Original Article

Association of serum sphingosine-1-phosphate with forced expiratory volume and absolute eosinophil count in asthma patients

Muhammad Rashid, Mudassar Khan, Muhammad Jamal, Nadia Awan, Muhammad Waseem

Sahiwal Medical College, University of Haripur, University of Health Sciences Lahore, Pakistan

Objective: To investigate the association of serum sphingosine-1-phosphate with forced expiratory volume and absolute eosinophil count in asthmatic patients.

Methodology: In this cross sectional comparative study, 36 Asthmatic patients and 36 non-asthmatic healthy adults as control were selected through convenient sampling. Forced Expiratory Volume (FEV1), Absolute Eosinophil Count (AEC) and serum Sphingosine-1-phosphate (S1P) of all patients and control were measured.

Results: Mean FEV1 value of asthmatics (49.30±14.79) was significantly lower than mean FEV1 value (92.8±5.9) of controls, while mean AEC (0.23±0.1) and S1P (1942.74± 426.30) were significantly higher in asthmatic patients than mean AEC (0.07±0.02) and S1P (940.44±210.9) of controls (p=0.001). Serum S1P levels in asthmatic patients were negatively associated with FEV1 (r^2 = -0.808), while, serum S1P levels were positively associated with AEC ($r^{2=}$ 0.674) in asthmatic group. Area under the curve in ROC analysis was 0.98, cut off value of S1P with 91.7% sensitivity and 83.1% specificity was 1251 nmol/L for asthma.

Conclusion: Serum S1P levels were negatively associated with the FEV1 and positively associated with ACE. (Rawal Med J 202;45:771-774).

Keywords: Asthma, eosinophil, inflammation, sphingosine-1-phosphate.

INTRODUCTION

Bronchial asthma is a common chronic inflammatory disease of respiratory tract.[1] It is associated with hyper responsiveness of airways and leads to repeated attacks of chest tightness, wheezing, breathlessness and coughing, specially at night and morning.[2] It is caused by the accumulation of inflammatory cells such as eosinophil and mast cells in airways.[3] It is diagnosed with clinical history and impaired lung function test according to severity of asthma.[4] According to National Asthma Education and Prevention Program (NAEPP), asthma is classified as intermittent, mild persistent, moderate persistent and severe persistent based on the individual's symptoms and spirometer data.

Symptoms included in the classification are daytime symptoms and nighttime awakenings.[5] About 0.3 billion asthmatic patients are present all over the world.[6] Its prevalence increases by 50% every decade,[7] and 100 million new cases will be added to this population by 2025.[8] Asthma prevalence has been found to vary from 0.7 to 11.9% across Asia.[9] The prevalence of asthma in Pakistan is 4.3% in adults.[10] Forced Expiratory Volume (FEV1), serum IgE levels, eosinophil counts, Fractional Exhaled Nitric Oxide (FENO) levels, and sputum eosinophil's counts are used to diagnose asthma.[11]

Eosinophil count (EOS) can reflect the degree of airway inflammation in acute asthma attacks and FEV1 Is an indicator of airways obstruction.[12,13] Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite that plays important roles in allergic responses, including asthma and anaphylaxis, the incidence of which is rising worldwide especially in industrialized urban populations.[14] Studies show that S1P is associated with airway inflammation during asthma attacks.[15] This study aimed to explore the association of S1P with FEV1 (a marker of air ways obstruction in asthma) and absolute eosinophil count (a marker of air way inflammation) in asthmatic patients.

METHODOLGY

This cross sectional study was conducted at Asia Diagnostic Center, Islamabad, Pakistan with the collaboration of Pulmonology Clinic Sahiwal from January to August 2019 after getting approval from Ethical Approval Committee of Medical Lab Technology Department, University of Haripur. Sample size was calculated by a formula for comparison of two means.[16] Calculated Sample size was 36 for each group. Thus, total of 72 participants from 18 to 65 years of age group were enrolled in present study by convenient sampling. Those with the history of parasitic infection, chronic obstructive lung disease and allergic reactions were excluded from the study.

FEV1 were performed and blood samples were collected in EDTA while gel tube for the analysis of Absolute eosinophil count (AEC) and S1P. AEC was performed on BC 5000 Mindary a five part differential hematology analyzer. S1P levels were performed by using SIP ELISA Kits from My Bio Source and readings were obtained at 450nm by bio rad micro plate reader model 680.

