



Journal of Medical Sciences

ISSN 1682-4474

science
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JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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J. Med. Sci., 8 (4): 378-383
15th June, 2008

Incidence of Paraproteinaemia in Hyperproteinaemic Patients Visiting the Chemical Pathology Unit of the Komfo Anokye Teaching Hospital

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The aim of this study was to determine the incidence of paraproteinaemia in patients found to be hyperproteinaemic at Komfo Anokye Teaching Hospital (KATH) and to find out the prevalence of Bence Jones Proteins (BJP) in the urine samples of such patients. The study involved 90 patients (48 males, 42 females) who reported to the Chemical Pathology Unit at KATH for protein estimation and were found to have total protein levels above 82 g L^{-1} using the ATAC 8000 Chemistry autoanalyzer. The initial screenings were done using serum protein electrophoresis. Serum protein electrophoresis revealed monoclonal bands in 11 (12.2%) of the patients and out of these 6.67% were found to have BJP in their urine. 57.8% had polyclonal increase in the γ region, 14.4% had hypoalbuminaemia, 6.6% gave normal electrophoretic pattern and 8.8% had either increase in α_2 proteins or beta protein or beta increase with reduced albumin or decrease in all plasma proteins and a higher frequency of monoclonal gammopathy in females than in males. The study revealed a high incidence of IgG monoclonal gammopathy compared to IgA and IgM monoclonal gammopathies. There is high incidence of paraproteinaemia among hyperproteinaemic patients reporting at the Hospital. Serum protein electrophoresis should therefore, be routinely performed on all samples found to have high protein level. This will enable early treatment of patients with monoclonal gammopathies with the appropriate replacement therapy.

Key words: Paraproteins, monoclonal gammopathies, serum protein electrophoresis, bence-jones proteins, immunoparesis, monoclonal gammopathy of uncertain significance

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INTRODUCTION

The paraproteinaemias which are also known as B-cell or plasma cell dyscrasias or monoclonal gammopathies comprise a heterogeneous group of diseases [including lymphoma, leukemia, multiple myeloma and monoclonal gammopathies of uncertain significance (MGUS)] characterized by the presence in serum or urine of a monoclonal immunoglobulin also referred to as a paraprotein.

Paraproteins are the earliest described tumour markers and remain an essential part of the investigation, diagnosis and monitoring of patients with B cell dyscrasia (Sheldon and Riches, 2001).

There are several situations in which a paraprotein may be identified and these scenarios have differing clinical significance. At one extreme there may be an overtly malignant clonal proliferation of plasma cells resulting in multiple myeloma or solitary plasmacytoma (skeletal or extramedullary). At the other extreme there may be a low level paraprotein ultimately classified as monoclonal gammopathy of undetermined significance (MGUS) which may be of little clinical relevance (Cook and Macdonald, 2007).

Recently published population based studies from Minnesota have clarified the incidence of paraproteinaemia in the general population. In individuals aged >50 years the overall incidence of a paraprotein is 3.2%; this varies with age [age 50-59, 1.7%; age >70, 5.3%] (Kyle *et al.*, 2006). There is also an ethnic variation with previous reports noting the age adjusted prevalence of MGUS being threefold higher in African Americans than the white population (Landgren *et al.*, 2006).

In 2001, 5 cases of lymphoma (4 of the patients being between the ages of 5 and 10 and the other 16 years), 38 cases of leukemia (with 35% of these patients ranging in age from 10 to 19 years) and 10 cases of (MGUS) were reported at the Oncology Unit of the Komfo Anokye Teaching Hospital (KATH) (Opere-Sem and Bedu-Addo, unpublished data).

In 2002, 42 cases of leukemia (25% of the subjects being between 4 and 9 years) and 5 cases of multiple myeloma were reported at KATH (unpublished data).

From January to May 2003, three cases of lymphoma and 19 cases of leukemia (with 35% of them ranging in age from 5 to 9 years) were reported (unpublished data). From the above data, it is observed that the incidence of these malignant diseases at KATH is high and has early onset.

