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Protein Isotypes and Laboratory Characteristics of Multiple Myeloma

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Abstract

Multiple myeloma (MM) is a B-cell malignancy that is characterized by monoclonal expansion and accumulation of plasma cells in the bone marrow. The abnormal plasma cells in MM cell clones produce an abnormal immunoglobulin which is called a monoclonal protein (M protein) and free light chain proteins designated as kappa or lambda. A descriptive study was carried out at the University Teaching Hospital in Lusaka, Zambia. Serum protein electrophoresis, immunofixation, complete blood count, calcium, albumin, urea, lactate dehydrogenase and creatinine were assessed on confirmed Multiple myeloma patients. Our results showed IgG (80%) and IgA (20%) as the only protein types of MM in indigenous black Zambians at the University Teaching Hospital. The predominant light chain of IgG(66%) and IgA(13%) was Kappa. It was further observed that Albumin and Haemoglobin levels were low, Lactate dehydrogenase and Creatinine levels were high. This study showed that IgG and IgA were the common isotype protein of MM in blood of patients presenting at UTH and the predominant light chain of IgG and IgA was Kappa.

Keywords: Serum proteins, Multiple myeloma, Ig G, Ig M, light chain, heavy chain.

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Introduction

Multiple myeloma (MM) is a B-cell malignancy that is characterized by monoclonal expansion and accumulation of plasma cells in the bone marrow [1]. The abnormal plasma cells in MM cell clones produce an abnormal immunoglobulin which is called a monoclonal protein (M protein) and free light chain proteins designated as kappa or lambda [2].

The M-Protein is a tumor marker specific for monoclonal gammopathies because it reflects the clonal proliferation of immunoglobulin [3]. The normal immunoglobulin is comprised of the heavy and light chain. In regards to the type of immunoglobulin produced, MM can be classified into IgA MM, IgD MM, IgE MM, IgM MM, IgG MM, light chain MM and non-secretory MM [2]. The best method for detecting M-protein is high resolution gel electrophoresis. M-Protein is observed as a localized band which is frequently usually seen on the gamma or beta globulin region, it may also be seen on the alpha 2 globulin region but this is rare [4]. To characterize MM in respect to the protein produced, immunofixation is also an important tool used.

The most common type of M-protein found in MM is IgG followed by IgA and light chain only, and renal failure and bone disease appear to be more frequent in these patients [3]. IgD MM is less common and is characterized by detection of small levels of M-protein, patients have extra medullary disease and an advanced stage of the disease with a median age of 52 years and had shorter survival rates [5].

IgE MM is less common in all MM cases [6]. Patients tend to be found to have bone pain, renal failure, hyperglycemia and bence jones proteinuria. A hall mark of IgE MM is t [11; 14] (q13; q32). MM is diagnosed by examining the bone marrow showing plasma cell infiltration of 10% or more, detection, and quantification of monoclonal protein in either serum or urine by (Serum Protein Electrophoresis (SPEP), urine protein electrophoresis (UPEP), immunofixation (IFE)) except in patients with true non secretory MM, and evidence of end organ damage [7]. The diagnosis should bemade by looking at whether the patient is symptomatic or asymptomatic to follow each protocol correctly. Other tests done to evaluate patients with MM in the laboratory include a complete blood count, chemistry profile, Beta -2 microglobulin, cytogenetics, imaging techniques (Magnetic resonance imaging (MRI), positron emission tomography (PET), computerized tomography (CT)) and Immunophenotypic studies.

Materials and Methods

Selection of participants and specimen collection - Blood was collected with informed consent from confirmed MM patients reporting for management at the Cancer Disease Hospital(CDH) and Haemato-oncology clinic at the University Teaching Hospital (UTH), Lusaka, Zambia. Blood samples were collected from research participants via venipuncture using the Evacuated Tube System (ETS) following the Clinical Laboratory Standards Institute (CLSI) order of draw.

Specimen Preparation and Storage – In the laboratory,blood specimen in Ethylene diaminetetra acetic acid (EDTA) and in plain tubes were collected. Blood in plain container was centrifuged at 1500 rpm for 15 minutes to separate serum from the blood cellular components. Only serum was carefully collected from the vacutainers using pasture pipettes and transferred to 2ml cryovials with sealable screw caps, which were stored in a freezer at -20^{o}C until the specimens were required for analysis.

Serum protein electrophoresis -serum samples were analyzed according to the manufacturer's recommendation and assay procedures qualitatively for monoclonal protein using the SAS-MX electrophoresis chamber which is intended for the separation and quantification of serum proteins by Agarose gel electrophoresis.

Serum protein immunofixation - serum samples were analyzed according to the manufacturer's recommendation and assay procedures by the SAS-MX electrophoresis chamber which is intended for the separation and identification of monoclonal gammopathies by Agarose gel electrophoresis.

Complete blood count- whole blood samples were analysed on Sysmex XT 4000 Haematology analyzer according to the manufacturer's recommendation and assay procedures for the automated analyzer. All test protocols were calibrated and controls ran before samples could be assayed.

