



Topical Intranasal Corticosteroids Compared with Systemic Steroids in the Treatment of Eustachian Tube Dysfunction in Children

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Using corticosteroids in treatment of ETD has been the focus of various studies in which many of them suggested that either topical intranasal corticosteroids or systemic oral steroids are helpful in the management of ETD. The aim of the work was for comparing the efficacy of topical intranasal corticosteroids with that of systemic oral steroids in the treatment of ETD.

Patients and Methods: prospective trial on 100 consecutive patients in the age group of 6- 12 years with an intact TM as to be documented on otoscopic examination and with an ETD as to be documented with a tympanogram type C. Subjects were allocated equally into two groups **group 1:** had intranasal corticosteroids and **group 2:** had systemic oral steroids. Data such as ear complaint, patient history, general investigation, otorhinolaryngological clinical check, tympanometry, pure tone audiometry and treatment, if taken were collected from all the patients.

Results: The decrease in tympanograms type C after treatment was correlated with the increase in type A tympanograms which indicate complete resolution of the condition in both tested groups of both treatment arms. Difference between the two study groups regarding tympanogram type C normalization shows no statistically significant difference between each treatment arm. Pure tone audiometry results indicate an improvement in the subjects HL after management in both examined groups, but results weren't statistically significant.

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Conclusions: Using corticosteroids, whether oral or intranasal, in the management of ETD is effective in resolving the condition, but there is no significant difference between the two in the outcome results and so oral steroid complications could be avoided by using local steroid spray.

Keywords: Intranasal; systemic; steroids; ETD.

1. INTRODUCTION

The Eustachian tube (ET) (also known as the pharyngotympanic tube) links the middle ear cavity to the nasopharynx. It performs unique tasks and may be considered an organ. It performs the following functions: pressure equalisation and ventilation of the middle ear, muco-ciliary cleaning of middle ear discharges, and defence of the middle ear from noises, pathogens, and secretions from the nasopharynx [1].

Abnormal or impaired ET functions (ETD) may lead to pathogenic changes in the middle ear. Several mechanisms for ETD have been hypothesized. Congestion of the nasal mucosa may result in oedema and ETD spreading retrogradely. Inadequate muco-ciliary function, whether innate or as a consequence of allergy or other inflammatory etiologies, may result in secretion retention, obstructing the ET. Aeroallergen inhalation followed by direct allergic inflammation inside the ET may result in venous engorgement and mucus hypersecretion, thus obstructing gas exchange in the middle ear area [2].

ETD is a common disease in children as the ventilator performance of children's ET is less efficient than that of adults. Also, ETD and middle ear abnormality due to upper respiratory tract infections being riskier to children younger than 2 years as compared with ones older than 2 years and also adults. Therefore, children are at highest risk for developing ETD complications such as acute otitis media compared to adults [3].

Corticosteroids are a family of steroid hormones generated in the adrenal cortex. They also include their synthetic equivalent hormones. It impairs carbohydrate, fat, and protein metabolism, and possess anti-inflammatory, immunosuppressive, anti-proliferative, and vasoconstrictor properties. Anti-inflammatory actions are mediated through trans repression of inflammatory mediators and induction of anti-inflammatory mediators (transactivation). Immunosuppressive actions are achieved by direct action on T-lymphocytes, which

suppresses delayed hypersensitivity responses. Vasoconstriction is mediated by the inhibition of inflammatory mediators such as histidine [4].

Using corticosteroids in the treatment of ETD has been the focus of various studies in which many of them suggested that either topical intranasal corticosteroids or systemic oral steroids are helpful in the management of ETD [5].

The aim of the work was to compare the effectiveness of topical intranasal corticosteroids with that of systemic oral steroids in the management of ETD.

2. PATIENTS AND METHODS

This prospective study which was done on 100 consecutive participants in the age group of 6-12 years with an ETD as to be documented with a tympanogram type C - TM confirmed by otoscopic examination but with ETD as confirmed by a tympanogram type C. Participants were selected from the outpatient clinic of the Otorhinolaryngology Department of Tanta University hospital, between April 2019 till April 2020.

Craniofacial syndromes, cleft palate and developmental delay, obstructive adenoid hypertrophy, ossicular chain discontinuity, TM atelectasis and subjects with sensoryneural HL were excluded.

