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Study of surfactant level in cases of Otitis Media with Effusion

Tahia Hashem Saleem[1], Esam A. Abo El-magd[2], Ahmd Gaber AbdElraheem[3], Zaki F. Aref[3], Mahmoud S. Khalefa [2]

1. Biochemistry Department, Faculty of Medicine, Assuit University.
2. ENT Department, Faculty of Medicine, Aswan University.
3. ENT Department, Faculty of Medicine, South Valley University.

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ABSTRACT

Introduction: Otitis media with effusion (OME) is a common disease characterized by the retention of fluid and inflammatory by-products in the middle ear without any clinical symptom of acute infection .***Objective:*** To evaluate the level of surfactant in patients with otitis media with effusion. Methods: The surfactant system of the middle ear was examined biochemically in cases of otitis media with effusion and compared to control group. A total of 60 patients with otitis media with effusion compared to 20 normal volunteers.

Results: Biochemical analysis of the nasopharyngeal aspirate around the Eustachian tube opening in control group which obtained endoscopically through the nose revealed the presence of phospholipids constituting surfactant with phosphatidylcholine constituting 73.25% of the total phospholipids. Biochemical analysis of the effusion aspirate in cases of otitis media with effusion revealed a highly significant decrease in the total phospholipids compared to normal control group and also a significant change in the phospholipid profile.

Conclusion: significant biochemical changes in the surfactant system of the middle ear are an evident finding in cases of otitis media with effusion. This suggests a possible role for surfactant deficiency in the aetiopathogenesis of cases of otitis media with effusion.

INTRODUCTION:

Otitis media with effusion (OME) is the most common inflammatory disease of the middle ear, characterized by the presence of fluid in the middle ear cavity, behind an intact tympanic membrane. Typically, there are no signs or symptoms of acute infection. OME mainly affects children at a critical age of speech and language development, with hearing loss as the major complaint. OME is generally considered as a benign condition with high percentage of spontaneous recovery. [1,2] However, if OME persists for more than 3 months, it is defined as chronic otitis media with effusion (COME). For COME the guidelines of the National Institute for Health and Care Excellence (NICE)

recommend the insertion of transtympanic ventilation tubes (TVT) or the fitting of hearing aids when surgery is contraindicated.[3] several risk factors have been associated with OME i.e previous acute otitis media, hereditary, parental smoking, attending day care centres, bottle feeding and autumn season.[4,5] The pathogenesis includes inflammation of the middle ear mucosa and an accumulation of fluid within the middle ear. The disturbance of the excretory function is due to mechanical obstruction of the Eustachian tube and/or mucociliary dysfunction of the tubotympanum. The pathogenesis is not fully understood, especially the reasons for failure of mucociliary clearance of the middle ear. It is not clear whether the cilia function normally in the middle ear and ET in the chronic phase of OME. [6]

Flisberg et al. (1963)[7] were the first to suggest that the presence of surface tension-lowering substances (surfactants) might influence the function of the ET. Several recent studies investigated the efficacy of surfactant therapy on the resolution of OME.[8] Although surface-active substances may be helpful in resolving acute OME due to infection and inflammation, persistent OME can develop due to a variety of pathological conditions related to the structure of the ET. [6]

Material and Methods

Over a period of 12 months from December 2015 to December 2016, 60 patients with otitis with effusion aged from newborns to 20 years and 20 healthy volunteers were used as controls, were included from ENT outpatient clinic of South Valley University hospital. All patients and controls gave written informed consent before entering the study and the study protocol was approved by the ethical committee of the faculty of Medicine, South Valley University.

The diagnosis of otitis media with effusion was made in our study group on the basis of the following clinical findings in the form of dull tympanic membrane, loss of cone light, loss of landmarks of the eardrum, blue drum, and/or alteration in the mobility of tympanic membrane. Every patient had complete ear, nose and throat examination. All cases had detailed assessments aided by X-ray of soft tissue neck (lateral view) for adenoidal enlargement and an audiologic assessment. All patients were subjected to tympanometric screening (Immittancemeter-Interacoustics-Automatic AZ26, Denmark). Patients subjected to surgical management in the form of myringotomy and ventilation tube insertion (grommet), myringotomy and adenoidectomy or myringotomy and adenotonsillectomy according to the predisposing factor. Samples of middle ear effusions were collected using sterile syringe during the puncture of tympanum or tympanostomy tube placement. The amount, colour and character of middle ear effusions were recorded. These middle ear effusions were used for measurement of total and differential phospholipids.

For control group, Samples collected from nasophyrengeal secretions around Eustachian tube opening under endoscopic guidance through the nose and used for measurement of total and differential phospholipids (surfactant).

