



RESEARCH

EFFECT OF TRANSTYMPANICALLY APPLIED GASTRIC CONTENT ON DISTORTION PRODUCT OTOACOUSTIC EMISSIONS IN RABBITS

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SUMMARY

Purpose: Gastro-oesophageal reflux is a common problem in children and even in adults. Many otolaryngological problems were linked to reflux induced inflammation. Reflux has been implicated as a culprit in otitis media with effusion mostly in children.

Materials and Methods: Sixteen rabbits were used. Animals were divided into two groups. One group received its own gastric content and the other group received saline transtympanically for twenty days. Hearing levels were evaluated with distortion product otoacoustic emissions (DPOAE) on day 0 and day 27. Animals were sacrificed on day 27 and histological analyses of middle ear were performed.

Results: Epithelial thickness of the middle ear mucosa, capillary vasodilatation and inflammatory cell count were all significantly increased in the gastric-content group compared with the control group ($(p = 0.002, \chi^2 = 12.667)$ ($p = 0.004, \chi^2 = 11.200$), $p < 0.001$ respectively). The comparison of pre and post procedure difference of (signal to noise ratio) SNR levels between gastric content and control groups revealed statistically significant difference in 0.5 and 1 kHz frequencies ($p = 0.017$, $p = 0.017$ respectively).

Conclusion: Transtympanically applied gastric content is found to induce middle ear inflammation and decrease in cochlear reserve in 0.5 and 1 kHz frequencies.

Keywords: Laryngopharyngeal Reflux, otoacoustic emissions, gastric juice, hearing loss

TRANSTİMPANİK UYGULANAN GASTRİK İÇERİĞİN TAVŞANLARDA OTOAKUSTİK EMİSYON ÜZERİNDEKİ ETKİSİ

ÖZET

Amaç: Gastroezofajial reflü çocuklarda ve hatta erişkinlerde sık görülen bir sorundur. Birçok otolaringolojik sorun reflü tarafından tetiklenen inflamasyonla ilişkilidir. Reflü, çocuklarda effüzyonlu otitis media nedenlerinden biri olarak gösterilmektedir.

Hastalar ve Yöntemler: Bu çalışma Dokuz Eylül Üniversitesi Hayvan laboratuvarında gerçekleştirilmiştir. 16 erişkin tavşan üzerinde çalışılmıştır. Hayvanlar iki gruba ayrılmıştır. Yirmi gün boyunca bir gruba kendi mide içeriği, diğer grupta transtimpanik salin verilmiştir. İşitme seviyeleri distorsiyon produkt otoakustik emisyon (DPOAE) ile 0 ile 27 günlerde ölçülmüştür. Hayvanlar 27 gün sakrifiye edilmiş, orta kulakları incelenmiştir.

Bulgular: Orta kulak mukozasının epitelyal kalınlığı, kapiller vazodilatasyon ve inflamatuvar hücre sayıları gastrik grupta istatistiki olarak anlamlı bulunmuştur. ($(p = 0.002, \chi^2 = 12.667)$ ($p = 0.004, \chi^2 = 11.200$), $p < 0.001$, sırasıyla) Pre – postop SNR seviyeleri 0,5-1 kHz seviyesinde istatistiki olarak anlamlı fark bulunmuştur. ($p = 0.017$, $p = 0.017$)

Sonuç: Transtimpanik verilen gastrik içerik orta kulak inflamasyonuna yol açmakta ve 0,5 ve 1 kHz seviyesinde işitme kaybına yol açmaktadır.

Anahtar Sözcükler: Laringofaringeal Reflü, otoakustik emisyonlar, gastrik sıvı, işitme kaybı

INTRODUCTION

Gastro-oesophageal reflux (GER) is common in children and is associated with morbidity and mortality¹. In the first few months of life (0-3 months), postprandial reflux is a physiological event which gradually decreases, disappearing by one year of age². The passage of gastric contents from the oesophagus into the respiratory and otolaryngeal tracts is extra-oesophageal reflux.

