University of Zambia
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Characterization of Multiple Myeloma at the University Teaching Hospital (UTH), Lusaka

By

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A Dissertation Submitted to the University of Zambia, in Partial Fulfilment of the Requirements for the Master of Sciences Degree in Pathology (Haematology)

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Declaration

This work/dissertation in its present form has not been submitted or accepted previously for the award of a degree or diploma in this or any tertiary institution, and is not being submitted for a degree or diploma in any tertiary institution or for another degree or diploma at this institution. I declare that this Dissertation contains my own work except where specifically acknowledged. I further declare that I followed all the applicable ethical guidelines in the conduct of the research. This dissertation has been prepared in accordance with the Masters of Sciences in Pathology (Haematology), University of Zambia guidelines.

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Certificate of Approval

The University of Zambia approves this Dissertation on "CHARACTERIZATION OF MULTIPLE MYELLOMA AT THE UNIVERSITY TEACHING HOSPITAL".

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Abstract

Context: Multiple myeloma (MM) is the second most common blood cancer after lymphomas, thereby accounting for 10% of all haematological malignancies. The incidence of MM is related to age with the condition being more common in the elderly. There is a higher incidence and mortality rate among blacks.

Aims: To determine the biological and clinical characteristics of MM at the University Teaching Hospital in Lusaka, Zambia.

Methods and Results: A descriptive study was done at the University Teaching Hospital in Lusaka, Zambia. Record files of 46 patients diagnosed with MM from 2008-2015 April were reviewed to determine the clinical and radiological features at presentation .The median age at diagnosis was 53.0 (range 32-81 years). From 46 patients, 25 (54%) were men and 21(46%) were females. The most frequent and common clinical, laboratory and radiological features in order of occurrence were osteolytic lesions, back ache, bone pain, anaemia, pathological fractures, chest pain and fatigue. Females had a higher proportion of fatigue than men with (P= 0.036). It was also observed that pathological fractures in individuals with osteolytic lesions were statistically significant with (P=0.001).

Serum protein electrophoresis, immunofixation, complete blood count, Lactate dehydrogenase, urea, creatinine, albumin and calcium were assessed in only 15 patients as others died and were lost to follow up. Our results showed that the only types of MM present were IgG (80%) and IgA (20%). It was also observed that LDH and creatinine values were high, albumin and HB values were low and calcium, WBC, RBC, HCT and platelet values were normal.

Conclusion: This study showed that IgG and IgA were the common isotype protein of MM in blood of patients presenting at UTH. The predominant light chain of the IgG and IgA was Kappa. It was further shown that osteolytic lesions which are a radiological feature of MM were the most frequent feature of patients presenting with MM at UTH, Lusaka Zambia.

Acknowledgements

First and foremost I would like to thank my principal supervisor, Dr. Trevor Kaile, the head of department of Pathology and Microbiology at UNZA, whose dedication to produce quality MSc. Pathology Graduates with emphasis on excellence in research has been of great encouragement and help. His guidance and understanding in the Department of Pathology and Microbiology has been invaluable.

I would also like to thank Dr. Hamakwa Mantina on his guidance, correction, understanding, and most importantly, his patience during my research which has also helped in the production of this dissertation.

I would also like to thank Professor Chintu and Dr Kowa whose technical contributions and mentorship was paramount to understanding the biological characteristics of multiple myeloma. I also show utmost gratitude to Dr. Korolova and Dr Ben Andrews whose assistance on research methodologies and statistical analytical tools are the core elements that have given meaning to the research findings.

Since the commencement of this research, there have been numerous lecturers, graduate students, laboratory staff, and administrative staff members who have contributed to the work of my research and to them I give gratutide: Panji Nkhoma, Musalula Sinkala, Jonas Zimba, Graham Chianzu, Mubanga Mutale, Webby Mbuzi, Pauline Okuku, Beauty N'gandu, Moses Nkandu, Reggison Phiri, Mwindulula Mudenda, Reuben Zulu, C. L. Musonda, D. Sichone, Mrs N. Mazombwe among others.

I would like to take this opportunity to thank the University of Zambia for all of the funding they provided me with in order to make this dissertation possible. I also thank many others who have been of help over the course of this research, the Department of Pathology and Microbiology and the Cancer Diseases Hospital (CDH) at UTH - Lusaka and many departments around the University of Zambia. I would also like to thank my husband, Brian and my family for being there for me in so many ways.

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List of Abbreviation

BREC	:	Biomedical research ethics committee
MM	:	Multiple myeloma
M-protein	:	Monoclonal protein
MGUS	:	Monoclonal gammopathy of undetermined significance
UTH	:	University Teaching Hospital
UNZA	:	University of Zambia
VDJ	:	Variable diverse and joining
IL-6	:	Interleukin 6
VEGF	:	Vascular endothelial growth factor
LDH	:	Lactate dehydrogenase
HB	:	Haemoglobin
HCT	:	Haematocrit
WBC	:	White blood cells
RBC	:	Red blood cells
CD	:	Cluster of Differentiation
FISH	:	Fluorescence in situ hybridization
ISS	:	International staging system
Ig	:	Immunoglobulin
USA	:	United States of America
MRI	:	Magnetic resonance imaging
PET	:	Positron emission tomography
CT	:	Computer tomography
MFC	:	Mutiparameter flow cytometer
NCAM	:	Neural cell adhesion molecule
FGFR3	:	Fibroblast growth factor receptor 3
MMSET	:	Myeloma SET domain protein
SPEP	:	Serum protein electrophoresis
UPEP	:	Urine protein electrophoresis
IFE	:	Immunofixation
IgA	:	Immunoglobulin A

IgM	:	Immunoglobulin M
IgG	:	Immunoglobulin G
IgE	:	Immunoglobulin E
IgD	:	Immunoglobulin D

Chapter 1

Introduction

1.1 Background

Multiple myeloma (MM) is the second most common blood cancer after lymphomas, thereby accounting for 10% of all haematological malignancies (Kyle *et al.,* 2004).

There have been published descriptive studies of MM incidence and survival by race. Prior data from the Statistics Epidemiology and End Results (SEER) program and multiple risk factor intervention trial have shown consistently higher incidence and mortality rate among blacks (Altekruse *et al.,* 2010). This is also supported by studies from the USA that have shown that the incidence of MM in African Americans is two to three times more frequently compared with European Americans and that of other ethnic groups (Landgren *et al*., 2009).

The incidence of MM is related to age with the condition being more common in the elderly. The age of onset of the disease is different in developed and developing countries. In developed countries, the median age at diagnosis is 62-65 years and about a decade less in developing countries (Altekruse *et al.,* 2007). Recent statistics show an increase in incidence in individuals below 55 years of age, most of them being under the age of 40 years old at the time of diagnosis (Alexander *et al*., 2008).

It has been observed that there are gender differences in the incidence of MM and recent statistics and several studies indicate that MM is more common in males than females (Coleman *et al*., 2008).

There are various clinical features of MM, and these include bone disease, hypercalcemia, renal failure, haematological abnormalities and increased susceptibility to infections.

