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Original Article

Does the balance of nitric oxide and L-arginine play a role in the development of allergic rhinitis?

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Abstract

Objective: The aim of the present study was to determine the mechanisms that play a role in the etiopathogenesis of seasonal allergic rhinitis (SAR). We investigated whether amino acids are effective in SAR development. The present study was conducted to investigate the presence of an alteration by comparing the serum free amino acid levels between SAR patients and healthy controls.

Methods: Forty-four patients with SAR were enrolled in the study group (Group 1), and thirty-three healthy volunteers were enrolled in the control group (Group 2). Group 1 consisted of the patients who had elevated specific IgE antibodies against at least one of the following, tree, grass, mold spores or mite, according to the allergen- specific IgE antibody test (the inhalant allergen test). They had at least one SAR symptom and had been diagnosed with SAR for at least two years. Levels of twenty-five serum free amino acids in both groups were measured by LC-MS/MS system. Serum nitric oxide (NO) levels were measured by griess reaction in both groups. Nitrate and nitrite levels were used as markers for NO measurement.

Results: The serum free L-arginine level was found to be significantly lower in group 1 (60,96±22,99 ng/ml) as compared to group 2 (86,27±31,35 ng/ml), (p: 0,047). A significant increase was observed in the levels of NO at group 1 (220.64±22.44 pmol/ml) compared with group 2 (165.84±19.32 pmol/ml), ($p < 0.05$).

Conclusion: We think that arginine/NO balance should be taken into consideration in the investigation of the physiopathology of SAR and potential novel therapeutic options.

Keywords: Arginine, human serum amino acids, mass spectrometry, nitric oxide, seasonal allergic rhinitis.

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INTRODUCTION

Allergic rhinitis (AR) is a common disease which peaks in the young and influences many individuals in every age group (1, 2). Its prevalence has increased during the recent decade. The World Health Organization (WHO) estimates that 400 million individuals suffer from AR worldwide (2-4). The disease is often neglected or misdiagnosed, diagnosis is overlooked, or it is treated incorrectly. This condition not only harms health but also leads to social costs. Allergic rhinitis is clinically important although it does not lead to mortality because the disease leads to many complications, it is an important risk factor that makes asthma control challenging, and it influences the quality of life and productivity at work and at school (1). Allergic rhinitis is an inflammatory process in the nasal mucosa which is typically IgE-mediated, triggered by environmental allergens, and characterized by the presence of inflammatory cells in sub-mucosa (5). One or more of the following symptoms, which are reversible spontaneously or with treatment are seen in the course of the disease including rhinorrhea that lasts at least one hour in a day, nasal itching and sneezing, and nasal obstruction. Various AR forms were systematized based on various criteria over a number of years. AR was classified in terms of the symptoms, the duration of the symptoms, the severity of the symptoms expressed by the patients, and the pathophysiology of the disease. AR was classified as seasonal, perennial, and episodic according to symptoms. Seasonal allergic rhinitis (SAR) develops at only during certain periods of the year and is influenced by plant pollens like grass, cereals, trees, and bushes (6).

Allergic rhinitis is characterized by eosinophil accumulation in the tissues (7, 8). However, the contribution of eosinophils to this process depends on their migration from circulation to the inflammation site (9). The mechanisms responsible for eosinophil migration could not be totally understood; however, it was possibly induced by various mechanisms including cytokines, chemokines, and adhesion molecules (8).

Nitric oxide (NO), which is known to be an important inflammatory agent, mediates various atopic diseases and is produced in nasal airways in relatively large amounts in patients with AR (10). Nitric oxide is produced by a mammalian cell from L-arginine and oxygen by an enzyme family known as NO synthases (NOS). Three NOS forms are available, including neuronal (nNOS or type I), endothelial (eNOS or Type III), and inducible (iNOS or Type II). nNOS and eNOS are structural, and iNOS is induced by some cytokines like bacterial lipopolysaccharide or tumor necrosis factor-alpha, interleukin-I, and interferon (8). Incubation of rats (11) and human (12) has shown that eosinophils alleviate eosinophilic chemotaxis with in vitro NOS inhibitors, and NO plays a role in cell migration and thereby AR (8). NO, which is a potent vasodilator, controls nasal capacitance and vasodilation, mediates acute congestion triggered by allergens, and thereby leads to nasal obstruction by decreasing openness (13).

