INTRODUCTION

Food allergy is an immune system reaction that occurs soon after eating a certain food. Even a minute amount of the allergy-causing food can trigger symptoms and signs such as digestive problems, hives or swollen airways. In some people, a food allergy can cause severe symptoms or even a life-threatening reaction as anaphylaxis. In practice, however, more frequently physicians encounter patients with "atypical" or delayed adverse reactions to food which are not Type I IgE dependent reactions, and in whom conventional allergy tests are usually unhelpful ***(Gupta et al., 2013)***.

Food allergies are usually characterized as IgE mediated ("immediate") or non-IgE mediated ("delayed"); the latter are presumed to be cell mediated ***(Boyce et al., 2010)***.

Specific diagnosis of food allergy, particularly IgE-mediated reactions, traditionally relies on skin tests and on the determination of allergen specific IgE. However, allergy specialists are often confronted with unclear and inconclusive results, e.g. due to cross-reactivities, or non-IgE mediated allergies. Double-blind, placebo-controlled food challenges are still the gold standard in food allergy diagnostics, against which all other approaches should be

verified. However, in vivo provocation tests with food allergens carry the risk of inducing severe allergic reactions and are costly and time consuming ***(Scarpellini and Tack, 2012).***

There is a far less understanding of non-IgE mediated food allergy than IgE-mediated FA and its clinical relevance is likely under-estimated in most cases. This is in part due to delayed onset of symptoms and hence difficulty in making the clinical association between offending food and clinical symptoms. The lack of easily accessible diagnostic measures also contributes to the problem ***(Jyonouchi 2008).***

Food additives and naturally occurring food chemicals are attributed by many patients as the etiology of their symptoms. Studies have assessed the potential for food additives and food chemicals to cause adverse symptoms, but conclusive research is difficult to conduct due to a number of factors. Some of these factors include difficulty of performing double blind placebo controlled challenges using foods or varying dosages of individual additives, setting and applying inclusion criteria, standardizing the outcome and ruling out potential co-factors ***(Kang et al., 2014)***.

A wide range of adverse reactions have been attributed to the consumption of these added or natural food chemicals. The most common clinical features are chronic urticaria or angioedema. Symptoms can also include atopic eczema, flushing, hypotension, abdominal pain, diarrhoea and asthmatic reactions, and occasionally severe (anaphylactic) reactions ***(Vally and Misso, 2012)***.

The majority of food-additive reactions are not IgE mediated, with many occurring up to 24 hours after challenge hence making IgE (immediate hypersensitivity) reactions unlikely. Some food additives cause direct mast cell and basophil activation with histamine release by mechanisms as yet unexplained ***(Cardinale et al., 2008)***. The activation of Arachidonic acid lipoxygenase pathway is thought to be the basis for leukotriene release seen in adverse reactions to tartrazine, sodium benzoate and nitrates. This cysteinyl-leukotriene release that has been well documented in food-additive intolerance would explain the variable timing of reactions seen. Provocation testing for food-additive intolerance has been difficult to standardize, and results of challenges vary greatly from study to study ***(Zhang et al., 2014)***.

To date, no reliable diagnostic tests for food-additive intolerance have been developed. We know that IgE does not play an important role in these reactions; therefore,

skin-prick testing and RAST testing results have been non-conclusive. Various IgG ELISA tests and leucocytotoxic tests promoted recently have proved equally disappointing. Direct challenge testing is the only available test with any reliability at present ***(Kang et al., 2014)***.

The cellular allergen stimulation test (CAST) which determines leukotriene release from blood leucocytes has shown some value in the diagnostic process. CAST simulates in vitro the reaction between the patients' leukocytes and the antigen. CAST measures the production of liberated and newly synthesized sulphido-leukotrienes, which are mediators in the immune response to the antigen ***(Boumiza et al., 2005)***.

Aim of the Work

The aim of this work is to evaluate the efficacy of CAST (cellular allergen stimulation test) in diagnosis of allergy to food additives.

Food Allergy

Definition

Food allergy is an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food. An adverse reaction is a term indicating any untoward reaction that occurs following ingestion of a food or food additive. It may be the result of toxic or nontoxic reactions. Toxic reactions occur in any exposed individual following ingestion of a sufficient dose. Nontoxic reactions depend on individual susceptibilities and may be immune mediated (food allergy or food hypersensitivity) or non–immune mediated (food intolerance). Food intolerances comprise most adverse food reactions and are categorized as enzymatic, pharmacologic, or idiopathic. Food allergies are usually characterized as IgE mediated ("immediate") or non-IgE mediated ("delayed"); the latter are presumed to be cell mediated ***(Boyce et al., 2010)*.**

IgE-mediated reactions are characterized by an acute onset of symptoms generally within 2 hours after ingestion or of exposure to the trigger food typically involving the skin, gastrointestinal tract, and respiratory tract. Allergic sensitization occurs when food-specific IgE antibodies are produced by plasma cells that have differentiated from

allergen-specific B lymphocytes. The specific IgE antibodies bind to the surface of tissue mast cells and blood basophils, and on re-exposure to the food, antigenic proteins in the food bind to these cell surface-bound IgE antibodies, which triggers the release of symptom-causing mediators, such as histamine and leukotrienes ***(Prescott and Allen, 2011).***

