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Homogeneous immunoglobulins in Ghanaians living in Accra, Ghana

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Summary

Serum screening for homogeneous immunoglobulins (H-Ig) was done on 149 apparently healthy Ghanaians (aged 17 – 95 years) and 73 sick subjects who presented at the Korle-Bu Teaching Hospital from October 1999 to September 2000. Sera were screened by agarose gel electrophoresis and those with equivocal results were confirmed by immunoelectrophoresis. Immunoglobulin classes (IgG, IgA and IgM) and Bence Jones proteinuria were measured and determined by single radial immunodiffusion method and heating respectively. Total protein, albumin, calcium, uric acid, urea and creatinine were estimated on ACE chemistry autoanalyser. The laboratory profile of 5 Ghanaians with a picture of multiple myeloma and one with monoclonal gammopathy of undetermined significance drawn from the sick subjects (6 of 73) are presented. None of the 149 healthy subjects studied in three age groups (17-40; 41-64 and $65 years) had H-Ig, and their serum IgG, IgA, IgM, urea, creatinine, uric acid, calcium, total protein and albumin levels were within the normal range. H-Ig were present in sera of 6 out of the 73 sick subjects (8.2%); 5 of them (4 males, 1 female) presented a picture of multiple myeloma. Three of these 5 patients had IgG, and the others IgA paraproteinaemia. All 5 patients had immunoparesis which was absent in the 6th patient (a male) who also had IgA paraproteinaemia (< 10 g/L), active bone marrow with < 2% mature plasma cells and no renal involvement. Results of bone marrow examination supported a diagnosis of multiple myeloma in the 3 patients with IgG paraproteinaemia, but were not available for the other two. Bence-Jones proteinuria was found in 2 (both with IgG paraproteinaemia) of 4 patients (50%) available for testing. Renal involvement was indicated in the 5 patients with a picture of multiple myeloma as urea and creatinine levels were significantly raised.

Keywords: *Homogeneous immunoglobulins, Ghanaians*

Résumé

Les serums de 149 Ghaniens sain agés entre 17-95 ans et 73 patients étaient examinés pour l'immunoglobine(Hg-Ig) au centre Universitaire hospitalier de Korle-Bu d'Octobre 1999 a décembre 2000 par la méthode d'électrophorése à gel d'agarose et ceux ayant les résultats équivoques confirmés par l'immunophorése. Les diverse immunoglobines et la proteinurie de Bence Jones étaient determiné par la méthode simple d'immunodiffusion radiale. Les proteines totale, calcium , albumine, acide urique et créatinine étaient estimés par l'autoanalyse ACE. Les résultats de 5 Ghaniens ayant de multiple myélome et un ayant la gamopathie monoclonale de significance non précise a ces patients étaient presentés. Aucun des 149 sujects volontaires n'avaient l'Hb-Ig et les autres paramétres ci-dessus étaient entre valeur normale. L'Hb-Ig était present chez 6 des 73 patients (8.2%) parmi lesquels 5 males avec des multiples myélomes . Trois des 5 avaient l'Hb-Ig et d'autres IG-A paraproteinemique et tous immunoparésiques a l'exception du 6 ieme paraproteinemique (10g/l), les cellules osseuse active(<2%) et sans participation rénale. Les résulats de la moélle osseuse supportaient ce diagnosie de multiple myélome chez 3 patients ayant l'IgG paraproteinemique. La proteinurie de Bence Jones était identifiée chez 2 des 4 patients (50%) testés avec l'IgG paraproteinemique. La participation rénale était identique chez 5 patients ayant de multiple myélome d'un taux d'urée et de créatinine significativement elevé.

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Introduction

Monoclonal B-cell proliferative disorders, otherwise known as monoclonal gammopathy, are accompanied by an electrophoretically homogeneous immunoglobulin component (H-Ig) of a single isotype in the serum. The occurrence of monoclonal gammopathies increases with age [1] and attains a frequency of about 20% in the general population above 95 years of age [2]. Whereas Ligthart et al [3] detected no monoclonal gammopathies in a control group of healthy young subjects, they found its frequency in aged individuals to range from 11% in the "optimally healthy" to 38% in sick elderly people. They observed that most of the cases were related to immunodeficiency, with a clear association of the occurrence of monoclonal gammopathies of this type with the health status.