Extra-oesophageal reflux is implicated in various otolaryngologic diseases such as laryngitis, subglottic stenosis³, chronic sinusitis⁴, and otitis media³. Given anatomic and physiologic immaturity of Eustachian tube in children, they are prone to otitis media with effusion (OME) compared to adult population. Extra-oesophageal reflux can cause inflammation of Eustachian tube, impaired mucociliary clearance, obstruction of the tube and stasis of secretions and eventually effusion. Pepsin was demonstrated in middle ear of patients with otitis media with effusion⁵. A recent study O'Reilly et al demonstrated pepsin in the middle ear cleft of 20% of paediatric patients with otitis media undergoing tympanostomy tube placement, compared with 1.4% of controls⁶.

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Hearing loss caused by OME can be evaluated using otoacoustic emissions (OAE). Studies indicate that cochlear reserve can be monitored especially with distortion product otoacoustic emissions (DPOAE) in patients with OME^{7,8}.

Here we use an experimental reflux induced middle ear inflammation model to evaluate the effect of gastric content induced inflammation on hearing levels and cochlear reserve.

MATERIAL and METHODS

All animals were treated in accordance with protocols approved by the Animal Care and Use Committee of Dokuz Eylul University. The study was conducted at the animal care facility of Dokuz Eylul University Faculty of Medicine. Sixteen healthy adult New Zealand rabbits (2271 to 3182 g each) with no external or middle ear pathology were used.

The animals were anesthetized by intramuscular administration of Xylazine (Basilazin, 5 mg/kg; Animedica GmbH, Senden-Bosensell, Germany) and ketamin (Ketalar, 50 mg/kg; Eczacıbaşı Warner Lambert, Istanbul, Turkey). DPOAE (Vivosonic Integrity V500 Toronto, Ontario, Canada) measurements were made using tympanometry probes adjusted to fit in the rabbit ear canal. Baseline DPOAE measurements were taken from the ears before the procedure. The cochlear activity of ear of all rabbits was examined in day 0 and day 21, using DPOAEs in sedated animals. Once the probe was placed with a good seal in the ear canal, the DPOAEs were measured. Equilevel primary tones f1 (65 dB) and f2 (55 dB) were fixed at f1/f2 = 1.22, and DPOAEs were measured at seven different frequencies ranging from 0,5 to 8 kHz (0,5, 1, 2,3, 4, 5, and 8 kHz). By calculating the difference between the distortion products and noise standard deviations, the signal-noise ratios (SNR) for each frequency were found. Two DPOAE measurements were obtained on days 0, and 21 in order to detect any possible deterioration in cochlear activity. All measurements were recorded in a quiet room.

Under microscopic observation, a tympanic perforation was created using a pick. Gelfoam was inserted through the perforation to prevent tympanic membrane closure. After the surgical procedure, the animals were divided into two groups of eight each. The rabbits received either their own gastric contents (gastric-content group, n = 8) or saline (control group, n = 8) transtympanically injected with a 23-gauge dental syringe, once daily (0.1 mL/day) for 20 days. On day 27, the rabbits were euthanized by

injection of a lethal dose of pentobarbital sodium and isopropyl alcohol. After decapitation, the temporal bones were harvested.

Formalin-fixed, paraffin-embedded samples of tissues from control and experimental rabbits were prepared by routine paraffin embedding procedures. In brief, tissue samples were fixed in 10% formalin for 24 to 48 h, dehydrated in a graded series of ethanol, cleared in xylene, and embedded in paraffin. Paraffin blocks were serially sectioned at a thickness of 5 µm and prepared for histochemical staining. Serial sections were obtained from middle ear tympanic membrane to middle ear promontorium mucosa. For histological assessment, sections were stained with hematoxylin and eosin (H&E). The sections observed under a bright-field microscope (BX 40; Olympus, Tokyo, Japan). Control samples were processed in an identical manner. Two blinded observers independently and scored the H&E stained sections for epithelial thickness, capillary vasodilatation and number of inflammatory cells.

