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Levels of complement components, immunoglobulins and acute phase proteins in plasma during aging in Nigeria

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Summary

Plasma samples from Nigerians aged 6 - 95 years were examined for their content of complement components (C3, C4, factor B-Bf), immuloglobins (IgG, IgA, IgM IgD) and acute phase proteins (transferrin, albumin, C-reactive protein - CRP, alpha-2-macroglobulin). Albumin, was estimated colorimetrically and the other components by the single radial immunodiffusion techniques. No significant age-related changes in mean values of the four immunobulins and the four acute phase proteins could be demonstrated. Also, the mean values for C3 and Bf did not change significantly with age but C4 values rose significantly with increasing age (r - 0.232: $P < 0.01$).

Keywords: *Complement, immunoglobulins, acute phase proteins, aging Nigerians.*

Résumé

La contenance plasmatique en complement (C3, C4, facteur B1-Bf), immoglobiline (IgG, IgA, IgM, IgD) et les proteines de la phase aigue (transferrine, albumine, proteine de reactif-C-PRC, macroglobuline alpha-2). L= albumine etait estime par la methode colorometrique et les autres composants par la technique de radiale unique d'immunodiffusion. Aucune difference significative velie aux ages en volueure moyenne de quatre immunoglobuline et de 4 proteines de la phase aiguen avait ete demontree. De la meme maniere, la voleur moyenne de, C3 et Bf n' avaient pas change significativement avec l'age, mais les valeures de C4 avaient augmente'significativement avec l'augmentation d'age (r - 0.232, $P < 0.01$).

Introduction

The influence of aging on specific and non-specific mechanisms of the host defence systems have been studied [1-4]. Aging has a polymorphic effect on the immune system [5]. This underscores the complexity of the mechanisms responsible for age-induced changes, and the magnitude of the task of relating individual immunologic indices to susceptibility to infection in the aged. It is possible that susceptibility to infection can be reduced in old individuals by modulating their immunologic responsiveness.

This study examines the possibility of using one or more of the proteins; complement components (C3, C4, Bf), immunoglobulins (IgG, IgA, IgM, IgD) and acute phase reactants (transferrin, albumin, CRP, alpha-2-macroglobulin); to index the aging process in Nigerians.

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Subject and methods

A total of 170 Nigerians were assessed for the levels of C3, 186 for C4 and 115 for factor B. One hundred and seventy-two individuals were studied for their plasma immunoglobins G and A, 159 for IgM and 58 for IgD. One hundred and twenty-two had their samples analysed for transferrin, 121 for albumin, 62 for alpha -2- macroglobulin and 150 for C-reactive protein. The samples (3.0 ml) were collected between August 1990 and December 1991 in Ibadan, Nigeria. Blood 1.5 ml was mixed with ethylene diamine tetra-acetic acid (EDTA) anti-coagulant in plastic containers and centrifuged at 800XG for 5 minutes. The remaining 1.5 ml of blood was collected in plastic container with heparin at 10 units per ml, and centrifuged. Both EDTA-Plasma and heparin plasma samples were stored at -20°c until analysed. EDTA-plasma was thawed only once for use in complement estimations.

The socio - economic status of the subjects was determined by the method described by Williams [6] which is based on the level of formal education, occupation and income. All the subjects were fully ambulatory and living independently. Their health status was subjected to exclusion criteria including infection, inflammation, malignancy and diabetes. The laboratory tests leading to exclusion included a haematological examination (leukocyte count with differentials) and serological screening of samples for amoebiasis, hepatitis-B virus infection and salmonellosis. The subjects in the different age groups had comparable levels of total plasma protein, albumin, transferrin and C3 B components used to index nutritional status. The methods employed and the results obtained in the analysis of exclusion indices have been reported previously [7].

C3, C4, Bf, IgG, IgA, IgM, IgD, transferrin, alpha-2-macroglobulin and CRP were measured by the single radial immunodiffusion method of Fahey and Mckelvey [8] as modified by Salimonu et al [9]. Commercial monospecific antisera to human C3c, C4 Bf, IgG, IgA, IgM, IgD, transferrin, alpha 2-macroglobulin and CRP (Serotec, Oxford, England) were used respectively. The levels of C3, C4, IgG, IgA and IgM as well as transferrin, CRP and alpha-2-macroglobulin were measured against the same commercial serum standards (Behringwerke Ag, Marburg, Germany). Bf was assayed against a locally pooled plasma standard. IgD levels were quantified against the World Health Organisation standard 67/37 [10]. Plasma albumin concentrations were determined by the brilliant cresol green method [11]. Briefly,) 0.02 ml of the test plasma was added to 4.0 ml of a working solution of bromocresol green (BCG) dye. Also 0.02 ml of albumin standard (3.1%) solution was added to 4.0 ml working BCG solution. The absorbances of tests and standard were read at 630 nm against a reagent blank.