Statistical Analysis: SPSS version 22 was used for data analysis. Independent t-test was used to compare values of FEV1, ACE and SIP. $p<0.05$ was considered significant.

RESULTS

Out of 72 participants, 36 were controls and 36 were asthmatics. Among total, 37 were males and 35 were females of 18 to 65 years of age group. Comparison of FEV1, S1P and AEC between control and asthmatic patients was performed by independent t-test (Table 1). FEV1, ACE and S1P were significantly different in asthmatic patients as compared to controls ($p<0.001$).

Table 1. Comparison of FEV1, S1P and AEC in controls and asthma patients.

Variable	Groups	N	Mean	Std. Deviation	P-Value
FEV1	Control	36	92.8056	5.90796	<0.001
	Asthmatic	36	49.3056	14.47129	
Absolute Eosinophil Count	Control	36	.0700	.02084	<0.001
	Asthmatic	36	.2336	.10556	
S1P	Control	36	940.4444	210.92307	<0.001
	Asthmatic	36	1942.7222	426.30965	

Fig 1. Correlation of S1P to FEV1.

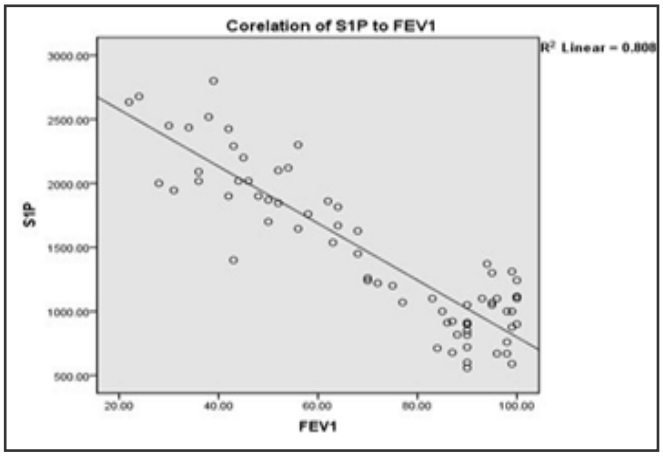


Fig 2. Correlation of S1P to AEC.

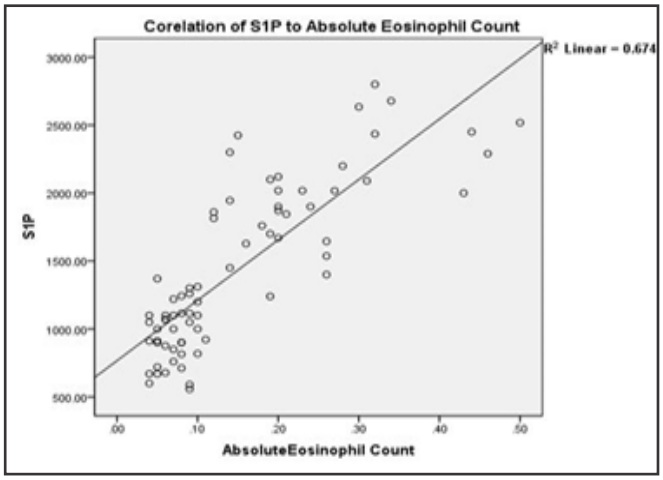
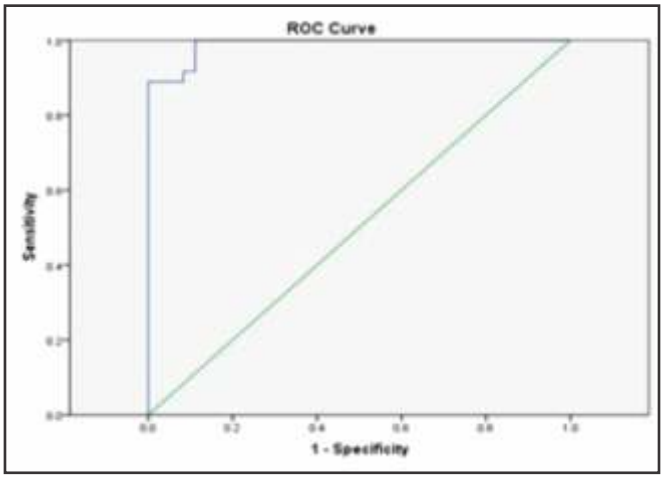


Fig 3. ROC analysis of S1P.