Non–IgE-mediated immunologic reactions (cell mediated) include food protein–induced enterocolitis, proctocolitis, and enteropathy syndromes. These conditions often present with abdominal complaints, such as vomiting, abdominal cramps, diarrhea ***(Sackeyfio et al., 2011).***

Epidemiology

Understanding the prevalence of food allergy in different regions in the world is vital for measuring the impact of food allergy for implementing prevention strategies ***(Martin et al., 2013*).**

Accurate determinations of food allergy prevalence are difficult because factors such as allergy definitions, variations in study populations, different methods of prevalence estimation, geographic variation, ages, dietary exposure, and other factors influence the estimate ***(Sicherer, 2011)***. A review of the literature concluded that food allergy affects between 1-10% of the population. It

remains unclear whether the prevalence is increasing. A number of recent studies provide high estimates of food allergy. Gupta et al study estimated that 8% of children have food allergy, 2.4% have multiple food allergies, and approximately 3% experience severe reactions ***(Gupta et al., 2011)***.

Prevalence of food allergy in Egypt is still not clear. Interestingly, a study was conducted on 100 children in Cairo (Egypt) diagnosed to have allergic diseases and found positive skin prick tests with peanut extract in seven children (7%). Specific IgE results of these children ranged from 17-24 kUA/L. The 7 children sensitized to peanut had positive family history of allergic diseases. Six of the 7 children consented to oral challenge studies and 3 were confirmed to have peanut allergy. Of the other children, 10 had confirmed allergy to other foods including egg allergy in 2, fish in 3, cow's milk in 2, sesame in 1, banana in 1, and prunes in 1. Nine of these ten children were not sensitized to peanut, however, one of them was sensitized to both peanut and bananas. In their conclusion, the authors stated that peanut allergy in Egypt is underestimated and that the sensitization rates may be even higher than previously thought ***(Hossny et al., 2011)***.

There are a number of theories regarding the apparent increase in prevalence of food allergies, especially

peanut allergy, however, definitive answers to the causes are still lacking. Postulated hypotheses have focused on hygiene, dietary fat, antioxidants, vitamin D, and dual-allergen-exposure (eg, initial exposure to a food allergen via a non- oral route, such as the skin). Although, there are some data in support of these hypotheses to explain the increasing risk of asthma and allergic rhinitis, there are limited data regarding the role for these hypotheses in explaining the increased prevalence of food allergy ***(Berin and Sampson, 2013).***

- Hygiene hypothesis-Early life exposure to infectious pathogens, as well as normal gut microbiota, may influence the development of the immune system away from a Th2 response. Better hygiene, through decreasing microbial exposure, may lead to an increase in atopic disease ***(Berin and Sampson, 2013).***

- Dietary fat hypothesis-This hypothesis postulates that decreased consumption of n-3 fatty acids (eg, omega-3 fatty acids) and increased ingestion of n-6 fatty acids (eg, vegetable oils) leads to greater production of IgE through the influence of prostaglandin-E2 ***(Berin and Sampson, 2013).***

- Antioxidant hypothesis-This hypothesis argues that antioxidants found in fresh fruits and vegetables, such as

vitamin C and beta-carotene, have protective anti-inflammatory effects. Dietary patterns that include more processed foods, and less fresh products, may therefore increase susceptibility to allergy ***(Berin and Sampson, 2013).***

- Vitamin D hypothesis-Vitamin D has been shown to have immune-modulatory effects. Proposed but unproven theories suggest a role for both vitamin D excess and deficiency in the development of allergic disease ***(Berin and Sampson, 2013).***

- Dual-allergen-exposure hypothesis-This theory proposes that sensitization to a food is more likely to occur in a young child if the initial exposures are low-dose and via the cutaneous route rather than high-dose and via the gut ***(Berin and Sampson, 2013).***

- Food processing hypothesis-How foods are processed may also affect allergenicity. This can lead to varying prevalence in different cultures. As an example, roasting and emulsification of peanut to make peanut butter may alter the proteins and allow them to be presented to the immune system in a manner that may be conducive to promoting allergic responses. Rates of peanut allergy are lower in countries where peanuts are primarily boiled rather than roasted ***(Sicherer and Sampson, 2007).***

- Other hypotheses - Staphylococcus aureus - derived enterotoxins commonly cause food contamination. Staphylococcal super antigens are also associated with atopic dermatitis. In a mouse model, oral administration of staphylococcal enterotoxin B (SEB) along with egg or peanut antigen results in Th2 polarized responses. Oral challenge with allergen triggers anaphylaxis. Expression of TGF-beta and regulatory T cells is impaired. Feeding high doses of antigen restores tolerance ***(Ganeshan et al., 2009)*.**

Risk factors and common allergens

Risk factors

A plethora of risk factors are thought to influence food allergy, including gender (more common among male children), race (increased among Asian and black children compared with white children), genetics (familial associations, HLA, and specific genes), atopy (comorbid atopic dermatitis), vitamin D insufficiency, dietary fat (reduced consumption of omega-3 polyunsaturated fatty acids), reduced consumption of antioxidants, increased use of antacids which reduce digestion of allergens, obesity (being an inflammatory state), increased hygiene, and the timing and route of exposure to foods ***(Osborne et al., 2012)***.