Participants were allocated into two main groups with respect to the method of treatment, they were enrolled by the investigators and assigned to one of the two treatment arms until each treatment arm reached the required subject numbers: **Group 1:** 50 subjects received single dosage of 50 micrograms mometasone furoate/spray in each nostril once daily. The parents of the subjects were instructed on how to use the nasal spray for optimum delivery of the medication to the ET orifice. **Group 2:** 50 subjects received systemic oral steroids; oral prednisolone 1 mg/kg/day in single morning daily dose.

All patients underwent the following: full data taking, complaint and present history, analysis of

the patient's complaint with emphasis on ear fullness, ear clogging sensation and decrease hearing acuity, onset, course, duration, site of the affected ear whether it is unilateral or bilateral, factors provoking and relieving the condition, history of taking any medications for the condition such as, topical or systemic decongestant, corticosteroids or any other drug history.

Questionnaire to assess the ETD symptoms done by ETD Patient Questionnaire (ETDQ-7), (Fig. 1) [6].

Based on indications of ETD, ETDQ-7 consisted of seven questions, with a response of "1" indicating no problem and "7" representative a risk problem. An overall high score marked a more potential disease [6]. In ETDQ-7 participants were questioned if they had pressure, ache in the ears, a sense of congested or muffled hearing, ear symptoms associated with sinusitis or the common cold, crackling noises or tinnitus in one or both ears throughout the preceding month. In this scale, the decreased total score was 7 and the highest was 49. All participants were instructed to response the ETDQ-7 questionnaire according to their symptoms appear in the previous month. The questionnaire was explained and answered by the subjects with the assistance of their caregivers. A subject getting a score < 14.5 was

considered normal, and one with a total score ≥ 14.5 was considered symptomatic.

2.1 Investigation

1.Tympanometry: All subjects were subjected to tympanometry to detect any infection in the middle ear and ETD was confirmed if the result showed tympanogram type C. A tympanogram of type A was characterised as one with a peaked pressure value less than -100 kPa. Tympanograms with peaks and pressure values more than -100 kPa were classified as type C. Tympanograms that were not peaked or were flat were classified as type B.

2.Pure Tone Audiometry: It was carried out mainly to exclude the possibility of coexistence of sensory – neural HL in the tested subjects, and to confirm the ETD in the subjects with mild to moderate conductive HL presented on the audiogram.

2.2 Treatment

Subjects in the two treatment arms received the medications for two weeks only to prevent side effects of long-term use, in the follow up visit after treatment the patients were again subjected to (ETDQ-7), general and otorhinolaryngological examination, tympanometry and pure tone audiometry.

ETDQ-7 Questions Related to Eustachian Tube Dysfunction Symptoms Present in the Past Month and the Scoring System from 1 to 7. ^a							
Over the Past 1 Month, How Much Has Each of the Following Been a Problem for You?	No Problem		Moderate Problem			Severe Problem	
1. Pressure in the ears?	1	2	3	4	5	6	7
2. Pain in the ears?	1	2	3	4	5	6	7
3. A feeling that your ears are clogged or "under water"?	1	2	3	4	5	6	7
4. Ear symptoms when you have a cold or sinusitis?	1	2	3	4	5	6	7
5. Crackling or popping sounds in the ears?	1	2	3	4	5	6	7
6. Ringing in the ears?	1	2	3	4	5	6	7
7. A feeling that your hearing is muffled?	1	2	3	4	5	6	7

Abbreviation: ETDQ-7, Eustachian Tube Dysfunction Questionnaire.
^aMinimum score = 7, maximum score = 49.

Fig. 1. ETD Patient Questionnaire (ETDQ-7) [6]

2.3 Statistical Analysis

Analysis was done by SPSS version 20.0. (Armonk, NY: IBM Corp). Quantitative variables were presented as mean and standard deviation (SD), range (minimum and maximum), median and interquartile range (IQR). Parametric variables were compared between the two groups utilizing unpaired Student's t-test. Non-parametric variables were compared in two groups by Mann Whitney test and to compare variables within the same group by Wilcoxon signed ranks test. Qualitative variables were described as frequency and percentage (%) and were analysed by using the Chi-square test, Fisher's exact test McNemar and Marginal Homogeneity Test when appropriate. A two tailed P value < 0.05 was measured as statistically significant.

3. RESULTS

All patients' demographics, risk factors and clinical data of both groups showed in Table 1.

There was no significant difference between the two study groups regarding the subjects age or gender, Table 1.