Malignant paraproteinaemia is found in multiple myeloma, Waldenstrom's macroglobulinaemia and heavy chain diseases. However serum H-Ig, especially in elderly persons, may also reflect benign proliferative disorders in the immune system; which are more frequent than the malignant ones [4]. Benign monoclonal gammopathy may

be transient and may result from environmental factors such as chronic antigenic stimulation [3]. Paraproteinaemia in apparently healthy people has been termed "essential" paraproteinaemia while "idiopathic paraproteinaemia" or monoclonal gammopathy of undetermined significance (MGUS) are terms used to refer to benign paraproteinaemia.

Renal involvement is a major complication of multiple myeloma, particularly in advanced disease with most patients developing severe renal failure [5]. One of the commonest causes of death in myelomatosis is renal failure which is particularly common in patients with prominent Bence Jones proteinuria and is due to the precipitation in the distal and collecting tubules of bulky protein casts [6].

This study evaluates the frequency and nature of homogeneous immunoglobulin components in healthy and sick Ghanaians in Accra.

Materials and methods

Subjects

Serum samples were obtained from blood (5ml) collected from each of 149 apparently healthy Ghanaians (92 males, 57 females) aged 17 – 95 years (45.54 ± 20.19) at the Korle-Bu Teaching Hospital, Accra. Of these, 99 were blood donors at the National Blood Transfusion Service, Korle-Bu and 50 were consulting the Eye Clinic for non-infectious and non-inflammatory conditions such as cataract and glaucoma. Blood donor participants were screened and found to be normotensive, have haemoglobin concentration within normal limits, and negative for hepatitis-B surface antigen in serum. Sera were similarly obtained from 73 sick Ghanaians who were suspected to be suffering from multiple myeloma or other lymphoproliferative disorders. These patients were either presenting at the Outpatient Clinic or admitted to the Haematology Department of the Korle-Bu Teaching Hospital during the period October 1999 to September 2000. Serum samples were investigated either fresh or after storage for 3-9 months at –20°C.

Methods

Films 3-5cm in length were made of aspirated bone marrow by means of a smooth-edged glass spreader at the bed side. Differential counts were made in the cellular trails behind marrow fragments which drag behind the spreader. Bone marrow films were fixed in methanol for 20 minutes and then stained with Leishman [7].

Initial screening for H-Ig was done by agarose gel electrophoresis (The Binding Site Limited, Birmingham, England). One percent agarose prepared in barbitone buffer (pH 8.6) was used in casting the gel to give a layer 1mm thick. The same buffer was used for electrophoresis, which was done at 20V/cm. After electrophoresis, the gel was fixed for 10 minutes in a solution made up of saturated aqueous picric acid (750ml) and glacial acetic acid (150ml). Excess picric acid was removed in a bath of ethanol for 5 minutes. The gel plate was reduced to a thin film by pressing between filter papers and air-drying in an oven at about 50°C. Staining of the gel was done for 5 minutes in Naphthalene Black (comprising methanol – 450ml, deionised water- 450ml, glacial acetic acid – 100ml, naphthalene – 5g) and destaining in a mixture of Methanol (1125ml), deionised water (1125ml) and glacial acetic acid (250ml) for about 10 minutes.

Immunoelectrophoresis was done using an oligo-specific polyclonal sheep antiserum against human IgG, IgA and IgM (The Binding Site Limited, Birmingham, England). Immunoglobulins G, A and M were measured by the Single Radial Immunodiffusion method as described previously [8]. Commercial standard sera and monospecific sheep antisera to human IgG (ã chain), IgA (a chain) and IgM (m chain) were used. Bence-Jones proteinuria was assessed, by its ability to precipitate between 40 - 60°C and to redisolve above 60°C and reprecipitate on cooling to 60°C using the heating test [9].