Myeloma cells cause damage to the bones and cause bone loss that interferes with the normal process of bone repair and growth. Bone disease occurs in 80 to 90% of MM patients. The development of bone disease can result in pain, pathological fractures, spinal cord compression and hypercalcemia (Terpos and Dimopoulous 2005).

Hypercalcemia results when there is an increase in bone resorption and calcium leakage out of the bones (Oyajobi, 2007). Up to 30% of MM patients present with hypercalcemia, this can present with central nervous system dysfunction (confusion, coma and obtundation), muscle weakness, pancreatitis, constipation, thirst, polyuria and renal failure.

Renal failure is seen in 20% to 30% of MM patients at the time of diagnosis and investigations (Eleutherakis-papaiakovou *et al.,* 2007). Recent statistics show that this damage is as a result of damage caused to renal tubules by free light chain accumulations (Bence Jones protein). When light chain accumulates in the distal tubules, tubular casts are formed and obstructive nephropathy occurs. This phenomenon is called myeloma kidney (Cohen *et al*., 2009). Other factors known to cause renal damage in MM is the hyperviscosity from excessive amounts of M protein in the blood, dehydration, hypercalcemia, nephrotoxic drugs and infections (Penifield 2006).

The growing number of myeloma cells can also interfere with the production of all types of blood cells (WBC, RBC and platelets) leading to anaemia, leukopenia and thrombocytopenia. Anemia is seen in 70% of patients at time of diagnosis and it is usually normocytic normochromic type of anaemia (Kyle *et al*., 2008).

Multiple myeloma is associated with an increased incidence of early infection especially bacterial infections mostly pneumococcal. This is related to the deficits in the body's humoral and cellular immunity. It has been reported that 10% of patients die of infective causes within 60 days of diagnosis (Augustson *et al.,* 2005).

The diagnosis of MM has been recently standardized (WHO, 2008). It is diagnosed by examining the bone marrow which shows 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 (Dimopoulos *et al*., 2011). The diagnosis should be made 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.

This study had aimed at determining the characteristics of MM detected at UTH in order to understand the biology, natural history and pathogenesis of the disease.

1.2 Statement of the Problem

Worldwide, it is estimated that 86,000 new cases of MM occur annually accounting for 0.8% of all new cancer cases and 63,000 people are reported to die from MM each year accounting for 0.9% of all cancer deaths (Parkin *et al*., 2005; Ferlay *et al*., 2010).

There is little data on the characteristics of MM in Zambia. It has however been observed that there is a steady increase in the number of myeloma cases diagnosed at UTH. This observation is supported by data obtained from the bone marrow register of the UTH Haematology laboratory from which it was found that there were 3 new MM cases diagnosed in 2010, 3 MM cases in 2011, 13 cases in 2012 and 12 cases from January to October 2013. In the absence of more data, one cannot be certain if the increased number of cases diagnosed at UTH is a true rise in incidence or just an increase in the awareness of the condition as a result of improved diagnostic criteria. Further, we do not understand the biology, natural history and pathogenesis of MM in indigenous black Zambians presenting at UTH.

1.3 Justification of the Study

This study will describe the characteristics of MM at UTH and the information generated will help in understanding the natural history, biology of the disease and the common isotype protein of MM involved in the disease.

The information generated will also provide knowledge on the cost of performing a full diagnostic workup on a patient with MM and this information could be used in the planning and budgeting for the management of patients with MM.

1.4 Literature Review

Multiple myeloma is a multistep process whose etiology is unknown. However, efforts are being undertaken to determine the etiology of hematological malignancies in general and of MM in particular (Boffeta *et al.,* 2007).

1.4.1 Pathogenesis of MM

1.4.1.1 B-cell differentiation

Normal B cell differentiation occurs in early antigen independent and late antigen dependent stages. This differentiation from stem cells to plasma cells later brings about the production of plasma cells and memory B cells (Fairfax *et al*., 2008).

During antigen independent differentiation, the precursor B cells undergo VDJ rearrangement resulting in the expression of surface IgM, and then mature into naïve resting B cells that circulate in the blood and lymphoid tissue. However, when B cells come across an antigen they aggregate in the germinal centers (spleen and lymph nodes) and undergo somatic hyper mutation and immunoglobulin class switch producing high affinity immunoglobulin.

1.4.1.2 Chromosomal abnormalities and Translocations

In MM, there are some chromosomal abnormalities that occur, and these causes loss or gain of a specific chromosomal region that include partial deletion of chromosome 13, loss of the short arm of chromosome 17 and gain of chromosome region 1q (Hanamura *et al*., 2006).

Chromosomal abnormalities then lead to translocations in MM patients that are categorized as primary early chromosomal translocations and secondary late translocations. Primary early chromosomal translocations occur at immunoglobulin switch region on chromosome 14(q 32.33) along with partner genes such as cyclin D1 (chromosome 11q13), cyclin D3 (chromosome 6p21), fibroblast growth factor receptor 3 (FGFR3)/ Myeloma SET domain protein (MMSET) (chromosome 4p16, and C-Maf chromosome 16q23) leading to deregulation of oncogenes. C-MAF helps MM cell proliferation and binds to the bone marrow stromal cells. Inhibition of FGFR3 is said to promote plasma cell differentiation and induce apoptosis (Trudel *et al*., 2006).

MMSET and FGFR3therefore, lead to immortalizations of plasma cells. Secondary late translocations and gene mutations that are implicated in the disease progression include complex karyotypic abnormalities, the activation of neuroblastoma rat sarcoma (NRAS) and Kirsten rat

sarcoma viral oncogene (KRAS) mutations in FGFR3 and TP53 and inactivation of cyclin dependent kinase inhibitors 2A and 2C.

1.4.1.5 Abnormal Chromosomes

Patients with MM are classified as being hyperdiploid (chromosome number ranging from 48 to 74) or non hyperdiploid (chromosome number less than 48). Hyperdiploidy is as a result of trisomy that involves chromosome 3, 5, 7, 9, 11, 15, 19 and 21 (Chng *et al*., 2007). Non Hyperdiploidy patients can be hypodiploidy (have less than or equal to 44 chromosomes), near-diploid (have 45-46 chromosomes) or near-tetraploid (have more than 75 chromosomes).

1.4.1.3 Bone marrow microenvironment

There is an interaction of MM cells and the bone marrow micro environment (Kyle *et al.,* 2009). Bone marrow microenvironment is made up of extra cellular matrix proteins such as vitronectin, collagen, and laminin. It also has hematopoietic stem cells, immune cells, bone marrow endothelial cells, bone marrow stromal cells, osteoclasts and osteoblasts. MM cells adhere to bone marrow stromal cells and extracellular matrix (Giuliani *et al.,* 2001).

1.4.1.4 Cytokines

Various cytokines are involved in the disease causation and progression of MM. Some of the cytokines involved are interleukin-6 (IL-6), insulin like growth factor (IGF) and vascular endothelial growth factor (VEGF).

