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Immunoglobulin classes, complement factors and circulating immune complexes in chronic sinusitis patients

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Summary

Serum concentrations of immunoglobulin classes (IgG, IgA and IgM), complement factor B (FB), 4th complement factor (C4) and circulating immune complexes (CIC) were determined in 34 Nigerians with chronic sinusitis and 37 apparently healthy age and sex matched controls by single radial immunodiffusion and polyethylene glycol precipitation methods, respectively. The mean serum IgG, IgA, C4, FB and CIC were significantly reduced in chronic sinusitis patients compared with the controls. The mean serum levels of IgG, IgM, C4 and FB were higher in male chronic sinusitis patients compared with female patients. In contrast the mean serum level of IgA was significantly reduced in chronic sinusitis patients. Only IgG and IgA showed significant correlation with the duration of chronic sinusitis. These findings in Nigerian patients suggest immuno-deficiency as one of the causes of chronic sinusitis in Nigerians, therefore routine serum immunoglobulin evaluation is recommended and if found deficient immunoglobulin replacement therapy is advocated for the treatment of such chronic sinusitis.

Keywords: *Chronic sinusitis, immunoglobulins, complement factors, immune complexes.*

Résumé

Les méthodes de la précipitation de glycol en polythylene et l'immunodiffusion en radial sont employées respectivement pour determiner les classes (IgG, IgA et IgM) de concentration d'immunoglobulin, le complément facteur B (FB), 4ème complément facteur (C4) et les compleses immunitaires d'échange dans 37 Nigérians en bonne santé équilibré en genre et âge servant comme le groupe de contrôle et 34 d'autre souffrant de sinusitis chronique. La moyenne sérum IgG,IgA, C4, FB et CIC sont considérablement réduit chez les malades des sinusitis chronique par contre au groupe de contrôle. La moyenne sérum IgG, IgM, C4 et FB sont plus élevée chez les hommes souffrant de sinusitis chronique en comparaison avec les malades feminins. Au contraire, le niveau de moyenne sérum d'IgA est considérablement reduit chez les malades de sinusitis chronique. Ces decouvertes chez les malades nigérians suggèrent que la déficience immunologiqu fait partie des causes de sinusitis chroniques chez les Nigérians. Par conséquent, l'évaluation routinaire d'immunoglobulin de sérum est recommandée et au cas d'une déficience, une thérapie est suggerée pour le traitement d'un tel sinusitis chronique.

Introduction

Sinusitis has been defined as an inflammation of the mucous membranes in the paranasal sinuses due to inadequate drainage secondary to mechanical or physical obstruction, bacterial infection or allergy [1]. Although sinusitis occur frequently in all ages and varies from acute to chronic states, mortality due to sinusitis had decreased significantly because of effective medication and surgery. Immunoglobulin deficiencies have been identified and are related to increased susceptibility to respiratory tract infections [2]. Most patients with sinus diseases are not immuno-deficient, however a proportion of patients with chronic sinusitis unresponsive to medical and/or surgical therapy may be immunodeficient [3].

The symptoms of sinusitis in the immunodeficient patients and immunocompetent individuals are similar thereby creating difficulty in differentiating these two groups [3]. However, most immunodeficient patients with sinus diseases have frequent reoccurrence of sinusitis despite antibiotic therapy, leaving them with the option of surgical procedure which rarely has long-term benefit [4].

The trends of sinusitis had been studied, but the immunological basis of chronic sinusitis was not considered [1]. A previous study by Armenaka et al determined the levels of only immunoglobulin classes (IgG, A & M) and IgG subclasses, but the levels of these immunoglobulins were not related with the duration of sinusitis or other aspect(s) of humoral immunity determined [5].

The present study was designed to estimate serum immunoglobulin classes (IgG, and A and M), Factor B (FB), C4 and circulating immune complexes (CIC) in Nigerians with chronic sinusitis and relate these with duration of the disease.

Materials and methods

Patients

Thirty-four patients (16 males and 18 females) with chronic sinusitis aged between 6-75years (38 ± 20yrs) were selected for this study between March, 1998 and August, 2000. The age ranges for males and females were 10-73 years (39±14yrs) and 9-75 years (36 ± 20yrs), respectively. The diagnosis of sinusitis was based on clinical and radiological evidence as previously described [1] This was corroborated by histological diagnosis whenever specimens were taken during various surgical treatments. Patient with chronic sinusitis secondary to anatomic obstructions, allergy, mucociliary clearance abnormalities and/or acquired immunodeficiency were excluded from the study.

Controls

Thirty-six healthy (18 males and 19 females) volunteers aged between 9 and 70 years (40 ± 23yrs) of the same socio-cultural background with the chronic sinusitis patients served as controls. The control males aged between 12 and 70 years (41 ± 25yrs) and control females aged between 9 – 66 years (39 ± 20). None of the subjects was taking oral corticosteriod and none had ever received allergen immunotherapy.