Total protein, albumin, calcium, uric acid, urea and creatinine were estimated spectrophotometrically on ACE Chemistry Autoanalyser (Schiapparelli Biosystems, BV, ENI Diagnostics Division, Woerden, The Netherlands). The ACE Total protein Reagent, in which copper ions complex with the peptide bonds of protein under alkaline conditions to form a violet-coloured compound, was used. The amount of the violet complex is proportional to the increase in absorbance measured bichromatically at 544nm/692nm.

The method employed by the ACE Albumin Reagent Assay is based on the binding of albumin to bromocresol green (BCG) dye. When serum is added to the reagent, pH changes turn the dye from yellow to green. The green complex of albumin with BCG is read bichromatically at 629nm/692nm.

The ACE Calcium Assay is a dye binding method where calcium forms a bluish-purple complex with Arsenazo in an acid medium. The amount of coloured complex formed is quantified by determining the absorbance of the reaction mixture bichromatically at 647nm/692nm.

Uric acid measurement by the ACE method is an enzymatic procedure in which uric acid is oxidized by uricase to yield allantoin and hydrogen peroxide which reacts with 3,5-dichloro-2-hydroxy-benzenesulphonic acid and 4-aminophenazone to produce a chromogen. The abosorbance of the chromogen solution is measured bichromatically at 505nm/692nm.

Quantitative determination of Urea concentration in serum using the ACE BUN/UREA Assay utilizes the enzyme urease to hydrolyse urea to ammonia and carbon dioxide. It then reacts the ammonia with 2-oxoglutarate and NADH in the presence of glutamic dehydrogenase to give glutamic acid and NAD^+. The absorbance is measured bichromatically at 340nm/647nm. In the ACE Creatinine Assay, the amount of a red-orange coloured complex formed by creatinine with alkaline picrate during a fixed time interval is determined at 505nm.

One way analysis of variance was employed to test the significance of differences in laboratory parameters among age groups in healthy subjects.

Results

Homogeneous immunoglobulin components were not found in any of the 149 healthy subjects studied in the 17-40 years, 41-64 years and ≥65 years. These subjects had serum IgG, IgA, IgM, urea, creatinine, uric acid, calcium, total protein and albumin levels that were within normal limits (Table 1). The mean IgG levels were 18.57 ± 3.70 g/L for subjects 17–40 years old, 18.66 ± 4.45g/L for people 41-64 years old and 19.80 ± 3.39g/L for those ≥ 65 years old. For IgA, the mean concentrations were 2.88 ± 1.02g/L, 3.59 ± 0.99g/L and 3.32 ± 1.03 g/L respectively; and for IgM the levels were 2.30 ± 1.20g/L, 2.40 ± 1.26g/L and 1.89 ± 0.77g/L respectively. One way analysis of variance revealed no significant differences in these parameters among the age groups except for IgA, calcium and albumin. Bonferroni adjustment pinned the significant difference for IgA to only the comparison between 17-40/41-64 years age groups (-0.19, -1.23). For calcium, only the 41-64/≥65 years age groups comparison was significant (+0.193, +0.007); and for albumin both 17-40/≥65 and 41-64/≥65 years age groups comparisons were significant (+4.495, +1.886 and +3.821, +0.619 respectively).

The sera of 6 out of the 73 sick subjects (8.2%) contained H-Ig. Three of the 6 had IgG paraproteinaemia and the other 3 had IgA paraproteinaemia. Five of the 6 patients (4 males, 1 female) had a suppression of immunoglobulin isotypes other than the paraprotein (Table 2). The patient without immunoparesis had IgA paraproteinaemia <10g/L and active bone marrow with <2% mature plasma cells. This patient had elevated total

Table 1: Laboratory parameters (mean ± 1 s.d. serum levels) in 149 apparently healthy Ghanaians screened for paraproteinaemia

