ORIGINAL ARTICLE

Altered serum sphingosine 1 phosphate level in food allergy and its association with disease severity

Sphingosine 1 phosphate and food allergy

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Summary

Background. Allergy is a multifactorial disease in which variant genetic and environmental factors are involved. Pathophysiology of food allergy (FA) and its natural history are poorly understood till now. Novel biomarkers and curative treatment options are strongly required. We aimed to study the significance of serum sphingosine 1 phosphate (S1P) level in food allergy and assess its possible correlation with disease severity. **Methods.** Study conducted on fifty participants diagnosed to have food allergy and another fifty group matched individuals who served as a control group. **Results.** The mean food allergy severity score of patients was 2.64±1.06, in which nearly two thirds (70%) are moderate, 18% are mild and about 12% of them are severe. A statistically significant increase in median value of S1P was found in patients with food allergies when compared to the control group. The best S1P cut-off level for the prediction of food allergy was (122.35 ng/L), with a sensitivity of 97.9% and a specificity of 95.7%, with a (97%) accuracy. Regarding Spearman's rho correlation analysis, a positive significant correlation was found between serum S1P level and Food allergy severity score. **Conclusions.** Sphingosine 1 phosphate (S1P) serum level can be a diagnostic marker for food allergy and its modulation could be a modality treatment for food allergies.

Key words

Food allergy; markers; S1P; sphingosine 1 phosphate.

Impact statement:

Despite previous research, the pathophysiology of food allergy is still unclear. Understanding the role of S1P in food allergy may lead to the development of new therapeutic options.

Introduction:

Prevalence of allergic diseases is around 25% worldwide, in which their treatment is mainly focusing on identifying and avoiding allergens, which is not always possible (1). In addition, for patients with cross-reactivity or multi-sensitization, treatments are often suboptimal, affecting their quality of life and inducing an economic burden (2).

Over the past years, research has increasingly focused on lipids, particularly sphingolipids which play variety of roles in immune regulation, inflammation and cell functions. These sphingolipids have been studied in different diseases as neurological disorders, cancer, autoimmunity, and infections. Besides, sphingolipid metabolites, in particular sphingosine-1-phosphate (S1P), has been linked to various immune processes and is found to play a role in the development of allergic diseases (3,4).

S1P plays an essential role in cell growth, survival, inflammation, and tissue remodelling (5,6). It has been shown to assist in IgE mediated sensitization by activating invariant Natural Killer T cells (iNKT) and promoting T2 cytokine production (1). However, its role in the pathogenesis of food allergy (FA) remains unknown.

S1P also regulates immune cell migration (5,7) including macrophages and mast cells (MCs), to the sites of inflammation for tissue repair contributing to allergic responses (8). Additionally, S1P production by MCs contributes to the T2 response, which is key mediator in allergy (10) and its depletion during systemic anaphylaxis has been linked to cardiovascular shock suggesting it could be a diagnostic marker for allergy (11,12).

As well as, in the intestine, sphingolipids can be found in large quantities, mostly because of their abundance in food such as meat, milk, and eggs (13) which is associated with an increased population of plasma cells in the colon, and development of FA (14). Peculiarly, over 50% of all plant allergens are lipid carriers as

source of sphingolipids (15), which may act as adjuvants and shift the immune system toward a T2 response (16). Hence, we aimed to investigate one of the metabolomic profiling panel named sphingosine 1 phosphate in food allergy and assess its possible correlation with disease severity and Outcome.

Material and Methods:

A case-control study conducted on fifty participants (Group 1) diagnosed to have food allergy according to GA2LEN guidelines, 2022 **(17)** attending Ain Shams University Allergy clinic over a period of twelve months. Another fifty healthy individuals (age and gender-matched) were enrolled and served as a control group (Group II). We included adult patients diagnosed with Ig-E mediated food allergy proved by allergy tests and elimination diet. Patients with conventional risk factors for AsCVD or with autoimmune diseases and/or on immunosuppressive therapy, patients with any end organ failure, mast cell disorders, or other causes of elevated IgE level were excluded. We also excluded patients already receiving long-term systemic immunotherapy for allergy or receiving any medications could affect the test results, and patients with any other allergic disorders, or non- IgE mediated food allergy. Informed consent was obtained from all participants. The study was approved by the local ethical committee of our university. All data collected will remain confidential and will be used for the research only.