Assays

Consents of the subjects were sought before the collection of the blood samples. Ten (10ml) of blood was collected from each subject by venepuncture and allowed to clot. After the clot had retracted at room temperature (20°C) the serum was separated

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and M) and complement (C4 and FB) measurements, were kept at - 20°C until needed for the assay. The circulating immune complexes were estimated immediately after serum separation. Immunoglobulin classes (IgG, IgA and IgM); complement factors (FB and C4) were estimated by single radial immunodiffusion technique of Fahey and Mckelvey [6] A volume of an optimally diluted nonspecific anti-serum was mixed with noble agar and poured on a glass plate. Wells of equal diameter were cut in the antibody, agar mixture. The wells were filled with test or standard serum. The plates for IgG measurements were incubated at 37°C for 3 hours. Those of IgA, Ig, EB and C4 were placed at room temperature (20 °C) for 18 hours. After incubation the diameters of the precipitin rings were measured with mircometer eyepiece.

Levels of CIC were assayed using the polyethylene glycol precipitation method of Haskova *et al* [7]. Polyethylene glycol (PEG) 6000 solution was added to serum in borate buffer to give a final concentration of 3.7% (P.E.G.) and 1 in 3 dilution of serum. After incubation at room temperature the immune complex concentrations were measured at 450nm wave length using a spectrophotometer (MRI00Iplus).

Statistical methods

The student's t-test was used for checking the levels of significance in the concentrations of immunoglobulin classes, complement factors and CIC (Tables 1-3). Pearson's correlation coefficient was used to correlate the levels of the immunological parameters with the duration of sinusitis (Table 4).

Results

As shown in Table 1, the mean levels of IgG, IgA and IgM in the male chronic sinusitis patients were significantly different from those in female patients; whereas only mean IgM value in female controls was significantly higher than that of male controls. The mean values of IgG and IgA in male and female patients were significantly reduced compared with controls, while mean IgM values in different sexes of the patients were significantly raised compared with the controls. The overall mean values of IgG, IgA and IgM in the patients were significantly different in the patients compared with the controls.

Table 1: Serum IgG, IgA and IgM levels in different sexes of chronic sinusitis patients and controls.

	Chronic Sinusitis		Controls			
Males	Number	Values (mg%)	Number	Values (mg %)	t-value	p-value
IgG	16	2007±287*	18	2400±211	5.5	<0.01
IgA	16	200± 76*	18	286± 89	3.1	<0.01
IgM	16	388± 85*	18	205± 40	7.6*	<0.01
Females						
IgG	18	1752± 281	19	2489± 289	7.9	<0.01
IgA	18	248± 80	19	293±93	2.0	<0.05
IgM	18	309± 80	19	269± 42	2.0	<0.05
Both sexes						
IgG	34	1989± 245	37	2445±242	7.9	<0.01
IgA	34	223± 85	37	289± 90	4.4	<0.01
IgM	34	352± 80	37	222± 75	6.9	<0.01

P-values <0.05 is significant
**Significantly-different from the females.*

There were no significant differences in the mean levels of C4, FB and CIC in male controls compared with female controls whereas C4 and FB were significantly elevated in male chronic sinusitis patients compared with female patients. The mean values of C4, FB and CIC were significantly different in both male or female patients compared with corresponding sex of the controls (Table 2).

Table 2: Serum C4, FB and CIC levels in different sexes of chronic sinusitis patients and controls.

	Chronic Sinusitis		Controls			
Males	Nunbers	Values (mg%)	Number	Values (mg %)	t-value	p-value
C4	16	16±7*	18	24±9	3.0	<0.01
FB	16	13±3*	18	17±3	4.0	<0.01
CIC	16	10±5*	18	16±6	3.1	<0.01
Females						
C4	18	10± 5	19	26±10	5.3	<0.01
FB	18	9±4	19	18±6	5.3	<0.05
CIC	18	11±4	19	19±4	6.2	<0.05
Both sexes						
C4	34	13±6	37	26±9	7.5	<0.01
FB	34	11±3	37	17±4	6.7	<0.01
CIC	34	10±5	37	17±5	5.8	<0.01

P-values <0.05 is significant
**Significantly different from the females.*

Table 3 shows that the mean levels of IgG and IgM in patients belonging to the 21 – 40 years age group were significantly raised compared with that of the patients in the 0 – 20 years age group. The mean levels of IgA in patients of 21 – 40 years age group and above 41 years age group were significantly different from that of patients in 0 – 20 years age group.

Table 3: Serum IgG, IgA, IgM, C4, FB and CIC in chronic sinusitis patients belonging to different age groups.