	Age Group (years)				
Parameter	17 – 40	41 – 64	⊕65	F	F_{05}
Total protein (g/L)	82.52 ± 4.61 n = 77	81.44 ± 6.56 n = 31	80.54±5.81 n=41	- 0.237	3.00
Albumin (g/L)	41.15 ± 2.60 n = 77	40.18 ± 2.71 n = 31	37.96±3.25 n=41	17.26 3	3.00
Calcium (mmol/L)	2.37 ± 0.16 n = 77	2.44 ± 0.13 n = 31	2.34±0.20 n=41	3.704	3.00
Uric acid (mmol/L)	318.66 ± 70.52 n = 77	344.32 ± 75.80 n = 31	308.76±81.55 n=41	-21.83	3.00
Urea (mmol/L)	4.02 ± 1.18 n = 77	4.34 ± 1.48 n = 31	4.22±1.17	0.818 n=41	3.00
Creatinine (mmol/L)	101.57 ± 15.42 n = 77	98.23 ± 16.39 n = 31	99.76±14.56	0.567 n=41	3.00
IgG (g/L)	18.57 ± 3.70 n = 71	18.66 ± 4.45 n = 31	19.80±3.39	1.438 n=40	3.00
IgA (g/L)	2.88 ± 1.02 n = 76	3.59 ± 0.99 n = 31	3.32±1.03	6.160 n=40	3.00
IgM (g/L)	2.30 ± 1.20 n = 76	2.40 ± 1.26 n = 31	1.89±0.77	2.400 n=40	3.00

Normal range values for adult Ghanaians issued by the Department of Chemical Pathology, University of Ghana Medical School are: Total protein, 60.0-86.0 g/L; Albumin, 36.0-52.0 g/L; Calcium, 2.12-2.62 mmol./L; Uric acid, 120-420 :mol./L; Urea, 2.0-7.0 mmol./L; and Creatinine, 60-120 :mol./L. The range of normal values of IgG (11.3-26.5 g/L), IgA (1.1-5.3 g/L) and IgM (#4.4 g/L) adult Ghanaians have been published [10].

Bonferroni adjustment at 90% confidence level gave the following result: for albumin, AB: +2.405, -0.465; AC: +4.495, +1.886; BC: +3.821, +0.619; for calcium, AB: +0.014, -0.154; AC: +0.106, -0.046; BC: +0.193, +0.007; and for IgA, AB: -0.190, -1.230; AC: +0.040, -0.920; BC: +0.850, -0.310.

Table 2: Laboratory results in 6 patients with paraproteinaemia

No	Patient	Age (yrs)	Sex	BJP (in urine)	EP	IEP	IgG (g/L)	IgA (g/L)	IgM (g/L)	Urea (mmol/l)	Creatinine (µmol/L)	Ca (mmol/L)	Uric (µmol/L)	TP (g/L)	Albumin (g/L)
1	11R	60	F	Nd	M-protein	IgG paraprotein	18.70	0.09	0.17	nd	nd	nd	nd	nd	nd
2	18R	48	M	Positive	M-protein	IgG paraprotein	43.80	0.55	0.45	20.0	1163	2.04	733	125	26.2
3	38R	58	M	positive	M-protein	IgG paraprotein	37.00	0.28	0.54	9.1	220	2.55	725	132	23.7
4	69R	51	M	negative	M-protein	IgA paraprotein	12.00	12.45	0.41	25.7	433	2.00	2356	134	35.4
5	72R	54	M	negative	M-protein	IgA paraprotein	8.50	13.65	0.27	7.4	132	2.00	457	128.3	28.5
6	112R	68	M	Nd	M-protein	IgA paraprotein	12.75	9.06	1.17	4.6	109	2.09	340	99.8	32.6

protein but slightly reduced level of albumin and normal levels of urea, creatinine, uric acid and calcium. Mature plasma cells were >10% in bone marrow films for the 3 patients with IgG paraproteinaemia. Bone marrow examination was not done for the other 2 patients with IgA paraproteinaemia. Bence-Jones protein was found in the urine of 2 of the 4 patients tested (50%). Both Bence-Jones protein positive patients had IgG paraproteinaemia. The levels of urea (range: 7.4 – 25.7 mmol/L), creatinine (range: 132–1163 mmol/L) and uric acid (range: 457-2356mmol/L) in serum were raised in all of the patients with a picture of multiple myeloma whose sera were available for testing. These patients had elevated serum total protein (range: 125–134 g/L), reduced serum albumin (range: 23.7–35.4 g/L) and normal serum calcium (2.00–2.55 mmol/L) concentrations.

