The Seasonal Risk of Food Allergies in Albania: Focus on Fish and Crustaceans

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Abstract: Introduction: Food allergies, particularly to seafood, are becoming increasingly common worldwide, posing significant health risks to individuals across all age groups. Allergic reactions to seafood can be severe, including symptoms such as oral allergy syndrome, urticaria, angioedema, gastrointestinal distress, and anaphylaxis. The primary allergens in fish and shellfish are heat-stable proteins that remain active even after cooking. While most allergic reactions are immediate, some may occur hours after consumption, particularly in individuals allergic to mollusks. This study focuses on assessing the risk of seafood, in the Albanian population by analyzing the prevalence of specific IgE antibodies across different seasons. Understanding these patterns is crucial for improving diagnosis, management, and prevention strategies for food allergies in Albania
Materials and methods. The study included a total of 353 participants from different age groups across the Albanian Republic. Blood samples were collected from July to December 2023 to measure specific IgE antibodies against food allergens using the Polycheck reagent, which tests for 30 allergens.
Results and discussions: To facilitate data analysis, the test results were organized into three seasons: summer (July-August), autumn (September-October), and winter (November-December).
Tuna had the highest prevalence in the summer season (27.05%), followed by crab (13.11%), while shrimp had the lowest (0.82%) IN Summer season.
In autumn, crab had the highest prevalence (7.48%), followed by tuna and shrimp (both at 2.8%).
During the winter season, crab again had the highest prevalence (5.65%), followed by clam shell (4.03%). No positive IgE cases were identified for codfish.
Using SPSS version 21, significant correlations were found between:
Tuna and Codfish: (p=0.000)
Shrimp and Crab: (p=0.035)
Conclusion: The prevalence of food allergies to fish and crustaceans varies significantly by season in the Albanian population. These findings suggest the need for greater awareness and education for consumers and healthcare professionals on the importance of early diagnosis and management of food allergies

Keywords: Seafood Allergy,IgE Antibodies,Seasonal Variation,Albanian Population

I. INTRODUCTION (HEADING 1)

Many people are concerned by seafood allergies, which are mostly related to three groups: Arthropoda (shrimp and crab), Mollusca (squid and mussels), and Chordata (fish)[1].
These allergies are primarily caused by high molecular weight proteins that hold their heat stability even after cooking[2]. It is well-known that these proteins can result in allergic reactions, which can vary in severity from minor symptoms like urticaria and oral allergy syndrome to severe and potentially fatal anaphylaxis[3].
These allergic reactions usually happen fast, usually within two hours of ingestion. Still, up to eight hours after eating[4].Some people—especially those allergic to shellfish—may develop late-phase symptoms. IgE-mediated hypersensitivity can also result from simple inhalation or skin contact with seafood allergens, while ingestion is the most prevalent cause.

[1] Shellfish Allergens: Cross-reactivity Within the Shellfish Group

One of the major allergens in shellfish is tropomyosin, a pan-allergen that exhibits significant cross-reactivity due to its molecular similarity across different shellfish species[6].This means that if you're allergic to one type of shellfish, you're likely to react to others as well. Besides tropomyosin, newer allergen classes like arginine kinase, myosin light chain, and sarcoplasmic calcium-binding proteins have also been identified[7].Interestingly, there's a notable cross-reactivity between shellfish allergies and dust allergies, particularly in children, due to the shared presence of tropomyosins[8].

[2] Fish Allergens: Cross-reactivity Within the Fish Group

Fish have the presence of powerful allergens, such as parvalbumin (especially the beta subtype). These 12 kD calcium-binding proteins are the major allergens, resistant to both heat and digestion[9].
Baltic cod parvalbumin, Gad c 1, was the first to be identified and since then, different isoforms have been found in commonly consumed fish such as cod, carp and herring[10].The high structural similarity of parvalbumins in different fish species means that fish-allergic individuals often react to multiple fish species[11].
Interestingly, dark-muscled fish such as tuna and swordfish tend to have lower levels of parvalbumins and thus are less allergenic compared to their white-muscled counterparts such as cod, salmon and pollock[12].
However, cod stands out as one of the most allergenic types of seafood due to its high content of parvalbumin[13].Other important fish allergens include beta-enolase, aldolase, and aldehyde phosphate dehydrogenase[14].

