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Determining the effect of antigens prepared from kiwi fruit on allergic patients in Basra province, Iraq

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Abstract
This study aimed to determine total IgE in subjects under study and controls an estimation of the specific IgE antibody response against kiwifruit antigen by ELISA assay. There are many studies on against kiwifruit in other countries of the world, but this study differs in sample type and Geographical location. A total of one hundred twenty blood samples from allergic patients (41 Males and 55 female) with age group (15-69) years were tested by direct and indirect Enzyme-linked immunosorbent assay for total and specific IgE antibodies against kiwifruit antigens. In our result, we show that the total IgE 100>IU/ml for patients had a higher rate of (72.9%) with significant difference ($p<0.01$) between total IgE 100< IU/ml, the sera positivity against kiwifruit had a higher rate of (72.9 %) compared with control. Female and first age group ≥40 had a higher rate of sera positivity against allergens. At rate of (83.6%, 64.6%) respectively. Also, the rustles showed relationship between total (IgE<100IU/ml) and specific had higher rates in Sera negative kiwifruit allergens were (53.8%) compared with sera positivity against kiwi fruit allergens a rate of (27.1%) while the relationship between total (IgE>100 IU/ml) and specific IgE had higher rates in sera positivity against kiwifruit allergens at a rate of (78.6%) compared with sera negative kiwifruit allergens at a rate of (21.4%).

Keywords: *Antigens; ELISA assay; IgE; kiwifruit*

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Introduction
Food allergy is a serious public health problem and triggered by an aberrant immune response elicited by the oral administration of antigens. Systemic exposure to an antigenic stimulus leads to the development of specific antibodies and of cell-mediated immunity [1], [2]. Kiwi fruit one of the sources of food allergy [3, 24, 25. 26]. Lead to type I hypersensitivity in both children and adults Symptoms of kiwifruit allergy vary from mild symptoms localized to the oral mucosa to severe systemic reactions including urticaria, angioedema, anaphylaxis and gastrointestinal symptoms, particularly in young children [4].The allergen has the property of inducing the immune system to produce IgE antibodies and triggering allergic symptoms in a sensitized individual[5]. Recently identified and characterized kiwi allergens[6]. This study aimed to determine total IgE in subjects under study and controls. An estimation of the specific IgE antibody response against kiwifruit antigen by ELISA assay.

Materials and Methods
Patients: Collected 3ml from96 patient's random during the period from July 2017 to November 2018, (Male 41 and females55) with age group from (15-69) years.Also collected blood samples from 24 people who have nosuffering from allergy symptoms of kiwifruit was considered as a control group. Both patients and control group agreed to participate in the trial.The protocol for the research project has been approved by a protocol for the research project has been approved by an Iraqi ministry of health in constitutes Ethics Committee in 14, Jan, 2018.The total IgE concentration determined in all samples according to the manufacturer's protocol by IgE ELISA kit (Demeditec/Germany).Preparation of kiwifruit proteinextracts was prepared essentially as described by [7]. Determine kiwifruit proteinconcentration according to the protocol Kit (Rockford, IL, U.S.A.) Protein Reagent assay.

Estimation Specific IgE (manual ELISA technique):
Evaluation specific IgE for kiwifruit antigen in all sera depend on ELISA assay according to [8] must determine the optimal dilution forreagent, serum and antigen before adding to wells. Each well of ELISA plates will be Coated with 100 µl from 50 µg/ml of the kiwi fruit antigens diluted, After coating incubated at 4°Cwashing the plate with diluted (PBS, pH 7) containing (0.05 %) tween 20 immediately. The serum sample was diluted into the following dilution (1/2 to 1/128), (100µl) of each was added antigen dilution starting from the second well in the first vertical row. Then cover seal was applied and the plate was incubated at 37 ºC for two hours. The plate was taken out of the 37ºC incubation and washed. The conjugate IgE-HRP diluted two (1/100 to 1/6400) and added (100µl) to all wells and incubated one hour at (37 ºC). After that wash the plate four times, added 50 µl substrates (TMB) to each well and incubation at 37 ºC, added (50µl) to stop the reaction and read the plate by an ELISA plate reader at weave 450nmsame ELISA procedure was performed on (120)serum samples. The best concentration-selected of sera (1/2 µl/ ml) and conjugate (1/100 µl/ ml).A cut-off value of the IgE titer in the tested samples estimated according to [9], ten serum samples were taken from volunteer individuals who were not exposed to Kiwi fruit Protein antigens. These samples were considered as negative controls.
Cutoff value=X + (3* standard deviation)
Statistical analysis: Uses SPSS software to assess for statistical significance of data.

