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Original article

Evaluation of Mucosal Associated Invariant T-Cells (MAIT) in Childhood Asthma

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Abstract

Background: Bronchial asthma is a worldwide problem. It is a chronic inflammatory disease characterized by airway hyperresponsiveness and obstruction. Various immune cells are involved in asthma development, severity and response to treatment. Mucosal Associated Invariant T- cells (MAIT) cells have been suggested to be involved in the pathogenesis of bronchial asthma. We aimed at assessing activated MAIT cells in children with asthma and evaluating their role in disease pathogenesis, severity, and control. **Methods:** A case-control study included 15 bronchial asthma and 10 apparently healthy, age and sex-matched children as controls. All participants were subjected to complete blood count (CBC) and peripheral blood smears examination to assess eosinophilia, serum total IgE levels, and immunophenotypic analysis for peripheral blood mononuclear cells to assess the frequency of circulating MAIT cells (CD3+ CD161high Vα7.2+) and using monoclonal anti-human CD69 as an activation marker for MAIT cells. **Results:** MAIT cells were significantly less in asthmatic group compared to healthy control group. Activated (CD69+) MAIT cells at 3.8% were found to be a good predictor of childhood asthma with 73.3% sensitivity, 60% specificity and 68% accuracy. Activated MAIT cells were detected in 46.7% of cases, they were significantly higher in the fair controlled group, while the inactivated (CD69-) MAIT cells were significantly higher in cases with good and poor asthma control ($P < 0.05$). **Conclusion:** MAIT cells are decreased in asthmatic patients' peripheral blood without any association with disease severity nor control suggesting they may be involved in asthma pathogenesis.

Keywords: Bronchial asthma, MAIT cells, asthma severity, asthma control.

Introduction

Bronchial asthma is one of the most common chronic childhood disorders. It is a public health problem, that has a significant economic burden on societies due to its effects on the child, his family, and the health care system.[1] Bronchial asthma is a complex disease caused by the interaction between genetic, environmental, and other factors.[2]. It is typically characterized by eosinophilic inflammation, initiated by the alarmins secreted after a trigger in bronchial epithelium. Alarmins include TSLP, IL-25 and IL-33. This inflammation is then sustained by the release of cytokines as IL-4, IL-5, and IL-13 from innate and adaptive immune cells including T helper 2 (Th2) cells, invariant T cells, natural killer (NK) cells, eosinophils, basophils, and innate lymphoid cells type-2 (ILCs-2).[3]

Mucosal-associated invariant T (MAIT) cells are one of the innate-like T lymphocytes that are highly abundant in human blood and mucosal tissues of intestine and lungs.[4] MAIT cells represents about 10% of peripheral blood T lymphocytes.[5] They are activated by T-cell receptor (TCR) signals, cytokine signals independent of TCR, or by a combination of both.[6] However, in either mechanisms; upon activation, MAIT cells rapidly proliferate, secrete proinflammatory cytokines and other substances leading to lysis of the infected cells.[7] MAIT cells within the mucosal tissues of lungs promote an antimicrobial defenses through secreting IFN-γ, TNF-α, IL-17, perforin, and granzyme B.[8]

The role of MAIT cells in bronchial asthma has been studied previously and it was found that a higher frequency of circulating MAIT cells is associated with lower risk of developing bronchial asthma in young children and with higher frequency of IFN-γ-producing CD4+ T cells, suggesting a possible protective role of MAIT cells as children getting older.[9] This protective role has been proved when MAIT cells-deficient OVA-induced asthma murine model had exacerbated eosinophilic airway inflammation what suggests the protective role of MAIT cells against type 2 airway inflammation in murine asthma model.[10] However, the IL-17-producing MAIT cells (MAIT-17) were found to be positively correlated with the frequency of exacerbations and inversely related to symptoms in school-age children with bronchial asthma.[11]

Thus, we aimed in this study at investigating the frequency of activated MAIT cells in patients with childhood bronchial asthma and evaluating their role in disease pathogenesis, severity, and control.