Allergens in Processed Seafood
Seafood processing techniques, such as heating, smoking, and chemical treatments, can significantly alter the allergenicity of fish and shellfish proteins[15].Surprisingly, cooking can sometimes increase the allergenicity of seafood. This happens through several mechanisms, including protein denaturation, exposure to new epitopes, aggregation, and chemical modifications, making the allergens even more potent than in their raw form [16]
Understanding the complexities of seafood allergies and the various factors that influence their severity can help individuals better manage their conditions and avoid potentially dangerous reactions. Whether through careful dietary choices or by being mindful of cross-reactivities, those with seafood allergies can navigate their world a bit more safely.

Laboratory diagnosis
The diagnosis of seafood allergy is based on the patient's accurate medical history, skin prick test(SPT), quantification of specific IgE antibodies, and in some cases oral challenge tests^1^. However, diagnosing seafood allergies can be challenging for clinicians, as a positive SPT or the presence of specific IgE antibodies is not necessarily indicative of clinical susceptibility^2^.
Medical History
A thorough medical history is crucial in diagnosing seafood allergies. Patients should provide detailed information about any previous allergic reactions, including the type of seafood consumed, symptoms experienced, and the timing of the reaction. Additionally, clinicians may inquire about any associated factors such as exercise, alcohol consumption, or concomitant medication use, which can influence the severity of allergic reactions.
Skin Prick Test (SPT)
The SPT involves introducing a small amount of allergen extract into the skin, typically on the forearm or back, and observing for any signs of an allergic reaction, such as redness, swelling, or itching. While a positive SPT indicates sensitization to the allergen, it does not necessarily confirm clinical allergy. Therefore, SPT results must be interpreted in conjunction with the patient's medical history and other diagnostic tests.
Quantification of Specific IgE Antibodies
Measurement of specific IgE antibodies to seafood allergens through blood tests, can provide additional diagnostic information. Elevated levels of specific IgE antibodies indicate sensitization to the allergen but do not always correlate with clinical allergy. As with SPT results, specific IgE levels should be interpreted in the context of the patient's medical history and other clinical findings.

Oral Challenge Tests
Oral challenge tests involve administering increasing doses of the suspected allergen under medical supervision and monitoring for any allergic reactions. These tests are considered the gold standard for confirming a diagnosis of seafood allergy, as they directly assess the patient's tolerance to the allergen. However, oral challenge tests carry a risk of triggering severe allergic reactions and should only be performed by trained healthcare professionals in a controlled clinical setting.
In summary, the diagnosis of seafood allergy requires a comprehensive approach that includes a detailed medical history, skin prick testing, quantification of specific IgE antibodies, and, in some cases, oral challenge tests. Clinicians must carefully interpret diagnostic test results in conjunction with the patient's clinical presentation to accurately diagnose and manage seafood allergies.

MATERIALS AND METHOD

A.Sample collection
In this study, 353 individuals participated, ranging in age from 6 months to 78 years. Some of these individuals were asymptomatic, while others exhibited various clinical signs suggestive of allergic reactions. To assess their immune

response, a 5ml venous blood sample was collected from each participant using a butterfly vacuum system, with blood drawn from forearm veins utilizing a 21G vacuum system.

Upon collection, all blood samples were meticulously identified and homogenized at least five times to ensure consistency. Subsequently, each sample was allowed to set for 30 minutes at a controlled temperature range of 18–25ºC. Following this incubation period, all tubes underwent centrifugation at 1500g/3500RPM for 10 minutes at the same temperature range (18–25ºC).

Furthermore, before further analysis, all samples were carefully inspected for the presence of hemolysis and lipemia to ensure the integrity of the collected blood specimens. This rigorous sample processing protocol aimed to maintain the quality and reliability of the data obtained from the study, providing a solid foundation for the subsequent analysis and interpretation of the results..

B.Analyzing the sample

The measurement of specific IgE was performed with the Indirect ELISA method using Policheck LOT 18 REF.WB05 reagents. The analysis of the tests was carried out according to the laboratory protocols related to pre-analytical, analytical and post-analytical phases. The laboratory tests were carried out in the Genius Shpk laboratory.Reagents in use were stored at 2–8 °C and protected from contamination.