Study design:

All participants were subjected to a comprehensive history taking including family history and clinical examination. Detailed history of food habits, assessment of food allergy severity score (revised Consortium for Food Allergy Research (CoFAR) Grading Scale **(18):** 5 levels ranging from generalized urticaria, localized angioedema, rhinitis, and abdominal pain (grade 1) to death (grade 5) were recorded. Pulmonary function test was conducted when required. Skin prick test and serum specific IgE level for the most common encountered food allergens has been carried out for the participants with history of food allergy. Serum Sphingosine 1 phosphate level by ELIZA was done for all participants.

Laboratory investigations:

Peripheral blood serum samples were collected from each subject. Samples were separated by centrifugation at 3000×g for 20 min, and their serum aliquots were stored at -20 °C within 1 h of collection for further investigation. Complete blood count using EDTA blood samples on Sysmex XN 1000 for CBC parameters and ESR using modified Westergren method. CRP was measured using Cobas C, Roche diagnostics, TSH using electrochemiluminescence on Cobas E411 and ALT, AST, HBA1C and creatinine on Cobas C501. Serum specific IgE level were measured using ELISA, DRG diagnostics.

Serum Sphingosine 1 phosphate level

It was measured by sandwich ELISA KIT, Human Sphingosine 1 phosphate, SIP (Bioassay Technology Laboratory, E1860Hu, China), assay was done according to manufacturer's instruction.

Skin Prick Test

We performed the skin prick test using the most conventional aeroallergen extracts. The result was interpreted after 15-20 minutes. It was considered justifiable when the difference in mean wheal diameters was at least 1 mm between the histamine (positive) and saline (negative) controls. Patient is considered sensitized to that peculiar allergen when a wheal diameter is ≥ 3 mm more than the negative control (19).

Statistical methodology

The Statistical Package for Social Science (IBM SPSS version 23) was used in this research to analyse collected data. The quantitative data was shown as means, standard deviations, and ranges when they displayed a parametric distribution, and as medians with interquartile ranges (IQR) when they displayed a non-parametric distribution. For qualitative data, they were shown as percentages and numbers. The groups were compared using the Chi-square test and/or Fisher exact test. To compare two different groups, the independent t-test was used with quantitative data and parametric distribution, whereas the Mann-Whitney test was used for non-parametric distribution. The receiver operating characteristic curve (ROC) was used to identify the optimal cut off point and its sensitivity, specificity, positive predictive value, negative

predictive value, and area under the curve (AUC). The allowable margin of error was set at 5%, while the confidence interval was set at 95%. As a result, the P-value was considered significant at the level of 0.05.

Results:

Regarding the demographic data, the two studied groups were comparable regarding age and gender as shown in **table I.** None of the participants had history of anaphylaxis. About 16% of patients were reactive for high salt diet, 10% of them were reactive to either banana, or milk, four cases were tested positive for each of eggs, mango, Tomatoes + Strawberry and Tomatoes + high salt diet, fish showed positive reaction in 6% of cases and other cases showed positive reaction for cocoa, Wheat+ strawberry, cocoa+ wheat, Sea food or sea food+ Shrimps, Milk+ High salt diet and Mango + fish. The mean Food allergy severity score was 2.64±1.06, two thirds of the studied patients (70%) were moderate, nearly 18% of them were mild and about 12% of them were severe.

Routine laboratory investigations:

Table II demonstrated that the mean of serum total IgE level was 262.69 (IU/L) with median 210 (145.75-271.5), the mean TLC was 7.68 mm3, the mean Neutrophils was 4.85 mm3 , the mean Lymphocytes was 2.33 mm3 with median 2.05 (1.8-2.8), the mean Eosinophils was 0.02 with median 0.1(0.03-0.2), the mean ESR was 8.44 mm/hr, the mean CRP was 4.05, the mean HbA1C was 4.94%, the mean TSH was 1.83 mIU/L, the mean Creatinine was 0.74, the mean ALT was 16.58 U/L and the mean AST was 16.8 U/L.

