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DIAGNOSTIC RELIABILITY OF INTRAOPERATIVE FIBEROPTIC LARYNGOSCOPIC REFLUX FINDING SCORING AND ESTIMATION OF MIDDLE EAR EFFUSION PEPSINOGEN-1 LEVELS IN CHILDREN WITH CHRONIC OTITIS MEDIA WITH EFFUSION

[1]Naslshah G. H. Kazem MD, [1]Mohamed Elsayed MD, [1]Mohamed H. G. MD, [2]Azza M. El-Bermawy MD, [3]Ola GH Kazem MD and [4]Lamiaa Z Mobarak MD

[1]Department of Otorhinolaryngology, Faculty of Medicine, Benha University.
[2]Department of Medical Biochemistry, Faculty of Medicine, Benha University.
[3]Department of Pharmacology, Faculty of Medicine, Benha University.
[4]Department of Tropical Medicine, Faculty of Medicine, Benha University.

***Corresponding Author: Naslshah G. H. Kazem MD**

Department of Otorhinolaryngology, Faculty of Medicine, Benha University.

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ABSTRACT

Objectives: To estimate pepsinogen-1 (PG1) levels in middle ear effusion (MEE) and serum of children had bilateral chronic otitis media with effusion (OME) and to evaluate reliability of intraoperative laryngoscopic determination of Reflux Finding Score (RFS) for presence and severity of laryngopharyngeal reflux (LPR). **Patients & Methods:** Thirty children with OME were asked to complete the Reflux Symptom Index (RSI) scoring sheet with a score >5 is strongly suggestive of LPR. Patients older than 6 years underwent upper gastro-esophageal endoscopy (GEE) for GERD grading according to the Los Angeles classification of esophagitis. After induction of anesthesia and prior to tracheal intubation all patients underwent laryngeoscopic examination for laryngeal manifestations of GERD and RFS scoring >11 is strongly suggestive of LPR. Then, patients were intubated and underwent myringotomy with insertion of Gromet's tube after collection of MEE samples. MEE and serum samples were collected for ELISA estimation of Human PG1 levels. **Results:** PG1 was detected in MEE of 23 patients (Group A), but none in MEE of 7 patients (Group B). RSI score was non-significantly higher, while RFS score was significantly (p=0.034) higher in group A compared to group B. Serum PG1 levels were non-significantly higher in patients of group B than group A. Mean PG1 levels in MEE of total and Group A patients were significantly (p=0.002, 0.001, respectively) higher than mean serum levels, while was significantly (p=0.001) lower than mean serum levels in Group B. FRS scorings showed positive significant correlation with PG1 levels of MEE, body mass index (BMI) and RSI scorings. PG1 levels of MEE showed positive significant correlation with RSI scores and BMI, while showed negative significant correlation with age and male gender. **Conclusion:** Measurable amounts of PG1 were detected in 76.7% of studied patients with significantly higher levels than serum levels. RSI and RFS could define patients with LPR. Obesity prevalence was 70% and showed positive significant correlation with RSI and RFS scoring and with PG1 levels estimated in MEE. Upper GEE may be unnecessary invasive investigation for children with OME unless indicated.

KEYWORDS: chronic otitis media with effusion, Middle ear effusion pepsinogen-1 level, Reflux Finding Score, Obesity.

INTRODUCTION

Otitis media with effusion (OME) is a common problem facing general practitioners, pediatricians and otolaryngologists. Otitis media with effusion (OME) is defined as chronic inflammation of the middle ear mucosa characterized by the retention of fluid behind an intact tympanic membrane within the middle ear space without signs or symptoms of an acute ear infection.[1] The most common complication of OME is hearing loss, and it is the commonest cause of hearing loss in children.[2]

The persistence of the middle ear effusion (MEE) for at least 3 months is defined as chronic otitis media with effusion (COME) which is a multifactorial disease. The causes of COME include Eustachian tube dysfunction, upper respiratory tract infection, insufficient aeration of the mastoid cells, mucociliary clearance abnormalities, adenoid disease, allergic rhinitis, and immunologic disorders.[3]