Very little has been done on the characterization of immunoglobulins (Igs) and the diagnosis of the paraprotein in Ghana. The treatment, monitoring and prognosis of paraproteinaemia, especially myelomatosis, however depend on the early detection of a paraprotein.

It was Bence Jones who recognized the potential importance of the abnormal urine protein in diagnosis of what he described as *Mollitis ossium* and what we now know as multiple myeloma (Bence, 1847).

Electrophoresis is the only reliable method for the detection of paraproteins in serum and or urine (Sheldon and Riches, 2006). Agarose electrophoresis is the most common method in use in many laboratories for the detection of paraproteins in serum and urine (Riches and Hobbs, 1988).

The aim of this research was therefore to determine the incidence of paraproteinaemia in, hyperproteinaemic patients reporting at the Komfo Anokye Teaching Hospital and the prevalence of Bence-jones proteins in the urine, of such patients.

MATERIALS AND METHODS

This was a hospital-based study carried out during the period between June 2002 and May 2003. Ninety patients (48 males and 42 females), who consecutively visited the Chemical Pathology Department of the Komfo Anokye Teaching Hospital for protein estimation and were found to have total protein level above 82 g dL⁻¹, were selected for the study. Total protein analysis was performed using the chemistry analyzer ATAC 8000.

Five milliliters of venous blood samples were collected from these patients and separated within 2 h to obtain sera.

One percent of agarose gel was prepared in barbitone buffer of pH 8.6. This was poured into a U-frame mould to give an agarose layer of 1 mm thickness and stored at 4-8°C overnight before use. After creating the sample application sites, 2.5 µL of undiluted sample was applied into each slit. A normal control sample, as well as a marker was applied alongside the samples. In a barbitone buffer of pH 8.6, the samples were subjected to electrophoresis at a constant voltage according to Jeppson *et al.* (1979). The run was stopped when the marker had travelled 5 cm from the starting point. The gel was fixed in picric acid, pressed and dried by hot air oven and stained with either Schwarz B stain or Ponceau S stain, dried again and was ready for interpretation.

The heat precipitation test (Cheesbrough, 1991) was used to screen for Bence Jones proteins.

RESULTS AND DISCUSSION

There is scanty literature on the characterization of Ig and the diagnosis of paraproteins in Ghana, particularly KATH and very little work has been done so far on the incidence of paraproteins in Ghana. Paraproteins are characteristic of malignant diseases of B-cells, the most common of which is multiple myeloma. Others include Waldenström's macroglobulinaemia and Franklin's

