8) allergen extracts were detected in the 18 children (34.6%) showed positivity in Prick-test. The most frequently detected allergens were house dust mite, ragweed, grain mix and mould mix at 66.6%, 44.4%, 33.3% and 27.7% rate of occurrence, respectively.

4.3.3. Total IgE concentration in the serum

The serum total IgE concentration exceeded the reference level in only 5 children (9.6%).

4.3.4. Allergen specific IgE positivity

Two-third of the children (35 cases, 67.3%) showed allergen specific IgE positivity.

4.3.5. Comparison of our patient group to the control group based on the results of allergen specific IgE positivity

The allergen specific IgE positivity (for both the nutritive and the inhalative allergens) was significantly higher in the OME group than in the control group.

5. DISCUSSION

The development and persistence of MEE in OME in children is a complex, still not clearly known process, which could be characterized by a chronic inflammation based on immunologic mechanisms. The chronic inflammation, independently from the initial stimuli, is maintained by inflammatory cells and their mediators. Examination of either the components of the MEE (such as inflammatory mediators, cells or particles of viruses) or the whole body of the child (such as allergy) help us to understand the role of the contributing factors and the process of fluid development more precisely.

According to our A/G we can distinguish two characteristic types of MEE.

MEE samples in group B, in which A/G was higher than 0.7, contained elevated albumin concentration. The fact that albumin comes from peripheral blood gives an evidence, that elevated albumin concentration is a result of an increased local microvascular permeability and extravasation of albumin into the interstitial tissues and the middle ear cleft. This process is sustained by all vasoactive inflammatory mediators, such as histamine, prostaglandins, activated complements, arachidonic acid metabolites and immune complexes,

which can change the permeability of the capillaries in the mucosal lining of the middle ear. These mediators are derived from granulocytes, partly from neutrophils activated by a bacterial infection, and partly from eosinophils and mast cells accumulated in the middle ear mucosa as a result of an allergic reaction. Activation of these cells by cytokines IL-8 or IL-4 leads to the release of storage enzymes, chemoattractants and vasoactive agents. Elevated albumin concentration in MEE in this group denotes transudation as dominating mechanism in the development of effusion.

We assume that in group A, where A/G was less than 0.7, the type of effusion was an exudate. Low albumin concentration in this group can be explained by normal local microvascular permeability for albumin. This effusion type could most probably be characterized by a high number of mononuclear T-cells, B-cells, monocyte, macrophages and a higher amount of immunoglobulin-G produced by activated B cells.

We found a significant correlation between A/G value and cytokines present in MEE. Low A/G associates with high TNF-α and IFN-γ levels, whereas high A/G ratio correlates with elevated IL-4 and IL-10 levels.

Our observations suggest that elevated concentration levels of proinflammatory cytokines (TNF-α and IFN-γ) in group A reveal a dominance of B and Th1 lymphocytes in the process of OME. In contrast, when a dominantly higher albumin concentration is measured in MEEs (group B), the high concentration of IL-4 and IL-10 suggests the presence of activated Th2 lymphocytes. The first

type could be hypothetically identified as the secretory form of OME, while the other could be equivalent with the persistent MEE after an acute infection form of OME.

Virus detection has shown that 34.7 % of MEE samples from children with OME are respiratory virus positive by PCR. The most common viruses were enteroviruses (29.3%), followed by rhinoviruses (13.3%) and human bocavirus (2.7%).

The high prevalence of rhinovirus positivity is in line with earlier studies. Rhinovirus RNA can remain detectable by PCR up to 8 weeks in MEE and if the child had a rhinovirus infection during the weeks preceding surgery, the possibility of coincidental positive rhinovirus findings cannot be excluded. It is, however, a limitation of our study that we do not have information on respiratory symptoms of the children for the pre-surgical period as long as eight weeks. In contrast, we have found the prevalence of enterovirus in MEE samples as high as 29.3%. Mere detection of virus nucleic acid in the MEE does not, therefore, necessarily indicate a causal relationship between virus infection and the concurrent disease.

The newly discovered HBoV mostly identified in acute respiratory infections. Our team was the first to detect HBoV in the MEE in OME. We have find a very low prevalence (2 positive samples from the total 75; 2.7%), which can be interpret with the chronic feature of the disease. Otherwise HBoV is detected in fewer than 8% of acute respiratory exudates.

It is interesting to note the tendency that a higher number of virus positive samples were found in the mucous than in the serous

effusion group. Experimental studies showed that inflammatory cytokines (especially TNF-α) significantly increased the production of mucopolysaccharides by the epithelial cells in culture from the mucosal lining of the middle ear. Viral nucleoproteins effectively stimulate inflammatory T cells to produce TNF-α and other proinflammatory cytokines.

A number of previous studies provide evidence for the combined occurrence of OME and allergy.