Statistical analysis

SPSS 16 for Windows was used for the statistical analysis. Histochemical staining differences were evaluated using the contingency-table analysis using the χ^2 test. Fisher's exact test was used when numbers in one of the cells were < 5. SNR level intragroup differences were evaluated with Wilcoxon signed rank test, and inter group differences with Mann-Whitney U test. The statistical significance level was established at P < 0.05, and confidence intervals were 95%.

RESULTS

Histological findings

In the control group, microscopic observations of the H&E-stained sections confirmed that the middle ear space contained simple squamous epithelium, consistent with bone. In the gastric-content group, microscopic observations of the H&E-stained sections revealed thickened lamina propria and epithelium lining; increased numbers of active fibroblasts, collagen fibres, and inflammatory cells; and vascular dilatation (Figure 1). Scores for epithelial thickness (normal, 1; mildly thickened, 2; and thickened two-fold, 3; and capillary vasodilatation (none, 1; mildly increased, 2; and increased, 3) were assigned for each group (Figure 2). Epithelial thickness of the middle ear mucosa, and capillary vasodilatation were all significantly increased in the gastric-content group compared with the control group (3 X 2 contingency-table analysis using the χ^2 test, confidence interval % 95) [(p =



0.002, $\chi^2 = 12.667$) ($p = 0.004$, $\chi^2 = 11.200$), and ($p = 0.01$, $\chi^2 = 9,143$) respectively). Inflammatory cells were significantly increased in gastric content group compared with the control group (Mann-Whitney U test, $p < 0.001$) (Figure 3)

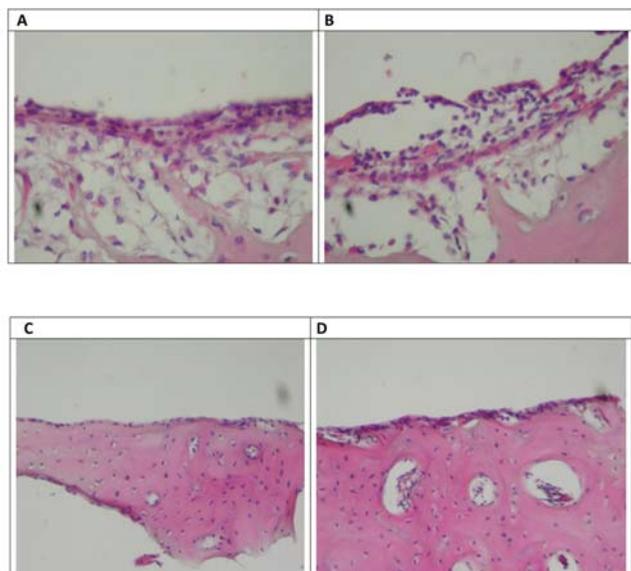


Figure 1: H&E-stained sections of the middle ear from rabbits in the gastric-content and control groups. (A-B) Control group. (C-D) Gastric-content group. Thickening of the epithelium, increased numbers of active fibroblasts and collagen fibers in the lamina propria, increased capillary dilatation, and increased number of inflammatory cells is seen. Ep: epithelium. Lp: lamina propria. Cp: capillary. B: bone.

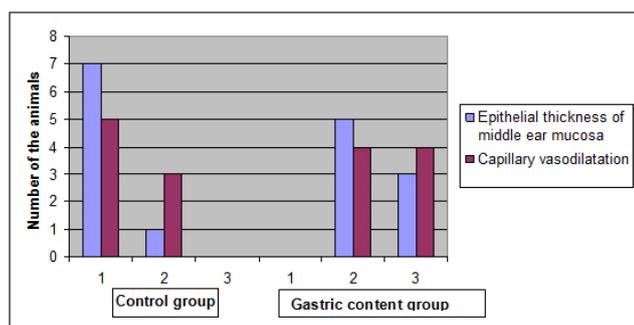


Figure 2: A graphic showing comparison of the histopathological scores of the control and gastric-content groups. The scoring of each parameter was as follows: Epithelial thickness of the middle ear mucosa: normal, 1; mildly thickened, 2; or thickened two-fold, 3. Number 2. Capillary vasodilatation: none, 1; mildly increased, 2; or increased, 3.