Arginine is accepted to be the biological precursor of NO. In vivo and in vitro studies have shown that arginine is a substrate in NO synthesis in almost all cells, and the arginine-NO synthesis pathway have been defined in the related literature (14). The relationship between many biomolecules (amino acid, metabolite, enzyme, hormone, etc.) and diseases has been investigated in scientific studies to better understand the pathophysiology of the diseases. Amino acids, which have many important functions like protein synthesis, play an important role in these biochemical events; thus, many studies that elucidate the relationship between some diseases and amino acids are available. The relationship between serum/plasma amino acid profiles and diabetes mellitus (15), liver and kidney diseases (16-18), metabolic syndromes, sepsis (19), nasal polyposis with allergic etiology (20), and various cancer types including hepato-cellular carcinoma, breast cancer, lung cancer, colon cancer, renal cell carcinoma, cervical intra-epithelial neoplasia, and COVID-19 have been investigated biochemically (15, 21-29). In amino acid analysis, liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) has been considered as very

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important to performing bioanalytical analysis with speed, selectivity and sensitivity (30). It was proposed that the obtained data could be used as a marker for diagnosis and the course of the disease or treatment effectiveness.

Based on the data mentioned above, it was suggested that alterations could be present in the serum free amino acid profiles of patients with SAR. Serum free amino acid profile alteration was investigated in patients with SAR by using the LC-MS/MS method. Thus, with this study, it was aimed to examine the serum amino acid profile in SAR patients and to investigate the contribution of amino acids and their metabolites in the development and progression stages of the disease. It was observed that the serum arginine level decreased statistically significantly compared to the control samples. Afterwards, whether there was a change in nitric oxide level or not, it was studied in the same samples that were backed up.

MATERIALS AND METHODS

Study population

Ethics committee approval was obtained from Erzurum Regional Research and Training Hospital for this case-control study (BEAHKAEK 2020/11-123). Forty-four SAR patients aged 20-40 years who were admitted to the Ear-Nose-Throat (ENT) Department of Erzurum Regional Research and Training Hospital were enrolled in the study group (Group 1), and thirty-three healthy volunteers were enrolled in the control group (Group 2). Healthy individuals who were matched for age, gender, and body mass index (BMI) were included in the control group (Table 1). A detailed medical history was obtained from all participants, and basic rhinology examination, nasal endoscopic examination, and routine ENT examinations were performed on all participants. Group 1 consisted of the patients who had been diagnosed with SAR for at least two years, who had elevated specific IgE antibodies against at least one of tree, grass, mold spores, or mite according to the allergen-specific IgE antibody test (the inhalant allergen test), and who had had at least one symptom of SAR. Exclusion criteria were smoking and/or alcohol use; the presence of acute or chronic sinusitis, atopic dermatitis, asthma, nasal polyposis, or chronic medication us; a history of systemic or topical steroid use for any reason during the last months; pregnancy or lactation; the presence of upper respiratory tract infection during the study; or the presence of hepatic, renal, hematologic, cardiovascular, metabolic, neurologic, psychiatric disorders or malignancies.

Table 1. Demographic data of groups 1 and 2.

Parameters	Group 1 (n=20)	Group 2 (n=20)
Male	11	10
Female	9	10
Age	31,45	32,60
BMI (kg/m^2)	24.3	25.1

Serum Free Amino Acid Measurement

For all participants, blood samples were taken from the ante-cubital vein into gel-containing biochemical tubes following one night of fasting. Biochemical tubes were centrifuged at 3500 rpm for 10 min. The samples were divided into two equal pieces for any retest need and these samples were stored at -80°C until the day of analysis. On that day, the serum samples were taken from the -80°C environment and prepared for analysis after reaching room temperature. 50 µl of serum samples from the study and control groups was transferred into Eppendorf tubes. Vortex was done for 10 seconds by adding 50 µl of internal standard into it. 700 µl of amino acid solvent solution (Mobile phase A, Mobile phase B, v:v, 1:4) was added to each tube. After each