Phospholipid assessment: aspiration of 0.2 ml was enough for the analysis. The organic solvents, chloroform, methanol, methylacetat and n-propanol were of analytical grade (E.Merck,

Darmstadt). The phospholipid standards, phosphatidylcholine (p-6638), sphingomyelin (S-7004), phosphtidylethanolamine (P-9137), phosphatidylinositol (P-5766),phosphatidylserine (P-6641) and the detection reagent 8-anilino-1-naphthalene sulphonic acid, ammonium salt, practical grade (A-3125) were purchased from Sigma, St Louis.

Extraction of phospholipids: In the extraction of phospholipids, about 250µl of the rinsing were pipetted into a 10 ml disposable test tube containing three ml chloroform-methanol. The mixture was shaken vigorously for three minutes and the aqueous and organic phases were separated by centrifugation for five minutes at 2000 g; the aqueous (upper) layer was discarded, the organic layer was transferred to a 5 ml Pyrex beaker, and evaporated in a 60º C water bath, using a hair-dryer. After drying, the residue was re-dissolved in 50 µl of chloroform-methanol (1:1 volume/volume). Samples were spotted onto precoated silica gel using two-dimensional thin layer chromatography (TLC). The first phase solvents consisted of chloroform-methanol-acetic acid-saline (50:25:8:4) whereas the second phase was chloroform-methanol-acetic acid-saline (50:7.5:8:2). Separation was carried out on 20× 30 cm plates in solvent saturated chambers. Thirty to 50µl of the extracts were spotted onto the silica gel plates, whereas control standards of synthetic phospholipids were spotted parallel to the rise of each solvent. After development, the plate was removed from the tank and dried under a stream of warm air until solvent removal was complete. To visualize the spots of phospholipids, the plate was dipped into a 0.2 per cent aqueous solution of the detection reagent. To remove excess reagent, the plate was placed face downwards on lint-free tissue paper. When inspected in ultraviolet light at 365πm, the spots appeared yellow-green on a dark, uncoloured background that was only faintly fluorescent.

Phospholipids quantitation: The spots were scraped into Pyrex tubes. To each tube 0.3 ml 10 N sulphuric acid and 0.1 ml hydrogen peroxide (100 volume) were added. The tubes were then heated at 180ºC for 30 minutes. A further 0.1 ml hydrogen peroxide (100 volume) was added and the tubes reached at 180ºC for a further 30 minutes. To the digestion products in the Pyrex tubes, 4 ml distilled water was added. This was mixed thoroughly and centrifuged using a GallenKampbench for five minutes to pellet the silica gel. To 2 ml of the supernatant was added 2 ml of the reducing reagent solution and these mixed thoroughly. The tubes were heated at 80ºC for 15 minutes to develop the final colour and the optical density was measured at 820 πm using a Pye-UnicamSp 500 or a Cecil spectrophotometer.

Estimation of total phospholipids: total phospholipids were estimated in the extract by using Kits supplied by Sigma St Louis and the amount counted per ml of the extract. By using the percentage of different kinds of the phospholipids, quantitation of each parameter was also counted.

Statistical analysis: Data entry and data analysis were done using SPSS version 19 (Statistical Package for Social Science). Data were presented as number, percentage, mean, standard deviation and median. Chi-square test was used to compare between qualitative variables. Mann-Whitney test was used to compare quantitative variables

between groups in case of non-parametric data. P-value considered statistically significant when $P < 0.05$.

for all these tests, the level of significance (P-value) can be explained as:

1-No significance $P > 0.05$
2-Mild Significance $P < 0.05$
3-Moderate significance $P < 0.01$
4-High significance $P < 0.001$.

Results:

Eighty participants were enrolled in this study, 49 were males and 31 were females they were divided into two groups., group A (60) patients of otitis media with effusion and group B (20) control group. A total of 36 (60%) patients were under the age of five years, while 18 (30%) were between 5-10 years. The remaining 6 (10%) patients were between 10-20 years while in group B (control) 12 (60%) subjects were under the age of five years and 5 (25%) were between 5-10 years and 3 (15%) were between 10-20 years. In group A Patients included were 24 (40%) females and 36 (60%) males while in control group B there were 13 (65%) males and 7 (35%) females.

All these cases belonged to the middle and low socio-economic group. Unilateral ear disease was diagnosed in 11 (18.3%) patients while the others 49 (81.7%) had bilateral ear involvement.