C. Features of the analysis

- Sample material: Serum
- Analysis duration: Manual procedure: 2.5 hours,
- Lower limit of detection: 0.15 kU/l
- Specification: human IgE; no cross-reaction with IgG, IgA, IgD, IgM
- Necessary materials
- IT equipment: Personal computer (Windows 7 or higher versions, Net Framework 3.5), printer, flatbed scanner (search for recommended models or check at www.polycheck.de)
- Biocheck Imaging Software (BIS): for analysis, calculations and reports
- Laboratory equipment: shaker (30 rpm), pipette 200 -1000µl; (1ml) to dispense the WASH solution
- Demineralized water for the preparation of WASH solution

D.Test Method and Principle

The Polycheck® Screening Assay is an immunoassay for the quantitative measurement of allergen-specific IgE in serum. The respective allergens are coated separately on the strips as well as the calibrator in a holder which is fixed in the well of the Polycheck® cassette. During incubation of the patient's serum, allergen-specific IgE binds to the respective allergens.

Unbound serum components are removed by washing. Anti-IgE antibodies labeled with monoclonal ligands bind to allergen-bound IgE. Unbound antibodies are removed by washing. Enzyme-tagged anti-ligands bind to immune complexes; excess enzyme conjugate is removed by washing.

The substrate solution is added and specially bound enzymes convert the colorless substrate into a dark precipi-tate. The color intensity of the lines is proportional to the corresponding concentration of allergen-specific IgE in the patient's serum.

With the help of Biocheck Imaging Software (BIS) and a computer, Polycheck® cassettes are interpreted. Each single allergen will be identified and, according to the calibrator curve present in each cassette, the concentration of each specific allergen IgE is quantified.

E.Test Performance

- All test components should be at room temperature and mixed well.
- Use only the reagent parts supplied with the current kit.
- Powder wash buffer should be diluted with demineralized water at least 30 minutes before use.

F.Avoids foaming.

- Test cassette membranes should not be allowed to dry during analysis.
- All incubation steps are performed at room temperature (18 – 24 °C) and with continuous shaking.
- For the correct interpretation of the test results, a flatbed scanner with a CCD sensor and a scanning resolution of 600 DPI should be used.

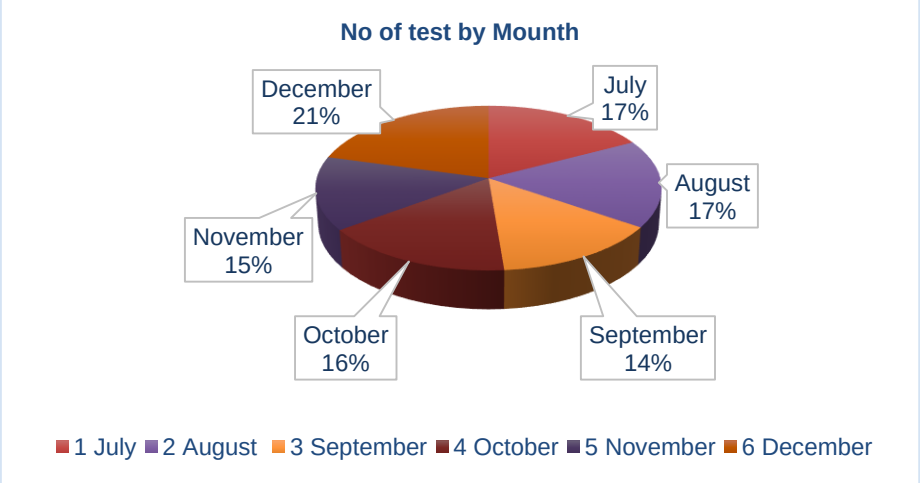
RESULTS

The data of this study includes the time period July- December 2023. In total, there are 353 samples analyzed in the laboratory.

Table 1. Table 1 shows the distribution by month (July-December) of the number of specific IgE tests. There is an increase in the number of tests in December

Distribution of tests by month			
No.	Month	No. of tests by month	Total in %
1	July	61	17.28
2	August	61	17.28
3	September	50	14.16
4	October	57	16.15
5	November	51	14.45
6	December	73	20.68
Total		353	100.00

They are followed by the graphic presentation, that shows the distribution in the percentage of tests performed during different months.



Graph 1. This graph shows the distribution in % of tests by month. From the graph, the highest % in several tests belongs to December (21%).

For easier processing of laboratory data, these data have been organized by season. The Summer season includes July and August, the Autumn season includes September and October, and the Winter season includes November and December.

Table 2. Prevalence of positive IgE in Summer season 2023

Summer season (n=122)				
Allergen	**July**	**August**	Total	Percentage %
Clam shell	4	2	6	4.92
Shrimp	0	1	1	0.82
Crab	6	10	16	13.11
Tuna	12	21	33	27.05
Codfish	2	0	2	1.64

The analysis of laboratory data reveals that Tuna has the highest prevalence of IgE positivity during this period, accounting for 27.05% of the cases. Crab follows as the second most prevalent, with 13.11% of the cases showing IgE positivity. In contrast, Shrimp has the lowest prevalence, with only 0.82% of the cases testing positive for IgE. This data highlights the significant variability in allergic responses to different types of seafood, underscoring the need for targeted allergen management and patient education for those most affected by these allergens.

