SERUM IMMUNOGLOBULIN AND COMPLEMENT PROFILES IN BRONCHIAL ASTHMA IN LIBYANS

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SUMMARY: Serum immunoglobulin and complement profiles were studied in 48 patients with bronchial asthma (BA) and in 26 healthy Libyans as controls (CS). Of the 48 patients, 26 were extrinsic (BA-E) and 22 were intrinsic (BA-I) asthmatics. In CS, 96% had normal levels of immunoglobulin E (IgE) (≤ Mean ± + 2 SD:134 iu/ml) and immunoglobulin D (IgD) ≤ Mean ± 2SD:2.7 mg/dl). Only 4% of CS had abnormal IgE (> 134 iu/ml) and IgD (>2.7 mg/dl levels. On the contrary, majority of patients had elevated levels of both IgE (> 134 iu/ml : 71% BA, 81% BA-E, 59% BA-I) and IgD (>2.7 mg/dl: 78% BA, 88% BA-E, 63% BA-I). However, some patients had normal levels of both IgE (≤ 134 iu/ml: 29% BA, 19% BA-E, 41% BA-I) and IgD (≤ 2.7 mg/dl : 22% BA, 12% BA-E, 37% BA-I) and yet they were asthmatics. The chi square (χ^2) test revealed that these distributions of patients in relation to normal and abnormal levels of IgE and IgD were significant as compared to CS. The majority of asthmatic attacks may therefore be explained as due to IgE-mediated mechanisms and the rest possibly due to other mechanisms. The probable role for elevated serum IgD as antiidiotypic (anti-IgE) antibody was discussed in the light of knowledge currently available in this are of research.

Key Words: Bronchial asthma, Immunoglobulin, Complement.

INTRODUCTION

Bronchial asthma is characterized by repeated episodes of reversible partial obstruction of the bronchi caused by a combination of oedema, increased secretion and muscle contraction. Although the pathogenesis of these lung symptoms is not simple, the relationship between atopy and asthma is often close. The presence of a reaginic substance in the serum of atopic patients was first reported by Prausnitz and Kustner (21) and it was identified as immunoglobulin E (IgE) by Ishizaka and Ishizaka (12). Subsequently, a correlation between elevated serum IgE level and atopic disease has been shown in several studies (13, 16). However, IgE level in patients with bronchial asthma had been shown to vary considerably according to extrinsic or intrinsic pattern of the disease (6). Also, the short-term sensitizing IgG-antibody described by Parish has been thought to be responsible for some cases of skin test negative asthma (2,20). Recent evidence has suggested that activation of the complement pathway can occur producing mediators, e.g. C5a and leukotriene B4, which can directly cause bronchospasm (26). Literature survey had indicated that little work was done or reported on serum immunoglobulin and complement levels in Libyan asthmatic patients as well as in healthy Libyans. The serum immunoglobulin and complement profiles were therefore studied in 48 Libyan patients with bronchial asthma (BA) as well as in 26 healthy Libyans as control subjects (CS). The observations made are reported in this communication and discussed in the light of present knowledge in this field.

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MATERIALS AND METHODS

Subjects: Forty eight patients with Bronchial Asthma (BA) as per the definition of American Thoracic Society (1) were chosen for the study. There were 23 males and 25 females, between the ages of 17 and 68 years. At the time of the study, all of the patients had active disease and were on bronchodilator therapy. Those who were on long term steroid therapy and those dependent on steroids were excluded from the study. None of the patients had any other co-existing significant disease like diabetes, cardiac disease, renal and liver dysfunction. The patients with overlapping signs and symptoms of bronchitis, those with evidence of infection and pregnant females were excluded. After a thorough clinical evaluation, the patients were classified into extrinsic (allergic) and intrinsic (idiosyncratic) groups employing the following criteria (4,5,18) : Patients with onset of illness before the age of 30 years, seasonal or intermittent attacks with relief in between, with positive history of known external allergens and positive family history of bronchial asthma or other atopic disorders were placed in the extrinsic (BA-E) group. While the intrinsic (BA-I) group consisted of patients with onset of symptoms over the age of 30, with perennial or chronic nature of illness; no history of known external allergens; absence of other associated allergic disorders (like Rhinitis, Hoyfever, urticaria, eczema) and negative family history of bronchial asthma. The 26 control subjects (Sex:17 male, 9 female; age 17-65 years) were obtained from the blood bank and out patient department of the hospital after a through clinical examination (Table 1).