Diminished hearing, clogged ears and common cold were the most common symptoms that subjects described as their problem, in group 1, 18% complained about diminished hearing, 54% experienced clogged ear sensation, while 28% had common cold symptoms. In group 2, 33% complained about diminished hearing, 28% experienced clogged ear sensation while 39% had common cold symptoms.

Table 1. Comparison between the two study groups according to demographic data

	Group I (n = 50)		Group II (n = 50)		Test of sig.	p
	No.	%	No.	%		
Age (years)						
6 – 8	23	46.0	25	50.0	$\chi^2=$ 0.306	0.858
8 – 10	17	34.0	17	34.0		
10 – 12	10	20.0	8	16.0		
Min. – Max.	6.0 – 12.0		6.0 – 12.0		t=0.325	0.746
Mean ± SD.	8.70 ± 1.96		8.58 ± 1.73			
Median (IQR)	9.0 (7.0 – 10.0)		8.50 (7.0 – 10.0)			
Gender						
Male	33	66.0	28	56.0	$\chi^2=$ 1.051	0.305
Female	17	34.0	22	44.0		

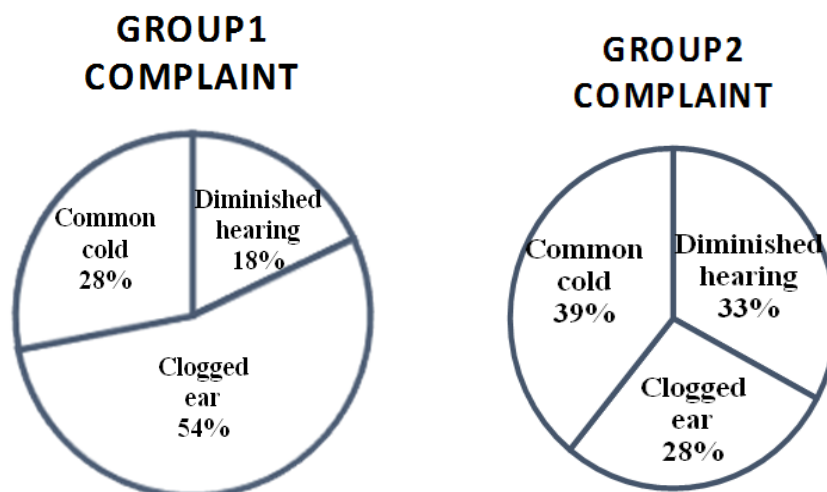


Fig. 2. Distribution of ETDQ-7 scores for each individual question

Table 2. Comparison between the two studied groups according to ETDQ7 total score

ETDQ7	Group I (n = 50)		Group II (n = 50)		Test of sig.	p
	No.	%	No.	%		
Before						
<14.5	10	20.0	1	2.0	$\chi^2=$	0.004*
≥14.5	40	80.0	49	98.0	8.274*	
Min. – Max.	9.0 – 35.0		14.0 – 29.0		U=	0.026*
Mean ± SD.	19.46 ± 6.28		21.32 ± 3.90		928.50*	
Median (IQR)	17.50 (15.0 – 24.0)		21.0 (18.0 – 24.0)			
After						
<14.5	33	66.0	34	68.0	$\chi^2=$	0.832
≥14.5	17	34.0	16	32.0	0.045	
Min. – Max.	7.0 – 21.0		8.0 – 23.0		U=	0.319
Mean ± SD.	12.66 ± 3.97		13.30 ± 3.70		1106.0	
Median (IQR)	11.50 (9.0 – 16.0)		12.50 (11.0 – 16.0)			
p₁	<0.001*		<0.001*			

χ^2 : Chi square test, U: Mann Whitney test, p: p value for comparing between the studied groups, p₁: p value for Wilcoxon signed ranks test for comparing between before and after in each group *: Statistically significant at p ≤ 0.05, Group I: receive the intranasal corticosteroids, Group II: receive the systemic oral steroids.