Gastro-esophageal reflux (GER) occurs when gastric contents reflux into the esophagus or oropharynx and

produce symptoms. It has been linked to the development of many airway disorders: croup, chronic cough, laryngeospasm, laryngeomalacia, vocal cord nodules, asthma, reactive bronchoconstriction, apnea, sudden infant death syndrome, rhinitis, sinusitis, subglottic stenosis, and glottic granuloma[4]

The possible relationship between GER and OME has been studied over recent years. GER is thought to cause inflammation of the nasopharynx, dysfunction of the Eustachian tube, and impairment of the mucociliary clearance, thus, increasing the incidence of OME.[5]

There is a strong association between GER and pharyngolaryngeal reflux as factors leading to respiratory disease, manifested as dysphonia, wheezing, coughing, recurrent laryngitis, bronchial obstruction, laryngospasm and apparent life-threatening events. These manifestations can be mild or severe and may sometimes put the patient's life at risk.[6]

Pepsinogen is secreted mainly by stomach mucosa cells. The process of pepsinogen activation requires cleavage of 44 amino acids from the primary structure of pepsinogen to transform it into active pepsin form in acidic conditions. Pepsinogen is completely deactivated in alkaline environments such as the middle ear cavity, so pepsin is not detected in the middle ear under normal conditions, but pepsinogen may be activated to pepsin under stimulation of hydrochloric acid in gastric juice during reflux.[7]

The current study aimed to estimate pepsinogen-1 levels in effusion fluid and serum of children had chronic otitis media with effusion (OME) and to evaluate the reliability of intraoperative laryngoscopic determination of Reflux Finding Score (RFS) as a test for presence and severity of laryngopharyngeal reflux (LPR)

PATIENTS AND METHODS

The current study was conducted at Otorhinolaryngology Department, Benha University Hospital in conjunction with Medical Biochemistry Department, Faculty of Medicine, Benha University since June 2012 till Aug 2013. The study protocol was approved by Local Ethical Committee. After obtaining written fully informed parents' consents, concerning diagnostic procedures including upper gastrointestinal (GI) endoscopy for signs of gastro-esophageal reflux disease (GERD), all children presenting with clinical manifestations of bilateral chronic otitis media (COM) were enrolled in the study.

Prior to clinical examination, patients during the attendance of their mothers were asked to complete the Reflux Symptom Index (RSI) score.[8] sheet about how much the included problems affected the patient within the last month. The sheet included nine problems each was graded 0 if there was no problem till five indicating severe problem and a score >5 in the proper clinical situation is strongly suggestive of laryngopharyngeal reflux (LPR) (Table 1).

Then, all patients underwent full otorhinolaryngological examination including clinical examination with concern of GERD-related manifestations. Tympanometry and pure-tone audiometry was performed to confirm diagnosis of otitis media with effusion (OME) and to evaluate the condition of hearing. Patients older than 6 years underwent upper GI endoscopy for GERD grading according to the Los Angeles classification of esophagitis[9] as table 2.

Table 1: The reflux symptom index (RSI).[8]

Problem	Evaluation score
1. Hoarseness or a problem with your voice	0 = no problem 5 = severe problem Total score range: 0-45
2. Clearing your throat	
3. Excess throat mucus or postnasal drip	
4. Difficulty swallowing food, liquids, or pills	
5. Coughing after you ate or after lying down	
7. Troublesome or annoying cough	
8. Sensations of something sticking in your throat or a lump in your throat	
9. Heartburn, chest pain, indigestion, or stomach acid coming up	

Table 2: Los Angeles Classification of Esophagitis.[9]

Type	Description
A	One (or more) mucosal break ≤5 mm that does not extend between the tops of two mucosal folds
B	One (or more) mucosal break >5 mm-long that does not extend between the tops of two mucosal folds
C	One (or more) mucosal break that is continuous between the tops of two or more mucosal folds but that involves <75% of the circumference
D	One (or more) mucosal break that involves at least 75% of the esophageal circumference