Materials and methods

Study design. This case-control study was approved by the local

ethics committee of Faculty of Medicine, Benha University in accordance with the ethical guidelines of the 2004 Declaration of Helsinki (Research Ethics Committee No. MD 19-12-2018). Informed consents have been obtained from parents / guardians of all participants before enrolment in the study.The study was performed from September 2020 to February 2021. The studied patients group included 15 bronchial asthma cases (11 males and 4 females) with mean age 8.07±2.78 years attending Pediatrics Department, Benha University Hospital. Bronchial asthma was diagnosed according to the Global Initiative for Asthma (GINA)[12] criteria with the presence of one or more symptoms including wheezing, shortness of breath, chest tightness and cough. Any case was excluded if age was less than 2 years or more than 18 years, suffered from any chest illness other than asthma (e.g., pneumonia, bronchitis), had any systemic chronic illness (e.g., liver, renal, or endocrine diseases). The control group included 10 apparently healthy, age and sex-matched children with mean age 7.80±2.45 years with negative history of bronchial asthma, allergy, atopy or other pulmonary diseases.

Methods. A detailed history was obtained from all enrolled children including history of bronchial asthma (symptoms, disease onset, frequency of attacks, current and previous medications), history and family history of atopy (rhinitis, conjunctivitis, eczema and/or asthma), and history of other illnesses or medical conditions. Comprehensive clinical examination for diagnosis and assessment of asthma severity and control according to the GINA 2023 [12] was conducted.

Laboratory investigations. All participants were subjected to complete blood count (CBC) and Leishman-stained peripheral blood smears examination to assess eosinophilia, serum total IgE levels, and immunophenotypic analysis for peripheral blood mononuclear cells on BD FACSCanto™ Flow cytometer to assess the frequency and activation of circulating MAIT cells using the following monoclonal antibodies; anti-human CD3-APC, anti-human CD161-PerCP, anti-human TCR V-alpha 7.2-PE and anti-human CD69-FITC (BioLegend, (USA. The gating strategy used was side scatter vs CD3+ to get T-lymphocytes, then MAIT cells were defined as CD3+ CD161high Vα7.2+ in gated T-cells. CD69 was used as a marker for MAIT activation.

Statistical Analysis:

Obtained data were arranged and statistically analyzed using SPSS v.20.0 for windows (SPSS Inc.,2011. Chicago, IL, USA). Quantitative data were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR). The independent Student's (t) test was used to compare the means of two groups of normally distributed variables while Mann Whitney (U)

test was used for non- normally distributed variables. The Kruskal Wallis test (KWT) was used to compare the medians of more than two independent groups of non-normally distributed variables. Qualitative data were expressed as absolute frequencies (number) and relative frequencies (percentage). Percent of categorical variables were compared using Chi-square (X^2) test. Spearman's correlation coefficient (r) was calculated to assess the relationship between various studied variables. The validity of activated MAIT cells as a predictor for childhood bronchial asthma was tested by Receiver Operating (ROC) curve. All tests were two sided. P-value <0.05 was considered statistically significant.

Results

This case-control study was conducted on 15 children with bronchial asthma and 10 apparently healthy age- and sex-matched control subjects with age ranged between 5 and 15 years. The demographic and clinical characteristics of the studied groups are shown in **Table 1**.

Disease history in the studied asthmatic patients were evaluated to assess the frequency of asthmatic attacks, disease severity and disease control according to the GINA 2023. About one third of cases (33.3%) had one attack per two weeks, 26.7 % had one attack per one month, and 20% complained of one attack per three weeks. Regarding asthma severity, more than half of cases (53.3%) had mild asthma, 20% had moderate asthma and 26.7% were with severe asthma. Asthma control was assessed by Asthma Control Test (ACT) which revealed more than half of studied cases (53.3%) were good controlled, 26.7% were fairly controlled and 20% showed poor control of their disease.

All participants underwent laboratory investigations including complete blood count (CBC), serum total IgE levels and immunophenotyping to identify and analyze MAIT cells. Mucosal Associated Invariant T-cells (MAIT) were significantly higher in control subjects than asthmatic cases (P 0.044) while their activation marker CD69 did not show difference between both groups (P 0.739). **Table 2**

The role of activated MAIT cells to predict childhood asthma was evaluated by using ROC curve analysis. It was performed to test the predictive power of activated MAIT cells (CD69+) for childhood asthma as the best CD69+ MAIT cut-off level for the prediction of childhood asthma was (3.8%), which had 73.3% sensitivity, 60% specificity, 73.3% positive predictive value, 60% negative predictive value and a 68% accuracy. **Fig.1**

Immunophenotypic identification of the activated MAIT cells (CD69+) revealed that 46.7% of cases had

activated MAIT cells while 53.3% of cases had inactivated MAIT cells.