Table 3. Comparison between the two studied groups according to tympanogram types

	Tympanogram type	Group I (n = 50)		Group II (n = 50)		χ^2	MC ^p
		No.	%	No.	%		
Right	Before						
	A	20	40.0	25	50.0	1.166	0.625
	B	2	4.0	2	4.0		
	C	28	56.0	23	46.0		
	After						
	A	37	74.0	41	82.0	1.203	0.589
	B	2	4.0	2	4.0		
C	11	22.0	7	14.0			
p₁	<0.001*		<0.001*				
Left	Before						
	A	12	24.0	17	34.0	2.768	0.238
	B	6	12.0	2	4.0		
	C	32	64.0	31	62.0		
	After						
	A	34	68.0	37	74.0	2.091	0.404
	B	6	12.0	2	4.0		
C	10	20.0	11	22.0			
p₁	<0.001*		<0.001*				

χ^2 : Chi square test, MC: Monte Carlo, p: p value for comparing between the studied groups p₁: p value for Marginal Homogeneity Test for comparing between before and after in each group, *: Statistically significant at p ≤ 0.05

In group 1, ETDQ7 total score of the tested 50 subjects was (mean 19.46 ± 6.28 SD) in the first visit before having the medication, in the check-up visit after two weeks of medication ETDQ7 total score was (mean 12.66 ± 3.97 SD), 40 subjects 80% had a total score ≥14.5 in the first visit before having the medication, whereas only 17 34% subjects had a total score ≥14.5 in the check-up visit after two weeks of medication; therefore, there was a statistically significant

score improvement when comparing between before and after management in this group.

In group 2, ETDQ7 total score of the tested 50 subjects was (mean 21.32 ± 3.90 SD) in the first visit before having the medication, in the follow up visit after two weeks of medication ETDQ7 total score was (mean 13.30 ± 3.70 SD), 49 subjects 98% had a total score ≥14.5 in the first visit before having the medication,

whereas only 16 32% subjects had a total score ≥ 14.5 in the check-up visit after two weeks of medication, also there was a statistically significant score improvement when comparing between before and after management in this group.

The decrease in tympanograms type C after treatment was correlated with the increase in type A tympanograms which indicate complete resolution of the condition in both tested groups of both treatment arms.

Difference between the two study groups regarding tympanogram type C normalization shows no statistically significant difference between each treatment arm, Table 4.

In group 1, 15 subjects 30% experienced H.L in the right ear, and 11 subjects 22% experienced H.L in the left ear before having the medication. In the follow up audiometry only 3 subjects 6% experienced H.L in the right ear, also only 3 subjects 6% experienced H.L in the left ear. In group 2, 9 subjects 18% experienced H.L in the right ear, and 15 subjects 30% experienced H.L in the left ear before having the medication. In the follow up audiometry only 7 subjects 14% experienced H.L in the right ear, also only 8 subjects 16% experienced H.L in the left ear. Pure tone audiometry results indicates an improvement in the subjects HL after management in both examined groups, but results weren't statistically significant.

Table 4. Comparison between the two studied groups according to tympanogram type (C) Per-ear

Type c tympanogram	Group I (n = 100)		Group II (n = 100)		χ^2	p
	No.	%	No.	%		
Before	60	60.0	54	54.0	0.734	0.391
After	21	21.0	18	18.0	0.287	0.592
p₁	<0.001*		<0.001*			

χ^2 : Chi square test, p: p value for comparing between the studied groups, p₁: p value for McNemar test for comparing between before and after in each group, *: Statistically significant at $p \leq 0.05$, Group I: receive the intranasal corticosteroids, Group II: receive the systemic oral steroids.

Table 5. Comparison between the two studied groups according to pure-tone audiometry

Audiometry	Group I (n = 50)		Group II (n = 50)		χ^2	p	
	No.	%	No.	%			
Right	Before						
	Normal	41	82.0	35	70.0	1.974	0.160
	HL	9	18.0	15	30.0		
	After						
	Normal	47	94.0	43	86.0	1.778	0.182
	HL	3	6.0	7	14.0		
p₁	1.000		0.500				
Left	Before						
	Normal	39	78.0	35	70.0	0.832	0.362
	HL	11	22.0	15	30.0		
	After						
	Normal	47	94.0	42	84.0	2.554	0.110
	HL	3	6.0	8	16.0		
p₁	1.000		1.000				

χ^2 : Chi square test, p: p value for comparing between the studied groups, p₁: p value for McNemar test for comparing between before and after in each group, Group I: receive the intranasal corticosteroids, Group II: receive the systemic oral steroids.

4. DISCUSSION

ETD is often characterised by symptoms and indicators of middle ear pressure dysregulation, including ear fullness, popping and crackling noises, ear pain, muffled hearing, and tinnitus, which indicates a problem with the ET's ventilatory function in conditions of normal atmospheric pressure, ETD may be due to functional obstruction, dynamic impairment (e.g., muscular failure) or anatomic blockage (115,120). ETD can be a mechanism for middle ear disease and is linked with TM retraction, OME and chronic otitis media. Therefore, detecting and diagnosis of ETD is of great importance [7].