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sample was vortexed for one min, centrifugation was done at 4°C for eight min. The supernatant was separated and filtered by using 0.45 µm filters. Samples were measured with the LC-MS/MS system (Agilent 6460 Triple Quadropol, USA). Conditions for chromatography and mass spectrometry of LC-MS/MS, which was used for separating and defining the amino acids, are presented in Tables 2 and 3. Twenty-five amino acids defined as the result of the analysis are listed with their correlation coefficients and regression equations in Table 4.

Table 2. Solvent Composition Schedule during the gradient elution for LC-MS/MS.

Time **Flow: 0.7 mL/min**	***Change Solvent Composition** **A**	 **B**
1.00 min	78.00 %	22.00 %
4.00 min	70.00 %	30.00 %
5.00 min	70.00 %	30.00 %
5.10 min	22.00 %	78.00 %
9.00 min	22.00 %	78.00 %

*A: %3 Formic Acide–%5 Methanol–30 mM Ammonium Formate, B: Acetonitrile

Table 3. Mass conditions.

Parameters	**Value (+)**	**Value (-)**
Gas Temp (°C)	150.00	150.00
Gas Flow (L/min)	11.00	11.00
Nebulizer (psi)	40.00	40.00
SheathGasHeater	375.00	375.00
SheathGasFlow	11.00	11.00
Capillary (V)	2000.00	0.00
Vcharging	0.00	0.00
Injection Volume (µl)	1.00	
Ion source	AJS ESI	
Ion Mode	Positive	

Serum Nitric Oxide Measurement

Serum NO levels were measured by the griess reaction as previously described (31) and proposed by Keles MS (32). NO measurement is difficult because of its brief half-life. Therefore, nitrate and nitrite levels, which are stable end products of nitric oxide metabolism, were used as markers. NO levels were expressed as pmol/ml.

Statistical Analysis

Statistical analyses for NO measurument were performed using IBM SPSS 20.0 package program (SPSS for Windows, SPSS Inc., Chicago, IL, USA). An independent sample t-test was used for a comparison of mean differences between the groups. Analyses were done using the IBM SPSS 20.0 package program. Student's t test were applied to compare groups, and data were expressed as the mean±SD. A p level of <0.05 was accepted as statistically significant.

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Table 4. The correlation coefficients and the regression equations of serum free amino acid values.

Amino Acids	LR	*r*
1-Methyl-L-Histidine	y= -1.824848E-005x2 + 0.010764x - 0.023209	0,999429547
3-Amino Isobutyric acid	y= 0.003120x + 0.013203	0,995779956
3-Methyl-L-Histidine	y= 0.079485x - 0.344579	0,999437832
Beta-Alanine	y= 0.010029x + 0.004655	0,99926485
DL-5-Hydroxy Lysine	y= 0.002175x - 0.002046	0,999802015
Ethanolamine	y= 0.456542x + 5.731842	0,996284407
Gamma-aminobutyric acid	y= 0.010590x + 0.011764	0,999485107
L-2-Aminobutyric acid	y= 0.124358x + 0.095294	0,999732909
L-Alanine	y= 0.001987x - 0.017717	0,999604882
L-Anserine	y= 0.004788x - 0.004086	0,997479288
L-Arginine	**y= 0.010204x + 0.002139**	**0,997033234**
L-Asparagine	y= 0.013283x - 0.016439	0,998770169
L-Carnosine	y= 2.124417E-005x2 + 4.770619E-004x - 0.002777	0,998006027
L-Citrulline	y= 0.015528x + 0.019890	0,998370407
L-Cystine	y= 0.009640x + 0.011147	0,99948961
L-Glutamic acid	y= 0.015541x + 0.016491	0,99956412
L-Glutamine	y= 0.005484x + 0.047163	0,99968459
L-Glycin	y= 0.002507x - 0.053966	0,99829716
L-Histidine	y= 0.322855x + 5.240337	0,99853341
L-Isoleucine	y= 0.001014x + 0.007671	0,997839361
L-Leucine	y= 3.818289E-004x - 7.426710E-004	0,999561954
L-Lysine	y= 0.018360x - 0.096683	0,999438878
L-Methionine	y= 0.020414x - 0.013773	0,999163956
L-Ornithine	y= 0.017106x - 0.003227	0,9972577
L-Phenylalanine	y= 0.014849x - 0.058219	0,999340222
L-Proline	y= 0.005180x - 0.042782	0,99956426
L-Serine	y= 0.011543x - 0.102119	0,999504997
L-Threonine	y= 0.009038x + 0.015851	0,999756685
L-Tryptophan	y= 0.024978x - 0.042477	0,999125613
L-Tyrosine	y= 0.010546x - 0.021475	0,999668265
L-Valine	y= 0.002746x - 0.011662	0,999579547
Taurine	y= 8.956307E-005x - 8.085060E-004	0,99806888
Trans-4-hydroxy L-proline	y= 0.002025x - 0.006136	0,995686311