Table 1: Mean ("1s.d.) plasma concentrations of complement components in difference age groups

Age group (Years)	C3 (g/L) 170	C4 (g/L) 188	Factor B (% pooled serum) 115
6-25	0.04±0.10 n=47	0.25±0.08 n=47	105±44.0 n=20
26-45	0.64±0.13 n=48	0.32±0.20 n=54	108±35.9 n=42
46-65	0.60±0.12 n=43	0.36±0.20 n=47	106±37.0 n=26
>65	0.61±0.10 n=32	0.33±"0.21 n=40	102±33.1 n=27
r	- 0.118	0.232	-0.065
p	>0.10	<0.01	>0.20
F	1.79	2.99	0.14
$F_{.05}$	2.68	2.68	2.68

Bonferroni adjustment gave the following results for c: 6-25/26-45: -0.16, +0.02; 6-25/46-65: -0.21, -0.02; 6-25/>65: -0.18, +0.02; 26-45/46-65: -0.13, +0.05; 26-45/>65: -0.11, +0.09; 46-65/>65: +0.13, -0.07.

Table 2: Mean (±1s.d.) plasma concentration of the immunoglobulins in different age groups

Immunoglobulins (g/L)	Age group (in years) 6-25	26-45	46-65	>65	r	p	F	$F_{.05}$
IgG (n=172)	16.95±3.76 n=51	14.58±3.35 n=46	16.88±5.25 n=43	16.16±5.34 n=32	-0.005	>0.20	2.92	2.68
IgA (n=172)	1.84±0.93 n=51	1.90±0.44 n=46	2.02±0.97 n=43	2.23±1.23 n=32	+0.143	>0.05	1.79	2.68
IgM (n=159)	2.89±1.67 n=49	2.37±2.00 n=44	2.07±1.10 n=37	2.14±1.33 n=29	-0.152	>0.05	2.29	2.68
IgD (n=58)	0.06±0.04 n=13	0.08±0.14 n=16	0.02±0.02 n=13	0.11±0.22 n=16	+0.167	>0.20	1.53	2.76

Bonferroni adjustment gave the following results for IgG: 6-25/26-45: +4.52, + 0.22; 6.25/46/65: +2.26, - 2.12; 6-25/>65: + 3.17, - 1.59; 26.45/46-65: -4.54, -0.06; 26-45/>65: - 4.01, +0.85; 46-65/>65: +3.18, - 1.74.

Results

The mean ("1s.d) ages of those whose samples were included in the C4 estimation were 15.9 ± 8.2 (6-25 years old), 35.3 ± 6.3 (26-45 years old), 55.0 ± 5.2 (46-65 years old) and 72.9 ± 6.4 (>65 years old). The mean age ("ls.d) for the 26-45 years old people included in the IgG measurement was 35.6 ± 6.2 Subjects of different socioeconomic standing were evenly distributed among the age groups (x^2 = 19.12; $P > 0.05$). C3 and Bf mean values (Table 1) did not change significantly with age ($P > 0.10$ in both cases). However, C4 levels demonstratesignificant increase with rising age (r = 0.232; $P<0.01$). The mean plasma concentration of the immunoglobulins (Table 2) reveals no significant age-related changes for any of the four immunoglobulins ($P>0.05$ in all cases). However, IgA levels tend to increase; and IgM values to decrease with age. Also, there was no significant change with age ($P>0.10$ in all cases) in the 35.6 ± 6.2 mean concentrations of all the four acute phase proteinsassessed (Table 3).

Table 3: Mean (±1.s.d.) Plasma concentrations of complement components in different age groups.

Acute phase proteins (g/L)	Age group (in year) 6-25	26-45	46-65	>65	r	p	F	F_{05}
Transferrin (n=122)	2.30±0.93 n=20	2.45±1.22 n=34	2.32±1.28 n=36	2.74±1.32 n=32	-0.134	>0.10	0.84	2.68
Albumin (n=121)	40.1±10.4 n=41	43.6±10.3 n=23	29.6±9.8 n=28	38.8±9.8 n=29	-0.075	>0.20	1.09	2.68
Alpa-2-Macro Globulin (n=62)	3.63±0.74 n=15	4.08±0.83 n=16	3.09±0.71 n=15	4.09±0.84 n=16	-0.048	>0.20	5.65	2.76
C-reactive-protein* (=150)	.0.005±0.01 n=39	0.00±0.00 n=40	0.004±0.01 n=39	0.012±0.03 n=32	-0.038	>0.20	3.21	2.68

