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RAST-specific IgE to egg and milk in Nigerian asthmatic patients

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Summary
Food allergens have been described in studies in southern parts of Nigeria but rarely in northern parts of Nigeria. Furthermore, the role of specific antibody to such allergen moieties have not been determined. The aim of this study is to assess the role of two common food items in the aetiology of asthma in Zaria. RAST-specific IgE to milk and egg, which are two common foods in Nigeria, and skin prick tests were therefore studied in bronchial asthma patients and controls in Ahmadu Bello University Teaching Hospital, Zaria to determine their significance in the clinical manifestation of asthma patients. Total serum IgA, IgE and secretory IgA were also measured in patients and controls. RAST titres were poorly discriminating between controls and patients, although six patients had history of gastrointestinal symptoms after ingestion of egg while three patients had history of such symptoms, some with positive skin prick tests and high RAST-specific IgE titres. Asthmatic patients had significantly higher mean serum secretory IgA and total IgA levels than controls while total serum IgE levels were similarly distributed among patients and controls. It is suggested that provocation tests are mandatory to confirm bronchial allergic reactions to egg and milk and perhaps other food allergens in Nigerians in view of the non-discriminant RAST titres obtained in this study.

Keywords: *RAST-specific IgE, Egg, Milk allergens, asthma*

Résumé
Les substances allergènes ont été décrit dans les études au sud du Nigeria mais rarement au nord du Nigeria. Le rôle des anticorps spécifiques sur ces allergènes n'ont pas été déterminées. Le but de cette étude est de déterminée le rôle de deux aliments communs, ayant pour but d'évaluer le rôle de deux paramètres symptomatiques de l'asthme à Zaria. L'anticorps E spécifiques au RAST au lait et les œufs qui sont les aliments communs au Nigeria, qui sont deux substances communes au Nigeria, and tests d'un échantillon de la peau était cependant étudié chez les patients atteint d'asthme bronchial et des contrôles au Centre Universitaire Hospitalier d'Ahmadu Bello, Zaria pour déterminer leur importance dans les manifestations Clinique des patients asthmatiques. Ainsi étudié chez les patients asthmatiques et sains dans cet hôpital pour déterminer leur importance dans la manifestation clinique. Le taux du sérum en IgA, IgE et IgA sécrétoire étaient mesure chez les patients et le groupe sain. Les RAST titrés étaient moins discrimines entre les patients et les sujets sain, Bien que six patients avaient l'histoire des symptômes gastro-intestinaux après l'ingestion d'œufs alors que trois patients avaient l'histoire de tels symptômes, certain avec un test positif de la peau et haut spécificité des RAST-IgE titrès. Les patients asthmatiques avaient un taux moyen signicativement élevé de sérum sécrétoire IgA et le taux total IgA que les sujets sains alors que le taux du sérum en IgE était semblablement distribué parmi les patients et les sujets sain. Il est suggéré que la provocation des tests sont obligatoires pour confirmer les réactions d'allergies bronchiales aux œufs et au lait et certaines nourritures allergènes chez les Nigérians en vue d'obtenir les non-discriminants de RAST titrés dans cette étude.

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Introduction

High allergic reactivity previously unappreciated, exists in the tropics [1-3]. Food allergy defined as immunologically-mediated adverse reaction to food proteins including egg and milk is an important aetiological factor in hypersensitivity disorders like angioedema, eczema, urticaria, asthma, allergic rhinitis and gastro-intestinal disturbances [4]. This is a growing clinical problem that has been estimated to affect approximately 6% of children and 3-4% of adults in the U.S.A. [5-6]. It may occur locally in food-hypersensitive adults despite a lack of systemic food specific IgE [7], manifesting with elevated IgE in duodenal mucosa, increased numbers of mast cells, eosinophils, T lymphocytes (including CD4+ and CD8+) and up-regulation of Th2-cytokine expression in the duodenal mucosa of subjects with food allergy such changes being consistent with late-phase allergic inflammatory response in the gastrointestinal mucosa [8-9].