Sphingosine 1 phosphate level

A statistically significant increase in median value of S1P was found in patients with food allergy group (Group I) when compared to control group (Group II) as shown in **table III**. Analysis by the ROC curve was performed to test the predictive value of S1P for food allergy in which it revealed the best S1P cut-off level for the prediction of food allergy was (122.35 ng/L), which had a sensitivity of 97.9% and a specificity of 95.7%, predictive value for positive (PVP) = (96%), predictive value for negative (PVN) = (98%), and (97%) accuracy with confidence intervals (95% CI=0.981-1.0) (**Figure I**).

Sphingosine 1 phosphate and different disease parameters

As shown in **figure II**, although severe cases showed highest median of S1P level and mild cases showed the lowest S1P level but there was no statistically significant difference in S1P level between the different severity grades. Regarding Spearman's rho correlation between level of S1P and different parameters among cases group, a positive significant correlation was found between S1P level and Food allergy severity score only (**figure III**).

Discussion and Conclusions:

Food allergy is an immune-mediated disorder characterized by an exaggerated Th2 cytokine response upon exposure to specific food allergens. Its increasing prevalence highlights the need for further research into their underlying mechanisms and contributing factors (20). Despite previous substantial research, the mechanisms, risk factors leading to food sensitization remains unclear. Sphingolipids and their metabolites, mainly sphingosine-1-phosphate (S1P), has been proposed to play a main role in the induction and promotion of allergic diseases (21, 22). Additionally, polymorphisms in genes like ORMDL3, which regulate S1P homeostasis, have been proved to be linked to development of asthma (23,24).

S1P functions both as an intracellular signalling molecule and an extracellular mediator through its five membrane-bound receptors (S1PR1–5) (25). These receptors are found on the main effector cells involved in allergy mainly eosinophils, and mast cells in which the chemotaxis and effector responses of mast cells are regulated by both S1PR1 and S1PR2, in addition eosinophils display S1PR3 on their surface (8).

S1P is produced when sphingosine is phosphorylated by two sphingosine kinases, SphK1 and SphK2. Both Sph Ks have diverse tissue distribution and functions, and their activity is high in the lungs and small intestine while moderate in the spleen and stomach, suggesting its role in mucosal immunity (26).

Mast cells are potent producers of intracellular and secreted S1P. Although both SphK1 and 2 regulate S1P production in mast cells, the mechanism how they contribute to S1P production in mast cells is not well defined (27). S1P impacts allergic responses by influencing immune cell movement, activation, and function. Their levels were not only affected in anaphylaxis but were also found to be increased in asthmatic

patients after allergen challenge (28).

Upon cell activation, S1P can be released and act in an autocrine/paracrine manner. S1P regulates mast cell chemotaxis and activation through an autocrine loop. In allergic inflammation, the allergen cross-links IgE bound to FcεRI receptors, activating signalling cascades, including ERK, p38 MAPK, JNK, and PI3K (22). This process upregulates Th2 cytokines and activates SphK1, leading to increased S1P production. S1P may further promote Th2 inflammation by enhancing CD4+ T-cell migration to allergic tissues and activating mast cells, leading to the release of inflammatory medicators that worsen the allergic reaction (29,31).

Collectively, these data indicated that circulating S1P may have a significant role in the development of allergic diseases. However, evidence for a role of S1P in food allergy is scarce. Hence, we aimed to investigate the serum level of sphingosine 1 phosphate (S1P) in food allergy and assess its possible correlation with disease severity.

Our study was conducted on fifty participants proved to had food allergy by history, both skin prick test and specific IgE with elimination diet in comparison with another fifty-group matched healthy participants. Regarding the demographic data, the two groups were comparable regarding age and gender. The mean Food allergy severity score was 2.64±1.06 with median 2.6 (2.05-3.1) IQR, two thirds of the studied patients (70%) were moderate, nearly 18% of them were mild and about (12%) of them were severe.

While comparing the serum level of S1P between the two groups, a statistically significant increase in S1P level was found in the food allergy group when compared to the control group. Additionally, ROC curve revealed the best S1P cut-off level for the prediction of food allergy was (122.35 ng/L), with a sensitivity of 97.9% and a specificity of 95.7%, and (97%) accuracy.