S1P showed significant negative association with FEV1, which indicates that with increase of airways blockage, the levels of S1P are increased and

correlation between marker of airways blockage (FEV1) and S1P was -0.80 (Fig. 1). S1P showed positive association with AEC, which indicates that with increase of airways inflammation, the levels of S1P were increased and correlation between marker of airways inflammation (AEC) and S1P was 0.67 (Fig. 2). Area under the curve in ROC analysis was 0.98, cut off value of S1P with 91.7% sensitivity and 83.1% specificity was 1251 nmol/L for the diagnosis of asthma (Fig. 3).

DISCUSSION

Wheezing, shortness of breath, chest tightness or cough are the clinical features of asthma.[12] Asthma is currently diagnosed with clinical features as well as the lung function test s (FEV1) and with the markers of airways inflammation like IgE and Eosinophil count. FEV1 is considered as good diagnostic tool for assessment of airway obstruction in asthma.[13]

This study was a little effort to contribute in asthma diagnostic tools by purposing S1P as a diagnostic marker of asthma. Serum S1P levels were performed in asthmatic patients along with FEV1 and Absolute Eosinophil Count. All the test parameters were significantly different in asthmatic patients as compared to controls.[14] A study conducted by Zhao et al provided the findings that serum S1P level and peripheral blood EOS in different degree asthma groups were significantly increased, and FEV1 were decreased significantly and with asthma exacerbations, serum S1P levels and peripheral blood EOS were gradually increased.[15]

Similarly, in our study AEC and serum S1P levels in asthmatic patients were significantly higher with $p<0.001$. Several studies have shown that S1P is an important inflammatory mediator in the development of asthma and it play an important role in the disease mechanism.[16,17] It is clear that the level of S1P in the blood is highly expressed in acute asthmatic patients, as observed in our study.[18]

Serum S1P levels showed strong negative correlation (R= 0.889) with FEV1 in our study, similar association was observed by Zhao et al.[15] They observed correlation coefficient 0.81. S1P participates in the pathogeneses of asthma by degranulation of mast cell and inducing stress fiber formation in bronchial smooth muscles cells leading to airway hyper responsiveness.[19] As asthma worsens, FEV1 gradually decreases.[20] We observed that patients having more episode of wheezing, shortness of breath, chest tightness had decreased FEV1 while blood eosinophil count and S1P levels were high. S1P levels in our study were positively correlated with AEC with the correlation coefficient 0.67, which shows that S1P is also associated with airway inflammation.

ROC curve analysis was also performed in our study to find the cut of value of S1P to differentiate the asthmatic from controls. Area under the curve in ROC analysis was 0.98. Serum level of S1P at 1251 nmol/L has 91.7% sensitivity and 83.1 % specificity for the diagnosis of asthma. In other study, it was purposed 1181 with 88.2% sensitivity and 88% specificity.[17]

This study was conducted without gradation of patients according to severity level of asthma and patients were not tested for atopic and non-atopic state. A detailed study should be conducted with large sample size and proper grouping of patients according to atopic and non-atopic state as well as severity level of asthma to find more reliable results.

CONCLUSION

In this study we found that S1P levels were significantly higher in asthmatic patients and it was positively associated with absolute eosinophil count and negatively associated with forced expiratory volume.

Author Contributions:
Conception and design: Muhammad Rashid
Collection and assembly of data: Muhammad Jamal
Analysis and interpretation of the data: Nadia Awan
Drafting of the article: Mudassar Khan
Critical revision of the article for important intellectual content: Muhammad Rashid
Statistical expertise: Muhammad Rashid
Final approval and guarantor of the article: Muhammad Rashid
Corresponding author email: Muhammad Rashid: 4849487@gmail.com
Conflict of Interest: None declared
Rec. Date: Oct 4, 2019 Revision Rec. Date: Jul 19, 2020 Accept Date: Aug 20, 2020

REFERENCES

1. Kumbhare S, Pleasants R, Ohar JA, Strange C. Characteristics and prevalence of asthma/chronic obstructive pulmonary disease overlap in the United