**Detectable in 13%, 0%, 8% and 19% of subjects in the respective age groups.*
Bonferroni adjustment gave the following results: For alpha-2-macroglobulin: 6-25/26-45; -1.13, +0.23; 6-25/46-65; +2.13, -1.07; 6-25/.65: +0.22, -1.14; 26-45/46-65: +2.55, -0.57; 26-45/.65: +0.65, -0.67; 46-65/.65: -0.33, -.68. For CRP: 6-25/26-45: +0.014, -0.004; 6-25/46-65: +0.010, -0.008; 6-25/.65: +0.002, -0.009; 26-45/46-65, +0.005, -0.013; 26-45/.65: -0.003, -0.021; 46 B65/.65: +0.001, -0.017.

One way analysis of variance also showed that the gradual increase in C4 levels was significant in the 46-65 years age group ($F = 2.99$; $F_{.05} = 2.68$). The Bonferroni adjustment was used to evaluate the differences among C4 groups (6-25/26-45;-0.16 +0.02; 6-25/46-65;-0.21,-0.02; 6-25/>65:-0.18, +0.02; 26-45/46-65:-0.13, +0.05; 26-45/>65:-0.11, +0.09; 46-65/>65:+0.13,-0.07). There were no significant differences in C3 and Bf levels. Of the immunoglobins, only IgG showed significant difference among its mean values ($F = 2.92$; $F_{.05} = 2.68$). The difference was located to a dip in the 26-45 years age group. Alpha-2- macroglobulin and C-reactive protein also showed significant differences among their mean values ($F = 5.65$; $F_{.05} = 2.76$ and $F = 3.21$; $F_{.05} = 2$-68, respectively). The differences were located to a dip in the 46-65 years age group for the former and a dip in the 26-45 years age group for the latter.

Discussion

A lack of significant difference among the age groups in C3 and Bf levels suggests that there may be no age-associated differences in the complement system between ages 6 and 95 years. It would therefore be unlikely that the complement system is being activated in healthy subjects during aging. However, compensatory mechanisms can maintain normal levels of complement proteins as serum levels reflect a balance between catabolism and rate of biosynthesis [12]. Increased biosynthesis or reduced catabolism may be responsible for elevated C4 levels observed with increasing age. The few available studies in the literature show no gross deficiencies in complement levels; otherwise they show increases in the elderly [13,14]. Clinically relevant alterations in complement values are reductions except in inflammation when they are raised [12] Could the classical complement activation pathway be less effective with rising age while the alternative pathway remains intact or enhanced? Further studies should obtain C4 genotyping data as there are a number of genes which influence C4 levels.

The observation in this report that IgA level tends to increase with age and IgM values tend to decrease agrees with some previous reports [15,16] However, these tendencies did not reach statistical significance in the present study. Oyeyinka et al. [17] also reported increasing IgA concentrations with advancing age in Nigerians. Like the findings of Buckley and Dorsey [18, 19] which described a fall in mean concentrations of IgG after the age of 35 years in North Carolina, a significant dip in the 26-45 years age group (35.6±6.2 years) was observed in IgG mean values in this study. De-Greef et al. [4] also found that the contribution of IgG subclasses and the IgM and IgA classes to the pool of serum immunoglobulins remained relatively unchanged during the course of aging. Their most prominent observation of a continuous decline of serum IgD starting in young adults could not be confirmed in this study. The mean IgD values observed here are similar to those reported previously in Nigerians [20]. A general lack of age-related changes observed in the four immunoglobulins estimated suggests that they will not be important for the purpose of indexing the aging process.

The levels of transferrin, albumin, alpha-2-macroglobulin and CRP obtained with age in this study suggest that in healthy individuals, aging does not result in acute phase responses. They may therefore not be useful in indexing the aging process. CRP is an outstanding positive acute phase reactant in man, rising by up to a thousand-fold its basal plasma level in response to inflammatory stimuli [21]. It has been found useful as an indicator of active bacterial infection [22, 23] and may also serve as a helpful index of infection in the aged. There are no obvious reasons for the dip in the alpha-2-macroglobulin levels in the 46-65 years age group and for the dip in CRP levels in the 26-45 years age group. Rapin and Lagier [24] observed that serum albumin concentrations showed a statistically insignificant tendency to decrease with age. No significant change with age was noted in albumin and transferrin levels in this study. As transferrin concentration is decreased in malnutrition [25], it should prove useful in monitoring nutritional changes during aging. The lack of significant alteration in the levels of these acute phase reactants with age indicates that they will not be useful indices of the aging process.

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