* LR: Lineer regression equations, *r*: Correlation coefficient

RESULTS

The mean age was 31.45±4.59 in group 1 and 32.60±5.31 in group 2 (p:0.759). In group 1, 21 subjects were male and 23 were female; in group 2, 12 subjects were male and 11 were female. The female/male ratio was similar between the groups. The body mass index (BMI) (kg/m) was similar between the groups (Table 1). The serum free L-arginine level was found to be statistically significantly lower in Group 1 compared to Group 2 (Figure 1), and a significant alteration was not observed in the remaining 24 amino acids (Table 5). Serum NO level increased significantly in group 1 (220.64±22.44 pmol/ml) compared to group 2 (165.84±19.32 pmol/ml), ($p < 0.05$).

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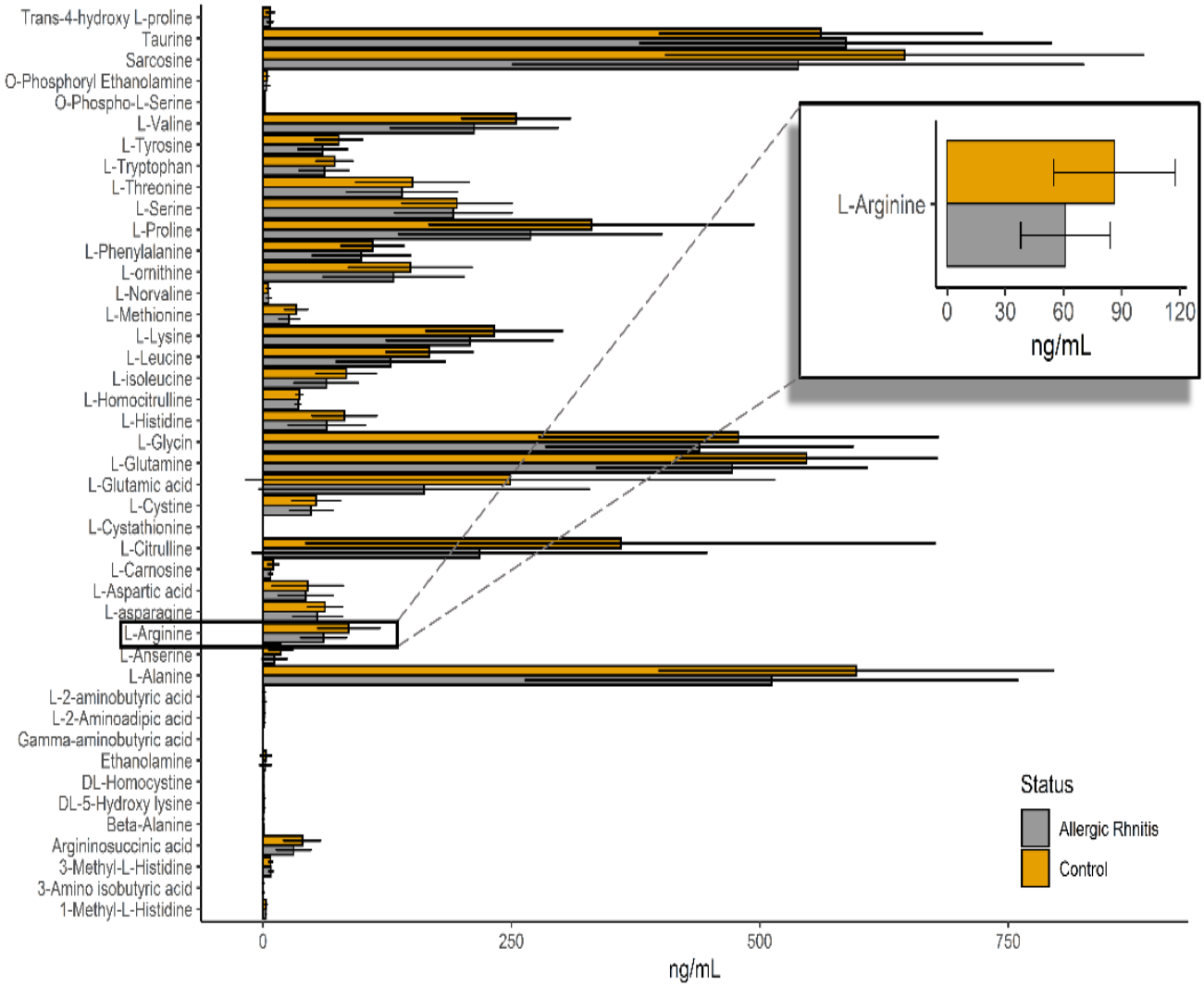