IL-6 is implicated in the growth of myeloma cells. It binds to gp40 that is expressed on most MM cell lines thereby inducing phosphorylation of gp130. Phosphorylation of gp130 activates the Ras/Raf/mitogen activated protein kinase MAPK Kinase and JAK2. These bring about the growth and survival of malignant clones (Dinarello, 2011).

Insulin-Like Growth Factor activates MAPK P13K/AKt signaling cascade in MM cells which is the known cause of proliferation of myeloma cells and drug resistance (Podar *et al*., 2001).

VEGF is produced by neoplastic MM cells and by BMSCs. Some studies suggest that it is involved in increased angiogenesis that makes the disease to be more fatal (Bruyne *et al.,* 2010).

1.4.1.6 Development of Monoclonal Gammopathy of Undetermined Significance to Multiple myeloma

It is now known that MM represents the late stage of an underlying plasma cell disorder that is preceded by MGUS. MGUS is characterized by serum M-protein concentration of <3g/dl, less than 10% bone marrow plasma cells, and the absence of end organ damage (Kyle *et al*., 2008).

1.4.1.7 Abnormal Immunoglobulin's (Proteins)

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. The M protein may be recognized depending on the heavy chain class that is involved but is different from the normal immunoglobulin because it is only comprised of either heavy or light chain only. The light chain may be designated as kappa or lambda. They precipitate and deposit, producing organ damage. The organ mostly affected is the kidney. When these monoclonal light chains appear in urine, they are called Bence Jones protein. Bence Jones protein causes renal lesions depending on the physical or chemical properties of the light chain.

The M-Protein is a tumor marker specific for monoclonal gammopathies because it reflects the clonal proliferation of immunoglobulin (Kyle et al., 2004). 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 (Longo *et al.,* 1998). To characterize MM in respect to the protein produced, immunofixation is an important tool used.

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 (Kyle *et al.,* 2003). Immunofixation is an important tool used to classify the proteins.

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 (Kyle *et al.,* 2008).

A study done by Pandey and Kyle in 2013 in a review of IgD and IgE invariants showed that IgD MM is characterized by detection of small levels of M-protein. The patients in this study had extra medullary disease and had an advanced stage of the disease. It was also observed that the patients with this type of MM have a median age of 52 years and had shorter survival rates.

Another study done by Talano *et al* in 2010 suggested that IgE MM is less common in all MM cases. 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).

Avet-Loiseau found that Ig translocation (11; 14) (q13; q32) is not just a hall mark of IgE MM but of IgM MM and non-secretory MM and though rarely seen, the patients tend to have renal failure.

1.4.1.8 Surface markers

B cells express a number of surface markers that are used to determine their developmental stage. The cell surface markers involved in MM are CD138, markers associated with the natural killer cells such as CD56/ neural cell adhesion molecule(NCAM), T cells CD28 and in most cases pan B cell markers, CD20, CD10 and CD19 have also been seen in a number of MM cases (Paiva *et al*., 2010).

1.4.3 Staging of MM

MM is classified according to the Durie-Salmon and International Staging System (ISS) criteria. These staging systems help in knowing the extent and the biological characterization of the disease.

The Durie-Salmon stage was developed over 30 years ago to provide practical ways of measuring MM tumor burden. Patients are categorized in three stages as stage I, stage II and stage III depending on the degree of anaemia, hypercalcemia, levels of serum or urine M- protein and also looks at the extent of the bone disease found in MM (Durie *et al*., 1975).

ISS is based on the concentration of Beta-2 microglobulin and albumin, which emerged from the multivariate analysis of clinical features from approximately 11,000 previously untreated MM patients as being predictive of the survival rate (Greipp *et al.,* 2005). In this staging system, patients are categorized in three stages (stage I, stage II and stage III);

1. Stage I, patients have a median survival of 62 months, Beta-2 microglobulin < 3.5mg/l and albumin > 3.5g/dl.
2. Stage II, patients have a median survival of 44 months and have neither stage I nor III.
3. Stage III, patients have a median survival of 29 months and Beta-2 microglobulin > 5.5mg/L.

1.5 Research Question

What are the characteristics and types of multiple myeloma in patients diagnosed at UTH?

2.0 Objectives

2.1 General objective

To characterize MM detected at UTH.

2.2 Specific objectives

2.2.1 To evaluate the clinical and radiological features of MM at presentation.

2.2.2 To describe the laboratory features of MM.

2.2.3 To determine the monoclonal protein isotype of MM.

Chapter 2

Materials and methods

3.1 Study Design

This study was a laboratory based descriptive study.

3.2 Study Site

The study was conducted at the University Teaching Hospital Haematology Laboratory in the Department of Pathology and Microbiology.

3.3 Target population

All female and male patients above 18 years of age previously diagnosed with MM and being managed at the haemato-oncology clinic and at the Cancer Disease Hospital and those being investigated for MM were included.

3.4 Sample Size

A sample size of 15 was used in this study for laboratory tests based on the data obtained from the bone marrow register of the UTH Haemato-oncology clinic which had a cohort of 28 multiple myeloma patients as of 28th October 2013, of which some were lost to follow up and some had died. 36 more record files of MM patients from 2008 to 2015 April were also reviewed to give a big sample size for evaluation of clinical and radiological features of MM at presentation, which added up to a total of 46 record file reviewed. The type of study was descriptive.

3.4.1 Sampling method

In this study, convenience sampling method was used in which consecutive individuals reporting to haemato-oncology UTH clinics and CDH clinics and found to meet the inclusion criteria (given below) were included into the study.

3.4.2 Inclusion Criteria

- Individuals previously diagnosed with MM that were being managed at the UTH haemato-oncology clinic and CDH clinic.
- Individuals being investigated for MM who had MM diagnosis confirmed.

3.4.3 Exclusion Criteria

- Individuals being investigated for MM who turned out not to have MM.
- Individuals who did not provide informed consent.

3.5 Data collection and processing

3.5.1 Clinical and Demographic data collection

All individuals who were previously diagnosed with MM and were being managed at the haemato-oncology clinic, Cancer Diseases Hospital and those who were being investigated for MM and had a confirmed diagnosis were enrolled in the study. Those that were being investigated for MM were confirmed by serum protein electrophoresis that showed the presence of a monoclonal protein. As the participants were being seen by the clinician, they were being informed and explained to about the study by the clinician who also provided them with the study information sheet. When the patient agreed to be part of the study, they were required to sign the consent form and then assigned a study number. Information on the patient's demographic data and clinical features was collected from the participants clinic file and compiled using a questionnaire.

3.5.2 Specimen collection

Blood samples were collected from research participants through venipuncture from the antecubital vein using the evacuated tube system. The Clinical Laboratory Standards Institute order of draw was followed, and the tubes were collected in the following order

I. Plain container for serum protein electrophoresis, immunofixation, albumin, calcium, creatinine, urea and lactate dehydrogenase.
II. Ethylenediaminetetraacetic acid (EDTA) for full blood count.

A total volume of 8 ml of blood was collected from every participant, 4 ml in the EDTA and 4 ml in the plain container. Upon collection, specimen tubes were labeled with the participants study ID corresponding to the one on the questionnaire.