Serum Specimens: The serum aliquots of BA and CS were obtained from the sample sent for routine laboratory analyses. They were stored at - 20°C until analyzed.

Determination of Serum Immunoglobulins and Complements: The serum levels of IgG, IgM, IgA, IgD, C3 and C4 were quantitated by radial immunodiffusion (RID) method of Mancini *et al* (17). The immunokits of bioMeriux, Marcy-1 'Etoile 69260 Charbonnieresles-Bains, France, were used for RID and the results were expressed as milligram per decilitre (mg/dl). The serum IgE concentration was measured by enzyme linked immunosorbent assay (ELISA) technique based on double antibody sandwich method of Hoffman (10). The IgE-Kits of bioMerieux, France, were used for ELISA and the results were expressed as international units per millilitre ($i\mu$/ml).

Statistical Analysis: The statistical significance of the results were evaluated by Student's t-test and Chi square (χ^2) test.

RESULTS

The details of the BA-patients and control subjects are stated in Table 1. Of the 48 patients, 26 and 22 patients were diagnosed as extrinsic and intrinsic asthmatics respectively.

Table 1: The age, sex, type of asthma, duration of asthma and other complications in Libyan patients with bronchial asthma (BA) and in control subjects (CS).

		Age	Sex	Type of Asthma	Duration of Asthma (yrs)
Patients	1	64	F	I	5
	2	40	F	E	12
	3	60	M	I	15
	4	60	M	I	10
	5	50	F	E	23
	6	33	F	E	13
	7	65	M	I	5
	8	60	F	E	25
	9	35	M	E	17
	10	18	M	E	3
	11	42	M	E	16
	12	50	F	E	25
	13	17	F	E	2
	14	65	F	I	8
	15	62	F	I	10
	16	40	F	E	12
	17	30	F	E	7
	18	34	F	E	9
	19	55	M	I	5
	20	22	M	E	3
	21	30	F	E	3
	22	28	F	E	5
	23	43	M	E	17
	24	63	M	I	7
	25	37	F	I	2
	26	70	M	I	5
	27	40	F	E	12
	28	65	F	I	15
	29	47	F	E	28
	30	55	M	I	3
	31	27	M	E	5
	32	23	F	I	2
	33	28	M	I	4
	34	38	M	E	8
	35	45	F	E	10
	36	47	F	I	12
	37	52	M	I	10
	38	30	F	E	4
	39	38	M	E	9
	40	35	F	I	11
	41	17	F	E	1
	42	65	M	I	8
	43	68	M	I	30
	44	20	M	E	2
	45	30	M	E	3
	46	60	F	I	15
	47	62	M	I	5
	48	68	M	I	25
n = 48		23M/25F			26E (10M, 16F) 221 (13M, 9F)
Controls:Twenty six healthy Libyan subjects (n=26 12 M/3F; Age:20-65 years) were chosen from the blood and out-patient as controls.					
n: Number of subjects: M: Male; F:Female; E: Extrinsic; I: Intrinsic					

Table 2: The serum immunoglobulin (IgG, IgM, IgA, IgD, IgE) and complement C3, C4) profiles in BA and CS.