After induction of anesthesia and prior to endotracheal intubation all patients underwent laryngeoscopic examination for evaluation of laryngeal manifestations of GERD and fulfilling prerequisites of Reflux Finding Score (RFS) as shown in table 3 and findings were scored for a collective score and a collective score >11 is

strongly suggestive of LPR.[10] Then, patients were intubated and underwent myringotomy with insertion of Gromet's tube after collection of middle ear fluid with suction cannula. About 2 ml of middle ear fluid were collected from each ear separately in sterile Eppindorff's tube and kept frozen at -20^{o} till further processing. Simultaneously, a blood sample was obtained under complete aseptic conditions and was centrifuged at 3000 rpm and serum was separated and kept frozen at -20^{o} till further processing using ELISA using Human pepsinogen I ELISA kit (KT-810, Epitope Diagnostics, Inc., 7110 Carroll Rd, San Diego, CA 92121, USA).[11]

Table 3: Reflux Finding Score (RFS).[10]

Finding	Evaluation scores
Subglottic edema	0: absent; 2: present
Ventricular obliteration	0: no; 2: partial; 4: complete
Erythema/hyperemia	0: no; 2: arytenoids only; 4: diffuse
Vocal cord edema	0: no; 1: mild; 2: moderate; 3: severe; 4: polypoid
Diffuse laryngeal edema	0: no; 1: mild; 2: moderate; 3: severe; 4: obstructing
Posterior commissure hypertrophy	0: no; 1: mild; 2: moderate; 3: severe; 4: obstructing
Granuloma/granulation	0: absent; 2: present
Thick endolaryngeal mucus/other	0: absent; 2: present

Statistical analysis

Obtained data were presented as mean±SD, ranges, numbers and ratios. Results were analyzed using One-way ANOVA with post-hoc Tukey HSD Test and Chi-square test (X^2 test). Correlation between demographic and clinical data and laboratory findings was conducted using Pearson's correlation coefficient. Statistical analysis was conducted using the SPSS (Version 15, 2006) for Windows statistical package. P value <0.05 was considered statistically significant.

RESULTS

The study included 30 patients with chronic OME; 12 males and 18 females with mean age 6.2±1.7; range: 3-9 years. Twenty patients were ≥6 years old with mean age of 7.2±1.1; range: 6-9 years and 10 patients were <6 years old with mean age of 4.2±0.8; range: 3-5 years. Twenty-one patients were obese, while 9 patients were of ideal weight with a mean BMI of 20.8±6.1; 10.2-32 kg/m^2. Twenty patients had upper respiratory disorders with a mean number of symptoms of 1.4±0.7; range: 1-3 symptoms. All patients had type B hearing affection.

Effusion was collected from 60 ears of the thirty patients; pepsinogen I (PG1) was detected in 46 effusion samples of 23 patients who were grouped as group A, while no PG1 could be detected in 14 effusion samples of 7 patients who were grouped as group B. Patients' enrolment data showed non-significant (p>0.05) difference between both groups apart from patients' height where patients of group B were significantly longer than those of group S. Details of patients enrolment data are shown in table 4.

Table 4: Patients' enrolment data.