Calcium, albumin, urea, lactate dehydrogenase and creatinine estimation- Serum samples were analysed on the Pentra 400 Chemistry Analyzer according to the manufacturer's recommendation and assay procedures for the automated analyzer. All test protocols were calibrated and controls ran before samples could be assayed.

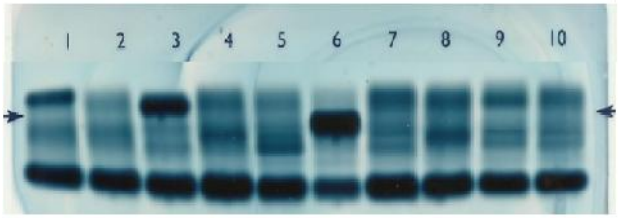

Figure 1: Serum protein electrophoresis detection of selected MM samples 1, Abnormal protein; 2, Normal protein; 3-10, Patient samples.

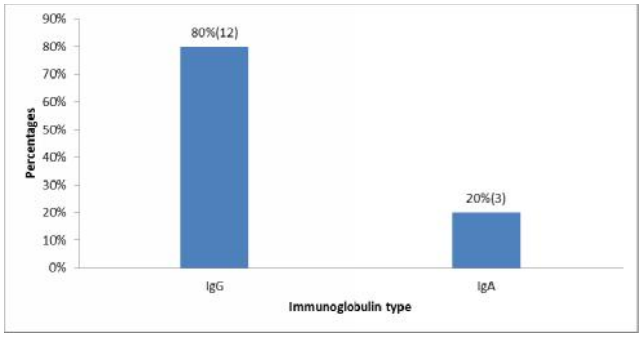

Figure 2: Distribution of protein isotypes of MM: IgG was the predominant Ig secreted by the MM.

Results

In total 15 confirmed MM participants were recruited. The study found that Multiple Myeloma participants had the abnormal protein (M protein) either IgG or IgA present in their serum qualitatively (Fig 1).Out of a total of 15 serum specimens analysed in the lab, 12 had IgG (80%) and 3 (20%) had IgA (Fig 2). 10(66%) out of the 12 IgG had IgG Kappa, 1(7%) had IgG and 1(7%) had IgG Lamda, 2(13%) Out of 3 IgA had IgA Kappa and 1(7%) had IgA Lamda (Fig 3-3e). The study further observed that Multiple myeloma participants had some normal values of haematocrit, white blood cells, platelets,

calcium and urea (Table 1).They also had high levels of creatinine and lactate dehydrogenase and low levels of haemoglobin and albumin compared to the normal reference ranges (Table 1).

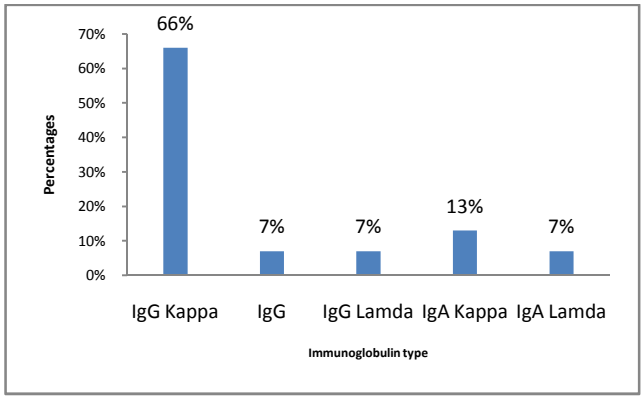

Figure 3: Distribution of IgG and IgA characteristics of MM: IgG-Kappa was the major isotype of IgG and IgA-Kappa was the major isotype of IgA.

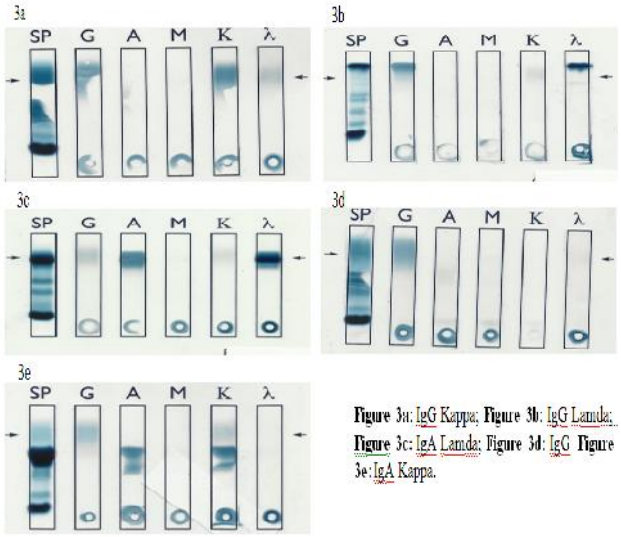

Figure 3a: IgG Kappa; Figure 3b: IgG Lamda; Figure 3c: IgA Lamda; Figure 3d: IgG Figure 3e: IgA Kappa.

Discussion

Multiple myeloma is plasma cell dyscrasia with a high degree of heterogeneity in its survival. The reason for this is the difference in its biological characteristics among patients.