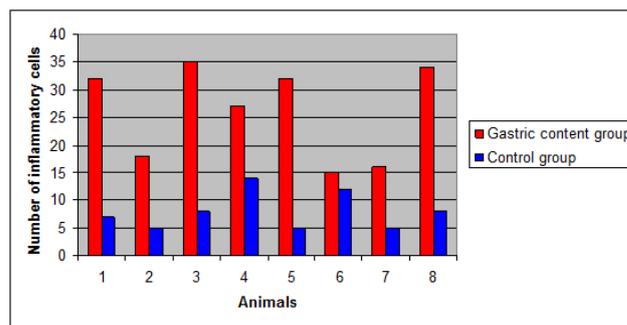


Figure 3: Comparison of the number of inflammatory cells in both groups (x400 magnification)

Audilogic Assessment

The comparisons of pre-procedure and post-procedure SNR levels are listed in Figures 4 and 5. In control group the differences of pre and post-procedure SNR levels in 0.5, 1, 3, 4, 5 kHz frequencies were found to be statistically significant ($p < 0.05$). In gastric content group the differences of pre and post-procedure SNR levels in 0.5, 1, 2, 3, 4, 5 kHz frequencies were found to be statistically significant ($p < 0.05$). The comparison of pre and post procedure difference of SNR levels between gastric content and control groups revealed statistically significant difference in 0.5 and 1 kHz frequencies ($p < 0.05$).

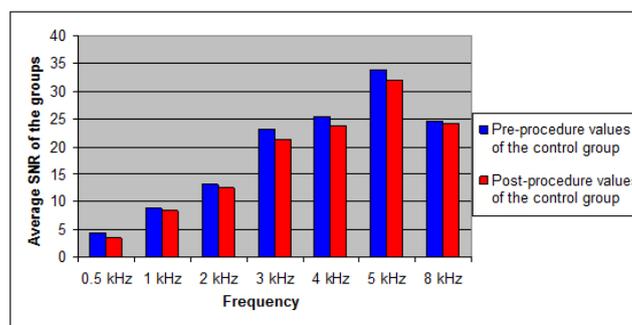


Figure 4: The comparison of pre-procedure and post-procedure signal noise to ratio (SNR) levels of the control group

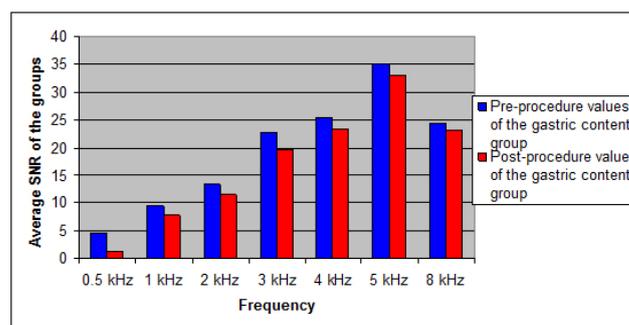


Figure 5: The comparison of pre-procedure and post-procedure SNR levels of the gastric content group



DISCUSSION

Otitis media with effusion is one of important cause of hearing loss in children. Extra-oesophageal reflux is one of the underlying culprits of OME⁹. In our study we create an experimental reflux model for evaluating pattern of gastric content induced hearing loss and assess the destructive effect of gastric content on cochlear reserve audilogically.

Heavner et al reported repeated exposure of pepsin in hydrochloric acid transtympanically leads to Eustachian tube dysfunction.¹⁰ Although they do not assess hearing status, Eustachian tube dysfunction is the most important factor in middle ear disease¹¹, and it can be speculated reflux can induce a chain of inflammatory events that lead to OME and hearing loss.