States. Ann Am Thorac Soc. 2016;13:803-1.
2. Tho NV, Park HY, Nakano Y. Asthma–COPD overlap syndrome (ACOS): A diagnostic challenge. Respirology. 2016;21:410-8.
3. Hirose K, Iwata A, Tamachi T, Nakajima H. Allergic airway inflammation: key players beyond the Th2 cell pathway. Immunol Rev. 2017;278:145-6.
4. Gao J, Zhou W, Chen B, Lin W, Wu S, Wu F. Sputum cell count: biomarkers in the differentiation of asthma, COPD and asthma–COPD overlap. Int J Chron Obstruct Pulmon Dis 2017;12:27-3.
5. Esden J, Pesta-Walsh N. Diagnosis and Treatment of Asthma in Nonpregnant Women. J. Midwifery Women's Health. 2019;64:18-7.
6. Croisant S. Epidemiology of asthma: prevalence and burden of disease. Heterogeneity Asthma. 2014;97:17-2.
7. Jin C, Shelburne CP, Li G, Riebe KJ, Sempowski GD, Foster WM, et al. Particulate allergens potentiate allergic asthma in mice through sustained IgE-mediated mast cell activation. J Clin Invest. 2018;121:941-5.
8. Sharifi L, Dashti R, Pourpak Z, Fazlollahi MR, Movahedi M, Chavoshzadeh Z, et al. Economic Burden of Pediatric Asthma: Annual Cost of Disease in Iran. Iran J Public Health. 2018;47:25-6.
9. Sharifi H, Ghanei M, Jamaati H, Masjedi MR, Aarabi M, Sharifpour A, et al. Burden of obstructive lung disease study in Iran: First report of the prevalence and risk factors of COPD in five provinces. Lung India. 2019;36:14-9.
10. Razzaq S, Nafees AA, Rabbani U, Irfan M, Naeem S, Khan MA, et al. Epidemiology of asthma and associated factors in an urban Pakistani population: adult asthma study-Karachi. BMC Pulm Med. 2018;18:18-4.
11. Asano T, Takemura M, Fukumitsu K, Takeda N, Ichikawa H, Hijikata H, et al. Diagnostic utility of fractional exhaled nitric oxide in prolonged and chronic cough according to atopic status. Allergol Int. 2017;66:344-50.
12. Guo C-L, Sun X-M, Wang X-W, Guo Q. Serum eosinophil cationic protein is a useful marker for assessing the efficacy of inhaled corticosteroid therapy in children with bronchial asthma. Tohoku J Exp Med. 2017;242:263-7.
13. Holloway JW, Hodgekiss C, Roberts G, Karmaus W, Zhang H, Ewart S, et al. Fixed Airways Obstruction In Smokers At 26 Years Of Age; An Early Indication Of COPD? J Allergy Clin Immunol. 2018;141:93-8.
14. Spiegel S. Targeting the S1P Axis and Development of a Novel Therapy for Obesity-Related Triple-Negative Breast Cancer. Virginia Commonwealth University Richmond United States; 2016.
15. Aoki M, Aoki H, Ramanathan R, Hait NC, Takabe K. Sphingosine-1-Phosphate Signaling in Immune Cells and Inflammation: Roles and Therapeutic Potential. Mediators Inflamm. 2016;2016:687-8.
16. Soe M, Sullivan K. Documentation of Sample Size For Comparing Two Means, 2005.
17. Zhao Y, Xu Y, Li S, Wei Y, Wang C. Role of serum S1P levels during asthma attack in the evaluation of asthma severity. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue. 2017;29:794-8.
18. Hekking P-PW, Wener RR, Amelink M, Zwinderman AH, Bouvy ML, Bel EH. The prevalence of severe refractory asthma. Ann Allergy Asthma Immunol. 2015;135:896-2.
19. Obinata H, Hla T. Sphingosine 1-phosphate and inflammation. Int Immunol. 2019;31:617-2.
20. Gomez-Munoz A, Presa N, Gomez-Larrauri A, Rivera I-G, Trueba M, Ordonez M. Control of inflammatory responses by ceramide, sphingosine 1-phosphate and ceramide 1-phosphate. Prog Lipid Res. 2016;61:51-6.
21. James B, Milstien S, Spiegel S. ORMDL3 and Allergic Asthma: from Physiology to Pathology. J Allergy Clin Immunol. 2019;43:44-9.
22. Lai W-Q, Wong WF, Leung BP. Sphingosine kinase and sphingosine 1-phosphate in asthma. Biosci Rep. 2011;31:145-50.
23. Accordini S, Cazzoletti L, Antó J, Cerveri I, Corsico A, Garcia-Aymerich J, et al. Asthma control and decline in FEV1/FVC ratio over 10 years in adults. Eur Respiratory Soc; 2018;34:59-2.