In this study we used the ETDQ7 to evaluate the ETD symptoms in the tested subjects, ETDQ7 is one of the Patient Reported Outcome Measures (PROMs). In case of ETD-related symptoms, they can act as a simple tool to recognise individuals with ETD, which can be an important tool for office [8].

McCoul ED et al. reported the reliability and validity of their questionnaire. The cut-off point for the investigation of ETD is ≥ 14.5 at 100% sensitivity and 100% specificity for categorizing a participant as having ETD, and to prevent recall bias, the ETDQ-7 only includes the symptoms that were present in the past month [6].

Many studies were designed to assess the accuracy of the ETDQ-7 for categorizing people with or without ETD, and they all confirmed the accuracy in the sensitivity and specificity of the questionnaire created by the authors [9,10].

Other studies stated that PROMs with regard to ETD such as ETDQ7 are not a disease specific and shows poor specificity even though the sensitivity remains high; questions of symptoms such as: earache, tinnitus, and muffled hearing cannot differentiate conditions with similar symptoms and not related to ETD; such as, series of trigeminal nerve pathologies, HL, temporomandibular joint (TMJ) dysfunction or inner ear pathologies such as Meniere's Disease. Also, when applying the questionnaire to children, especially the younger ones, it could be difficult for them to fully understand and answer its questions, despite the aid of their caregivers in explaining the questionnaire and the scoring system, which limits the

usefulness of the ETDQ-7 in this age group. So, ETDQ7 should not be used in the diagnosis of ETD, and just limit its usage in quantifying, documenting symptoms, and screen the course of the illness over time or following management [10,11].

In this study we used tympanometry as the main objective test to include subjects and to confirm their ETD, the tympanometry provides information regarding ET function, as the ET serves to ventilate the middle ear; a negative tympanogram pressure peak almost invariably indicates that the ET is not adequately ventilating the middle ear [12].

A type C pattern of tympanogram is indicative of negative middle ear pressure, as reflected by a negative pressure peak, and is indicative of ETD. Smith ME et al. (2018) stated that in ETD suspected participants supposed of having ETD with an intact TM without effusion, tympanometry should be applied as the first examination of ET function. This is suggested to increase the specificity of tympanometry. Therefore, if middle ear pressure measured by tympanometry is lower than -50 daPa participants can be investigated as having ETD without further testing [8].

Mucosal inflammation and edema play a major role in the development of ETD; One research examining the various causes of ETD discovered that 83% of patients had mucosal edema affecting the ET orifice. Additionally, 74% of patients exhibited reduced anterolateral wall motion, which was likely caused by the thickening of the inflamed mucosa. Steroids can have a positive effect on ETD through many mechanisms which include, inhibition of the associated inflammatory mediators, peritubal lymphoid tissue atrophy, and increased ET surfactant secretion [13,14].

In this study, subjects who received intranasal corticosteroids were found to have a statistically significant improvement in managing their ETD, when comparing between before and after the management according to the tympanometry results and patients' symptoms score questionnaire (ETDQ7) results.

This comes in agreement with Ma Y, et al. [15] who investigated patients with allergic rhinitis who develop ETD and presented that their ET function can significantly improve as nasal symptoms subside after 1 month of treatment

with mometasone furoate nasal spray and oral loratadine [15].

In that context, Shapiro, et al. [16] studied 45 children with Both allergic rhinitis and OME were studied prospectively to determine the efficacy of aerosolized nasal dexamethasone against placebo. At 1- and 2-week periods, normal middle ear pressures were more commonly detected in the dexamethasone group, indicating a short-term benefit; however, insignificance was discovered between the two groups when the trial concluded at week 3. The scientists found that aerosolized dexamethasone had some therapeutic effectiveness but suggested a two-week treatment duration [16].

Also, based on addressing the cause of ETD, our findings come in agreement with Cengel, et al. [17] who prospectively studied 122 children between the ages of 3 and 15 years with OME, who were awaiting surgery for adenoid hypertrophy or both. These authors observed a significant increase (42%) in the rate of resolution in children treated with daily intranasal mometasone furoate monohydrate for six weeks compared to those who received no therapy at all (14%). Additionally, they observed a substantial decrease in adenoid size among the treated children [17].