				Immunoglobulins and Complements (mg/dl)			
Subjects*	IgG	IgM	IgA	IgD	IgE*	C3	C4
Control Subjects (CS, n:26):							
Range:	800-1320	75-130	175-300	1.4-3.0	29-170	75-150	21-50
Mean ± SD:	1032 ± 148	109 ± 25	239 ± 38	1.9 ± 0.4	72 ± 31	128 ± 20	35 ± 11
Bronchial Asthma Patients (BA, n=48):							
Range:	900-1540	85-180	180-370	2.0-9.5	41-1400	80-126	20-66
Mean ± SD:	1165 ± 190	116 ± 22	251 ± 43	4.6 ± 328	120 ± 28	40 ± 12	
Bronchial Asthma Patients, Extrinsic (BA-E, n=26)							
Range:	900-1540	90-180	204-370	2.3-9.5	130-1400	80-152	20-66
Mean ± SD:	1217 ± 170	120 ± 19	265 ± 38	6.1 ± 1.6	470 ± 410	127 ± 22	37 ± 12
Bronchial Asthma Patients, Intrinsic (Ba-I, n=22)							
Range:	910-1420	85-150	180-300	2.0-6.1	41-360	80-186	21-59
Mean ± SD:	1105 ± 145	112 ± 17	235- ± 38	3.2 ± 1.0	154 ± 90	110 ± 30	42 ± 13

*n: Number of subjects; SD: Standard deviation; IgE: iu/ml

The results of the determination of serum immunoglobulins and complements are shown in Table 2 and Table 3. Among the various parameters, only IgD and IgE levels of BA were found to be significantly elevated than CS. When BA-patients were seggregated into BA-E and BA-I, it was observed that both IgD and IgE levels of BA-E were significantly higher as compared to both CS and BA-I (Table 3). The distribution of subjects in relation to their serum IgE and IgD levels grouped as within normal (≤ Mean + 2 SD) and above normal (> Mean +2 SD) are shown in Table 4. A large number of patients had higher serum levels of both IgE (> 134 iμ/ml) and IgD (> 2.7 mg/dl) compared to only 4% of CS. However, some patients had normal levels of both IgE (≤ 134 iμ/ml) and IgD (≤ 2.7 mg/dl) and yet they were asthmatics (Table 4).

Table 3: The statistical analysis by Student's t-test of the results stated in Table 2.

Groups Compared	P value						
	IgG	IgM	IgA	IgD*	IgE*	C3	C4
CS vs BA	NS	NS	NS	P<0.05	P<0.02	NS	NS
CS vs BA-E	NS	NS	NS	P<0.02	P<0.01	NS	NS
CS vs BA-I	NS	NS	NS	P<0.05	P<0.02	NS	NS
BA-E vs BA-I	NS	NS	NS	P<0.02	P<0.02	NS	NS

NS: Not significant (>0.05); *P<0.05: Significant.

Table 4: The distribution of subjects in relation to their serum levels of IgE (iu/ml) and IgD (mg/dl) grouped as within normal (≤+2 SD of CS) and above normal level (>+2 SD of CS) (From Table 2).

	IgE		IgD	
	Within normal (≤ 134 iμ/ml)	Above normal (>134 iμ/ml)	Within normal (≤ 2.7mg/dl)	Above normal (> 2.7mg/dl)
CS	25/26=96%	1/26=4%	25/26=96%	1/26=4%
BA	14/48=29%	34/48=71%	11/48=22%	37/48=78%
BA-E	5/26=19%	21/26=81%	3/26=12%	23/26=88%
BA-I	9/22=41%	19/22=59%	8/22=37%	14/22=63%

CS: Control Subjects; BA: Bronchial asthma patients;
BA-E: Bronchial asthma patients, Extrinsic;
BA-I:Bronchial asthma patients, Intrinsic.

These distributions of patients in relation to normal and abnormal levels of IgE and IgD were found to be statistically significant as compared to CS by Chi square (χ^2) test (Table 5).

