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ORIGINAL ARTICLE

Anti leukotrienes Effect on CD64 Expression on Monocytes in Children with Adenoid Hypertrophy

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ABSTRACT

Background: *Adenoid hypertrophy (AH) plays an important role in chronic rhinosinusitis and chronic otits media in children. Children may frequently have recurrent upper and lower respiratory tract infection.* ***Objectives:*** *To assess anti leukotrienes effect on CD64 expression on surface of monocyte cells in children complaining of adenoid hypertrophy.* ***Methodology:*** *This study was conducted on Patients diagnosed with adenoid hypertrophy at Otorhinolaryngology Department, Benha University Hospital, Egypt during the period from February 2018 to October 2018. Sixty patients were included and they were divided into two groups: group (1) 30 Patients who did not take antileukotrienes therapy (control group), and group (2) 30 patients who had commenced antileukotrienes therapy (case group). Samples of blood were taken from all patients to measure the level of CD64 by flow cytometry at Medical Microbiology and Immunology Department, Faculty of Medicine, Benha University before and after 3 months of antileukotrienes therapy.* ***Results:*** *Statistical data for CD64 levels revealed that at the comparison between CD64 levels at the beginning of this study and then after 3 months in group (1) were 157.63 and 141.64 respectively. There was non significant difference (P value =0.127) while at comparison between CD64 levels pre and post treatment in group (2) the level was 157.63 and 141.64 respectively. There was a significant difference (p value =0.003). The adenoid size had three courses in the study; stationary, regressive and progressive. None of patients in the group 1 showed regressive course regarding adenoid size. In group 2, one patient showed a progressive course while most cases (22 patients) had regressive course as they were improved on treatment. There was a highly significant difference in the different categories of adenoid size between the two groups (P value< 0.0001).* ***Conclusion****: Antileukotrienes therapy might be generally associated with decreasing levels of CD64 expression on surface of monocytes that lead to decrease the adenoid size in children complaining of adenoid hypertrophy..*

INTRODUCTION

Adenoid is an aggregation of lymphoid tissue placed in the roof and the posterior wall of nasopharynx. Adenoid hypertrophy is a common childhood disease. Adenoid when enlarged can obstruct the choana, especially during sleeping in a supine position. Symptoms caused by airway obstruction like mouth breathing; hyponasal speech and snoring in children are demonestrated [1].

Adenoid hypertrophy (AH) is considered to be one of the common problem in Pediatrics. They may create mechanical obstruction of Eustachian Tube (ET), so they are important in the pathogenesis of Otitis Media (OM). To make the diagnosis of adenoid hypertrophy (AH), recently nasal endoscopy is considered to be the gold standard even in children, because this technique is also able to identify a possible association between adenoid inflammation, infection and OM specially during infancy and early childhood [2] .

Moreover, AH plays an important role in chronic rhinosinusitis in children and chronic Otitis media in addition, children complaining of adenoid hypertrophy may frequently have recurrent respiratory tract infection both at upper and lower level [2].

Adenoid removed for airway obstruction and/or removed for recurrent infections have been studied to detect a possible mechanism to identify the cause of the chronicity and studies have reported the presence of bacteria organized into biofilms which provides germs with a greater ability to resist host defenses and antibiotics and may therefore promote the recurrences or the persistance of infections. Biofilm formation is

found on surfaces of adenoid, especially in children complaining of recurrent infections[3].

In addition, immune cells, that include macrophages, monocytes, are recruited and activated in children complaining of recurrent infections. Human cells of myeloid lineage contain 3 classes of receptors for the Fc portion of immunoglobuin G, designated FcyR1 (CD64), FcyR2 (CD16), which act in concert with complement receptors by providing an important link between the humoral and cell mediated immunity by functioning as key molecules in antigen presentation, phagocytosis, clearance of immune complexes, and release of inflammatory mediators [4].