Routine urine specimens were collected from research participants in a sterile urine container for Bence Jones protein.

Within 2 hours of collection, blood and urine was transported to the Haematology laboratory for processing.

3.5.3 Specimen Preparation and Storage

In the laboratory, each specimen serial number was recorded on to a compilation summary sheet. Plain tubes were centrifuged at 1500 rpm for 15 minutes to separate serum from the blood cellular components. Only serum was 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. Two cryovials for serum were stored for every participant. Urine was also stored in a freezer at -80 ^{o}C until specimens were required for analysis.

3.6 Quality Control

To ensure reliable results, quality control was performed on all the analytical instruments and analyzers to be used for any purpose during specimen analysis. Quality control included equipment calibrations and analytical control runs on every analyzer before each test analysis.

3.7. Specimen Analysis

3.7.1 Complete Blood Count Test Protocol

Complete blood count tests were determined using Sysmex XT 4000 haematology analyzer manufactured by Sysmex Europe- supplied by Sonergy Diagnostics-Lusaka. The machine counts the numbers and types of different cells (WBC, RBC, platelets) within the blood. It then prints out the results using an in built printer that is connected to it. The two main sensors used in this analyzer are light detectors and electrical impedance that passes through the blood thereby analyzing data about the size and aspects of light as they pass through the cells called font and light scatter.

3.7.1.1. Handling

The cap of the reagent cassette was removed and all foam removed using a Pasteur pipette before placing the cassette into the Sysmex XT 4000 haematology analyzer compartment. The analyzer was then calibrated after which controls were run and passed before running the specimens.

3.7.2. Calcium, Creatinine, LDH, Urea and Albumin Test Protocol

These were determined using the available test kits on the Pentra 400 Chemistry analyzer manufactured by Horiba ABX Pentra Europe- supplied by Germany available in UTH Clinical Chemistry Laboratory 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.

3.7.2.1 Handling

The cap of the reagent cassette was removed and all foam removed using a Pasteur pipette before placing the cassette into the refrigerated ABX Pentra 400 reagent compartment. The analyzer was then calibrated using ABX Pentra Multical after which ABX Pentra N Control and ABX Pentra P Control were run and passed before running the specimens.

3.7.3 Serum Protein Electrophoresis Test Protocol

Serum protein electrophoresis was assessed using the SAS-MX Chamber manufactured by Helena Biosciences Europe- supplied by Sonergy Diagnostics-Lusaka, Zambia which is intended for the separation and quantification of serum proteins by Agarose gel electrophoresis.

3.7.3.1 Reagent Preparation

The specimens, test cartridges and the controls (Abnormal serum and Normal serum) were all allowed to attain room temperature prior to performing the test. Lot numbers of all the test components (SAS-MX Serum Protein Gel, Abnormal and Normal serum controls, Tris/ Barbital Buffer Concentrate, Acid Blue Stain and Destain Solution Concentrate) were matched with each other and not mixed up during the procedure. The test cartridges were diluted as instructed in the test insert manual. Samples and controls were diluted in a 1:4 ratio (100 µl serum: 300 µl Tris barbital buffer) before use.

3.7.3.2 Assay Procedure

The SAS-MX Serum Protein gels were removed from the packaging and placed on a paper towel. The overlay was removed and the gel surface blotted with a blotter C after which the blotter was discarded. The sample application template was then aligned with the arrows at the edge of the gel and blotter A was placed on top of the template, and a finger rubbed across the slits to ensure good contact. Blotter A was then removed. 3µl of the controls (Abnormal serum and Normal serum) and samples were applied to each slit and allowed to absorb for 4 minutes. Whilst the samples were absorbing, 25ml of the buffer was poured into each inner section of the SAS-MX Chamber. Following sample absorption, the template was blotted with the blotter A and then both blotter and template were removed. The gel was positioned in the chamber,

Agarose side down, aligning the positive and negative sides with the corresponding positions on the chamber and electrophoresed at 80volts for 30 minutes. Following electrophoresis, the gel was dried at 65 degrees Celsius for 5minutes. The dry gel was immersed in acid blue stain solution for 10 minutes and destained in destain solution until the background was clear. The gel was washed in purified water and dried. The gels were evaluated qualitatively by visually inspecting for the presence and absence of the bands of interest.

3.7.3.3 Sensitivity and Specificity

The method is sensitive to 0.3g/L per band, determined as the lowest concentration of protein which was evident as a discrete band on the completed gel.

3.7.4 Serum Protein Immunofixation Test Protocol

Serum protein immunofixation was performed using the SAS-MX Chamber manufactured by Helena Biosciences Europe- supplied by Sonergy Diagnostics-Lusaka, Zambia which is intended for the separation and identification of monoclonal gammopathies by Agarose gel electrophoresis with the Helena Biosciences SAS-MX electrophoresis chamber.

3.7.3.1 Reagent Preparation

The specimens, test cartridges and the controls (Abnormal serum and Normal serum) were all allowed to attain room temperature prior to performing the test. Lot numbers of all the test components (SAS-MX IFE Gel, SAS-MX IFE Antisera Kit, Tris/ Barbital Buffer Concentrate, Acid Blue Stain and Destain Solution Concentrate, blotter A, B, C, D and X) were matched with each other and not mixed up during the procedure. The test cartridges were diluted as instructed in the test insert manual. Serum samples were diluted 1:1(50 µl serum: 50 µl saline solution) for the SP Lane and 1:9 (10 µl serum: 90 µl saline solution) for the immunoglobulin lanes before use.

3.7.3.2 Assay Procedure

SAS-MX IFE Gel was removed from the packaging and placed on a paper towel. The overlay was removed and the surface blotted with a blotter C after which the blotter was discarded. The sample application template was then aligned with the arrows at the edge of the gel and blotter A was placed on top of the template, and a finger rubbed across the slits to ensure good contact. Blotter A was then removed. 3µl of the samples were applied to each slit and allowed to absorb

for 5 minutes. Whilst the samples were absorbing, 40ml of the buffer was poured into each inner section of the SAS-MX Chamber. Following sample absorption, the template was blotted with the blotter A and then both blotter and template were removed. The gel was positioned in the chamber, Agarose side down, aligning the positive and negative sides with the corresponding positions on the chamber and electrophoresed at 120volts for 25 minutes. Following electrophoresis, the gel was placed into the incubation chamber containing a wet blotter and the antiserum application template was then positioned onto the gel surface. 1 µl of the appropriate IFE Control was applied to the wells in the gel, the incubation chamber closed and allowed to absorb completely. 2 drops of the appropriate antiserum were applied to the immunoglobulin lanes and even distribution of antiserum ensured in the lanes by rocking the gel. The gel was incubated at 25 degrees Celsius for 10 minutes. Following incubation, the antiserum template was removed by briefly washing the gel in saline solution with gentle agitation. The gel was placed on blotter D, Agarose side up, a blotter B wetted in saline then placed onto the surface of the gel followed by blotter X. The gel was pressed in an IFE supper press for 5 minutes, the blotters removed and the gel placed in saline for 4 minutes with gentle agitation. The gel was then placed on blotter D, Agarose side up. A blotter B wetted in saline solution was placed on to the surface of the gel followed by blotter a D and the gel pressed in an IFE super press for 1 minute. The blotters were removed and the gel dried at 65 degrees Celsius. The dry gel was immersed in acid blue stain solution for 4 minutes, destained in 2 washes of destain solution and washed briefly in purified water and dried.