In our study both in control and gastric content group show evidence of hearing deterioration. Except in frequencies 2 kHz and 6 kHz in control group and 6 kHz in study group, the intervention, regardless of inciting agent, seem to cause hearing loss in most of the frequencies. The difference of SNR levels between study and control group on the contrary, reveal statistically significant hearing loss in frequencies 0.5 and 1 kHz.

In a recent study, Tas et al reported 19 children (38 ears) with OME who are scheduled for myringotomy and/or ventilation tube insertion were evaluated with DPOAE⁸. They found that especially glue ear effusion can cause a reduction in emissions in 2, 4 and 8 kHz. A similar study performed on 43 children (85 ears) Yeo et al has concluded that middle ear effusion could affect the expression of DPOAE in frequencies 3 and 4 kHz¹². Both of these papers do not investigate the underlying factors that cause effusion.

In our study hearing loss observed nearly all frequencies measured in both groups might reflect the fact that perforated tympanic membrane and the fluids that fill the middle ear might result in deterioration of hearing status. Histological investigation revealed increased epithelial thickness of the middle ear mucosa, capillary vasodilatation and increased number of inflammatory cells compared to control group. As shown by histological evidence pronounced inflammation in study group might be responsible of low frequency hearing loss as compared to control group. This could be consequence of gastric content induced middle ear effusion.

It is known that ototoxic effects are initially seen in the high frequency region of the cochlea and lower frequencies are affected in the late stage of the ototoxic drug administration. Locally administrated povidine-iodine solution could cause transient otoacoustic emission disappear¹³. In rabbit ototoxicity model, Oghan et al assessed transtympanically administrated gentamicin and tobramycin with DPOAE and concluded that low frequencies (2 and 3 kHz) are more sensitive to the administration of topical gentamicin than to topical tobramycin¹⁴. The ototoxicity of topically applied agents is different between laboratory animals and humans. This may explained by the fact that in humans the round window is deeper and thicker than in small laboratory animals¹⁵. Statically significant decreased in cochlear reserve of the study group in frequencies 0.5 and 1 kHz were found. It can be speculated that gastric content might induce cochleotoxic effect on low frequencies. A recent study investigated ototoxicity of gastric reflux on rats. They prepared acidified gastric pepsin by the addition of HCl and deionized water to pepsinogen from porcine stomach and demonstrated that the acidified gastric pepsin caused statistically significant hearing loss in the experimental ears.¹⁶ The discrepancy between results might result from the methodology of the studies. In our study we used animal's own gastric content that resembles natural content of the laryngopharyngeal reflux.

A recent interesting study by Deppe et al.¹⁷ demonstrated that conductive hearing loss (CHL) alters the L2/L1 ratio that reaches the inner ear. As consequence of a CHL, the absolute DPOAE level alterations strongly depend on the L2,L1 combinations. They stated that as consequence of a CHL, DPOAE level can increase and concluded that Continuously increasing CHL values do not necessarily result in monotonically decreasing DPOAE level alterations.

There is some certain limitation of our study. Although we have histological examination of middle ear, there is no scanning microscopic examination of cochlea. A scanning electron microscopic examination of cochlea might support our speculation if gastric content is cochleotoxic. In our study hearing loss observed nearly all frequencies measured in both groups might reflect the fact that perforated tympanic membrane and the fluids that fill the middle ear might result in deterioration of hearing status. Histological investigation revealed increased epithelial thickness of the middle ear mucosa, capillary vasodilatation and increased number of inflammatory cells compared to control group. Apart



from the previous clinical studies mentioned our result show low frequency hearing loss. We have demonstrated that gastric acid induced middle ear effusion in an animal model result in a decreased SNR level in 0.5 and 1 Hz. Most of the clinical studies that evaluate the value of DPOAE in OME, did not investigate the inciting event that causes the effusion. We think that our results reflect a cochlear toxicity induced by gastric content.

CONCLUSION

Transtympanically applied gastric content can induce in middle ear inflammation and decrease in cochlear reserve in 0.5 and 1 kHz frequencies. This paper is the first study to evaluate gastric content induced ototoxicity. Further studies needed to investigate if gastric content is ototoxic or not.

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