Recurrent upper respiratory tract infection (Rhinosinusitis) was the most frequent predisposing factor accounting for 58.3 % of all cases; adenoids were diagnosed in 20% of patients, adenotonsillitis in 16.7%; tonsillitis were diagnosed in 5% of patients.Surgical intervention was done in the form of bilateral myringotomy and grommet tube application in combination to adenoidectomy were done to 20% and bilateral myringotomy with grommet tube application and adentonsillectomy were done to 16.7%, bilateral myringotomy and grommet tube application were done in 40%, bilateral myringotomy and grommet tube application and tonsillectomy were done in 5%,while unilateral myringotomy and grommet tube application were done in 18.3% (table1)

Table (1): Operations done

Operations done	No. (= 60)	%
Bil. myringotomy and grommet tube insertion and adenoidectomy	12	20.0
Bil. myringotomy and grommet tube insertion and adenotonsillectomy	10	16.7
Bil. myringotomy and grommet tube insertion and tonsillectomy	3	5.0
Bil. myringotomy and grommet tube insertion	24	40
Unilateral myringotomy and grommet tube insertion	11	18.3

Total phospholipids were significantly decreased in our 60 patients with comparison to 20 control group (table-2).

Table (2): Total phospholipids

Total phospholipids	Study (n= 60)	Control (n= 20)	P-value
Mean ± SD	436.97 ± 267.40	1244.00 ± 79.56	0.000*
Median (Range)	405.0 (140-830)	1240.0 (1100-1410)	

Differential phospholipids profile: significant decrease in phosphatidylcholine (lecithin) and non-significant decrease in phosphatidylethanolamine (cephalin)and non-significant increase in sphingomyline and significant decrease in other phospholipid in the patient group in comparison to normal control group **(**table-3-4-5-6)

Table (3): Level of Lecithin in the study group in comparison to control group

Lecithin	Study (n= 60)	Control (n= 20)	P-value
Mean ± SD	182.72 ± 106.85	911.20 ± 22.09	0.000*
Median (Range)	155.0 (60-373)	905.5 (880-970)	

Table (4): Level of Cephalin in the study group in comparison to control group

Cephalin	Study (n= 60)	Control (n= 20)	P-value
Mean ± SD	151.82 ± 130.41	212.80 ± 32.45	0.260
Median (Range)	105.0 (20-390)	208.5 (150-270)	

Table (5): Level of Sphingomyelin in the study group in comparison to control group

Sphingomyelin	Study (n= 60)	Control (n= 20)	P-value
Mean ± SD	65.43 ± 90.28	61.45 ± 27.20	0.254
Median (Range)	40.0 (10-700)	51.5 (24-103)	

Table (6): Level of other phospholipids in the study group in comparison to control group

Others	Study (n= 60)	Control (n= 20)	P-value
Mean ± SD	47.38 ± 32.18	58.50 ± 20.46	0.014*
Median (Range)	40.0 (8-140)	60.5 (16-81)	

Discussion

Otitis media with effusion is a very common problem in children. It denotes the presence of chronic effusion in the middle ear cleft without the acute symptoms of fever or severe otalgia. In our study we found that rhinosinusitis, adenoid hypertrophy and chronic tonsillitis were the most common predisposing factors (58.3%, 20%, and 5%; respectively). Khan and his colleagues., 2006 9 also reported that rhinosinusitis, adenoid hypertrophy and chronic tonsillitis were the most common predisposing factors (36.8%, 34.5%, and 13.8%; respectively). Joshua, 200810 reported that upper-respiratory tract infection was found to have a pronounced association with bilateral status of effusion at baseline. When inadequately treated, otitis media may lead to major functional limitations like hearing loss and impairment in development of speech and language 11

Most of our patients were of middle and low socioeconomic status that is in agreement with many recent studies. In India, Siddartha et al., 201211 reported that only 4% of OME cases belong to upper class and 96% to middle and lower classes. In our study, boys were more affected with OME than girls (60% Vs. 40%). This is in agreement with Kubba et al. 2000 12, Khan et al., 2006 9 and Erdivanili et al., 2012 13 who reported that boys are more likely to have OME than girls.

In our study, biochemical analysis of the nasopharyngeal aspirate in normal volunteers revealed the presence of phospholipids constituting surfactant, phosphatidylcholine, phosphatidylethanolamine, sphingomyline and other phospholipids.