DISCUSSION

The finding that 71% BA-patients (BA-E: 81%), BA-I:59%) had elevated levels of serum IgE (Table 4) supports the view that the majority of asthmatic attacks can be explained as mediated through IgE-activated mast cell

degranulation (13,16). Antigen-dependent cross linking of cell-bound IgE molecules with secondary bridging of IgE-Fc receptors had been shown to cause mast cell degranulation (11). This leads to release of mediators, either preformed (histamine, heparin, chmotoctic factors, tryptase, etc) or synthesized de novo as a result of activation (prostaglandings and leukotrienes), which act on the target tissue cells and produce the asthmatic symptoms. Therefore, it is quite important that IgE-response be regulated most efficiently which is dependent upon many factors including the interactions of helper and suppressor T-cells. Jarrett (14) proposed that an imbalance in helper and suppressor functions may underlie the atopic trait and a maturation defect of IgE-specific suppressor cells may be responsible. Marsh *et al.* (19) however suggested that antigen-specific Ir-gene for IgE-response exist which may be HLA-linked and individuals who possess this Ir-gene are expected to be atopic against that particular antigen. However, of considerable interest was the observation that a large proportion of patients (BA:78% BA-E: 88% BA-I: 63%) had significantly higher serum IgD levels in parallel with elevated serum IgE levels (Table 4). These distributions of patients were observed to be statistically

Table 5: The statistical analysis of the distribution of subjects in relation to normal or abnormal serum levels of IgE and IgD (From Table 4) by Chi square (χ^2) test.

	IgD		IgE*	
	χ^2	P	χ^2	P
CS vs BA	30.3596	< 0.001	36.2095	< 0.001
CS vs BA-E	31.5151	< 0.001	13.9822	< 0.001
CS vs BA-I	17.6040	< 0.001	4.5532	< 0.05
BA-E vs BA-I	3.851	< 0.05	4.1574	< 0.05

*P < 0.05 : Significant.

significant as compared to CS (Table 5). Although IgD was discovered long ago by Rowe and Fahey (22), there are few clues as to its role in the immune response or in host defense. Our observations led us to speculate therefore that excess IgD may be anti-idiotypic antibodies produced by the patient against IgE-idotypes. The beneficial implications of Jerne's (15) original concept of idiotypic-antiidiotypic interactions in clinical medicine had been recently reviewed (24). In our BA-patients, the proposed anti-idiotypic (anti-IgE) response may also be beneficial in many ways: some of the excess IgE may be neutralised and eliminated through IgE-anti IgE (IgE-IgD) complex formation and hence lesser amount of free IgE may be available to block Fc E^+T-cells (IgE-receptor positive T-cells). Consequently, more FcE^+T-cells will be available for activation by IgE-anti IgE complexes to generate isotype (IgE)-specific suppressor T-cells and these suppressor T-cells, in turn, may suppress IgE synthesis as shown by Hassner and Saxon (9). Immune complexes had been shown to induce idiotype-specific suppressor T-cells also (3). These crucial roles of T-cells in the regulation of IgE synthesis in vitro and in vivo have been well established (7, 8, 23).

In contrast, some of our patients had normal serum IgE ($\leq$ iμ/ml and IgD ($\leq$ 2.7 mg/dl) levels and yet they were asthmatics (Table 4). Hence asthmatic attacks in these patients could not be explained as due to mechanisms involving IgE and/or IgD. Assuming that mast cell degranulation plays a central role in asthma of all types, non-IgE-mediated mechanisms for activation of masi cells have to be found. Perhaps other immunoglobulin classes, complements and some non-immunological mechanisms may be involved. The IgG-antibodies, predominantly of IgG4-subclass, had been shown to be responsible for some cases of allergic asthma (2,25). But significant differences were not found in the present study for serum IgG, IgA, IgM, C3 and C4 levels between BA (BA-E and BA-I) and CS. These serum components were therefore not considered to be significantly involved in the pathogenesis of bronchial asthma in our patients. However, specific antibodies in any of these immunoglobulin classes or subclasses, anti-IgE specificity of elevated IgD, immune complexes and cellular immunological parameters have remained to be assayed in our future endeavour.

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REFERENCES

1. American Thoracic Society: Definition and criteria of bronchial asthma. Am Rev Res Dis 75:762-765, 1962.

2. Bryant DH, Burns MW, Lazarus L: Identification of IgG antibody as a carrier of reaginic activity in asthmatic subjects. J Allergy Clin Immunol 56:417-421, 1975.