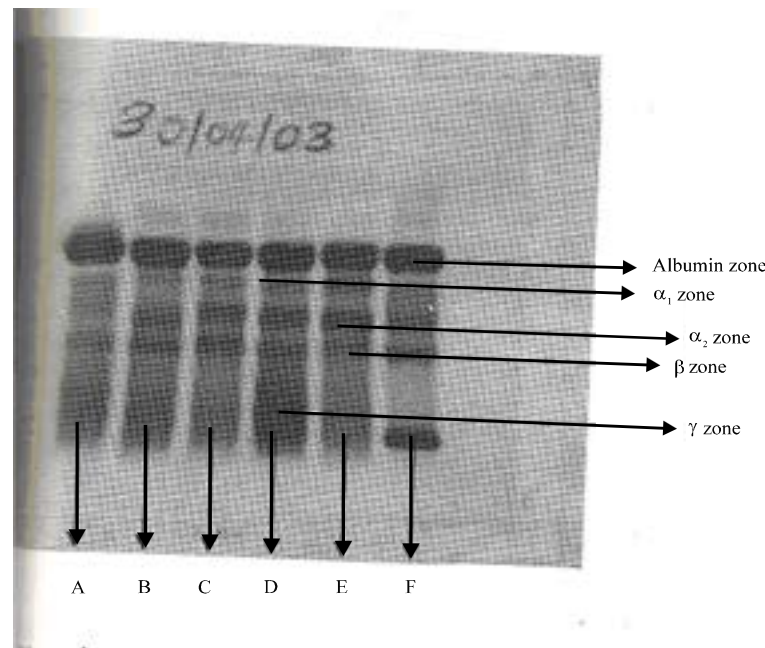


Fig. 1: Electrophoretic pattern showing different pattern with Amido Schwarz B stain. (A) normal pattern, (B) increased in α_2 and beta globulins, (C) increased in α_2 and beta globulins with polyclonal decrease in the gamma region, (D) increased in α_2 and beta globulins with broad diffuse polyclonal increase in the gamma region, (E) increased in α_1 , α_2 and beta globulins with polyclonal decrease in the gamma region and (F) decreased in α_1 , with increased α_2 and beta globulins with a paraprotein band in the slow gamma region

Table 1: Classification of the patients according to electrophoretic pattern

Type	No. (%)
Monoclonal+immunoparesis	11 (12.2)
Polyclonal	52 (57.8)
Hypoalbuminaemia	13 (14.4)
Others	8 (8.8)
Normal	6 (6.6)
Total	90 (100.0)

(heavy chain) disease. Paraproteins are occasionally seen in other malignancies of B-cell origin such as chronic lymphatic leukaemia (Marshall, 1988).

The classification of the patients according to electrophoretic pattern is shown in Table 1, as well as Fig. 1 and 2. Out of 90 samples selected for the electrophoresis, 11 (12.2%) were positive for monoclonal gammopathy with immunoparesis (Table 1). Seventy percent of the paraprotein bands were in the slow gamma region i.e., gamma-2 zone indicating the presence of IgG. Twenty percent in the fast gamma region corresponding to gamma-1 zone and probably indicating the presence of IgA and 10% was found between the beta and gamma-1 zone which probably also indicate the presence of IgM. This finding reveals a high incidence of IgG monoclonal gammopathy compared to IgA and IgM monoclonal gammopathies.

It also confirms the findings at Korle-Bu Teaching Hospital in Accra, Ghana, in 2001, which demonstrated that IgG and IgA constituted 80 and 10-15%, respectively (Unpublished data). However, the researchers at Korle-Bu were unable to demonstrate the presence of IgM monoclonal gammopathy which was explained as being due to the suppressive effect from spuriously elevated IgG.

The results of this study also correlated well with a preliminary study carried out at KATH in 1993 by one of the authors at KATH (Bedu-Addo, Unpublished data). Out of the 47 samples he studied, 6 (12.8%) had paraprotein, 38 gave polyclonal response and 3 gave normal responses. These data point out to the fact that the incidence of paraprotein is very high at KATH and it is consistent with the report of Landgren *et al.* (2006) that the incidence of MGUS is threefold higher in African-Americans than the white population.

The percentage values of IgG and IgA in monoclonal gammopathies correspond approximately to their physiological levels of 70-75 and 10-15%, respectively.

Figure 3 shows the rising incidence of monoclonal gammopathy with age. One out of the 11 patients with paraproteinaemia (i.e., one out of the total of 90, 1.11%)