The mechanisms thus include one or more of the hypersensitivity mechanisms of Gell and Coombs types I-IV, the activation of CD4+ T cells subsets that secrete cytokines and which are mainly Th2-like cells which produce interlelukin 4 and interleukin 5 (unlike Th1 pathway which produces gamma interferon). These interleukins recruit mast cells that further produce IL-4 which is an essential cytokine for IgE synthesis [10-12]. Food precipitants of asthma and allergy in Africans may include egg, milk, okro, groundnut, fish, *garri*, kolanuts, rice, yam, cassava, pepper, beans and oily food [13-16]. Studies of IgE specific antibodies in these patients are uncommon especially in Northern Nigeria where milk drinking is commonly practiced among the Fulani.

Prolonged breast feeding as occurs in tropical Africa and West Indies has been postulated to prevent or delay eczema and milk related hypersensitivity especially as breast milk cell supernatants from atopic donors have been shown to stimulate cord-blood IgA and IgE production in vitro [17-18]. The mechanism for this prevention or delay of eczema and milk related hypersensitivity is through IgE and IgA helping to bind and block intestinal allergens from initiating allergic reaction in the intestinal mucosal compartment. The present study was undertaken to determine the pattern of IgE-specific titres to egg and milk in northern Nigerians.

Material and methods

Patients

Sixty-six asthmatic patients attending the Asthma Clinic at Ahmadu Bello University Teaching Hospital, Zaria were studied along with twenty-two age and sex-matched controls. The patients were clinically stable at time of the tests. None of the patients was on oral or inhaled steroids which could affect the results of the study. A past history of reaction to milk or egg was sought in the patients and controls. Stool microscopy was studied to determine presence of parasites.

Clinical features of the patients

The duration of asthma was 6± 2.8, range 3-11 years. A positive family history of asthma was obtained in 12% of the patients. Precipitating factors volunteered included wood smoke (3 cases), exercise (2), dust (5), cool air (1) insecticides (1), and chest infection (10), while the other 44 patients could not volunteer obvious precipitating factors. Nocturnal attacks were frequent in ten patients. Rhinitis was reported in four patients. Four patients usually vomited while 2 others had abdominal upset whenever they are eggs. One patient vomited whenever he drank milk and two others had abdominal discomfort with milk. Two patients vomited whenever they ate fish. Flexural eczema was present in two patients. Emphysema was observed in six patients and two had cor pulmonale. Ninety-five percent of the patients had perennial asthma with marked exacerbations during the dusty harmattan months of October to April, and during the rainy season of July to September.

Controls

The controls were interviewed to exclude any past or present history of respiratory infections or any allergy. The controls were selected from the environment (rural or urban) from which the patients came. The number of controls were not as large as patients because of limited availability of reagents but the random selection of patients was to match groups of the patients according to age and sex. There was no history of asthma or allergy in any of the controls and the peak expiratory flow rates of the controls were within the normal range for our population. No control had any parasite in the stool at the time of recruitment.

Skin tests

Prick tests were carried out with Bencard reagents according to method of Pepys [19]. Immediate hypersensitivity prick tests using Bencard skin test solutions containing extracts of egg (white), egg (whole) and milk were performed in all patients and controls. Wheals more than 2mm greater than that of control were regarded as positive.

Total IgE

Blood was collected from all subjects and the serum stored at -20°C until analysed. Total IgE was measured using the radio-immunosorbent method according to original method of Ceska and Lundvist [20] using Phadebas Pharmacia reagents.

RAST (Radioallergosorbent test) for Allergen-Specific IgE

Specific serum IgE to egg (white), egg (whole) and milk were performed using the Pharmacia Phadebas kit [20,21]. The test was carried out as specified in the brochure with 50ul of test samples and controls. A primary incubation at room temperature for three hours was followed by overnight incubation at room temperature of radioactive tracer with paper disc complex. At the end of each incubation, the discs were washed three times with saline. The results were expressed as titres ranging from 0 to 4.