No significant association was observed between MAIT and activated MAIT cells with disease severity among the studied patients (P >0.05). However, the inactivated MAIT cells (CD69-) were significantly higher in patients with good and poor asthma control while the activated MAIT cells (CD69+) were higher among fair-controlled cases (P <0.05). **Table 3**

Persons' correlation analysis between MAIT cells, CD69+ MAIT cells and serum IgE level with relevant parameters among studied cases showed only a significant positive correlation between serum total IgE level with absolute eosinophil count (r 0.598, P 0.018). **Table 4**

Table 1. Demographic and clinical characteristics of the studied groups.

Characteristic		**Controls (n=10)**	**BA cases (n=15)**	***P*-value**
Age (years)		7.80±2.45	8.07±2.78	0.808
Sex	**Female**	4 (40.0)	4 (26.7)	0.484
	Male	6 (60.0)	11 (73.3)	
Personal history of atopy				
Negative		10 (100.0)	8 (53.3)	0.090
Conjunctivitis		0 (0.0)	1 (6.7)	
Eczema		0 (0.0)	2 (13.3)	
Rhinitis		0 (0.0)	4 (26.7)	
Family history of atopy				
Atopy	**No**	10 (100.0)	4 (26.7)	**<0.001**
	Yes	0 (0.0)	11 (73.3)	
Rhinitis	**No**	10 (100.0)	14 (93.3)	0.405
	Yes	0 (0.0)	1 (6.7)	
Eczema	**No**	10 (100.0)	13 (86.7)	0.229
	Yes	0 (0.0)	2 (13.3)	
Asthma	**No**	10 (100.0)	6 (40.0)	**0.002**
	Yes	0 (0.0)	9 (60.0)	

Data represented as mean ±SD or number (percent).

Table 2. Laboratory investigations among the studied groups

Characteristic	Controls (n=10)	BA cases (n=15)	*P*-value
Hb (g/dl)	12.3±1.15	12.36±1.48	0.915
WBCs (x10^3/µl)	7.81±1.8	8.49±3.07	0.534
Neutrophil (%)	56±6.62	44.2±11.84	**0.009**
Lymphocyte (%)	37.6±6.24	44.8±12.34	0.103
Monocyte (%)	3.9±1.52	6±2.3	**0.019**
Basophil (%)	0 (0-1)	0	0.574
eosinophil (%)	2.2±1.03	4.93±2.02	**0.001**
Eosinophils (cell/µl)	140.9±73.98	441.33±207.04	**<0.001**
Platelets (x10^3/µl)	278.6±65.86	212.4±40.35	**0.005**
IgE (IU/ml)	125 (92.25-150.5)	171 (73-655)	0.267
MAIT (%)	4.12±1.03	3.36±1.17	**0.044**
CD69 (%)	3.75 (1.8-6.9)	3.1 (1.9-8.5)	0.739

Data represented as mean ± SD or median (IQR) as appropriate.

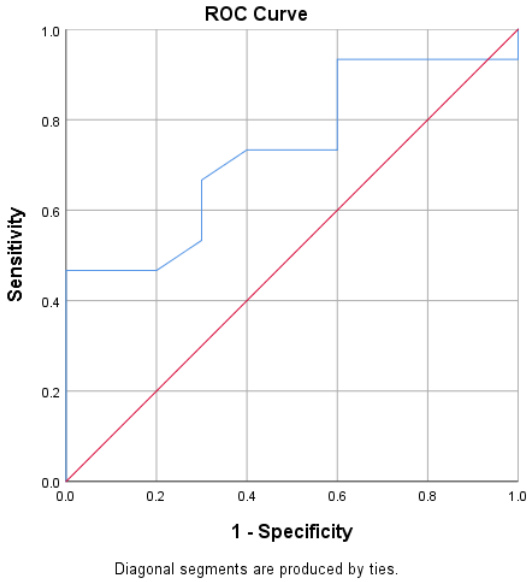

Figure 1. ROC curve of the predictive values of active MAIT cells in childhood asthma

Table 3. The association between MAIT and activated (CD69+) MAIT cells and bronchial asthma severity and control among studied cases.