Results

According to the IgE values, the result of this study showed that the total IgE level >100 increase the level of total IgE in allergic patients who are sensitive to the Kiwifruit antigens which prepared under study at a rate (72.9%) compared with control, with significant difference table (1). In table (2) the results of this study showed that overall Seropositivity against kiwifruit allergens at a rate (72.9%)also the result showed that females and first age groups had higher rates (57.3%, 67.7%) respectively. The results in a table (3, 4) respectively showed that (IgE<100IU/ml) had higher rates (53.8%) in Seronegative kiwifruit allergens compared with seropositivity against kiwifruit allergens with significant differences between them p< 0.05.

Table (1): Distribution of the total IgE level in current study samples.

Stat	Ex. No.	IgE<100IU/ml	IgE>100 IU/ml
patients	96	26(27.1)	70(72.9) *
Control	24	21(87.5)	3(12.5)
Total	120	47(39.2)	73(60.8)

*p< 0.01

Table (2): The Seropositivity & Seronegative against kiwifruit allergens according to sex and age

sex	Exam No.	Seropositivity against kiwifruit allergens No. (%)	Seronegative kiwifruit allergens No. (%)
Mal	41(42.7)	24(58.5)	17(41.5)
female	55(57.3)	46(83.6)	9(16.4)
Age group			
4≤0	62 (64.6)	42(67.7)	20(32.3)
>40	34(35.4)	28(82.4)	6(17.6)
Total	96(100)	70(72.9)	26(27.1)

Table (3): The relationship between total (IgE<100IU/ml) and specific IgE based ELISA in patients& control.

Stat	Ex. No (120)	IgE<100IU/ml No .(47)	Seropositivity against kiwi fruit allergens No. (%)	Seronegative kiwi fruit allergens No (%)
Patients	96	26(27.1)	12(46.2)	14(53.8) *
Control	24	21(87.5)	0	21(100)

*p< 0.0

Table (4): The relationship between total (IgE>100 IU/ml) and specific IgE based ELISA in all samples

Stat	Ex. No.	IgE>100 IU/ml	Seropositivity against kiwifruit allergens No(%)	Seronegative kiwifruit allergens No(%)
patients	96	70(72.9)	55 (78.6) *	15(21.4)
Control	24	3(12.5)	3(100)	0
Total	120	73(60.8)	58(79.5)	15(20.5)

*p< 0.05

Discussion:

Detection of total IgE is a good indicator, for now, the allergic response is mediated by IgE or non-IgE antibody [10]. Several studies have shown a high-level total IgE in food allergic patients as a study [11] showed increased level total IgE in food allergies at a rate (88.1%). While the study of [12] showed decreased levels of total IgE in food allergies at a rate (98.4 %) but [13] showed a relation between IgE levels to 11S and 7S proteins from kiwi. Kiwifruit has proteins similar to those of existing proteins from nuts and legumes, which react with specific IgE[14]. Kiwifruit is becoming one of the more common causes of food allergy with allergic symptoms ranging from mild oral allergy symptoms of anaphylaxis, in a pediatric and adult[15], [16].

Also, [17] observed a high incidence of allergy to kiwi fruit, also recently many studies identified similar IgE-binding epitopes on Act c 12 from golden kiwi fruit seeds [18, 27, 28]. The study of [19,20] was found the relationship between kiwi and peanut sensitivity in children and adults because the epitopes of the protein share with epitopes on homologous proteins, also [21] observed the sensitization to acted 1 antigen is a marker of severe kiwi allergy. Also [13]showed frequency allergy to kiwi fruit was high in a cohort and adults further a majority IgE-reactivity to proteins from kiwi. Addition the study of [10] showed total and specific IgE antibodies provide useful and complementary information on the immunological status and reflect the clinical activity in food-allergic patients. Several studies have reported elevated specific IgE in food allergy. Therefore, it is in accordance that IgE plays a central role in the pathophysiology of allergic disorders such as food allergy [22], in a study by [19] showed 65% of the patients have a kiwi allergy. The symptoms of kiwi sensitivity appear after two hours of digestion of the kiwi and the specific IgE antibody participates in a common kiwi sensitivity [23,24].To conclude, this study observed increasing allergenicity to kiwifruit in Iraqi people (adults & children) and the severity of the sensitivity to antigen relates to the level of IgE.

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