3.7.3.3 Sensitivity and Specificity

The majority of monoclonal proteins migrate in the cathodic, gamma region of the protein pattern, but due to their abnormal nature, they may migrate anywhere within the globulin region on protein electrophoresis. The monoclonal protein band on the immunofixation pattern will occupy the same position and shape as the abnormal band on the serum protein pattern. The abnormal protein is identified by the antiserum type it reacts with.

4.0 Statistical Analysis

Statistical analyses were performed using statistical package for social sciences (SPSS) version 20.0 and Microsoft excel. Fisher's exact test was used to test the association among clinical and radiological features and sex with a p-value of 0.05 chosen to indicate statistical significance.

Simple arithmetic mean and standard deviation were also used to calculate the mean concentrations of laboratory tests.

5.0 Ethical consideration and permissions

Permission to conduct the study was sought from the UTH Senior Medical Superintendent and CDH Senior Medical Superintendent. Permission to use equipment and facilities in the Department of Pathology and Microbiology in the UTH was sought from the Head of the Department of Pathology and Microbiology at UTH, and permission to use the equipment and laboratory facilities at the virology laboratory, chemistry laboratory and haematology laboratory was sought from the various Laboratory Managers at UTH.

Patient information and results were kept confidential and access to this information was restricted to the researcher and supervisors only. The questionnaire had captured the participants' file number, which was assigned a serial number hence specimen containers were identified by serial numbers. The file number was obtained for the purpose of retrieving clinical features at presentation.

The study participants were provided with an information sheet and given a thorough explanation and rationale of the research after which the participants had given written informed consent without duress. All the above mentioned was done in private on a one to one basis to avoid undue influence that may have affected or substituted the patient's will for that of any other persons. For some MM patients who were lost to follow up or died, their files were reviewed for the purpose of obtaining the age, sex and clinical features at presentation. This was done after seeking for permission from the CDH Senior Medical Superintendent.

The research proposal was submitted to the University of Zambia Biomedical Research Ethics Committee (UNZA-BREC) for approval and it was approved (REF. No. 006-06-14).

Chapter 3

Results

6.1. Serum protein electrophoresis

The study found that Multiple Myeloma participants had the abnormal protein (M protein) either IgG or IgA present in their serum qualitatively (Figures 1A and 1B) respectively.

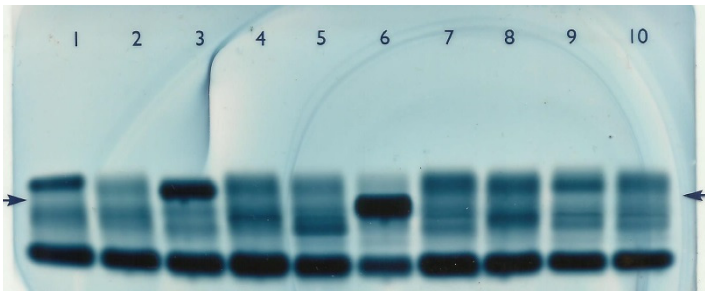


Figure 1A: Serum protein electrophoresis detection of selected MM samples. 1, Abnormal protein; 2, Normal protein; 3-10, Patient samples**.**

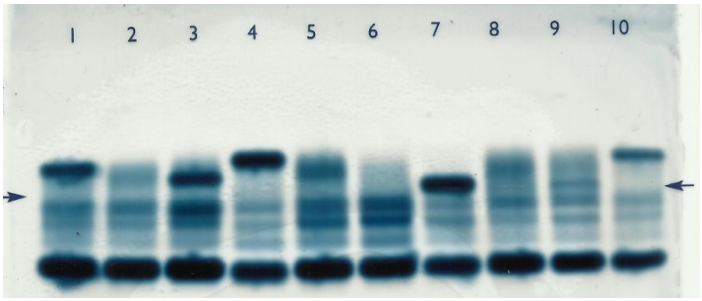


Figure 1B: Serum protein electrophoresis detection of selected MM samples. 1, Abnormal protein; 2, Normal protein; 3-10, Patient samples.

6.2. Serum Protein Immunofixation

Out of a total of 15 serum specimens analysed in the lab, 12 had IgG (80%) and 3 (20%) had IgA (Figure 2A).

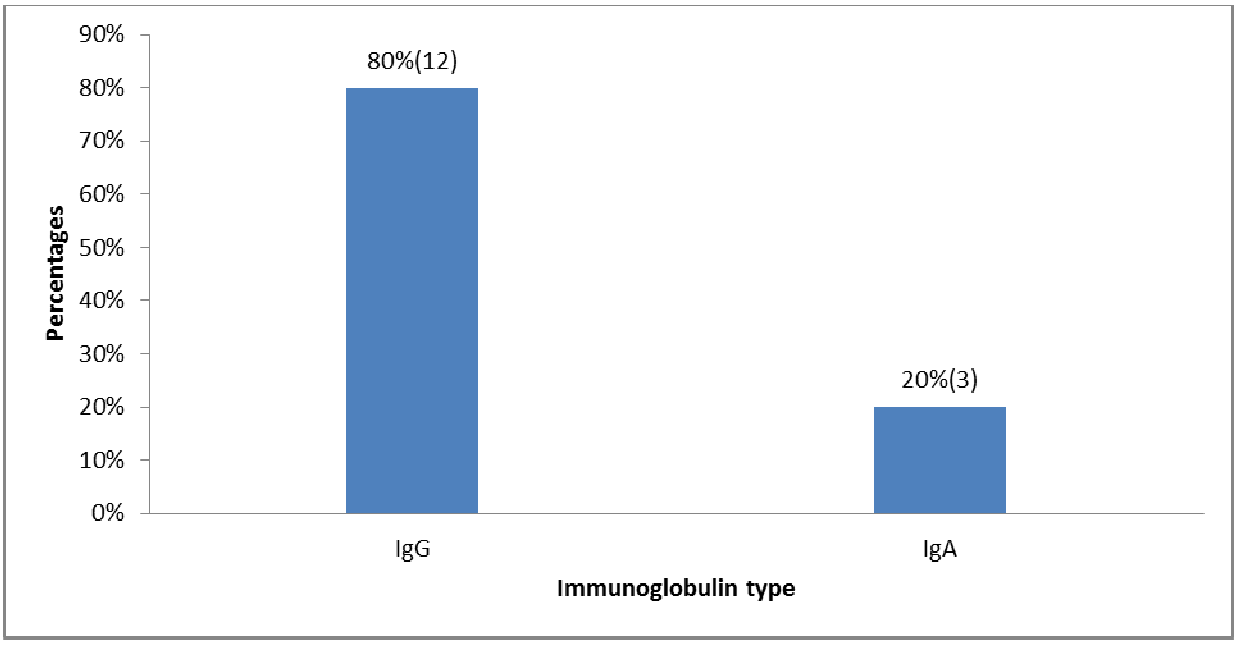


Figure 2A: Distribution of protein isotypes of MM: IgG was the predominant Ig secreted by the MM.