Table 3. Prevalence of positive IgE in Autumn season 2023

Autumn season (n=107)				
Allergen	September	October	Total	Percentage %
Clam shell	1	1	2	1.87
Shrimp	2	1	3	2.80
Crab	3	5	8	7.48
Tuna	1	2	3	2.80
Codfish	0	0	0	0.00

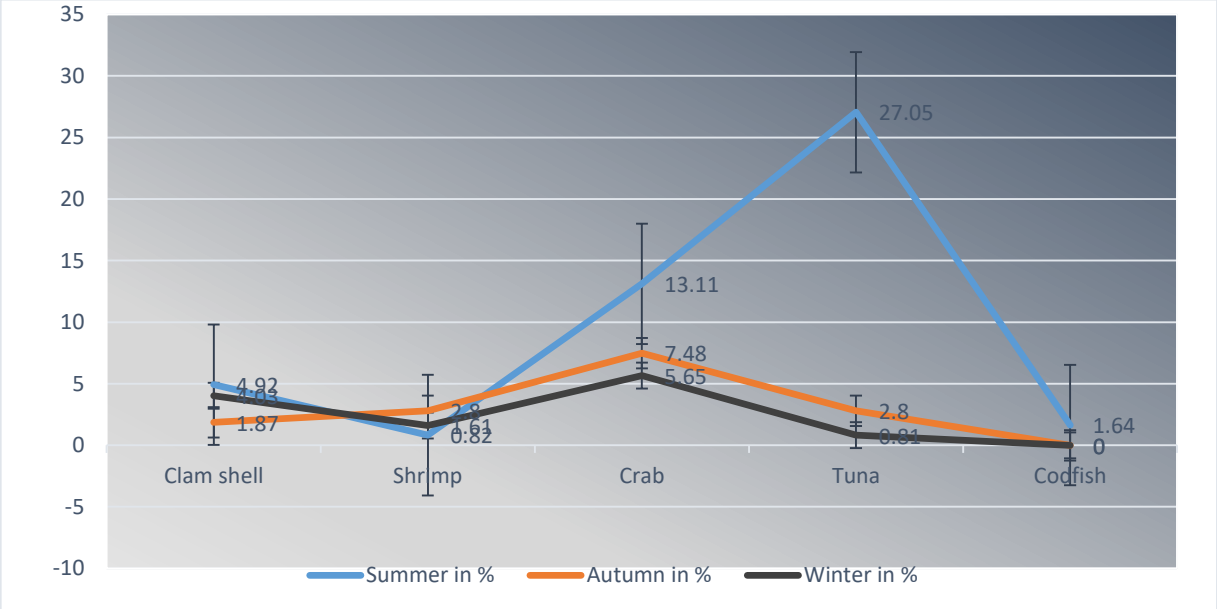
During the Autumn season, Crab was the allergen with the highest number of cases, accounting for 7.48%. This was followed by Tuna and Shrimp, each with 2.8% of the cases. This seasonal analysis emphasizes the prominence of Crab allergies during the autumn months, compared to other seafood allergens like Tuna and Shrimp, which have a relatively lower prevalence in the same period.

Table 4. Prevalence of positive IgE in Winter season 2023.

Winter season n=124				
Allergen	November	December	Total	Percentage %
Clam shell	1	4	5	4.03
Shrimp	0	2	2	1.61
Crab	2	5	7	5.65
Tuna	1	0	1	0.81
Codfish	0	0	0	0.00

During the Winter 2023 season, Crab emerged as the most frequent allergen, responsible for 5.65% of the cases. This was followed by Clam Shell, which accounted for 4.03% of the cases. Notably, no patients were identified with positive IgE responses to Cod during this period. This indicates that while Crab and Clam Shell were significant allergens in the winter months, Cod did not present any cases of IgE-mediated allergic reactions, highlighting a notable difference in allergen prevalence among different types of seafood during this season..

Table 4. The total percentage of positive IgE cases for various seafood allergens is expressed as follows.



The statistical software SPSS version 21 was used to process the laboratory test data in order to determine whether there is a relationship between the IgE of these groups.