Discussion

The evidence exists that the occurrence of monoclonal gammopathy is the expression of a defect directly related to the aging process, and is more frequent in association with active disease [3]. Defects of the immune system in ageing are most pronounced in the T cell system [11] and regulatory T cell defects are believed to be responsible for the increasing frequency of monoclonal gammopathy in ageing [12]. It has been shown that genetic factors play an important role [13, 14] while environmental factors such as chronic antigenic stimulation contribute to the development of benign monoclonal gammopathy [15-18]. Other previous data demonstrated that biological defects (in neutrophils and blood cell parameters) attributed to the ageing process could not be verified when the individuals studied were selected according to the stringent admission criteria of the 'Senieur' protocol [19, 20]; suggesting that such defects were due to infection and/or disease. No age-related increase in the prevalence of H-Ig was obtained in this study as none of the healthy subjects, including aged individuals had H-Ig.

Homogeneous immunoglobulin concentrations as low as 100-500 µg/ml can be detected by agar gel electrophoresis and immunoelectrophoresis [21] but it is possible that H-Ig below these concentrations could have been missed. However, the concentration of H-Ig in patients' sera was found to be in the region of 10mg/ml in another study [22] which also reported no H-Ig in the sera of 120 healthy children who were donors for allogeneic bone marrow transplantation. If the presence of H-Ig is unrelated to ageing in this population, its detection in serum of patients may serve as an indicator of impairment in the T-cell regulatory function; thereby necessitating follow-up and further investigations for underlying diseases and possible immunological disorders. It has been shown that T cell impairment, as present in the athymic nude mice, thymectomised mice, and irradiated and bone marrow reconstituted mice, was associated with a high frequency of H-Ig [13, 23]. The zero prevalence of H-Ig. obtained in healthy Ghanaians in this study, among whom multiple myeloma is not a rare finding, suggests that benign monoclonal gammopathy (usually accompanied by H-Ig in serum) may be unrelated to the development of multiple myeloma.

Homogeneous immunoglobulins were detected in 8.2% of the sick hospital patients studied with 5 of the 6 presenting a picture of multiple myeloma. Ligthart et al [3] found monoclonal gammopathy in 38% of a random in-patients group with IgG paraproteinaemia predominating. In the present study, IgG and IgA paraproteinaemia were equally prevalent. Although they [3] reported H-Ig - IgM isotype prevalence of 17%, no paraprotein of the IgM isotype was detected in these Ghanaians. This finding is in line with what the literature suggests in cases of multiple myeloma [4, 24]. The results indicate that more male than female Ghanaians have H-Ig in serum and may suffer from multiple myeloma. The reason for this difference between the sexes is not known. Renal involvement was indicated in all five Ghanaian patients with a picture of multiple

myeloma as urea and creatinine concentrations in serum were significantly elevated. Renal involvement is a major complication of multiple myeloma, particularly in advanced disease [25]. Bence Jones proteinuria was found in 50% of the patients tested in line with its expected general distribution in myeloma [24]. However, both patients with H-Ig of the IgG isotype tested were positive while both patients with the IgA isotype were negative. It is not clear, due to small number of samples, if the presence of Bence Jones proteinuria in these Ghanaians is related to the isotype of the myeloma protein.

The sixth patient with H-Ig appeared otherwise healthy from available clinical chemistry results. Monoclonal gammopathy of undetermined significance (MGUS) is characterised by a serum IgA paraprotein level less than 20 g/L with none or < 1 g/24hr Bence Jones protein in urine; less than 10% plasma cells in bone marrow and no hypercalcaemia or renal insufficiency [26]. The sixth patient may be suffering from MGUS, suggesting that a low prevalence of benign monoclonal gammopathy exists in the general Ghanaian population.

There are no obvious reasons for the rise in the serum IgA levels in the 41-64 years age group, and the dip in serum calcium levels in the ≈65 years age group. The decrease with rising age observed in albumin levels in Ghanaians in this study reached statistical significance in the aged. Rapin and Lagier [27] also observed serum albumin concentrations to decrease with rising age.

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