Human FcyR1 is constitutvely expressed on professional antigen presenting cells which express on their surface major histocompatibility complex class2 molecules. Membrane bound FcyR1 on antigen presenting cells has been found to be fundemental in mediating enhanced antigen presentation through internalization of antigen forming complexes with IgG. Thus, as antigen presentation is important for stimulating an effective immune response, the measurement of CD64 on monocytes may be useful in patients complaining of infectious disorders [5].

An antileukotriene, which include leukotriene modifier and leukotriene receptor antagonist, is a drug which acts as a leukotriene-related enzyme inhibitor or leukotriene receptor antagonist and so opposes the action of these mediators[6]. High numbers of LT receptors have been demonstrated in the tonsils of children complaining of obstructive sleep apnea. They reduce the apnea–hypopnea index and also reducing inflammation of adenoid and tonsils. Antileukotrienes may be important for children complaining of adenotonsillar hypertrophy because they have anti-inflammatory effects, which act to decrease adenotonsillar inflammation [7].

The aim of the present study is to assess anti leukotrienes effect on CD64 expression on surface of monocytes in children complaining of adenoid hypertrophy.

METHODOLOGY

Patients:

This study was conducted on Patients who were diagnosed with adenoid hypertrophy at Otorhinolaryngology Department, Benha University Hospital, Egypt, during the period from February 2018 to October 2018. Sixty patients were included in the study and they were divided into two groups: group (1) that included patients who did not take antileukotrienes therapy (control group – 30 cases), and group (2); which included patients who were commenced on antileukotrienes therapy (case group- 30 cases).

Inclusion criteria include:

Hypertrophied adenoid (size equal or more than 75% of retro palatal airway , no previous nasopharyngeal surgery and no sino nasal problems.

Exclusion criteria include:

Age more than 9 years or less than 3 years, allergic patients and patients with previous adenoidectomy. Approval of the ethical committee at Faculty of Medicine, Benha University was obtained and a written consent was taken from all parents of the included patients after describing and explaining the treatment strategy.

Pre-treatment data:

All sixty patients were subjected to a history taking, clinical examination, radiological and blood investigations Samples of blood were obtained from all patients to measure the level of CD64 by flow cytometry at Medical Microbiology and Immunology Department, Faculty of Medicine, Benha University. Flow cytometry is a technique that is used to identify and measure physical and chemical characteristics of a population of cells or particles. A sample that contain cells or particles was suspended in a fluid then injected into the flow cytometer instrument. The sample was focused to ideally flow one cell at a time through a laser beam and the light scattered is specific to the cells and to their components. Cells were usually labeled with fluorescent labels so the light is absorbed at first and then emitted in a band of wavelengths[8].

Treatment data including:

All cases of group (2) were on antileukotrienes as montelukast (4 mg daily) for a period about 3 months. The control group didn't not receive this kind of treatment.

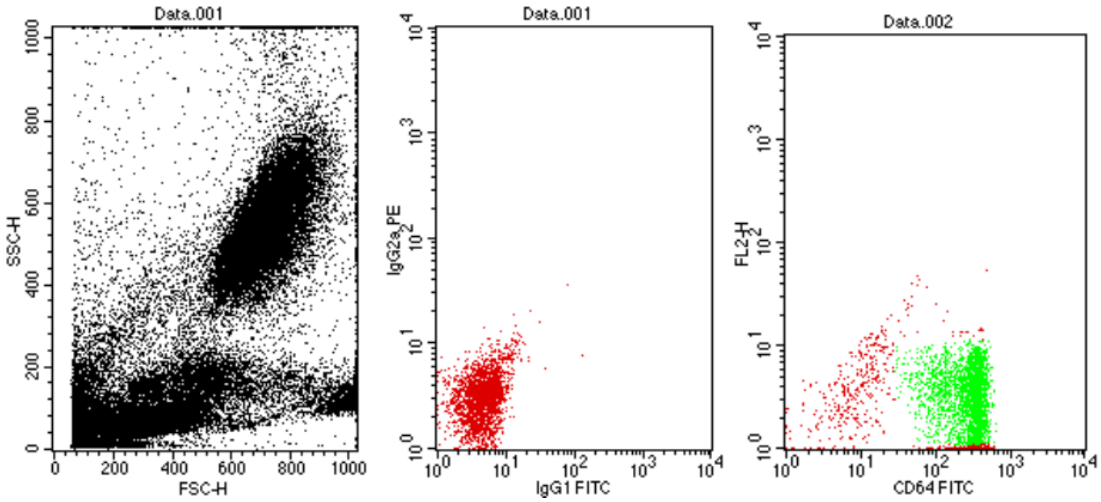
Post-treatment assessment:

