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Original Article

Using CD63 and CD203 in diagnosing food allergies

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Abstract

Food allergies are one of the most common diseases in recent times. Due to the increasing incidence of food allergies and the difficulty of diagnosing this type of allergy, the study aims to evaluate the role of the differential proteins CD63 and CD203 in diagnosing food allergies. The study was of 90 blood samples from people aged between 7–49 years old. It involves 70 blood samples from allergic people and 20 from healthy people, considered control samples. The allergy diagnosis was confirmed on 90 samples using the allergy type test using the diagnostic kit (Atopic Kit). The results brought to light that 30 samples were food allergy, 40 samples were not under food allergy, and 20 control samples were also not food allergy. Besides, the present study included the estimation of differential proteins CD203 and differential proteins CD63 in the serum of the studied samples using the ELISA technique. The results of the current study showed an increase in the concentration of CD203, as its average concentration in samples of people with food allergy reached a rate of 0.16+0.15 ng/mL, compared to the average concentration of control samples, which was at a rate of 0.14+0.05 ng/mL, with significant differences in the concentration of CD203 in people with food allergies, and control samples, at the probability level $P \leq 0.05$. Moreover, the study under question disclosed that differential proteins are essential criteria for measuring basophil activity and diagnosing food allergies. This study's conclusion is that CD203 and CD63 are important criteria for measuring basophil activity and diagnosing food allergies.

Keywords: food allergy, CD63, CD203, basophile cells.

Introduction

Food allergies constitute one of the most important chronic diseases in the world. it is considered the fourth chronic disease according to the classification of the World Health Organization [1]. Therefore, food allergies are a growing problem and a clinical dilemma in that allergic people suffer from allergies [2]. Food allergy indicators and symptoms usually appear within a few minutes to two hours after eating food containing the allergen, and in rare cases, the onset of symptoms may be delayed for several hours [3]. It is significant to indicate that evaluating basophil activation by changes in cell surface markers CD63 and CD203 is an important test to determine allergy to food [4]. The activity of basophils and mast cells is detected by measuring the concentration or determining the expression of markers of differential proteins CD63 and CD203 on the surfaces of these cells [5]. These proteins are found before their activation in the lysosomal membranes of basal and mast cells. Following the cell activation process, these proteins move to the surfaces of the cells [6]. The basophil activation test weighs the expression of activation markers, mainly CD63 or CD203c, on the basophil membrane after cross-linking IgE antibodies with allergens on the basophil surface [7]. Despite the scarcity of basophils, which constitute less than 3% of peripheral white blood cells, they are easily accessible cells, and their activity can be measured. Therefore, as indicated by Santos AF *et al.* [8], they are considered one of the best techniques available and are promising biomarkers for diagnosing food allergy. It is worth mentioning that the differential protein CD63 is a membrane protein of the LAMP family. The LAMP family comprises tetraspanin proteins often involved in vesicle fusion events [6]. The differential proteins in

basal cells CD203 and CD63 were found to be associated with histamine-containing granules [9].

Material and methods

Study design and setting

The present study was carried out on 90 human samples collected from venous blood, which included 50 blood samples from males and 40 blood samples from females within age groups ranging between 7–49 years old, residing in various Basrah Governorate. Human samples were randomly collected from people suffering from allergies. Special information was recorded for the samples of the current study according to a pre-prepared questionnaire form that included (name, age, gender, medical history, geographical location, type of allergy, duration of illness, appearance of symptoms, type of symptoms, and other diseases who suffer from).

Blood sample collection

Venous blood samples were drawn in a volume of 5 ml from each current study sample using a special single-use medical syringe. The samples were placed in test tubes free of anticoagulants. The blood serum was then separated at a speed of 3000 rpm for 5 minutes using a centrifuge and then divided. The serum obtained from the samples was divided into 5 replicates, A, B, C, D, and E, and was placed in Eppendorf tubes and kept frozen at a temperature of -4˚C until the time of use.

Diagnosing type of allergy (atopic kit)

The diagnosis of food allergy was confirmed in the samples of the current study using (Atopic Kit) as per the company's previously prepared instructions, IMMUNOLAB GmbH/China.

Estimation of differential proteins CD203 & CD63

The ELISA direct enzyme-linked immunosorbent test was used to measure the level of CD203 and CD63 in the serum of the people under study. It was conducted using a diagnostic kit prepared by China/Cloud-Clone Corp and Jordan/Al-shkairate establishment.

Results

The results in the current study show that the number of patients with food allergies was 30 samples at a rate of 33.3% out of a total of 70 samples compared with control samples (Table 1). Also noted in this study was that the percentage of males with food allergies was higher than that of females, which was 27.8%. Likewise, the second age group 17–26% had the highest rate of food allergies compared to other age groups at rate 14.4%, with a significant difference at the probability level of $p \leq 0.05$ (Table 2). Besides, the results showed that the percentage of samples allergic to food was 25 samples, representing 83.3% out of a total of 30 samples, and five were only three samples, representing 16.7%.

In addition, this study showed a significant increase in the concentrations of CD203 and CD63, as their average concentrations were 0.16+0.15 ng/mL and 1.05+0.71 ng/mL, respectively, in the samples of the people who suffer from food allergies compared with the control samples with significant different $p \leq 0.05$. (Tables 3 and 4).

Discussion

The results of the current study manifested that the number of patients with food allergies was 30 samples, at a percentage of 33.3%. The term food allergy is used to describe the harmful immune response to some foods [10]. Recently, the prevalence of food allergies

Table 1: Distribution samples of the current study according to sex and age groups.

Status	Patient samples examined Number (%)	Control samples Number (%)	Total
Sex			
Male	37 (41.1%)	13 (14.5%)	50 (55.5%)
Female	33 (36.7%)	7 (7.7%)	40 (44.4%)
Total	70 (77.8%)	20 (22.2%)	90 (100%)

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Table 1: Continued.