Data			Total (n=30)	Group A (n=23)	Group B (n=7)	P value
Age (years)	Frequency	<6	10 (33.3%)	9 (30%)	1 (3.3%)	NS
		≥6	20 (66.7%)	14 (46.7%)	6 (20%)	
	Mean (±SD)		6.2±1.7	5.9±1.7	7.3±1.5	NS
Gender	Males		12 (40%)	10 (33.3%)	2 (6.6%)	NS
	Females		18 (60%)	13 (43.4%)	5 (16.7%)	
BMI data	Weight (kg)		27±17.8	27.3±17.9	26.4±18.1	NS
	Height (cm)		129±21	122.7±20.5	147.3±14.9	=0.06
	BMI	Ideal	9 (30%)	6 (26.1%)	3 (42.9%)	NS
		Overweight	21 (70%)	17 (73.9%)	4 (57.1%)	
		Mean (±SD)	20.8±6.1	21.1±5.9	19.8±6.9	NS
Associated URT manifestations	Frequency	Yes	20 (66.7%)	16 (69.6%)	4 (57.1%)	NS
		No	10 (33.3%)	7 (30.4%)	3 (42.9%)	
	Type	Rhinorrhea	9 (30%)	8 (34.8%)	1 (14.3%)	NS
		Recurrent OM	8 (26.7%)	6 (26.1%)	2 (28.6%)	
		Laryngeal spasm	5 (16.7%)	4 (17.4%)	1 (14.3%)	
		Stret	5 (16.7%)	4 (17.4%)	1 (14.3%)	
		Otalgia	1 (3.3%)	1 (4.3%)	0	
	Number /affected patient		1.4	1.44	1.25	NS

Data are presented as numbers, mean±SD; percentages are in parenthesis; NS: Non-significant difference between both groups; URT: Upper respiratory tract; BMI: Body mass

Twenty-one patients had RSI of <5 and 4 patients had no symptoms; while only 5 patients had RSI of >5 for a mean RSI score of 3.2±2.3; range: 0-8 with non-significant (p>0.05) difference between both groups as regards frequency and mean score. Twenty-seven patients had RFS of <11; while only 3 patients had RFS of >11. However, mean RFS score was significantly (p=0.034) higher in patients of group A compared to group B. Details of patients reflux scoring data are shown in table 5 and figure 1.

Table 5: Patients' reflux scoring data.

Score		Total (n=30)	Group A (n=23)	Group B (n=7)	P value
RSI	0	4 (13.3%)	4 (17.4%)	0	NS
	<5	21 (70%)	14 (60.9%)	7 (100%)	
	>5	5 (16.7%)	5 (21.7%)	0	
	Mean (±SD)	3±2.3	3.1±2.5	2.7±1.3	NS
RFS	<11	27 (90%)	20 (60.9%)	7 (100%)	NS
	>11	3 (10%)	3 (21.7%)	0	
	Mean (±SD)	5.5±3.2	6.3±3.5	3.3±2.1	0.034

Data are presented as numbers & mean±SD; percentage are in parenthesis; RFS: Reflux Finding Score; RSI: Reflux Symptom Index.