Table 4. It is studied if the two varieties of tuna-cod fish and the shellfish that float between them correlate statistically.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	tuna codfish	-.06241	.15780	.00840	.04589	.07893	7.430	352	.000
Pair 2	shrimp crab	--.24125	2.14076	.11394	-.46534	-.01716	-2.117	352	.035

Based on the significance level of P≤0.05, statistical relationships were observed between Tuna and Cod (p=0.000) as well as between Shrimp and Crab (p=0.035). These findings suggest that there are significant associations between these pairs of seafood allergens, indicating potential shared allergenic properties or cross-reactivity among them.

Table 5. The statistical correlation between fish and shellfish and mollusks.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	clamshall - crab	.31147	9.84049	.52376	-.71861	1.34156	.595	352	.552
Pair 2	clamshall - tuna	.79161	8.74479	.46544	-.12377	1.70700	1.701	352	.090
Pair 3	clamshall shrimp	-.55272	10.09943	.53754	-.50447	1.60991	1.028	352	.305
Pair 4	clamshall codfish	-.85402	8.74052	.46521	-.06092	1.76897	1.836	352	.067

Based on the paired samples test results:

1. For the comparison between Clam Shell and Crab, the mean difference is 0.31147 with a standard deviation of 9.84049. The confidence interval for the difference ranges from -0.71861 to 1.34156. With a p-value of 0.552, there is no statistically significant difference between Clam Shell and Crab.

2. For the comparison between Clam Shell and Tuna, the mean difference is 0.79161 with a standard deviation of 8.74479. The confidence interval for the difference ranges from -0.12377 to 1.70700. With a p-value of 0.090, there is no statistically significant difference between Clam Shell and Tuna.

3. For the comparison between Clam Shell and Shrimp, the mean difference is 0.55272 with a standard deviation of 10.09943. The confidence interval for the difference ranges from -0.50447 to 1.60991. With a p-value of 0.305, there is no statistically significant difference between Clam Shell and Shrimp.

4. For the comparison between Clam Shell and Codfish, the mean difference is 0.85402 with a standard deviation of 8.74052. The confidence interval for the difference ranges from -0.06092 to 1.76897. With a p-value of 0.067, there is no statistically significant difference between Clam Shell and Codfish.

In conclusion, based on the provided data and analysis, there are no statistically significant differences between Clam Shell and any of the other seafood types (Crab, Tuna, Shrimp, and Codfish) at the specified significance level of 0.05. However, it's important to note that there are trends suggesting potential differences, especially with a p-value of 0.067 for the comparison between Clam Shell and Codfish, which might warrant further investigation with a larger sample size or different statistical methods.

DISCUSSION

The discussion surrounding seafood allergies presented in the text is both informative and insightful, shedding light on the multifaceted nature of this health concern and its broader implications for public health and clinical practice.

One key aspect of the discussion revolves around the diverse range of allergens present in seafood, categorized into three major groups: Arthropoda, Mollusca, and Chordata. By highlighting these groups, the text underscores the wide variety of seafood products that individuals may be allergic to, ranging from crustaceans like shrimp and crab to fish and fins. This classification lays the foundation for understanding the complexity of seafood allergies and the challenges involved in diagnosis and management.

Moreover, the text delves into the mechanisms underlying seafood allergies, emphasizing the role of high molecular weight proteins that exhibit heat stability even after cooking. This persistence of allergenic proteins poses challenges for individuals with seafood allergies, as they may experience allergic reactions even when consuming cooked seafood. Understanding the mechanisms driving these allergic reactions is crucial for developing effective diagnostic and management strategies.

The discussion also touches upon the issue of hidden allergens in food products, highlighting how seafood allergens can be concealed in various dishes and food products. This aspect underscores the importance of clear and accurate food labeling to ensure individuals with seafood allergies can make informed dietary choices and avoid potential allergens. Moreover, the mention of cross-reactivity among shellfish allergens and between different fish species underscores the complexities involved in diagnosing and managing seafood allergies effectively.

Furthermore, the text explores the diagnostic methods available for identifying seafood allergies, including medical history, skin prick tests, quantification of specific IgE antibodies, and oral challenge tests. While these diagnostic approaches are valuable, the text acknowledges the limitations and challenges clinicians face in interpreting test results accurately. This discussion highlights the need for a comprehensive diagnostic approach that considers clinical history alongside test results to ensure accurate diagnosis and effective management of seafood allergies.