Figure 1: Changes in amino acid levels in control and SAR patients

DISCUSSION

This is the first original research investigating the serum free amino acid profile in patients with SAR. In our study, the serum levels of 25 free amino acids were measured, and a significant reduction was observed in only one (L-arginine) amino acid in patients with SAR. Amino acids, which play a role in cell proliferation, gene expression and inflammatory responses, are related to many diseases and metabolic disorders (33-35). Amino acids have the potential to be an excellent biomarker for many disorders because they are regulator for protein synthesis and many metabolic pathways (20). Previously Celik et al demonstrated, an elevation in the levels of 15 amino acids in patients with nasal polyposis (20). Especially in inflammatory and immunologic diseased serum levels of amino acids is under investigation. In a guinea pig model of allergic asthma plasma level of symmetric dimethyl arginine and its ratio to total unmodified arginine, were found to be increased in sensitized guinea pigs compared to healthy controls (36).

Production of NO from L-arginine by the NO synthase family of isoenzymes plays an important role in the maintenance of airway tone (37). In our study the level of L-arginine was found to be decreased in SAR patients when compared to healthy ones. Also The levels of NO at patients with SAR increased when compared with that of healthy controls. SAR, which is an AR

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type that develops when allergens like plant pollens and mold spores are intensively found in the atmosphere. Allergens interact with basophils or mast cell-dependent specific IgE; triggers the degranulation of inflammatory mediators like prostaglandin, leukotriene, NO synthesis, and histamine, tryptase; and thereby leads to an inflammatory response in the nasal mucosa (35).

Table 5. Serum free amino acid values (ng/ml) in patients with SAR compared with controls.