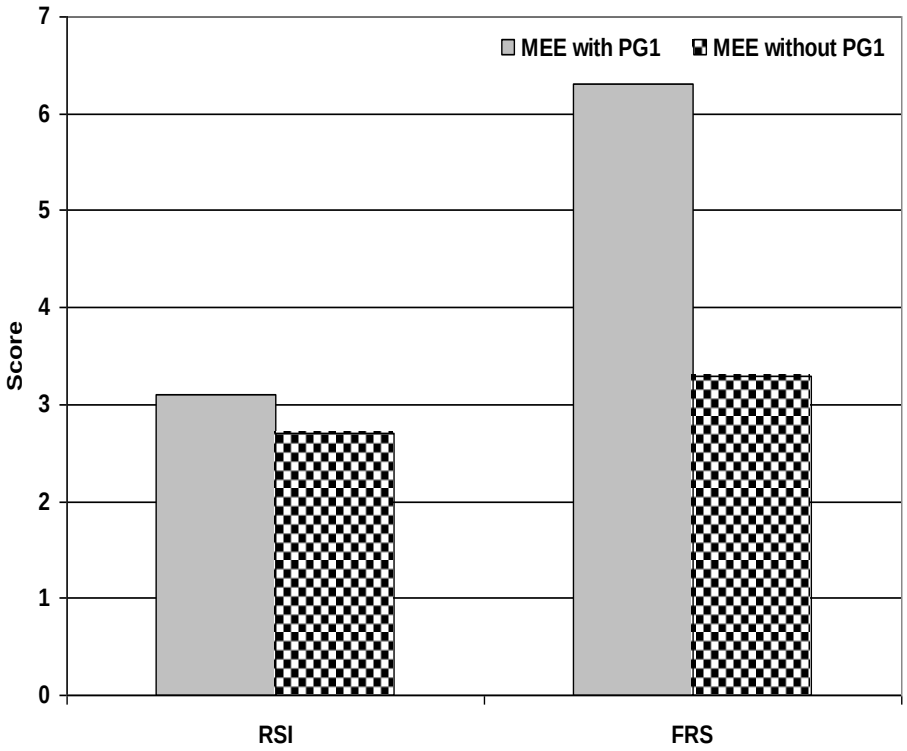

Fig. (1): Mean RSI and FRS scores of studied patients categorized according to detection of PG1 in their MEE

Mean serum PG1 levels was 41.7±22.3 ng/ml with non-significantly (p>0.05) higher serum PG1 levels in patients of group B (48.7±22.7 ng/ml) compared to those of group A (39.7±22.3 ng/ml). On contrary, PG1 levels in MEE of group A patients (109.3±60 ng/ml) was significantly (p=0.001) higher compared to that of group B (0 ng/ml) patients. Mean PG1 levels in MEE of total patients (83.8±70.3 ng/ml) was significantly (p=0.002) higher than mean serum level. However, mean PG1 levels in MEE of group A patients was significantly (p=0.001) higher than their serum levels. On the other hand, mean PG1 levels in MEE of group B patients was significantly (p=0.001) lower than their serum level (Fig. 2).

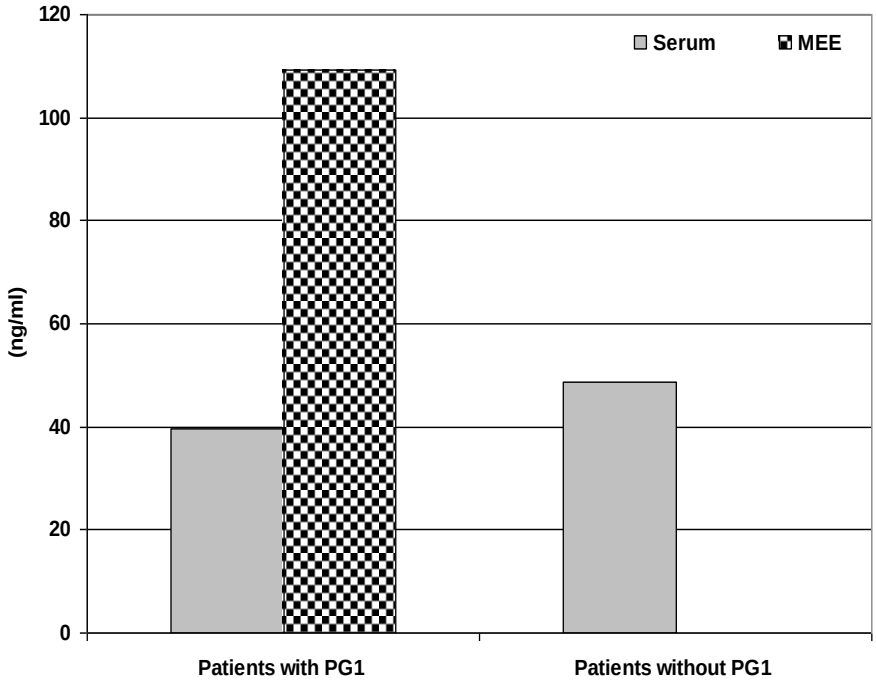

Fig. (2): Mean serum and MEE levels of PG1 estimated in studied patients categorized according to detection of PG1 in MEE

Mean PG1 levels in MEE of total patients showed positive significant correlation with RSI and FRS scores and with BMI, while showed negative significant correlation with age and male gender. BMI of studied patients showed positive significant correlation with FRS and negative significant correlation with male gender. FRS score showed positive significant correlation with RSI. On the other hand, correlations between other studied parameters were non-significant as shown in table 6.