		MAIT (%)	CD69+ MAIT	CD69- MAIT
Asthma Severity	**Mild (n= 8)**	2.85 (2.275-4)	3 (42.9)	5 (62.5)
	Moderate (n= 3)	2.9 (2.1-2.9)	2 (28.6)	1 (12.5)
	Severe (n= 4)	3.8 (3.3-6.0)	2 (28.6)	2 (25)
***P*-value**		0.195	0.680	
ACT	**Good (n= 8)**	3.2 (2.35-4)	2 (28.6)	6 (75)
	Fair (n= 4)	3 (2.9-3.7)	4 (57.1)	0 (0)
	Poor (n= 3)	2.1 (2.1-2.5)	1 (14.3)	2 (25)
***P*-value**		0.345	**0.043**	

Data represented as median (IQR) and number (percent).

Table 4. Correlation between MAIT cells, CD69+ MAIT cells and serum IgE level with relevant parameters among studied cases.

Variables		MAIT (%)	$CD69^+$ MAIT (%)	IgE (IU/ml)
Eosinophil (cell/µl)	***r***	0.179	0.491	**0.598**
	P	0.524	0.063	**0.018**
Asthma severity	***r***	0.465	0.166	-0.091
	P	0.081	0.555	0.747
ACT	***r***	0.224	-0.497	-0.426
	P	0.422	0.059	0.114

r: Correlation Coefficient

Discussion:

Asthma is a chronic inflammatory disease of the bronchioles, characterized by airway hyperresponsiveness and airway obstruction. It is a complex disease that involves several T-cells, B-cells, mast cells, eosinophils, dendritic cells, macrophages, chemokines, cytokines, and histamines that, when activated, can trigger the pathogenesis of asthma.[13]

Better understanding of disease pathogenesis helps in choosing the best treatment strategies and targeting causes of disease. Therefore, the current study was designed to assess MAIT cells and their activation in children with bronchial asthma and evaluate their role in disease pathogenesis and association with clinical and laboratory data.

MAIT cells, atypical subset of T-cells, have been suggested to have either protective role against airway inflammation and asthma, if biased

towards MAIT1 cells[9, 14] or pathologic role if biased towards MAIT17 cells.[11]

This study included 15 asthmatic children aged between 5 and 15 years. They were 11 (73.3%) males and 4 (26.7%) females with mean age 8.07±2.78 years. In addition, 10 age- and sex-matched healthy controls. Seven (46.7%) patients suffered from another atopic condition (e.g., conjunctivitis, eczema, or rhinitis). Eleven (73.3%) cases had a significant family history of atopy (P <0.001) with nine (60%) cases had significant family history of asthma (P 0.002). Asthma severity has been evaluated according to GINA 2023. We found that 53.3% of the studied cases had mild asthma, 20% had moderate asthma and 26.7% had severe asthma. Asthma control was assessed through asthma control test (ACT), that reveals 53.3% of cases were good controlled, 26.7% showed fair control and 20% were poorly controlled asthma.

Laboratory investigations were conducted to all participants, it was found that relative neutrophil and platelets counts were significantly elevated in control subjects than asthmatic patients (P 0.009 and 0.005, respectively), and relative monocytes, eosinophils and absolute eosinophil counts were significantly elevated in asthmatics than controls (P 0.019, 0.001 and <0.001, respectively). While neither hemoglobin concentration, total white blood cell count, relative lymphocytes and basophil counts and serum total IgE level showed significant difference between asthmatic cases and healthy controls. This was in line with **Yavuz et al**.[15] who demonstrated a significant elevation in eosinophil counts in asthmatic children compared to healthy controls and did not find any significant difference between asthmatic and healthy control groups regarding serum IgE levels. On the contrary to these results, another study reported that serum IgE was significantly elevated in asthma cases when compared to healthy controls.[16]