3. Caulfield MJ, Luce KJ, Proffitt MR, Gerny J: Induction of idiotype-specific suppressor T-cells with antigen-antibody complexes. J Exp Med 157:1713-1725, 1983.

4. Daniele RP: Pathophysiologic mechanisms in asthma. In: Pulmonary Diseases and Disorders. New York: McGraw Hill Book Company, p. 567-576, 1979.

5. Daniele RP: Asthma. In: Cecil's Text Book of Medicine. Philadelphia and London; W.B. Saunder's Company, p 359-363, 1982.

6. Godfrey RC: Respiratory disorders including atopy. In: Immunology in Medicine. London : Grune and Straton, p. 396, 1983.

7. Geha RS, Leung D: Cellular abnormalities in patients with elevated serum IgE levels. J Allergy Clin Immunol 78(5):995-999, 1986.

8. Geha RS, Reinherz E, Leung D, McKee (Jr.) KT: Deficiency of suppressor T-cells in the hyperimmunoglobulin E syndrome. J Clin Invest 68:783-791, 1981.

9. Hassner A, Saxon A: Isotype-specific human suppressor T-cells for IgE synthesis activated by IgE-anti - IgE immune complexes. J Immunol 132 (6): 2844-2849, 1986.

10. Hoffman DR: Estimation of serum IgE by an enzyme linked immunosorbent assay-ELISA. J Allergy Clin Immuno 151:303-307, 1973.

11. Ishizaka T : Analysis of triggering events in mast cells for immunoglobulin E-mediated histamine release. J Allergy Clin Immuo 67:90-96, 1981.

12. Ishizaka K, Ishizaka T: Identification of E antibodies as a carrier of reaginic activity. J Immunol 99:1187-1198, 1967.

13. Ishizaka K, Ishizaka T: Mechanisms of reaginic hypersensitivity: a review. Clin Allergy 1: 9-13, 1971.

14. Jarrett EEE: Activation of IgE regulatory mechanisms by transmucosal absorption of antigen. Lancet 11:223-225, 1977.

15. Jerne NK: The immune system (Network Theory). Sci Am 229(1):52-60, 1973.

16. Johansson SGO, Foucard T, Danneus A: IgE in human disease. In: Progress in immunology II, Vol 4, Amsterdam: North Holland, p.61, 1974.

17. Mancini G, Carbonare AO, Hereman JF: Immunological quantitation of antigens by single radial immunodiffusion. Int J Immunochem 2: 235-254, 1965.

18. McFadden ER, Feldman NT. Asthma: Pathophysiology and Clinical correlates. Med Clin N Am 61:1229-1239, 1977.

19. Marsh DG, Meters DA, Bias WB: The epidemiology and genetics of atopic allergy. N Eng J Med 305:1551-1559, 1981.

20. Parish WE: Short term anaphylactic IgG antibodies in human sera. Lancet II, 591-592, 1970.

21. Prausnitz C, Kustner H (1921): Studie ubec die Uberemdindlichkeit Zentralbl. Bakteriol (B). 86,160. In: Ummunology in Medicine. London: Grune and Straton, p.81, 1983.

22. Rowe DS, Fahey JL: A new class of human immunoglobulin I. A unique myeloma protein. J Exp Med 121:171-184, 1965.

23. Saxon A, Marrow C, Stevens RH: Subpopulations of circulating B cells and regulatory T-cells involved in vitro immunoglobulin E production in atopic patients with elevated serum immunoglobulin E. J Clin Invest 65:1457-1468, 1980.

24. Stevenson F: Idiotypes and diseases. Immunol Today 7 (10):287-288, 1986.

25. Van der Giesen M, Homan WL, Van Kernebeek G, Aalberse RC, Dieges PH: Subclass typing of IgG antibodies formed by grass pollen allergic patients during immunotheropy. Int Arch Allergy Appl Immunol 50:625-640, 1976.

26. Wedmore CV, Williams JT: Control of vascular permeability by polymorphonuclear leukocytes in inflammation. Nature 289:646-647, 1981.

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