To our mind, no studies till now have been conducted on S1P serum levels in food allergy except on animals. Accumulated data from previous studies in a murine model examining the role of S1P receptors on mast cell function support our results which demonstrated that S1P is necessary for mast cell migration to the gastric mucosa upon allergen challenge (7,29). In another study, when SphK-deficient mast cells were used, mast cell migration was impaired, and allergic responses were diminished (30). Also, pharmacological inhibition and genetic deletion of SphK or S1P1 receptors blocked mast cell migration toward antigens

while preserving chemotaxis to S1P itself, indicating an autocrine function for S1P in mast cell recruitment (7,31,32). In support for this concept, inhibition of S1P blocks migration of mast cells to antigen in vitro (29). Little is known about the individual role of these kinases in regulating immune cell function. Thus, sphingosine kinases are determinants of mast cell responsiveness, demonstrating their involvement in anaphylaxis (21).

S1P, by acting on its receptors (S1PR1–S1PR3), plays a crucial role in regulating mast cell migration, activation, and degranulation during allergic reactions. Specifically, S1P1 receptors on mast cells mediate chemotaxis, which directs mast cells toward the antigen site, where they subsequently release pro-inflammatory mediators. The S1P2 receptor, on the other hand, is involved in mast cell activation and degranulation. This process is essential in initiating and amplifying the allergic response. Furthermore, elevated levels of S1P could potentially increase the number of mast cells and eosinophils at the site of allergy, thereby contributing to the severity of food allergy symptoms. This mechanism is supported by our findings showing a positive correlation between serum S1P levels and the Food Allergy Severity Score only. In allergy, the allergen binds to at least two molecules of IgE, then IgE binds to the alpha subunit of FcεRI, resulting in cross linking of FcεRI. FcεRI then activates ERK, p38 mitogen-activated kinase (p38 MAPK), JNK, and PI3K (33,34,35), in addition to increasing the expression of Th2 cytokines (36, 37). FcεRI signaling can activate SPHK1, subsequently leading to S1P production (39). It is probable that Th2 cytokines could activate SPHK1 and increase the level of S1P in allergic inflammation.

Another study revealed that S1P is important for migration of CD4+ T-cells from the spleen to the intestine, resulting in antigen specific intestinal allergic hypersensitivity in another murine food allergy model. In this study, FTY720, a functional antagonist of S1P receptors, prevented allergic diarrhea and inhibited the infiltration of CD4+T cells as well as mast cells into the large intestine without impairing eosinophil infiltration (22). Importantly, intestinal epithelial cells express S1PRs, which regulate barrier function and, thus, could affect the transport of antigens through the intestinal mucosa (39).

Diesner and colleagues also investigated the effect of endogenous S1P production on food allergy induction using SphK1 and SphK2 deficient mice. S1P is linked to epithelial barrier function, so SphK1 and SphK2-

deficient mice exhibited reduced IgE responses upon oral sensitization, suggesting that S1P is crucial for oral allergen sensitization. (40). Moreover, acid suppression therapy, which impairs peptic allergen degradation, increases IgE-mediated food allergies, possibly via S1P-dependent mechanisms (41,42). SphK1 seems to be crucial for intestinal mast cell degranulation, while SphK2 is necessary for mast cell accumulation in the gastrointestinal mucosa (7,30).

Our findings also align with studies conducted in other allergic diseases as asthma and anaphylaxis, where increased levels of S1P were associated with enhanced mast cell activation and eosinophil infiltration. In asthma, for example, S1P levels were found to be elevated in bronchoalveolar lavage fluid after allergen challenge, correlating with eosinophil counts and disease severity. For instance, in a study by Kihara et al., S1P was shown to play a significant role in mast cell migration and eosinophil recruitment in asthma (43). Similarly, S1P is involved in mast cell-mediated inflammation, which is central to allergic reactions, not just in food allergy, but also in asthma and other allergic conditions like rhinitis. The role of S1P in these conditions supports the broader implication of S1P signalling in allergic diseases. Research by Schwab et al. further demonstrated that S1P, via its receptors, could regulate the migration and activation of immune cells, such as mast cells, in allergic rhinitis and asthma (44).