It was observed that phosphatidylcholine constituted 73.25% of the nasopharyngeal aspirate contents of phospholipids while sphingomyline 4.94 %, phosphatidylethanolamine constituted 17.11% and other phospholipids 4.7%. This means that phosphatidylcholine is the main constituent of the nasopharyngeal surfactant. This phospholipid profile corresponds with that observed by Ramadan et al., 2000 14 in nasal aspirate for assessment of surfactant level where they found that phosphatidylcholine constituted 75.35%. while phosphatidylethanolamine constituted 15.35% and sphingomyline 5.3% and other phospholipids 4%. This is also in agreement with Uhliarova and Calkovska ,.2012 15 in assessment of ET surfactant., they observed that biochemical analysis of the nasal aspirate in healthy individuals revealed the presence of phospholipids constituting surfactant as phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and other phospholipids. It was observed that phosphatidylcholine constituted 75% phospholipids of the nasal aspirate, while phosphatidyethanolamine constituted 15 %, sphingomyelin 5% and other phospholipids 4%. This is also in agreement with Hills 1984 16 and Viggo et al.,1988 17 in the Eustachian tube and nose analyses of normal cases.

In cases of otitis media with effusion the most significant finding was the significant decrease in the total phospholipids compared to normal volunteers this in agreement with Ghadiali et al., 2002 6 and Chul Ho Jang et al 2010 18.

Significant changes in the phospholipid profile were observed in cases of secretory otitis media with a significant decrease in phosphatidylcholine ($p<0.01$) and non-significant decrease in phosphatidyethanolamine ($p>0.05$) and non-significant increase in sphingomyelin ($P>0.05$) and significant decrease in other phospholipid ($P<0.01$) compared to normal volunteers.

These compositional changes phospholipid profile are similar to that observed by Ramadan et al., 2000 14., Van Golde et al., 1988 19., Gregory et al., 1991 20., and Gunther et al.,1996 21.in cases of primary atrophic rhinitis and in premature infants with neonatal respiratory distress syndrome and in acute pulmonary inflammations in adult respiratory distress syndrome and/or pneumonia. Van Golde et al., 1988 19.,explained these changes in the phospholipid profile by contamination of the surfactant pool by cellular membranes shed as a result of increased inflammatory cell injury.

As phosphatidylcholine is the main constituent and main active part of surfactant so these compositional changes will impair the surfactant function.

Grace et al.,1984 22, compared the phospholipids content of middle ear effusions resulting from ET obstruction in adult patients with that of children with secretory otitis media. In both groups, surface tension-lowering substances were isolated but the composition was different from adults having a higher amount of sphingomyelin., this is in agreement with our results where we found non-significant increase in sphingomyelin.

In other study done by Svane et al.,1988 23, a higher sphingomyelin/phosphatidylcholine ratio was present in children with secretory otitis media than in those without OM indicating a lower degree of surface lowering properties which is in agreement with our study.

Conclusion

Significant biochemical changes in the surfactant system of the middle ear are an evident finding in cases of otitis media with effusion. This suggests a possible role for surfactant deficiency in the aetiopathogenesis of cases of otitis media with effusion.

Summary:

Otitis media with effusion (OME) is the most common inflammatory disease of the middle ear, characterized by the presence of fluid in the middle ear cavity, behind an intact tympanic membrane. Typically, there are no signs or symptoms of acute infection. OME mainly affects children at a critical age of speech and language development, with hearing loss as the major complaint.

The aim of this study to assess and measure the total and differential phospholipids of surfactant in cases of OME and camper it with the results obtained from normal group. Eighty participants were enrolled in this study, 49 were males and 31 were females they were divided into two groups., group A (60) patients of otitis media with effusion and group B (20) control group. Patients subjected to surgical management in the form of myringotomy and ventilation tube insertion (grommet), and adenoidectomy or adenotonsillectomy according to the predisposing factor. Samples collected from middle ear effusion used for measurement of total and differential phospholipids (surfactant).For control group, Samples collected from nasophyrengeal secretions around Eustachian tube opening under endoscopic guidance through the nose

and used for measurement of total and differential phospholipids (surfactant).Recurrent upper respiratory tract infection (Rhinosinusitis) was the most frequent predisposing factor accounting for 58.3 % of all cases; adenoids were diagnosed in 20% of patients, adenotonsillitis in 16.7%; tonsillitis were diagnosed in 5% of patients.Total phospholipid were significantly decreased in our 60 patients with comparison to 20 control group($p<0.000$).

Differential phospholipids profile: significant decrease in hosphatidylcholine(lecithin) ($p<0.000$) and non-significant decrease in phosphatidylethanolamine (cephalin)and non-significant increase in sphingomyline and significant decrease in other phospholipid in the patient group in comparison to normal control group($p<0.014$). Significant biochemical changes in the surfactant system of the middle ear are an evident finding in cases of otitis media with effusion. This suggests a possible role for surfactant deficiency in the aetiopathogenesis of cases of otitis media with effusion

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