Table 6: Correlation coefficients between patients' demographic and clinical data and estimated PG1 levels in serum and MEE.

Parameters	MEE PG1		BMI		FRS	
	r	p	r	p	r	P
MEE PG1			0.403	0.027		
Serum PG1	0.247	NS	0.236	NS	0.356	NS
Age	-0.395	=0.031	-0.271	NS	0.127	NS
Male Gender	-0.350	=0.049	-0.425	0.019	-0.344	NS
RSI	0.366	=0.046	0.233	NS	0.393	0.032
FRS	0.507	=0.004	0.371	0.043		
URT manifestations	0.171	NS	0.197	NS	0.191	NS
Endoscopic scoring	0.235	NS	0.321	NS	-0.103	NS

"r": Pearson's correlation coefficient; NS: non-significant correlation; $p<0.05$: significant correlation; MEE: Middle ear effusion; PG1: Pesinogen-1; RSI: Reflux Scoring Index; RFS: Reflux Finding Score; URT: Upper respiratory tract; BMI: Body mass index

DISCUSSION

The current study yielded multiple findings; firstly, measurable amount of pepsinogen I (PG1) was detected in middle ear effusion (MEE) of 23 patients (76.7%), but none was detected in MEE of 7 patients and estimated PG1 levels in MEE were significantly higher than serum levels. These findings indicated that the source of PG1 in MEE is not the transudation from circulation into ME cavity. In line with these findings, **Nair et al.**[12] detected high PG level in MEE samples in 65.6% of studied patients and **Miura et al.**[13] out of systemic literature review reported that in children with otitis media (OM) mean pepsin/PG presence was 85.3% and of enzymatic activity was 34.2%. Also, **Abdel-Aziz et al.**[14] found the MEE levels of pepsin/PG were up to 4-540 times higher than plasma levels.

Luo et al.[15] found pepsin and PG levels in ME fluid of OM patients were significantly higher than in children assigned for cochlear implant without OM and than plasma levels and concluded that pepsin and PG in ME cavity may be associated with the pathogenesis of OME

in children. Also, **Doğru et al.**[16] found mean PG level was significantly higher in ME fluid than in serum of patients with MEE.

Recently, in 2016; **Buyruk et al.**[17] detected significantly higher PG level in ME fluids compared with serum samples and found highest PG level was in patients with purulent effusion; indicating a relation between PG level and complicated OM.

Secondly, gastro-esophageal endoscopy (GEE) detected mild manifestations of GERD in only 3 patients, thus indicating that the presence of PG1 in MEE in measurable amount is not necessarily related to GERD severity. In support of this, PG1 concentration in MEE showed a positive non-significant correlation with the presence of endoscopic GERD manifestations. Similarly, **Nair et al.**[12] reported lack of symptoms associated with GERD among children had OM with statistically insignificant association between GERD score and PG level.

However, review of literature showed that the association between presence and level of PG1 in MEE and presence and/or scoring of GERD is still a matter of controversy where **Miura et al.**[13] detected a mean prevalence of GERD in children with chronic OME of 48.4% and in children with recurrent acute OM (AOM) of 62.9% and concluded that the presence of pepsin/PG in ME could be attributed to physiologic reflux, but a cause-effect relationship between pepsin/PG in ME and OM is unclear. On the other hand, **Abdel-Aziz et al.**[14] reported a significant positive correlation between MEE pepsin levels and the number of pharyngeal reflux episodes measured by pH monitoring. Also, **Doğru et al.**[16] found MEE of 19% of patients were positive for H. pylori that showed a significant correlation with increased PG levels in MEE and concluded that these results support the role of laryngopharyngeal reflux (LPR) in the pathogenesis of OME. Such controversy could be attributed to limited number of parents accepting to subject their children to invasive investigations so the prevalence was discrepant and to the difference in the accuracy rate of these diagnostic investigations. However, the interference of patients' constitutional data leading to or associated with GRED may lead to varied findings on evaluation.