Hence, S1P need to be studied as a potential therapeutic target for treatment of allergic disorders. To date, food allergy therapy is mainly targeted at suppressing the symptoms. Given its multifaceted roles in allergy pathophysiology, inhibitors of SphK that block formation of S1P could also inhibit IgE/Ag-dependent cysteinyl-LT and cytokine production by preventing activation of the ERK pathway and AP-1-mediated transcription. SphK inhibitors have already been utilized to suppress tumour growth (45), in which administration of a monoclonal anti-S1P antibody to neutralize circulating S1P substantially reduced tumour progression in several xenograft and allograft models (46).

In conclusion, our study suggests that S1P plays an important role in the pathophysiology of food allergies, particularly in relation to the severity of the disease. Given its involvement in mast cell migration, activation, and degranulation, as well as its correlation with disease severity, S1P represents a promising biomarker for food allergy diagnosis and a potential therapeutic target. However, further research is needed

to explore the exact mechanisms of S1P in food allergy and to investigate its potential as a treatment target. Similarly, selective S1P receptor antagonists could also constitute a relevant strategy for the modulation of other allergic disorders. Future studies should focus on larger sample sizes, mechanistic insights into S1P's role in food allergy, and clinical trials to evaluate the efficacy of SphK inhibitors or S1P receptor antagonists as a therapeutic strategy for food allergies. The translational potential of targeting the S1P pathway for managing food allergies and other allergic conditions could significantly improve treatment outcomes in the future.

Limitations

This study may have some limitations. The relatively small sample size may reduce the generalizability of the findings. Additionally, our study focused only on serum S1P levels and did not explore the direct mechanistic pathways at the cellular level. Moreover, the cross-sectional design of the study limits our ability to infer causal relationships between S1P levels and food allergy severity. Future longitudinal studies are needed to establish a clearer understanding of how S1P levels fluctuate over time and contribute to the onset and progression of food allergies.

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Authors Contributions:

M.A, O.Z, R.G: conceptualization, methodology, project administration, writing - original draft. M.A, O.Z: investigation, validation. R.G: data curation, formal analysis. All authors: writing - review & editing.

Conflict of interest:

The authors declare that they have no conflict of interests.

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Table I: Patient's characteristics of both studied groups:

Variable			Group I (n=50)	Group II (n=50)	test	
					test	*P* value
Age (years)						
Mean ± SD.			31.2±9.09	32.96±1.4	-0.811 (t)	0.419
Range						
S	**Female**	N	32	30	0.170	0.680
e		%	64.0%	60.0%	(x^2)	
x	**Male**	N	18	20		
		%	36.0%	40.0%		

Independent t-Test (t)
Chi square test (X^2)

Table II: Laboratory investigations of Group I

Variables	Group I
T. IgE (IU/L)	
Mean±SD	262.69±173.93
Median (IQR)	210 (145.75-271.5)
TLC (mm3)	
Mean±SD	7.68±2.15
Range	(4.3-13)
Neutrophils (mm3)	
Mean±SD	4.85±1
Range	(3.2-7.7)
Lymphocytes (mm3)	
Mean±SD	2.33±0.68
Median (IQR)	2.05 (1.8-2.8)
Eosenophils (mm3)	
Mean±SD	0.21±0.47
Median (IQR)	0.1(0.03-0.2)
Basophils (mm3)	
Mean±SD	0.02±0.03
Median (IQR)	0.01(0-0.03)
ESR (mm/hr)	
Mean±SD	8.44±1.82
Range	(5-12)

CRP	
Mean±SD	4.05±2.32
Range	(0.8-9)
HbA1C (%)	
Mean±SD	4.94±0.32
Range	(4-5.5)
TSH (mIU/L)	
Mean±SD	1.83±0.71
Range	(0.42-3.9)
Creatinine (mg/dl)	
Mean±SD	0.74±0.18
Range	(0.5-1.1)
ALT (U/L)	
Mean±SD	16.58±5.38
Range	(8-31)
AST (U/L)	
Mean±SD	16.8±4.73
Range	(7-28)

Table III: Median value of serum sphingosine-1-phosphate (S1P) level among the studied groups

Variable	**Group I (n=50)**	**Group II (n=50)**	**tests**	
			test	***P* value**
S1P (ng/L) Median (IQR)	304.7 (179.9-641.5)	51.1 (39.2-77.3)	-8.267 (Z)	**<0.001***

Mann-Whitney Test (Z)

Figure I: ROC curve illustrating Predictive values of S1P level in food allergy prediction.