Immunophenotypic analysis of peripheral blood mononuclear cells was performed and MAIT cells were identified as $CD3^{+}CD161^{high}$ TCR V-alpha 7.2^{+} while CD69 was assessed as MAIT cells activation marker. We detected a significant increase in the frequency of MAIT cells in healthy control (4.12±1.03%) than in asthmatic cases (3.36±1.17%) (*P* 0.044), whereas the expression of activation marker (CD69) on MAIT cells did not differ among both groups (*P* 0.739). This agreed with a recent study that showed a decreased frequency of MAIT cells in adults with asthma compared to healthy controls, but the frequency of MAIT cells was found to decline with increasing disease severity. [17] Another report by **Mingzhu et al.**, stated that they found a higher mean of MAIT cells in healthy control (5.17±1.14%) than in asthmatic children (3.24±1.02%). But they

found lower MAIT cells in severe asthma cases (1.85±0.55%) compared to mild (3.92 ±0.69%) and moderate cases (2.73±0.12%), who were all significantly lower when compared to healthy control. [18]

The median (IQR) of MAIT cells in mild asthma cases was2.85 (2.275-4)%, 2.9 (2.1-2.9)% in moderate cases and 3.8 (3.3-6.0)% in severe cases.We found that median (IQR) of MAIT cells increased with increasing disease severity, although this did not reach statistical significance (*P* 0.195). This contradiction could be attributed to the small sample size, which could also explain the higher median of MAIT cells in severe cases. However, **Ishimori and colleagues** did not find any significant difference between asthma severity and MAIT cells in their studied asthmatic adults. [19]

In this study, there is no significant difference in MAIT cells as regard asthma control assessed by ACT. The MAIT cells median was 3.2% in good-controlled group, 3% in fair-controlled group and 2.1% in poorly controlled group (*P* 0.345). This agreed with **Wen et al.**, who reported that there was no correlation between MAIT cells frequency and ACT groups[17] and disagreed with **Ishimori et al**., who reported that ACT was significantly lower in severe asthmatic group when compared with mild/moderate groups. [19]

ROC curve analysis was performed to test the value of activated MAIT cells ($CD69^+$) in predicting childhood asthma, it defines 3.8% as the best $CD69^+$ MAIT cells cut-off with 73.3% sensitivity, 60% specificity, 73.3% positive predictive value, 60% negative predictive value and 68% accuracy.

In the current study, seven asthmatic children (46.7%) had activated ($CD69^+$) MAIT cells. They were three among mild cases with a median $CD69^+$ MAIT cells 3.1% (1.9-6.9), 2 among moderate cases with a median 6.5% (3.1-6.5) and 2 among severe cases with median $CD69^+$ MAIT cells 7% (0.65-18.6). However, this did not show ststistical significant difference (*P* 0.680 and 0.630).

To the best of our knowledge, none of the published studies evaluated $CD69^+$ MAIT cells in asthmatic children. However, activated MAIT cells have been studied in asthmatic adults. In one study the peripheral blood $CD69^+$ MAIT cells were less in adults with severe asthma than in mild/moderate cases. They reported their mean as 25.05±12.21 of total MAIT cells in severe cases and 31.88±15.28 in mild/moderate cases. [19]

Regarding asthma control, $CD69^+$ (activated) MAIT cells were significantly higher in fair-controlled group, while $CD69^-$ MAIT cells were significantly higher in cases with good disease control (*P* 0.043).

By studying the association between relevant laboratory tests and asthma severity, no significant differences were detected between relative, absolute eosinophils count and total serum IgE among asthma severity groups (*P* 0.740, 0.419, 0.662 respectively). This comes similar to another report that did not find significant difference in serum IgE levels among asthmatic children with various degrees of severity. [16] Meanwhile, **Elmeazawy and Shoeib** reported medians of relative eosinophil count of 2 (1.35–2.8) in mild, 2 (1.5–2.5) in moderate and 1.5 (1.45–2.4) in severe asthmatic groups with no difference in relative eosinophil counts between the asthmatic children and control groups. They also found a significant increase in serum total IgE medians with increasing disease severity as serum total IgE median was 80 (68.75-195) IU/ml in mild, 200 (90-400) IU/ml in moderate and 400 (78.5-1625) IU/ml in severe asthmatic group. [20]

Conclusion

In conclusion, as we demonstrated that MAIT cells are decreased in asthmatic patients' peripheral blood without any association with disease severity nor control. However, the activated (CD69+) MAIT cells are higher in fair controlled patients, while the inactivated (CD69-) MAIT cells were higher in mild and poor controlled patients. Thus, MAIT and activated (CD69+) MAIT cells may be involved in asthma pathogenesis, course, and response to treatment.

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