In our patient group, we have found a lower prevalence than in the literature; allergic rhinitis, asthma and dermatitis showed an 11.5%, 9.6% and 3.8% occurrence rate, respectively. The rate of the allergic rhinitis has not even reached the estimated average value of 20% of a similar population. Our 34.6% positivity rate of the skin Prick-test is similar to other studies, where this value ranged between 24% (11) and 100%

Elevated total IgE concentration was found in only 5 children (9.6%) of our patient group. According to earlier papers there was not shown difference in the total IgE level between groups of atopic and non-atopic OME children, which query the clinical value of routine measurement of the serum total IgE.

In our OME children group the occurrence rate of allergen specific IgE positivity was significantly higher ($p<0,001$) than in the control group with respect to both inhalative and nutritive allergens.

The frequent simultaneous presence of OME and allergy suggests that in allergy positive OME children local hypersensitivity

reactions may likely play a role in the development of middle ear effusion.

6. OUR NEW OBSERVATIONS

New observations have been established with the examination of the contributing factors and the patomechanism of development of OME.

Ad 1). We have recognised that based on the albumin and immunoglobulin-G concentration ratios in the effusion samples could be separated into two distinct groups. These groups are characterised with different cytokine profile and different potential patomechanisms. High albumin content correlates with elevated inflammatory cytokines (IL-4 and IL-10), whereas low A/G is associated with high levels of proinflammatory cytokines (TNF-α and IFN-γ). The first group is characterized by transudation, high Th1 and granulocyte cell number and decreased function of the Eustachian tube; whereas in the other group higher amount of activated B and Th2 cells could be observed, and exudation leads to fluid development.

Ad 2). We made the first observation about HBoV infection in the middle ear in OME. The low prevalence of HBoV (2.7%) doesn't allow us to establish any strong causative relationship between the two diseases.

Ad 3). Our epidemiologic study demonstrated that OME and allergy frequently occur together. We have find out, that the allergic background is more frequently present in the OME group than in the control group.

7. PUBLICATIONS, PRESENTATIONS

Underlying international publications in the theme of the dissertation:

1. **Rezes S**, Késmárki K, Sipka S, Sziklai I: Characterization of otitis media with effusion based on the ratio of albumin and immunoglobulin G concentrations in the effusion. Otol Neurotol 2007, **28(5)**: 663-7

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   Citation: 3
2. **Rezes S**, Söderlund-Venermo M, Roivainen M, Kemppainen K, Szabó Z, Sziklai I, Pitkäranta A: Human bocavirus and rhino-enteroviruses in childhood otitis media with effusion. J Clin Virol. 2009, **46(3)**:234-7

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   Citation: 1
3. **Rezes S**, Tóth L, Papp Z, Sipka S, Sziklai I: Evaluation of the relationship between allergy and otitis media with effusion in children. Eur Arch Otorhinolaryngol, 2010 (submitted for publication)

Underlying publications in the theme of the dissertation in Hungarian:

1. Késmárki K, Sipka S, Alexa M, Lakatos G, **Rezes Sz**, Szegedi Gy, Sziklai I: Dobüregi váladék immunológiai vizsgálata gyermekkori otitis media catarrhalis chronica serosa esetén. Fül-orr-gégegyógyászat 2001, **47(1)**: 28-32

2. **Rezes Sz,** Tóth L, Sipka S, Sziklai I: A gyermekkori otitis media catarrhalis chronica serosa (OMCCS) és az allergia összefüggésének vizsgálata. Fül-orr-gégegyógyászat 2009, **55(4)**: 165-69

Other international publications:

1. Papp Z, **Rezes S**, Jókay I, Sziklai I: Sensorineural hearing loss in chronic otitis media. Otol Neurotol 2003, **24(2)**: 141-4

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1. Papp Z, **Rezes Sz**, Jókay I, Sziklai I: Percepciós nagyothallás kialakulása idült gennyes középfülgyulladásban. Fül-orr-gégegyógyászat 2000, **46(2)**: 99-106
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1. **Rezes S**, Sipka S, Sziklai I: Inflammatory and immune proteins in the middle ear effusion of otitis media with effusion. 6th International Conference of PhD students, 12-18 Aug. 2007, Miskolc
2. **Rezes S**, Pitkäranta A, Sziklai I: Presence of human bocavirus and rhino-enteroviruses in otitis media with effusion in children. 8th International Conference of the ESPO, 8-11 June 2008, Budapest

Presentations in the theme of the dissertation in Hungarian language:

1. Késmárki K, **Rezes Sz**, Sipka S, Sziklai I: Serosus otitisben végzett allergológiai és immunológiai vizsgálatok klinikánkon (1998.01.01 - 2001.12.31). P 21. Magyar Fül-Orr-Gégeorvosok Egyesülete 37. Nemzeti Kongresszusa, Siófok, 2002. október 2-5.
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