Protein Isotypes: The findings of the study showed only 2 types of MM. The types of MM observed were IgG and IgA. IgG was the most common. IgG had Kappa and Lamda positive light chains, and there was one with no light chain detected using immunofixation in blood. IgA had Kappa and Lamda light chain positive.The findings of this study are in agreement with studies that say that IgG and IgA are the common types of MM.

The most common type of M-protein found in MM is IgG followed by IgA and light chain only, and renal failure and bone disease appear to be more frequent in these patients [3].Another study done in the USA also found that the common types of MM are IgG and IgA. IgG accounted for 61% of cases from the 787 patients and IgA accounted for 18% of patients which was then followed by Bence Jones proteinuria 6%, biclonal gammopathy 3.5% and IgD 0.5% [8]. Our findings are not in agreement with the findings of the study of in the USA that found that IgM MM, IgA MM and light chain MM accounts for 90% of all myeloma cases and 10% of IgG MM, IgE MM, IgD MM and non-secretory MM [2]. Our study showed a dominance of IgG MM over IgA MM. In another study done, it was reported that the mean age at diagnosis of IgG and IgA was 62 years with a slight predominance of male patients [9]. The mean age at diagnosis is at variance with our findings which observed 53years but our observations are in agreement with a slight predominance of male participants.

Another study observed that the clinical signs of IgD MM which include bone pain, weakness, fatigue and weight loss are also seen in IgG and IgA MM [10]. Management of IgG and IgA is similar to that of other isotypes [11]. Nevertheless, the monitoring of disease response to treatment of IgG and IgA MM may be different because of less excess antigens [12]. IgG and IgA MM has been reported to have a better prognosis than other types of MM [13]. However, these findings are in contrast to the findings of a study that reported that the survival of IgG and IgA MM are not different from IgD, IgE, IgM and light chain MM [14].

Relapse with the rising levels of free light chain and no change in paraprotein occurs in 5% of IgG and 15% of IgA MM [15]. In MM, the prognostic variables depend on the types of paraprotein produced, serum creatinine, calcium and percentage of bone marrow plasma cells predict prognosis for IgG and Bence Jones MM patients whereas HB and calcium together with the level of monoclonal protein were predictive for IgA MM [16].

Laboratory Characteristics

In this study, the mean values of laboratory tests done were calculated and it was observed that the values for Haematocrit, white blood cells, red

blood cells, platelets, calcium and urea were normal compared to the normal reference ranges.

The mean concentration values for HB was low (11.4g/dL) compared to the normal reference rang of 12 g/dL - 18 g/dL. This is similar to the findings of a study done in Nigeria [17]. Anaemia is a predominant feature of MM. This is usually seen as a result of low HB levels below the average. This is due to inadequate levels of erythropoietin, which are present in up to 50% of patients. To correct this, replacement therapy with recombinant erythropoietin is useful and has been shown to be effective in 80% of MM patients with a mean HB increase of 2 g/dL [18]. Anaemia may promote tumor hypoxia which is thought to impart resistance to irradiation and some chemotherapeutic agents and to cause malignant progression [19].

Albumin levels were also low (17.3 g/dl) compared to the normal reference range of 35 -50 g/dl. This is similar to a Chinese study that observed low albumin levels in MM patients [20]. The lower albumin concentration among patients may be due to a homeostatic mechanism controlling the plasma oncotic pressure causing changes in serum albumin [21]. Serum albumin is a significant prognostic factor that reflects the severity of disease progression and is an indirect indicator of increased levels of IL-6 which aid in myeloma cells to escape death. It has also been observed that low levels of albumin are correlated with high levels of Beta-2 microglobulin [20]. It may also indicate kidney injury which promotes protein loss through urine. This was supported by high creatinine levels in these patients.

It was also observed that LDH concentrations (229 IU/L) were high comparing to the normal reference ranges of 115 - 211 IU/L, and these findings are similar to the findings of a study done in Turkey [22]. LDH is a cytoplasmic enzyme which if the cell and membrane are damaged, is released into the extracellular area. Thus, it is an important marker used in the monitoring of disease progression and in MM, it is said to be correlated positively with Beta 2 microglobulin [23]. Elevated levels are observed rarely at onset of MM but increases as the disease progresses. As LDH gives an idea about the level of tumor mass, the increase of LDH during the course of disease may signify increased level of tumor or relapse [24].

Creatinine levels were above the normal reference range and this is similar to the findings of many studies. Creatinine levels are important, as they help to determine the extent of renal disease that is seen in MM. Renal disease in MM is present mostly as renal insufficiency and proteinuria. It results from the injurious effects of light chains to renal structures [25]. Patients presenting with renal failure have an early death rate due to renal failure [26]. It is therefore important to prevent renal failure [27] as this will improve survival [28].

Conclusion

This study showed that IgG and IgA were the common isotype protein of MM in blood of patients presenting at UTH. It was further observed that most of the patients presented with significant renal damage which attributed to hypoproteinaemia and anaemia with high levels of creatinine.

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