10 out of the 12 IgG had IgG Kappa, 1 had IgG and 1 had IgG Lamda, 2 Out of 3 had IgA Kappa and 1had IgA Lamda (Figures 2B-2Be).

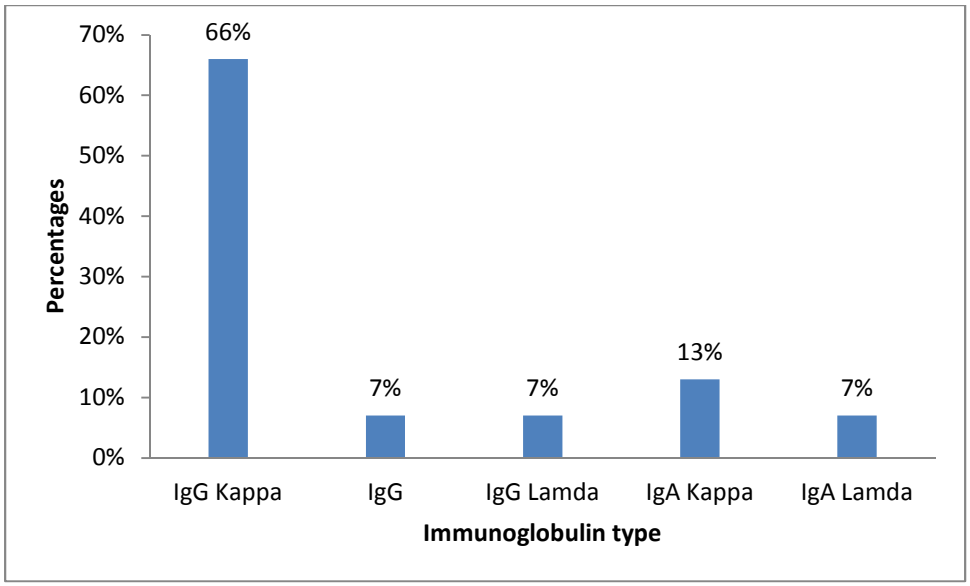
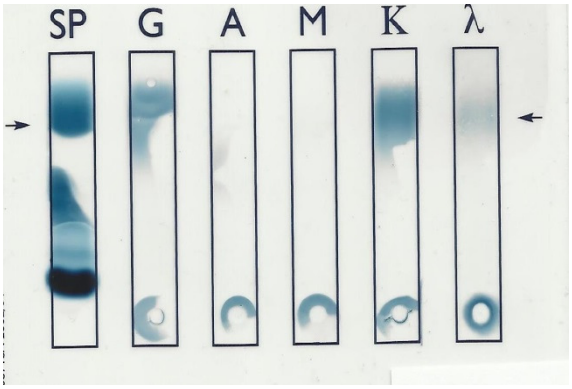


Figure 2B: Distribution of IgG and IgA characteristics of MM: IgG-Kappa was the major isotype of IgG and IgA-Kappa was the major isotype of IgA.

Figure 2Ba: Serum protein immunofixation detection of selected MM sample



SP- serum protein

G- IgG

A-IgA

M-IgM

K- Kappa light chain

λ- Lamda light chain

Figure 2B a: The band formation was seen on SP, G and K showing that the sample had serum proteins present distributed as heavy chain immunoglobulin gamma (IgG) and light chain kappa (K).

Figure 2Bb: Serum protein immunofixation detection of selected MM sample

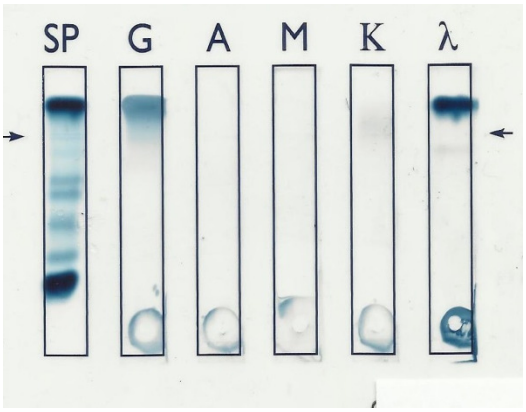


Figure 2B b: The band formation was seen on SP, G and ƛ showing that the sample had serum proteins present distributed as heavy chain immunoglobulin gamma (IgG) and light chain lamda (λ).

Figure 2Bc: Serum protein immunofixation detection of selected MM sample

Figure 2B c: The band formation was seen on SP, A and K showing that the sample had serum proteins present distributed as heavy chain immunoglobulin alpha (IgA) and light chain kappa (K).

Figure 2Bd: Serum protein immunofixation detection of selected MM sample

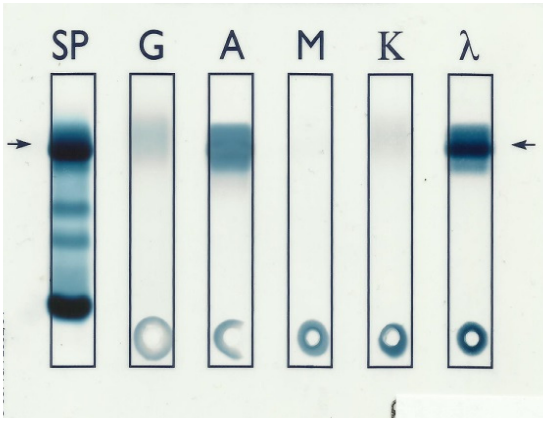


Figure 2B d: The band formation was seen on SP, A and ƛ showing that the sample had serum proteins present distributed as heavy chain immunoglobulin alpha (IgA) and light chain lamda (ƛ).

Figure 2Be: Serum protein immunofixation detection of selected MM sample

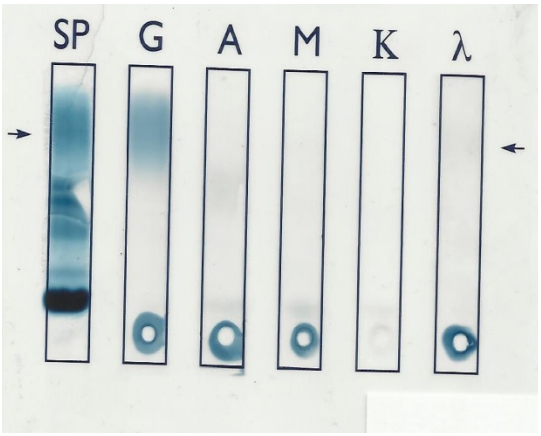


Figure 2B e: The band formation was seen on SP and G showing that the sample had serum proteins present distributed as heavy chain immunoglobulin gamma (IgG) and no light chain was detected.