Serum IgA and Secretory IgA levels

Total serum IgA and secretory IgA were determined with radial immunodiffusion technique according to the method of Mancini *et al* [22] in which antisera are incorporated into agarose and poured unto glass slides. Punctured holes are made in solidified agarose on the slide, then equal aliquots of sera to be measured are added, incubated overnight and the diameters of immune precipitated rings measured and results read out of a graph of standards. The results are expressed in international units per ml.

Statistical analysis

Statistical analysis was done with student t tests and chi-squared tests.

Table 1: Skin prick tests positivity in 66 patients and 22 controls

	% Patients (n=66)	% Controls (n=22)
Egg white	41	27
Egg whole	42	9
Milk	48	9

Results

The mean age of patients was 32 ± 3 years; range 19-50 years. The mean age of controls was 32 ± 2 years (range 18-49 years). The difference was not statistically significant (p>0.05). The male to female ratio was 1:1.1 in patients and 1:1 in the controls.

Total IgE

The geometric mean of total IgE in 40 patients tested was 527 ± 2.8 (range, 55 – 3520) iu/ml and 406.5 ± 3.4 iu per ml (range 28 – 3635) iu/ml in non-atopic controls. Differences were not statistically significant (P> 0.5, t test). Grouping of patients and controls according to range of total IgE in relation to urban or rural environmental dwelling of patients and controls shows that both high and low total IgE occurred in patients from any of the two environments (Table 2).

RAST IgE to milk and egg

Table 3 shows the RAST titres in 66 asthmatic patients and 12 controls.

Table 3: Antibody titre and percentage of positive subjects

Antibody titres	Egg		Milk	
	Patients (%)	Controls (%)	Patients (%)	Controls (%)
0	-	-	-	4
1	12	4	70	84
2	83	88	29	8
3	5	8	2	4
4	-	-	-	-
	100%	100%	100%	100%

Key: 0 – No detectable antibody
1 – Low 2 – Moderate 3 – High
4 - Very High
Differences between controls and patients are not statistically significant (X^2 test) p > 0.05

Table 2: Total IgE levels in patients and controls in urban and rural dwellers

Range of IgE (iu/ml)	No. of Patients	<100 iu/ml	>100-1000 iu/ml	>1000 iu/ml
Zaria (urban) patients	18	1	12	5
Zaria (urban) controls	15	4	8	3
Rural patients	22	3	14	5
Rural controls	7	-	5	2

Stool microscopy tests were positive for *Ascaris lumbiricoides* (2 cases), *Ancylostoma duodenale* (3), *Trichuris trichuria* (1), and *Schistosoma mansoni* (1).

Skin prick test

Table 1 shows the percentage of positive skin reactivity of patients and controls to egg (white), egg (whole), and milk.

Correlation of Skin Prick Tests and RAST titre

The agreement of skin test reactivity and RAST titre (2 or greater) in patients is shown in Table 4. 68% of the concordance results for milk allergen had both negative skin reativity and negative RAST. 50% of the discordant results for egg white allergen had positive RAST but negative skin prick test. 55% of the discordant egg

whole result had negative prick skin test and positive RAST titre. 21% of discordant milk result had negative prick test and positive RAST.

Table 4: Concordance between skin reactivity and RAST titre

	Concordance	Discordance
Egg (White)	50%	50%
Egg (Whole)	45%	55%
Milk	79%	21%

Patients with positive history of reactions

Table 5 shows the data for only the patients who volunteered abnormal reactions to milk or egg.