Food allergens

Major components of food allergens include proteins or glycoproteins with a molecular weight of 5-100 kDa and which have the ability to cross-link IgE receptors. Many allergens are enzymatically digested and denatured by the acidic environment of the stomach; however some are resistant to these conditions ***(Moreno, 2007)***.

A number of different forms of plant food allergies have been reported, including pollen-food allergy syndrome and latex-fruit syndromes, in which allergic symptoms are induced mainly in the mucous membranes after ingestion of the causative food ***(Price et al., 2015)***. The most comprehensively characterized allergen components associated with plant food allergies are profilins, seed storage proteins and pathogenesis related proteins such as non-specific lipid transfer proteins (LTPs). Collectively, these are called panallergens and are widely distributed among various plants and have cross reactivity with related plant species ***(Sinha et al., 2014)***.

Table (1): Members of panallergen families

panallergen family	plant allergen source							
	pollen			food				product
	trees	grasses	weeds	fruits	vegetables	legumes	nuts/seeds	latex
profilins	Bet v 2	Cyn d 12	Amb a 8	Act d 9	Api g 4	Gly m 3	Ara h 5	Hev b 8
	Car b 2	Lol p 12	Art v 4	Ana c 1	Cap a 2		Cor a 2	
	Cor a 2	Ory s 12	Che a 2	Cit s 2	Dau c 4		Pru du 4	
	Fra e 2	Phl p 12	Hel a 2	Cuc m 2	Lyc e 1			
	Ole e 2	Poa p 12	Mer a 1	Fra a 4				
	Pho d 2	Zea m 12	Par j 3	Lit c 1				
				Mal d 4				
				Mus xp 1				
				Pru du 4				
				Pru av 4				
				Pru p 4				
				Pyr c 4				
polcalcins	Aln g 4	Cyn d 7	Amb a 9					
	Bet v 3	Phl p 7	Amb a 10					
	Bet v 4		Art v 5					
	Fra e 3		Che a 3					
	Jun o 4							
	Ole e 3							
	Ole e 8							
	Syr v 3							
nsLTPs	Ole e 7		Amb a 6	Act c 10	Api g 2		Ara h 9	Hev b 12
	Pla a 3		Art v 3	Act d 10	Aspa o 1		Cas s 8	
			Hel a 3	Cas s 8	Bra o 3		Cor a 8	
			Par j 1	Cit l 3	Lac s 1		Jug r 3	
			Par j 2	Cit s 3	Lyc e 3			
			Par o 1	Fra a 3	Zea m 14			
				Mal d 3				
				Pru ar 3				
				Pru av 3				
				Pru d 3				
				Pru du 3				
				Pru p 3				
				Pyr c 3				
				Vit v 1				

(Mothes et al., 2004)

Cross Reactivity (CR)

Cross-reactivity (CR) is a phenomenon that occurs when reactivity to an antigen takes place due to an adaptive immune response to another antigen because of structural correlation between the two antigens ***(Bonds et al., 2008)***. The antigen–antibody reaction is based on the complementarity of the epitope with the idiotope ***(Villalta et al., 2010)***.

According to the World Health Organization guidelines for the prediction of allergenicity, sequence similarity of at least 35% in a fragment of 80 amino acids, or a complete identity with a peptide of 6-8 amino acids should be present between two allergens for cross reactivity to occur. On the other hand, mastocytes and basophils become activated, when IgE antibodies bound to the receptors of these cells recognize more than 2 epitopes with high affinity. Thus, CR between IgE and effector cells is unlikely if sequential similarity is less than 70%. Therefore, CR is an immunological phenomenon whose clinical manifestation–when this occurs–is the association of 2 or more allergies ***(Maloney et al., 2008)***.

The allergens responsible are usually homologous proteins belonging to specific families of molecules. Frequently, the reaction is caused by proteins that are

highly conserved from an evolutionary point of view and that, given their widespread presence, have been termed panallergens. Thus, we must take into account not only the taxonomic classification of organisms, but also the molecular classification of the allergens, as both play a key role in CR syndromes ***(Sinha et al., 2014)***.

Epitopes

The antigenic specificity of an allergen (mostly protein in nature) resides in restricted areas of the molecule, known as antigenic determinants or epitopes, which are recognized by the combining sites or paratopes of certain immunoglobulin molecules. Once an immunoglobulin has been shown to bind to an antigen, it becomes known as an antibody specific for that antigen. Epitopes can be classified based on more than one criterion. The principle classification is dependent on the site of epitope binding. Epitopes can bind antibody molecules both in their free form and as membrane-bound B cell receptors. These are called B-cell epitopes. On the other hand, T-cell epitopes are proteolytically cleaved peptides of the antigen that interact with the receptors of T cells ***(DeLano, 2002).***