6.3 Clinical and Radiological Features

46 Record files of patients diagnosed with Multiple myeloma from 2008 to April 2015 were reviewed. The median age at diagnosis was 53.0 (range 32-81 years). From 46 patients, 25 (54%) were men and 21(46%) were females. The study showed that the patients presented with 5

clinical parameters, 3 laboratory parameters and 2 radiological parameters (Table.1a-1c and figures 3A-3J).

6.3 Clinical and radiological features of MM at presentation

Table 1a: Clinical features of MM at presentation

Clinical features	Absolute count	Percentages
Back pain	27/46	59%
Bone pain	21/46	46%
Chest pain	12/46	26%
Fatigue	7/46	15%
Epistaxis	2/46	4%

Table 1a: Distribution of clinical features at presentation in order of occurrence from the highest to lowest.

Table 1b: laboratory features of MM at presentation

Laboratory parameters	Absolute count	Percentages
Anaemia	14/46	30%
Hypercalcemia	5/46	11%
Renal dysfunction	3/46	7%

Table 1b: Distribution of Laboratory features at presentation in order of occurrence from the highest to lowest.

Table 1c: Radiological features of MM at presentation

Radiological parameters	Absolute count	Percentages	Method of diagnosis
Pathological fractures	12/46	26%	X-ray and CT scan
Osteolytic lesions	30/46	65%	X-ray and CT scan

Table 1c: Distribution of Radiological features at presentation: osteolytic lesions were the most frequent feature.

Anaemia

Anaemia is defined as haemoglobin value less than the lower limit of normal for age and sex (12-18g/dl). 30% of total patients had anaemia. 8 (17.4%) females and 6 (13.0%) males had anaemia. Prevalence of anaemia between males and females was statistically insignificant with a p-value of 0.349 (Figure 3A).

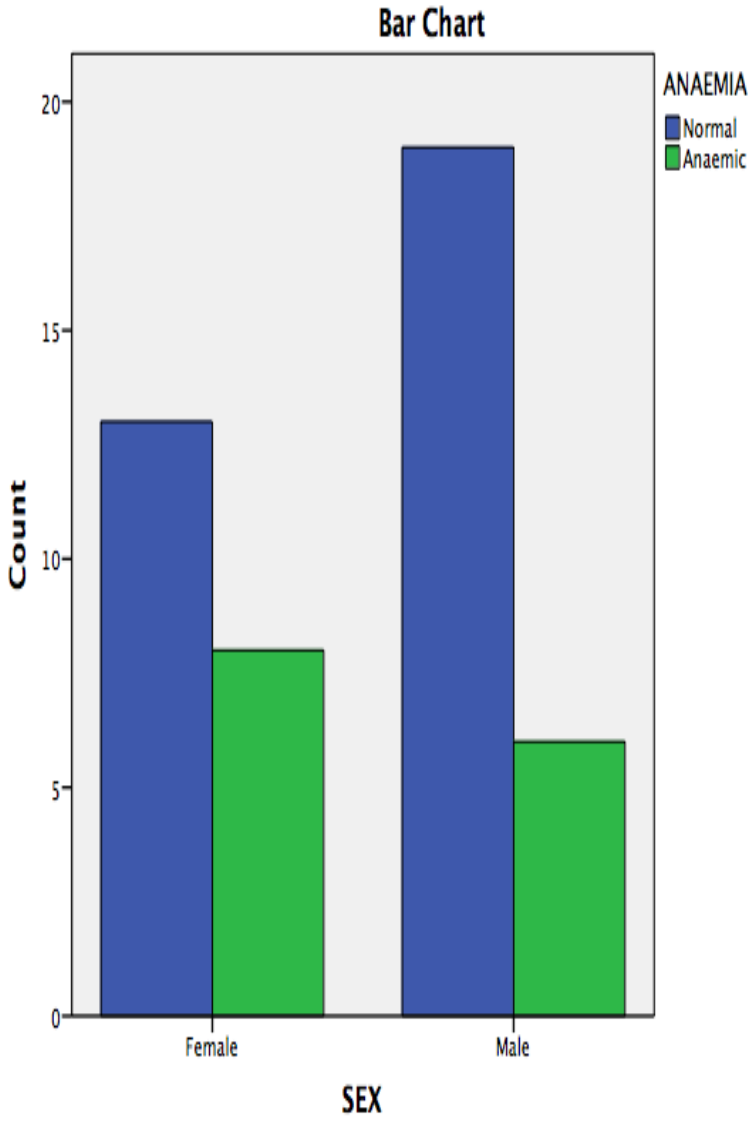


Figure 3A: Distribution of Anaemia between males and females: Anaemia was more in females compared to males.

Bone Pain

45% of total patients had bone pain.10 (47.6%) females and 11 (44.0%) males had bone pain. Prevalence of bone pains between females and males were statistically insignificant with a p-value of 1.000 (Figure 3B).

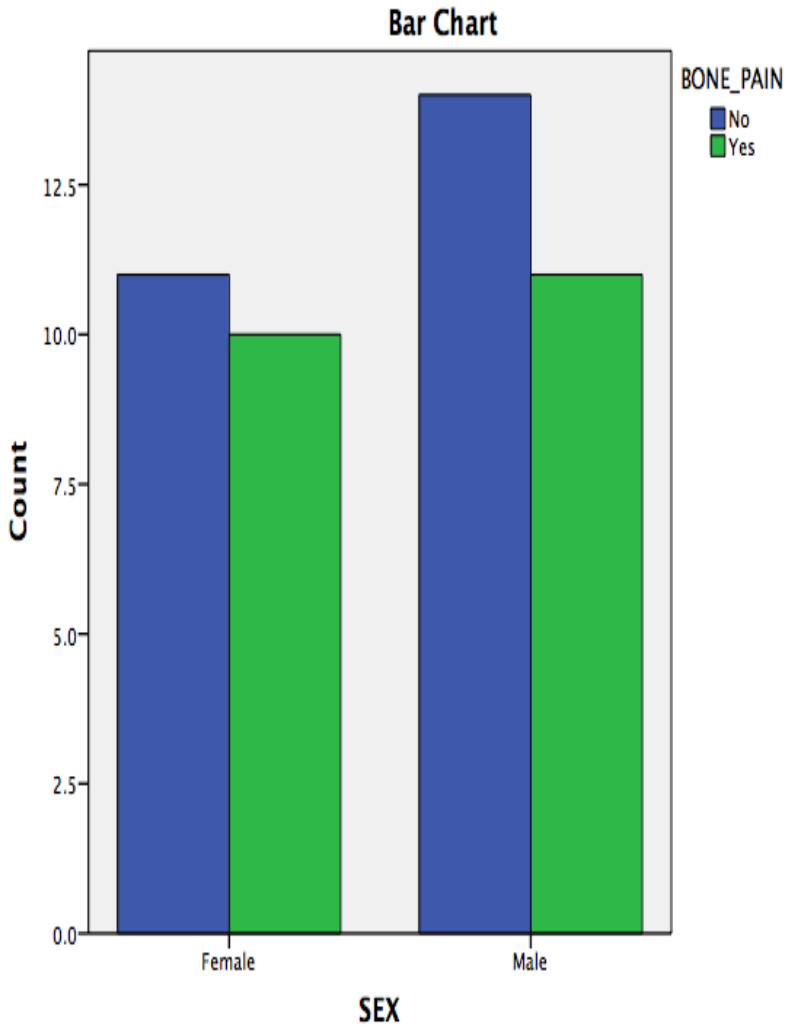


Figure 3B: Distribution of bone pain between males and females: Males had more bone pains.