Finally, the text concludes with a discussion on the statistical analysis of allergen relationships, highlighting significant associations between certain seafood allergens. This statistical insight provides valuable information for understanding the potential shared allergenic properties or cross-reactivity among different seafood allergens, informing clinical practice and guiding allergen management strategies.

In essence, the discussion surrounding seafood allergies in the text underscores the complexity of this health concern and the importance of a multifaceted approach encompassing understanding mechanisms, accurate diagnosis, effective management, and informed public policy to ensure the well-being of individuals with seafood allergies.

I. CONCLUSION

Seafood allergies represent a significant health concern, with allergens present across various groups such as Arthropoda, Mollusca, and Chordata . These allergies are primarily driven by high molecular weight proteins, which retain their allergenic properties even after cooking . The persistence of these allergens poses challenges for individuals, leading to a spectrum of allergic reactions ranging from mild to severe, including life-threatening anaphylaxis.Moreover, the issue of hidden allergens in food products complicates the landscape of seafood allergies, necessitating clear and accurate food labeling to facilitate informed dietary choices and prevent inadvertent exposure to allergens . Cross-reactivity among shellfish allergens and between different fish species further adds to the complexity of diagnosing and managing seafood allergies effectively .

The results of the study shed light on the prevalence of specific IgE responses to various seafood allergens across different seasons . Tuna emerged as the most prevalent allergen during the summer months, while crab showed prominence during the autumn and winter seasons . Statistical analysis revealed significant associations between certain seafood allergens, indicating potential shared allergenic properties or cross-reactivity among them .

Several factors could contribute to the observed results in the study:

1. **Seasonal Variation in Consumption Patterns**: Different seafood items may be consumed more frequently during certain seasons due to factors like availability, cultural practices, or culinary preferences. This could lead to varying levels of allergen exposure and prevalence of allergic reactions across seasons.

2. **Environmental Allergen Exposure**: Environmental factors such as pollen levels, humidity, or temperature fluctuations can influence the expression of allergic reactions. For example, higher pollen levels during certain seasons may exacerbate allergic responses in susceptible individuals, leading to increased prevalence of seafood allergies.

3. **Food Preparation Methods**: The way seafood is prepared and cooked can affect its allergenicity. Certain cooking methods may alter the structure or composition of allergenic proteins, making them more or less potent. Additionally, cross-contamination during food preparation could introduce allergens from other sources, impacting the prevalence of allergic reactions.

4. **Geographical Variations**: Seafood consumption patterns and allergen prevalence may vary geographically based on factors such as proximity to coastal regions, cultural dietary practices, and access to different types of seafood. These geographical variations could contribute to differences in allergic response prevalence across regions and seasons.

5. **Individual Sensitivities**: Each individual's immune system responds differently to allergens, leading to variations in allergic responses. Factors such as genetic predisposition, previous exposure to allergens, and overall immune health can influence an individual's likelihood of developing allergic reactions to specific seafood allergens.

6. **Cross-Reactivity and Shared Allergenic Properties**: Cross-reactivity between different seafood allergens or between seafood and other allergen sources (e.g., pollen or dust) may contribute to the observed prevalence of allergic reactions. Shared allergenic properties among closely related species or allergen families could lead to similar allergic responses to different seafood items.

7. **Underlying Health Conditions**: Individuals with underlying health conditions such as asthma or atopic dermatitis may be more susceptible to allergic reactions, influencing the prevalence of seafood allergies observed in the study population.

In the realm of diagnosis, various methods such as medical history assessment, skin prick tests, IgE antibody quantification, and oral challenge tests play pivotal roles. However, interpreting diagnostic results remains a challenge due to the nuances involved, highlighting the need for a comprehensive diagnostic approach that considers clinical history alongside test results .

Furthermore, the study underscores the importance of ongoing research and vigilance in addressing seafood allergies, given their potential impact on individuals' health and well-being. By addressing these aspects comprehensively, we can mitigate the impact of seafood allergies on individuals' health and well-being, ensuring a safer and more inclusive food environment for all.

ACKNOWLEDGMENT

A heartfelt acknowledgment is extended to Mirela Lika and Artan Trebicka for their invaluable contributions, unwavering support, and meticulous review throughout this endeavor. Their dedication, expertise, and commitment have significantly enriched the quality and depth of this work. Their contributions have not only enriched the content of this project but have also fostered a culture of collaboration, excellence, and mutual respect. Their willingness to share their expertise, provide constructive feedback, and offer guidance has been instrumental in navigating the complexities of this endeavor.

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