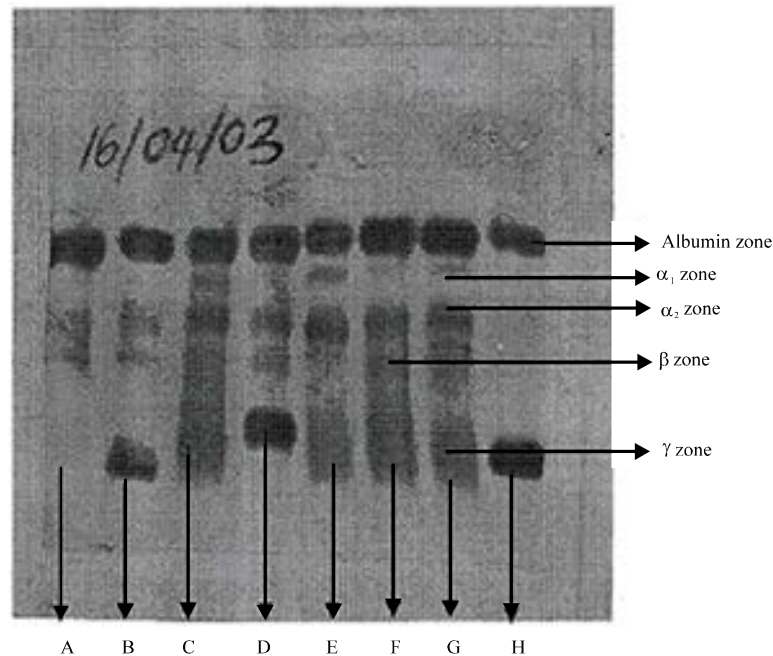


Fig. 2: Electrophoretic pattern showing different pattern with Ponceau S stain. (A) hypogammaglobulinaemia, (B) paraprotein band with immunoparesis, (C) increase in α_2 globulins, (D) paraprotein band in the fast gamma region, (E) decreased albumin and increased α_2 globulins, (F) decreased α_1 globulins, (G) decreased α_1 and beta globulins and (H) decreased albumin with immunoparesis and a protein band in the slow gamma region

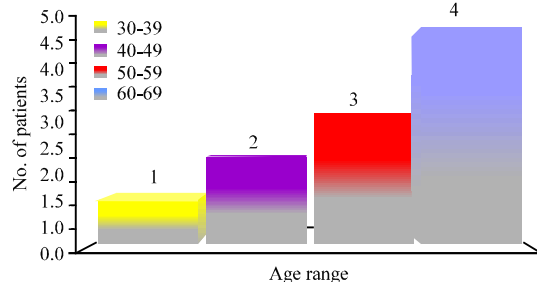


Fig. 3: Incidence of paraproteinaemia with rising age

was within the age group of 30-39; 2 (2.22%) within the age group of 40-49; 3 (3.33%) in 50-59 and 5 (5.5%) were within the age group of (60-69). This confirms other studies, which reported that the incidence of monoclonal gammopathy, is high in individuals above 50 years (Crawford *et al.*, 1987; Aguzzi *et al.*, 1992). These authors reported on the incidence of monoclonal gammopathy as 10% in patients older than 80 years and 3-5% among patients above 75 years, respectively. Similarly, Kyle *et al.* (2006) demonstrated that the incidence of paraproteinaemia in individuals aged >50 years was 3.2% but varying in age (age 50-59, 1.7%, age >70, 5.3%). Thus the incidence of paraproteinaemia in this study in the age 50-59 is about two times higher than that reported by Kyle *et al.* (2006). This again confirms the report of

Landgren *et al.* (2006) that the incidence of MGUS in African-Americans is much higher than whites.

The incidence of monoclonal gammopathy in this study was 5.5% for patients above 60 years. Further, data collected from the Oncology Department of KATH indicated that 65% of the patients were between the ages of 5-35 years. These suggest that paraproteinaemia starts at an earlier age in Ghana. These findings could be due to higher IgG levels in blacks than in whites.

Figure 4 reveals a higher incidence of monoclonal gammopathy in females as compared to males, although in this study the number of males outnumbered the females by a difference of six. This is similar to the observations made by Crawford *et al.* (1987) and Aguzzi *et al.* (1992) but in contrast to the report of Kyle *et al.* (2006) who demonstrated that the incidence of paraproteinaemia has preponderance in males (men: Women, 4.0: 2.7%).

57.8% of the patients had polyclonal gammopathy thus generalized (i.e., diffuse) elevation of Ig (Table 1). This is a very frequent finding in electrophoresis. This abnormality is seen in autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis; in liver diseases such as hepatitis and in infections which are common here (Cook and Macdonald, 2007).