Discussion

The finding of allergic reaction to local food items including egg, milk, okro, rice, yam, cassava, should prompt research into understanding the active moieties and their clinical application in the tropics especially in Africans [15,16]. Allergy to milk normally disappears within first two or three months of life although cases have been reported in adults [5,17]. Six out of 66 (9%) of our adult patients had gastrointestinal symptoms after ingesting egg while three adults (4.5%) had abdominal upsets after milk. The occurrence of secondary systemic respiratory reactions were however, not conclusively ruled out, in these patients. Such systemic allergic reactions can only occur if food allergens cross intestinal barrier tight junction between intestinal epithelial cells

Table 5: Clinical symptoms volunteered by patients and test results

	Age/Sex (years)	Symptom	RAST titre	Prick Skin Tests Egg White	Egg Whole	Total IgE
a). Egg	38/F	Vomits	2	+	+	517
	35/F	Vomits	2	+	-	704
	19/M	Vomits	2	-	-	347
	40/M	Abdominal discomfort	2	-	+	484
	35/F	Eczema/ Abdominal discomfort	3	+	+	484
	19/M	Vomits	2	+	+	891
b). Milk			RAST titre	Milk		
	38/F	Vomits	1	+		517
	35/F	Abdominal discomfort	1	-		704
	40/M	Abdominal discomfort	1	-		484

Key: + means positive
- means negative

However, none of these patients stated that they had respiratory symptoms after egg or milk was taken. Respiratory provocation tests were not carried out.

IgA and Secretory IgA

Mean serum IgA in 40 patients tested was 142.3 ± 60.9; range 47-347 iu/ml as compared to 96.2 ± 29.4; range 65 – 176 iu/ml in controls ($P<0.05$). Mean of serum secretory IgA in 34 patients tested was 645 ± 115, range 447 – 858 mg/L compared to 549.4 ± 50.8; range 510 – 624mg/L in controls ($P<0.05$).

where IgE receptors with low affinity are expressed in intestinal cells (isoform CD23a) which enhances transcytosis of allergen IgE complexes across intestinal cells [23-29] to mucosal immune system. This transcytosis results in secretion of chemokines, cytokines, reactive oxygen radicals and lipid metabolites with clinical manifestations [29].

Most studies of prevalence of cows milk allergy especially in children reported incidence figures between 0.38% - 7.5% [5]. Although good correlations have been obtained between the results of RAST tests for

egg white and clinical sysmptoms in Caucasians [30,31], high incidence of egg white specific IgE may also be detected in egg tolerant individuals [4,7,32]. Our results show that more of non-atopic controls had IgE egg specific titre above 2 titre although the difference was not statistically significant. Positive RAST to egg and to milk should be complemented in tropical practice with provocation tests if hyposensitization or dietary exclusion therapy is being contemplated. Although more of the asthmatic patients than controls had milk RAST titres above 2, the patients could not clearly link asthmatic symptoms with any milk ingestion. The majority of RAST titres to both egg and milk allergens were 1 or 2 titre in both asthmatic patients and controls with a poor discriminant value. High antigenic environmental load with cross-reacting antigens has been suggested as possible explanation for high positive skin tests in Zaria and the tropics [1,2], a hypothesis which may also explain the high positive RAST in controls and patients.

Soothill [37] and Soothill and others [34] observed a correlation between low serum IgA in the first month of life and subsequent development of allergic asthma. Spontaneous recovery later in life has been linked to recovery from transient IgA deficiency [35]. Mean serum IgA and secretory IgA levels were however higher in patients than controls and no IgA deficiency was observed. It had been postulated that deficient mucosal immunity may predispose to poor handling of food antigen with consequent allergic manifestations [10]. When produced by plasma cells in the lamina propria, IgA is transported to the intestinal luminal surface through epithelial cells with the addition of secretory piece to form secretory IgA which interacts with antigens to prevent their adherence and intestinal absorption. Milk precipitins and immune complexes involving food proteins have been detected in sera of patients with selective IgA deficiency [35,36], while IgE-antigen complexes have been shown to trigger inflammation via CD23 inflammatory cascade [29], which can be abrogated by blocking CD23 expression [37].