All cases underwent assessment including complete history taking, associated co-morbidities, clinical examination, endoscopic examination, imaging (digital X ray nasopharynx –CT nasopharynx). Blood samples were obtained for examination of the level of CD64 in cases and control groups after 3 months.

RESULTS

Study population characteristics:

In this study, the study cases were divided into two groups; the first group (group1–30 patients) is the control group who did not receive the treatment and the second group (group2–30cases) which included Patients who received anti leukotrienes therapy. Many variables such as gender, age, adenoid size and level of CD64 (shown in Figure 1) were examined. The mean age of patients in group (1) was (5.42±1.85) while in group (2) it was (6.2±1.92) with no Statistically significant difference (p=0.113).



Sample ID: case5
Tube: CD64
Quad % Gated X Geo Mean
UL 0.63 17.21
UR 1.13 115.33
LL 9.85 7.28
LR 88.39 259.65

Sample ID: case5
Tube: CD64
Gate % Gated

G1 100.00

G2 78.87

Fig. 1: CD flow cytometry to measure the levels of CD 64 expression on surface of monocytes

Statistical data for CD64 levels as shown in table 1 revealed that at the comparison between CD64 levels at the beginning of this study and then after 3months in group (1) were 115.02 ± 18.56 and 118.39 ± 16.89 respectively. There was non-significant difference (P value= 0.231) while by the comparison between CD64 levels pre and post treatment in group (2) The mean level of CD64 pre and post treatment were 157.63 ± 38.09 and 88.23 ± 40.28 respectively. There was a significant difference (p value=0.0001).

Table 1: COMPARISON between pre-CD64 &post-CD64 in group1 [control group] and group 2 [cases group].

	Group 1	**Group 2**
Pre-CD64	115.02 ± 18.56	157.63 ± 38.09
Post-CD64	118.39 ± 16.89	88.23 ± 40.28
Test of significance	t = 1.634	t = 34.924
Pvalue	*0.231 (non-significant)*	*< 0.0001* (highly significant)*

P value <0.001 (highly significant) ; >0.05(non-significant)

The adenoid size had three courses in the study (table 2) ; stationary, regressive and progressive. No patients in the group (1) showed regressive course regarding adenoid size. In group 2, one patient showed progressive course while most cases (22 patients) had regressive course as they improved on treatment. There was high significant of difference in the different categories of adenoid size between the two groups (P value< 0.0001).

Table 2: Comparison between courses of the adenoid size in the two study groups:

Adenoid size	**Group 1 Control group (N=30)**	**Group 2 Cases (N=30)**	**Test of significance**	**P value**
Stationary	17 (56.7%)	7 (23.3%)	$\chi 2$= 11.650	< 0.0001(highly significant).
Regressive	0 (0%)	22 (73.3%)		
Progressive	13 (43.3%)	1 (3.4%)		

DISCUSSION

Adenoid Hypertrophy (AH) represents a relevant problem for both the pediatrician and the ENT specialist. AH, which obstructs the nasal airway in children, is associated with numerous symptoms, including: snoring, nasal obstruction, oral breathing, rhinolalia clausa, restless sleep, hypersomnolence, and enuresis. AH is also considered to be the most common aetiology of obstructive sleep apnea and cardio-respiratory syndrome, with severe complications. Moreover, AH plays an important role in pediatric chronic rhinosinusitis, chronic otitis media and recurrent respiratory tract infection both at upper and lower level[9].