Additionally, Zhang L, et al. [18] did a systematic review of 6 RCTs included a total of 394 participants were included to evaluate the effectiveness of intranasal corticosteroids for enhancement nasal airway blockage in children with moderate to severe adenoidal hypertrophy. Five of the six studies found that intranasal corticosteroids were significantly effective in alleviating nasal blockage symptoms and decreasing adenoid size [18].

In case of OME, Karlidag et al. [19], reported an 8-week resolution rate of OME in children of 39% on a course of antibiotics plus nasal steroids, compared to 24% on antibiotics alone and 5% on no therapy. Each treatment arm included around 20 youngsters. However, the research sample was insufficiently large to be statistically significant [19].

Similarly, Tracy JM, et al. [20] in a study group of 61 children, we evaluated the benefit of adding intranasal steroids to an oral antibiotic regimen vs oral antibiotics plus placebo vs oral antibiotics alone for managing OME and discovered that steroid therapy resulted in a greater frequency of

effusion resolution as measured by otoscopy, tympanometry, and a symptom questionnaire at 1 and 2 months. At three months, the advantage was also seen, although it did not achieve statistical significance. The authors concluded that intranasal steroids may be a beneficial complement to antibiotic prophylaxis [20].

Also, Williamson et al. [21] in a study group of 200 children randomized to daily mometasone or placebo, found no difference in the number of days with HL associated with OME after three months of therapy, and after nine months of follow-up, there was still no benefit in the treatment group [21].

In this study, subjects who received systemic oral corticosteroids were also found to have a statistically significant improvement in managing their ETD, when comparing between before and after the management according to the tympanometry results and patients' symptoms score questionnaire (ETDQ7) results.

The effect of systemic oral steroids (primarily prednisone) on managing OME and ETD has been investigated many times in randomized controlled trials in the literature, and many suggested short-term benefits compared with non-steroid treatment [22].

Rosenfeld RM, et al. [23] did a meta-analysis of six randomized clinical trials in patients with OME (N = 264 children) and reported that oral steroids administered for 7–14 days (at doses ranging from 0.15 to 1.5 mg/kg per day) increased the rate of complete effusion resolution versus no steroid [23].

A systematic review by Butler and colleagues, et al. [24] of randomized clinical trials for using Systemic steroid treatment for OME provides more evidence of its effectiveness in resolving effusions in the near term. The odds ratio for OME persisting after short-term follow-up was 0.22 (95 percent confidence interval, 0.08 = 0.63) in children treated with oral steroids compared to a control, and 0.32 (95 percent confidence interval, 0.20 = 0.52) in children treated with oral steroids plus an antibiotic compared to a placebo plus an antibiotic [24].

Simpson SA, et al. [25] did a Cochrane database review, which involved 12 media to high-quality studies with a total of 945 participants. A pooled analysis of data utilising a fixed effect model for

OME resolution at a short term follow up (< one month) revealed that oral steroids had a meaningful impact in comparison to the control. At less than one month follow-up, oral steroids with antibiotics resulted in an improvement in OME resolution compared to placebo plus antibiotics. However, in the long-term, there was no evidence that steroid therapy can improve resolution of the retro-tympanic effusion or long-term HL, and therefore does not constitute a reference treatment for OME [25].

That was emphasized with a review by Berkman ND et al. [26], on the usage of oral steroids in the therapy of OME, which presented steroids to be of insignificant benefit either in resolution of the effusion or in enhancement of HL [26].

In this study, we found that there was no significant difference between intranasal corticosteroids and systemic oral steroids in the outcome results.

This come in agreement with the outcomes of a recent trial by Kadah SM, et al. [27] who compared the effectiveness of intranasal corticosteroids versus systemic oral steroids for treatment of OME in the appearance or disappearance of adenoidal hypertrophy in children and reported that both topical intranasal and systemic steroids are effective in the therapy of OME in children in the short term, without significant difference between the two techniques [27].

Further studies regarding the usage of corticosteroids, either oral or intranasal, in the control of ETD is recommended with including a larger number of subjects for having more accurate results. Also, a comparison between the efficacy of using oral or intranasal corticosteroids alone or in combination in managing ETD would be of a great value.

Further studies are recommended to find out ways to improve targeted therapy and medication delivery directly to the ET nasopharyngeal opening in managing the condition of ETD.

5. CONCLUSIONS

Using corticosteroids, whether oral or intranasal, in the management of ETD is effective in resolving the condition, but there is insignificantly different between them in the outcome results.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

A written informed consent was given by the relatives of participants.

ETHICAL APPROVAL

Full data of the procedures were approved by both the Institutional and ethical committees.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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