IgE levels in egg or milk allergic patients may be enhanced even by parasites unrelated to food allergens [38,39]. The IgE values in the patients that had gastrointestinal manifestations were in the moderate levels – levels which were also found in other asthmatic patients and even in controls. In conclusion, skin provocation tests in addition to oral food challenge are clearly mandatory to confirm allergic reactions to egg and milk and other food allergens in view of the non-discriminant results obtained with RAST titres as shown in this study.

Note: The Helsinki Declaration principles as revised was strictly adhered to in this work.

References

1. Warrell DA, Fawcett IW, Harrison BD, *et al.* Bronchial asthma in the Nigerian Savannah region. Quart J Med 1975; 325-336.
2. Lynch NR and Di Prisco-Fuenmayor MC. High allergic reactivity in a tropical environment. Clin Allergy 1984; 14: 233-240.
3. Onyemelukwe GC, Shakib F, Saeed TK, *et al.* RAST specific IgE in Nigerian Asthmatics. Ann Allergy 1986; 56(2): 167-170.
4. Golbert TM. Food allergy and immunological diseases of the gastrointestinal tract. In: Allergic diseases Diagnosis and Management ed. R. Patterson JB, Lippincott Company, Philadephia.
5. Sicherer SH, Munoz-Furlong A and Sampson HA. Prevalence of seafood allergy in the United States determined by a random telephone survey. J Allergy Clin Immunol 2004; 114: 159-165.
6. Sicherer SH, Munoz-Furlong A and Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digital dial telephone survey; a 5 year follow-up study. J Allergy Clin Immunol 2003; 112: 1203-1207.
7. Lin XP, Magnusson J, Ahlstedt S, *et al.* Local allergic reaction in food-hypersensitive adults despite lack of systemic food specific IgE. J Allergy Clin Immunol 2002; 109: 879-887.
8. Yang PC, Berin MC, Yu Li, *et al.* Mucosal pathophysiology and inflammatory changes in the late phase of the intestinal allergy in the rat. Am J Pathol 2001; 158: 681-690.
9. Wershil BK, Furuta GT, Wang ZS, *et al.* Mast cell-dependent neutrophil and mononuclear cell recruitment in immunoglobulin E induced gastric reaction in mice. Gastroenterology 1996; 110: 1482-1490.
10. Ricci M, Rossi O, Bertoni M, *et al.* The importance of Th2-like cells in the pathogenesis of airway allergic inflammation. Clin Exp Allergy 1993; 223: 360-369.
11. Ricci M and Rossi O. Dysregulation of IgE responses and airway allergic