Recent researches have concluded that there is a high CD64 expression on surface of monocytes in children complaining of adenoid hypertrophy. Children with size 4 adenoid hypertrophy had the highest CD64 expression levels. This significant association may be caused by the recurrence of airway infections that stimulate monocytes. Therefore, a vicious circle might start involving adenoid tissue enlargement, immune system stimulation, and airway infections. Pagella et al.[10] revealed that when comparing the levels of CD64 in 66 children complaining of adenoid hypertrophy with 30 controls, there was a significant expression of this antigen on monocytes of the group having hypertrophied adenoid. There was a difference of significant value in the expression of CD64 on monocytes (p-value = 0.0355) between patients and controls. Moreover, Children with enlarged adenoid showed higher values of CD64 expression. In our study, there was an over expression of CD64 in the cases group with mean of 157.63 ± 38.02 when compared with the control group (mean = 102.02.42 ± 18.56) ($p < 0.0001$).

Qian and Huang,[11] analyzed CD64 expression and other biomarkers for pyogenic infection in patients with COPD and healthy subjects. This study revealed that CD64 index was correlated directly with bacterial infection in patients complaining of COPD and showed better sensitivity than CRP, WBC, and other markers of inflammation. CD64-guided treatment also showed better outcomes when compared to clinical decision-making taken without CD64 guidance. CD64 index is a simple and quick biomarker for bacterial infection in these patients complaining of COPD and has important clinical significance.

Goldbart et al.[12] and Dayyat et al.[13] provided evidence that leukotriene receptor antagonists may be a therapeutic alternative in children with sleep disordered breathing avoiding adenoidectomy. The positive effect of antileukotrienes could be contributed to their anti-inflammatory activity. Therefore, these studies could support the thesis that there is an immune-mediated inflammation in the children complaining of AH .

Goldbart et al. [14] found that oral montelukast, administered over a period of 12 weeks in children with obstructive sleep apnea (OSA), effectively alleviated the severity of nocturnal respiratory disturbance, reduced the size of adenoid tissues, and significantly improve sleep symptoms. Furthermore, the treatment was not associated with any side effects and was well tolerated. The results of our study agreed with the finding of an open study performed by Cingi et al.[15] in American children over a period of 16 week.

Comparing these results to our case group, the adenoid size showed a dramatic decrease after receiving anti leukotriene treatment with decrease in the airway narrowing from 81.29% down to 60.89% obstruction post-treatment ($p < 0.0001$). The control group did not show that significant reduction as the case group ($p = 0.427$).

Goldbart et al.[14] reported also that Leukotriene modifiers could also be considered as a direct therapeutic option in mild OSA as an alternative to adenotonsillectomy. A recent placebo-controlled trial with oral montelukast in children complaining of mild OSA showed improvements of significant value in apnea index and in adenoid size **.**

The mean level of CD64 pre and post receiving antileukotrienes treatment was 157.63 and 141.64 respectively. There was a difference of statistically significant value between the case and the control group ($p= 0.003$). When looking to the results of our study, antileukotrienes therapy was effective in reduction of CD 64 expressions in children complaining of adenoid hypertrophy.

Kheirandish-Gozal [15] and his associates investigated the effect of montelukast on pediatric OSA. Montelukast when administrated for aperiod of 16 weeks effectively decreased the severity of the obstructive sleep apnea in children 2–10 years of age. These results support a therapeutic role for leukotriene modifiers in pediatric OSA provided that longterm trials confirm current findings

Schupper et al.[16] found that LT antagonists exhibited dose-dependent reductions in adenotonsillar cellular proliferation rates, with montelukast showing superior potency compared to the other antagonists tested, suggesting that LT-dependent pathways underlie components of the intrinsic proliferative and inflammatory signaling pathways and play a significant role in adenotonsillar hypertrophy in children

CONCLUSION

From this study we can conclude that antileukotrienes therapy might be generally associated with decreasing levels of CD64 expression on monocytes and decreasing the adenoid size in children complaining of adenoid hypertrophy. So we can

recommend the use of anti leukotrienes to decrease CD64 expression on surface of monocytes and to decrease adenoid size in children complaining of adenoid Hypertrophy.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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