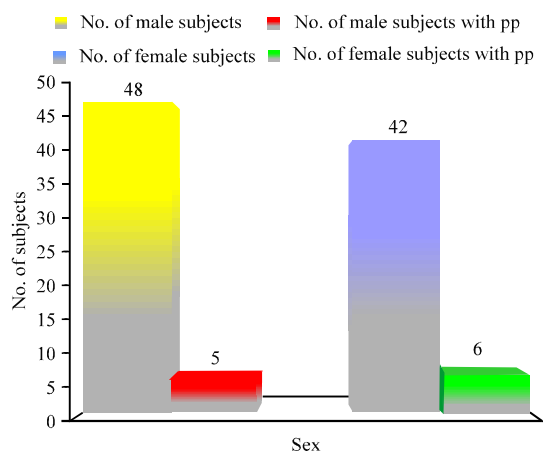


Fig. 4: Paraproteinaemia with preponderance in the female

14.4% of the subjects had hypoalbuminaemia (Table 1), which explains the body's compensatory mechanism of regulating albumin and globulin levels. Whenever, globulin increases, albumin decreases in response to controlling the level of total protein within normal limits. On the other hand, this could be due to cirrhosis of the liver which is usually characterized by a lowered albumin and a diffuse increase in gammaglobulins and β - γ -fusion due to the increase in IgA that occurs in some forms of the condition (Marshall, 1988).

About 6.6% of the subjects were found to have normal electrophoretic pattern. Although their protein level was found to be above the upper limit, their albumin and globulin levels were within the normal ranges. Others (8.8%) indicated, for example, increases in α_2 and β globulins (Fig. 1, pattern B); increased in α_1 , α_2 and beta globulins with polyclonal decrease in the gamma region (Fig. 1, pattern E); decrease in α_1 globulins (Fig. 2, pattern F) which is indicative of α_1 -antitrypsin deficiency. α_1 -antitrypsin is the major component of the α_1 -globulins and with deficiency, the band may be characteristically faint. Figure 2, pattern A shows a decrease in the γ -globulin band (hypogammaglobulinaemia) which may be seen in defects of humoral immunity involving IgG, the major component of the γ -globulins.

Bence Jones proteins namely, monoclonal light chains have usually been one of the biomarkers for a malignant process such as multiple myeloma and its related B-cell malignancies (Hannam-Harris *et al.*, 1980; Solomon, 1986). Out of the 11 patients with monoclonal gammopathies, 6 (54.5%) had BJP in their urine, i.e., an incidence of 6.67% of the total number.

In general, Bence Jones proteinuria with excretion of more than 1 mg/100 mL is of ominous significance (Hobbs, 1967).

In summary, the treatment, monitoring and prognosis of paraproteinaemia especially myelomatosis depends on the early detection of paraprotein band in electrophoretic pattern. This study has demonstrated a high incidence of monoclonal gammopathy in patients with hyperproteinaemia attending KATH.

Monoclonal gammopathy starts at an earlier age in Ghana. The increase in incidence of the monoclonal band with age is higher among females indicating that the elderly, particularly women are at risk of developing monoclonal gammopathy.

The study also demonstrated that 80% of subjects with paraproteinaemia had immunoparesis (i.e., the advanced state), meaning early detection will help in the identification of the Ig and appropriate replacement therapy given.

It is thus being recommended that serum protein electrophoresis be performed on all hyperparaproteinemic samples and classified. This will enable early diagnosis and treatment of paraproteinaemia as well as other pathological conditions such as nephrotic syndrome and liver disease.

Further, the paraproteins and BJP should be quantified since it may be impossible to comment on IgA and IgM concentrations from electrophoresis alone.

ACKNOWLEDGMENTS

We wish to thank Mr. Sarwel Kwalye Yeboah who technically helped to arrange and type this manuscript.

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