inflammation in atopic individuals. Clin Exp Allergy 1990; 20: 601-609.
12. Bahna SL, and Gandhi MD. Milk hypersensitivity 1 – Pathogenesis and symtomatology. Ann of Allergy 1983; 50: 218.
13. Hoffman DR, and Hadded ZA. Diagnosis of IgE mediated reaction to food antigens by radioimmunoassay. J Allergy and Clin Immunol 1974; 54: 165-171.
14. Neeguaye AR. Bronchial asthma in Ghananians – a study of 209 asthmatic patients. W Afr Med 1981; 1: 13-20.
15. Haddock HD and Onwuka SI. Skin tests in Nigerian asthmatics from the Equatorial forest zone in Benin Nigeria. Trans Roy Soc Trop Med & Hyg 1977; 71: 32-38.
16. Soyinka F. Skin tests in Nigerian asthmatics from the Equatorial forest Zone in Benin, Nigeria. Trans Roy Soc of Trop Med & Hyg 1978; 72: 107-115.
17. Allardyce RA and Wilson A. Breast milk cell supernatants from atopic donors stimulate cord blood IgE secretion *in vitro*. Clin Allergy 1984; 14: 259-265.
18. Donnally HH. The question of elimination of foreign antigen (egg white) in woman's milk. J Immunol 1930; 19: 15-23.
19. Pepys J. Atopy. Clinical aspects of Immunology 3rd Ed. PGH Gell RRA Coombs and PJ Lachman, pg. 877. Blackwell Scientific Publication, Oxford, London, Edinburgh, Melbourne, 1975.
20. Ceska M and Lundvist U. A new simple radioimmunoassay method for determination o f IgE Immunochem 1972; 9: 1021-1030.
21. Wide L, Bernich H and Hohansson SGO. Diagnosis of allergy by an *in vitro* test for allergen antibodies. Lancet 1967; II: 1105-1112.
22. Fahey JL, and Mckelvey EM. Quantitative determination of serum immunoglobulin in antibody – agar plates. J Immunol 1965; 94: 84-91.
23. Tu Y and Perdue MH. CD23-mediated transport of IgE/immune complexes across human intestinal epithelium: role of p38 mAFK. Am J Physiol Gastrointest Liver Physiol 2006: 9532-9538.
24. Tu Y, Salim S, Bourgeois J, *et al.* CD23-mediated IgE transport across human intestinal epithelium: inhibition by blocking sites of translation or binding. Gastroenterology 2005; 129: 928-940.
25. Montagnac G, Molla-Herman A, Bouchet J, *et al.* Intracellular trafficking of CD23: differential regulation in humans and mice by both extra cellular and intraceller exams. J Immunol 2005; 174: 3562-3572.
26. O'Neil DA, Porter EM, Elewaut D, *et al.* Expression and regulation of the human beta-defensins hBD-1 and hBD-2 in intestinal epithelium. J Immunol 1999; 163: 6718-6720.
27. Witholf T, Eckmann L, Kim JM, *et al.* Enteroinvasive bacteria directly activate expression of NOS and NO production in human colon epithelial cells. Am J Physiol 1998; 275: G564-571.
28. Eckmann L, Rudolf MT, Plasznik A, *et al.* D-myoinositol 1,4,5,6-tetrakisphosphate prodouced in humans intestinal epithelial cells in response to Salmonella invasion inhibits phosphoinositidie 3-kinase signaling pathways. Proc Natl Acad Sci, USA 1997: 9; 14456-14460.
29. Li H, Nowak-Wegrzyn A, Charlop-Powers Z, *et al.* Transcytosis of IgE – antigen complexes by CD23a in human intestinal epithelial cells and its role in food allergy. Gastroenterology 2006; 131: 47-58.
30. Aas K. The diagnosis of hypersensitivity to digested foods. Clin. Allergy 1978; 8: 39-42.
31. Bock SA, Lee WY, Remigo LK, *et al.* Studies of hypersensitivity reaction to food in infants and children. J Allergy and Clin Immunol 1978; 62; 327-332.
32. Schur S, Hyde JS and Wypych J. Egg white sensitivity and atopic eczema. J Allergy and Clin Immunol 1974; 54: 174-181.
33. Soothill JP, Stokes CR, Turner MW, *et al.* Predisposing factors and the development of reaginic allergy in infancy. Clin Allergy 1976; 6: 305-310.
34. Taylor B, Norman AP, Orgel HA, *et al.* Transient IgA deficiency and the pathogenesis of infantile atopy. Lancet 1973; II: 3-10.
35. Barret DJ, Bertani L, Wara DW, *et al.* Milk precipitins in selective IgA deficiency. Ann of Allergy 1979; 42: 73-81.
36. Hongxing Li, Chehade M, Wercheng Liu, *et al.* Allergen-IgE complexes trigger CD23-dependent CCL20 release from human intestinal epithelial cells. Gastroenterology 2007; 133(6)|: 1905-1915.

37. Cunningham – Rundles C, Brandeis WE, Good RA, *et al.* Milk precipitins, circulating immune complexes and IgA deficiency. Proc Nat Acad Sci 1978; 75: 3387- 3393.

38. Ventura A, Longo G and Tamburlini G. Reaginic hypersensitivity to cow's milk proteins. Helv Paediatrica Acta 1981; 36: 237-244.

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