Thirdly, 70% of studied patients were overweight compared to the cross-age and gender standard BMI with positive significant correlation between PG1 levels and BMI. In line with these findings, **Nelson et al.**[18] found children with a history of tympanostomy tube had a significantly higher risk of having a weight-for-length $\geq 95^{th}$ percentile. **Kuhle et al.**[19] suggested that there is a clear association between childhood obesity and OM. **Sidell et al.**[20] found nearly one-fourth of all children seeking health care are obese and reported a positive significant association between obesity and development of AOM.

Lee & Yeo[21] supposed that obesity may result in altered cytokine expression, GERD or fat accumulation, all may contribute to OME which may induce taste changes through ME cavity inflammation, thus contributing to obesity.

The obtained data spot light on a possibility of the presence of minimal gastro-esophageal sphincter (GES) incompetence which mostly occurs in overweight and obese people, especially on lying down, thus allowing escape of minute amounts of gastric juice with its PG1 content into the pharynx. Escaped juice may leak into ME cavity through the Eustachian tube or being inhaled into the larynx or irritating pharyngeal tonsils or nerves or being exhaled and irritating the nasal mucosa.

In support of these assumptions, 20 of studied patients had upper respiratory tract (URT) manifestations suggestive of the presence of LPR including with a mean number of about 1.4 symptom/ patients. Moreover, there was positive significant correlation between MEE levels of PG1 and scorings of both the Reflux Symptom Index (RSI) questionnaire and Reflux Finding Score (RFS) of intraoperative laryngoscopy and between BMI and both these scorings and with presence and multiplicity of the detected URT manifestations.

In line with these findings and relationships and with the provided assumption; **Habesoglu et al.**[22] found RFS was significantly higher in patients with unsuccessful tympanic membrane closure after myringotomy for OME than in patients with successful closure. **Luo et al.**[15] reported that pepsin and PG in ME cavity of OME patients may be originated from LPR that may be associated with the pathogenesis of OME in children.

In support of reliability of RSI and RFS scoring for LPR evaluation in patients with otorhinolaryngological disorders and associated URT manifestations suggestive of LPR; **Sone et al.**[23] reported that the prevalence of LPR symptoms evaluated by the RSI was significantly higher in OME patients than in the health checkup examinees and LPR symptoms were prominent in middle-aged men with a high BMI. **Li et al.**[24] examined children with URT manifestations using RSI, RFS, 24-hour pH monitoring and proton-pump inhibitors diagnostic therapy and found the positive diagnostic rates were 91.9%, 79%, 30.8% and 85.5%, respectively. **Spyridoulias et al.**[25] found salivary pepsin may be used as screening adjunct to supplement RFS in clinical workup of patients with extra-esophageal and URT presentations of reflux.

Recently, in 2016; **Verim et al.**[26] found LPR judged by high RSI and RFS scorings was associated with worse radiology and endoscopy scores in patients with chronic rhinosinusitis without polyposis. Also, **Min et al.**[27] found patients who underwent laryngeal microsurgery for benign laryngeal disease and had high RSI and RFS scoring showed significantly higher expression of

carbonic anhydrase III and heat shock protein 70, biomarkers for LPRD diagnosis, in specimens of mucosa of posterior commissure than those had lower RSI and RFS scorings.

CONCLUSION

Measurable amounts of PG1 were detected in 76.7% of studied patients with significantly higher levels than serum levels. Preoperative RSI and intraoperative laryngoscopic RFS could define patients with LPR. Obesity prevalence was 70% among studied patients and showed positive significant correlation with RSI and RFS scoring and with PG1 levels estimated in MEE. Obesity may be the start point for a vicious cycle ending in OME. Upper GEE may be unnecessary invasive investigation for children with OME unless indicated.

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