Status	Patient samples examined Number (%)	Control samples Number (%)	Total
Age group/year			
7–16	20 (22.2%)	5 (5.6%)	25 (27.8%)
17–26	31 (34.4%)	12 (13.3%)	43 (47.7%)
≥27	19 (21.2%)	3 (3.3%)	22 (24.5%)
Total	70 (77.8%)	20 (22.2%)	90 (100%)

has increased in most Western and Arab countries of the world, and this may be attributed to environmental factors and genetic predisposition, in addition to age and the local diet [11]. Although different types of foods can cause food allergies, the main food allergens responsible for most significant reactions include milk, eggs, peanuts, tree nuts, shellfish, fish, wheat, and soybeans, as well as additives and preservatives in some packaged foods [12]. Estimates of food allergies have varied according to many international and local studies. The percentage of food allergies ranged from 3% to 35% for milk, eggs, and peanuts, and about 3-4% for fruits and nuts.

From 0.1% to 1.4% for vegetables and less than 1% for wheat, soybeans, and sesame [13]. Likewise, many recent studies recorded only 11% of people are allergic to eggs and 19% to milk, such as the study by Skripak *et al*. In addition, there are various local studies in Iraq on food allergies, including the study of Raisan and Abdulla in 2012 against white and red meat, and the percentage was 13.3% for red meat and 14.8% for white meat [15]. Also recorded in 2019 a percentage 72.9% of people allergic to kiwi fruit [16], a percentage 36.49% of people allergic to peanuts.

Also, the average of concentrations CD203 and CD63, 0.16+0.15 ng/mL and 1.05+0.71 g/mL, respectively, in the samples of the people who suffer from food allergies. This indicates that the process of activating basal cells through degranulation and histamine release [17] observed that measuring the concentration of CD203 and CD63 is one of the best techniques to measure the expression of activation markers to neutralize food allergies [15] in species of fish [18] used surface markers for mast cells and basophils, CD203 and CD63, to evaluate their activation in response to a specific antigen in basal cell activation tests to measure food allergy, methods of cell activation, and the level of histamine conveyed an increase in the levels of CD203 and CD63 concentrations compared to the level of histamine release [19]. Also, Larsen *et al*. presented a study on testing the activation of basal cells and comparing it with the release of histamine in diagnosing peanut allergy [20]. The authors noticed a rise in the level of histamine in allergic people through the degranulation of mast cells

Table 2: Distribution of allergic patients and control group as sex and age groups.

Status	Allergic people Number (%)	Non-allergic people Number (%)	Control samples Number (%)	Total
Sex				
Male	25 (27.8%)	12 (13.3%)	13 (14.5%)	50 (55.6%)
Female	5 (5.5%)	28 (31.1%)	7 (7.7%)	40 (44.4%)
Total	30 (33.3%)	40 (44.4%)	20 (22.2%)	90 (100%)
Age group/year				
7–16	10 (11.1%)	11 (12.2%)	5 (5.6%)	26 (29%)
17–26	13 (14.4%)	17 (18.9%)	12 (13.3%)	42 (46.6%)
≥27	7 (7.8%)	12 (13.3%)	3 (3.3%)	22 (24.4%)
Total	30 (33.3%)	40 (44.4%)	20 (22.2%)	90 (100%)

Table 3: Concentration of CD203 in allergic, non-allergic, and control samples.

Differential proteins	Allergy samples (30 samples) Mean + standard deviation ng/ml	Confidence limit 95%		Non-allergic samples (40 samples) Mean + standard error ng/ml	Confidence limit 95%		Control samples (20 samples) Mean + standard deviation ng/ml	Confidence limit 95%		Probability
		Lowest value	Highest value		Lowest value	Lowest value	Lowest value	Lowest value	Lowes t value	
CD203	0.16+0.15	0.86	0.02	0.13+0.09	0.06	0.14+0.05	0.10+0.03	0.15	0.06	≤0.05

Table 4: Concentration of CD63 in allergic, non-allergic, and control samples.

Differential proteins	Allergy samples (30 samples) Mean + standard deviation ng/ml	Confidence limit 95%		Non-allergic samples (40 samples) Mean + standard error ng/ml	Confidence limit 95%		Control samples (20 samples) Mean + standard deviation ng/ml	Confidence limit 95%		Probability
		Lowest value	Highest value		Lowest value	Lowest value	Lowest value	Lowest value	Lowes t value	
CD63	1.05+0.71	3.44	0.57	0.89+0.79	2.70	0.01	0.64+0.43	1.25	0.09	≤0.05

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and the release of mediators; they deemed these to be immunological indicators for diagnosing food allergies [21] and detected an increase in the expression of basal cell activation markers and CD203 and CD63 when peripheral blood was examined *ex vivo*, significantly higher in people suffering from urticaria for unknown reasons compared to non-allergic people. It has also been evidenced in a study that measuring the expression of surface markers of basal cells, including CD203, is useful for detecting allergies [22]. Kim *et al*. calculated the expression of surface markers of activated basal cells, CD203 and CD63, and compared them with other types of activation markers and measured the level of stimulus time and histamine release as diagnostic indicators for diagnosing allergies in allergic people [23].

Conclusion

Both CD203 and CD63 are important criteria for measuring basophil activity and diagnosing food allergies. About 1/3 number of patients have food allergies. Females are less likely to have food allergies than males. The young age group has the highest rate of food allergies.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The approval for this study was obtained from the Ethics Committee of the Department of Biology, College of Education for Pure Sciences, University of Basrah (approval ID: 2 in 2020).

Consent to participate

Written informed consent was obtained from all the participants.

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