Amino Acids	***Group 1 (n=20)*** ***SAR*** ***Mean±SD***	***Group 2 (n=20)*** ***Control*** ***Mean±SD***	***p*-values**
1-Methyl-L-Histidine	5,14±3,67	5,86±5,74	0,733
3-Amino Isobutyric acid	3,29±2,60	1,94±1,27	0,152
3-Methyl-L-Histidine	7,79±2,19	7,59±1,80	0,823
Beta-Alanine	1,24±0,42	1,28±0,69	0,857
DL-5-Hydroxy Lysine	1,43±0,27	1,49±0,35	0,655
Ethanolamine	6,82±4,95	8,50±4,22	0,424
Gamma-aminobutyric acid	0,50±0,28	0,36±0,13	0,14
L-2-Aminobutyric acid	4,50±2,57	2,30±1,90	0,142
L-Alanine	511,93±247,98	596,97±198,48	0,4
L-Anserine	18,28±7,75	23,48±5,89	0,102
L-Arginine	**60,96±22,99**	**86,27±31,35**	**0,047***
L-Asparagine	54,71±24,99	62,28±17,57	0,436
L-Carnosine	7,73±1,10	10,51±4,50	0,075
L-Citrulline	29,41±14,89	34,31±10,71	0,402
L-Cystine	48,38±21,79	53,55±24,76	0,616
L-Glutamic acid	133,38±129,60	143,83±112,73	0,847
L-Glutamine	472,28±136,09	547,14±131,72	0,217
L-Glycin	439,24±154,59	478,58±200,78	0,619
L-Histidine	86,54±28,30	92,50±20,72	0,594
L-Isoleucine	63,64±32,53	83,67±30,52	0,163
L-Leucine	128,55±54,72	167,60±43,62	0,088
L-Lysine	208,08±84,08	232,73±68,92	0,474
L-Methionine	26,29±10,40	33,22±11,82	0,169
L-Ornithine	131,13±70,84	148,24±62,09	0,565
L-Phenylalanine	98,86±49,31	110,14±31,54	0,545
L-Proline	269,12±132,45	330,72±163,51	0,353
L-Serine	180,85±64,93	195,21±55,47	0,594
L-Threonine	139,89±55,58	150,56±57,26	0,67
L-Tryptophan	61,64±24,94	72,04±18,47	0,295
L-Tyrosine	60,04±24,74	76,13±24,15	0,149
L-Valine	212,26±84,46	251,90±57,58	0,229
Taurine	111,22±21,70	104,15±20.01	0,448
Trans-4-hydroxy L-proline	7,12±2,93	8,07±3,14	0,479

*.p<0.05

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The reason why L-arginine levels were decreased in SAR patients may be resulting from the imbalance in L-arginine-NO pathway. Previously Azedero et al. demonstrated that dietary arginine supplementation modulates the immune condition and inflammatory response (39), supporting our results that arginine levels decreased in SAR patients as a result of exaggerated NO production that may aggravate SAR. So, it might be beneficial to lower L-arginine intake patients with SAR to prevent excessive NO production. Also the increase in NO synthesis plays an important role in AR pathogenesis. It is known that the increase in NO production is very important during the development of symptoms such as nasal congestion, rhinorrhea and inflammation of the nasal mucosa (13, 36, 37, 39).

In patients with allergic rhinitis nasally exhaled NO concentrations found to be higher than orally exhaled NO concentrations (40-42). While the source of nasal NO is uncertain the increased concentration can be a result of its high levels in paranasal sinuses (43) Martin et al. demonstrated that nasal exhaled NO levels increase parallel to the seasonal and perennial rhinitis symptoms (44). Kharitonov et al. demonstrated that nasal exhaled NO levels were increased in both allergic rhinitis and asthma patients and this increase can be suppressed by nasal glucocorticoid application (42). Since NO production is suppressed with topical glucocorticoids (45), it has been suggested that the NO increase may result from iNOS expression in the nasal mucosa (40). As it is known, iNOS is an enzyme that catalyzes the production of NO from arginine and oxygen (8, 46). We can suggest that the decrease in serum arginine levels in patients with SAR is due to the increase in iNOS expression as a result of NO production. In line with our result previous data suggest an imbalance in L-arginine-NO pathway during SAR and shed light on this pathway as both diagnostic and therapeutic target.

CONCLUSION

We think that the measurement of the serum arginine and NO level could support the clinician in diagnosing patients who are challenging to diagnosis for SAR. We would also like to state that arginine/NO should not be overlooked for the investigation of SAR physiopathology and potential novel treatment methods.

Conflict of interest: The author declares that there is no conflict of interest.

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