Age group (yrs)	No	IgG(mg%)	IgA (mg%)	IgM (mg%)
0.20	9	1799±263	224±96	100±23
21-40	14	2184±968	251± 101*	210±100*
Above 41	11	1733±750	169±62*	205±83

**Significantly different from 0-20 years of age group (P<0.05).*
**Significantly different from above 40 years of age group (P<0.05).*

Both IgG and IgA in the patients showed significant correlation with duration of sinusitis (Table 4).

Table 4: Correlation of immunoglobulin classes (IgG, A and M), complement factors (FB and C4) and CIC with duration of sinusitis.

Immunoglobulin classes	Duration of sinusitis n = 34 r-value	p-value
IgG	0.47	<0.01(s)
IgA	0.33	<0.05(s)
IgM	0.10	>0.2 (ns)
C4	0.22	>0.2 (ns)
FB	0.11	>0.2 (ns)
CIC	0.19	>0.2 (ns)

r = Pearson's correlation coefficient
n = Number of pairs correlated
ns = Non - significant
s = Significant

Discussion

The finding of higher mean IgM level in female controls than in the male controls confirm previous reports of Aderenle *et al* [8] and Salimonu [9] The reason for the elevated IgM was connected with the observation that the X chromosome carries the gene affecting blood concentration of IgM [10] But the levels of C4 and FB were similar in both male and female controls, showing that the sources of immunoglobulins may be different from the sources of complement factors. In contrast, female chronic sinusitis patients had lower mean levels of IgG and IgM compared with their male counterparts, indicating that the disease may be more severe in female patients than the male patients. It is also possible that humoral immune responses during chronic sinusitis may be more depressed in females than males thus leading to lower IgG, IgM and complement (C4 and FB) levels in the females patients.

Reduced total mean IgG values in all chronic sinusitis patients compared with the controls in the present study may be related to reduced synthesis of certain subclasses of IgG. Low levels of IgG3 and IgG1 but normal level of IgG2 was found in chronic sinusitis and rhinitis [5] CIC activates complement pathways and are formed by specific combination of antigens and antibodies of IgM and IgG classes. In the present study, low mean level of CIC in chronic sinusitis patients might have been caused by reduced interaction between antigens from infectious agents and specific antibodies.

Because of reduced CIC levels in chronic sinusitis patients, activation of complement pathways may also be reduced, consumption of C4 and FB will be low, consequently serum levels of FB and C4 is expected to be raised. However in this study they were low. It may be suggested that formation of complement factors is also depressed in chronic sinusitis patients from the above findings.

IgA protects from respiratory disease by preventing the entry of foreign materials at the mucosal surfaces of the respiratory and gastro intestinal tracts. The suggestion is that relative mucosal deficiency of secretory IgA leads to abnormal entry of pathogens responsible for respiratory diseases. If mucosal IgA levels correspond to serum IgA, then low serum IgA as seen in these patients would lead to low mucosal surface IgA, thus predisposing them to recurrent respiratory tract infection such as chronic sinusitis.

Since IgA is involved in activation of complement via alternative pathway, significant reduction of IgA in chronic sinusitis might lead to reduced complement activation via alternative pathway.

It is possible that increased synthesis of IgM to compensate for low levels of IgG and IgA may explain the observed higher mean levels of IgM in chronic sinusitis patients.

A simultaneous high mean serum IgM found in association with low mean serum IgG had been attributed to impairment of T-cell-dependent switch in antibody production at least in the minimal change [11]. It is possible that this basic abnormality in the T-cell function would render the immune system defective in producing IgG antibody to post-primary immunogenic stimulation. The observation of high serum IgM in chronic sinusitis leads to a likelihood of a fundamental process irrespective of cause; adequate understanding of this defect would certainly lead to a better appraisal of chronic sinusitis due to immunodeficiency. Raised IgM in chronic sinusitis patients may also be explained from the fact that classical pathway of complement activation is reduced since IgM is usually consumed during this cascade as a result of immune complex formation.

The observation of reduced mean levels of Ig classes (IgG, A and M) in chronic sinusitis patients between 0 and 20 years group and above 40 years group compared with those in 21 – 40 years group shows that chronic sinusitis due to immunodeficiency may be prevalent at the extremes of age. The present findings support the previous observation that immunity in healthy humans decline at young and old ages [12] These also agrees with findings of decreased FC receptor – mediated phagocytosis, reduced ingestive and digestive abilities of neutrophils, drop in the functional capability to produce IL 2 by peripheral blood lymptocytes in aged people [13,14,15]. Leslie *et al* also described a fall with age in the immunoglobulin levels [16]. This is further supported by significant correlation between serum IgG and IgA with duration of chronic sinusitis.

It is therefore concluded that IgG and IgA could be used as index of chronic sinusitis and that immunodeficiency is a feature of chronic sinusitis in South-western Nigeria and hence routine immunoglobulin evaluation is recommended.

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