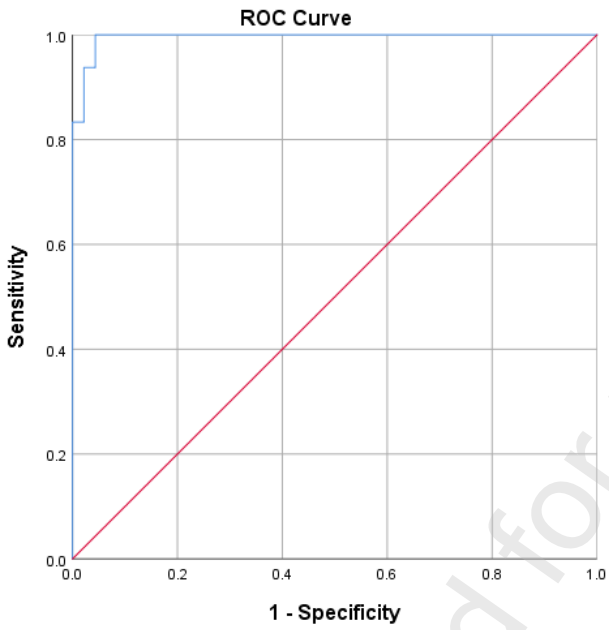

Figure II: Box plot illustrating the relation between S1P levels and severity grade of food allergy

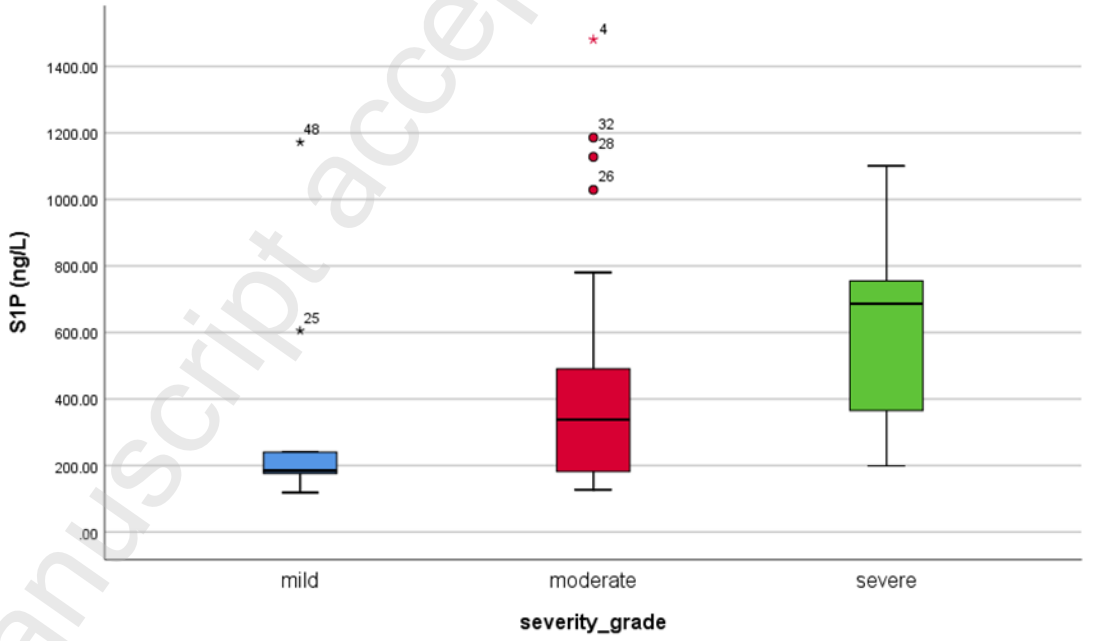

Figure III: Scatter diagram illustrating correlation between level of S1P and different parameters among group I