Hypercalcemia

Hypercalcemia is defined as calcium value above 2.55 mmol/L (0.8-2.2mmol/L). Hypercalcemia was only present in 5 (10.9%) male participants. Prevalence of hypercalcemia in males was statistically insignificant with a p-value of 0.054 (Figure 3C).

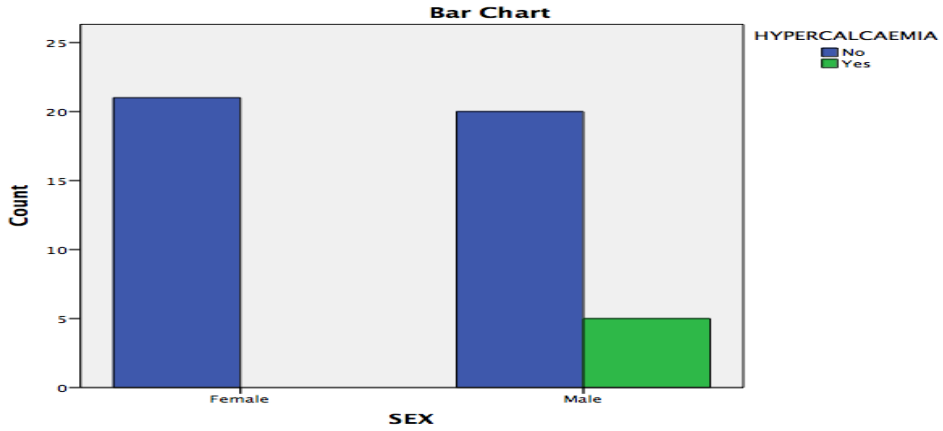


Figure 3C: Distribution of hypercalcemia between males and females: Hypercalcemia was only present in males.

Fatigue (Weakness)

A total of 15% participants had fatigue. 6(13.0%) females and1 (2.2%) male had fatigue. Females had a significantly higher proportion of fatigue than males with p-value = 0.036 (Figure 3D).

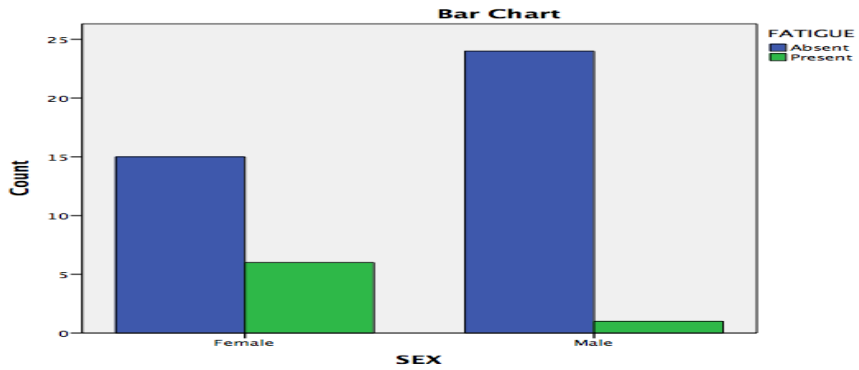


Figure 3D: Distribution of fatigue between males and females: Females had more fatigue.

Chest pain

A total of 26.1% had chest pain as a symptom, 6(13.0%) females and 6(13.0%) males. Prevalence of chest pains between females and males were statistically insignificant with a p-value of 0.749 (Figure 3E).

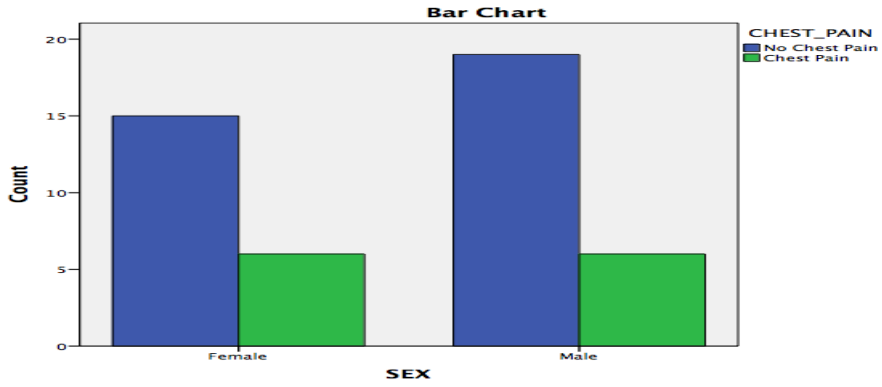


Figure 3E: Distribution of chest pain between males and females: Chest pain was equally distributed between males and females.

Pathological fractures

Total of 26.1% had pathological fractures.4 (19.0%) females and 8 (32.0%) males. Prevalence of pathological fractures between females and males were statistically insignificant with a p-value of 0.502 (Figure 3F).

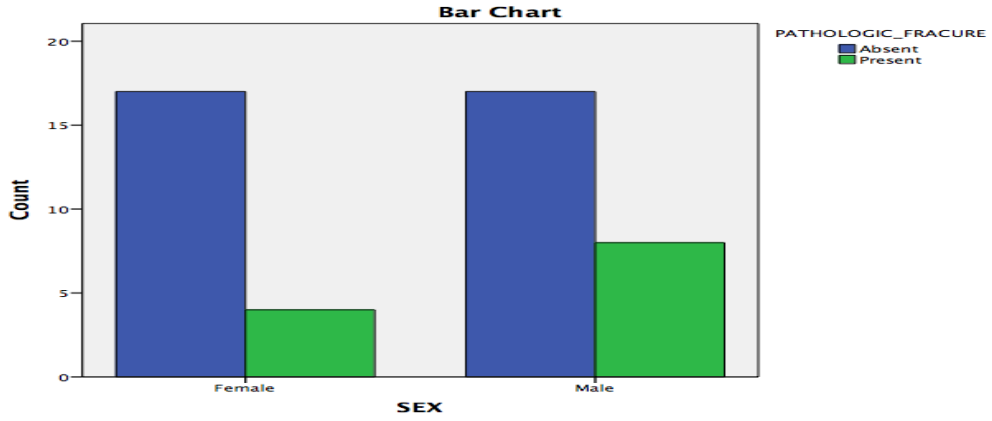


Figure 3F: Distribution of pathological fractures between males and females: Pathological fractures were more in males.

Osteolytic lesions

Total 65.2% of the patients had osteolytic lesions, 15 (32.6%) females and15 (32.6%) males. Prevalence of osteolytic lesions between males and females were statistically insignificant with a p-value of 0.538 (Figure 3G).

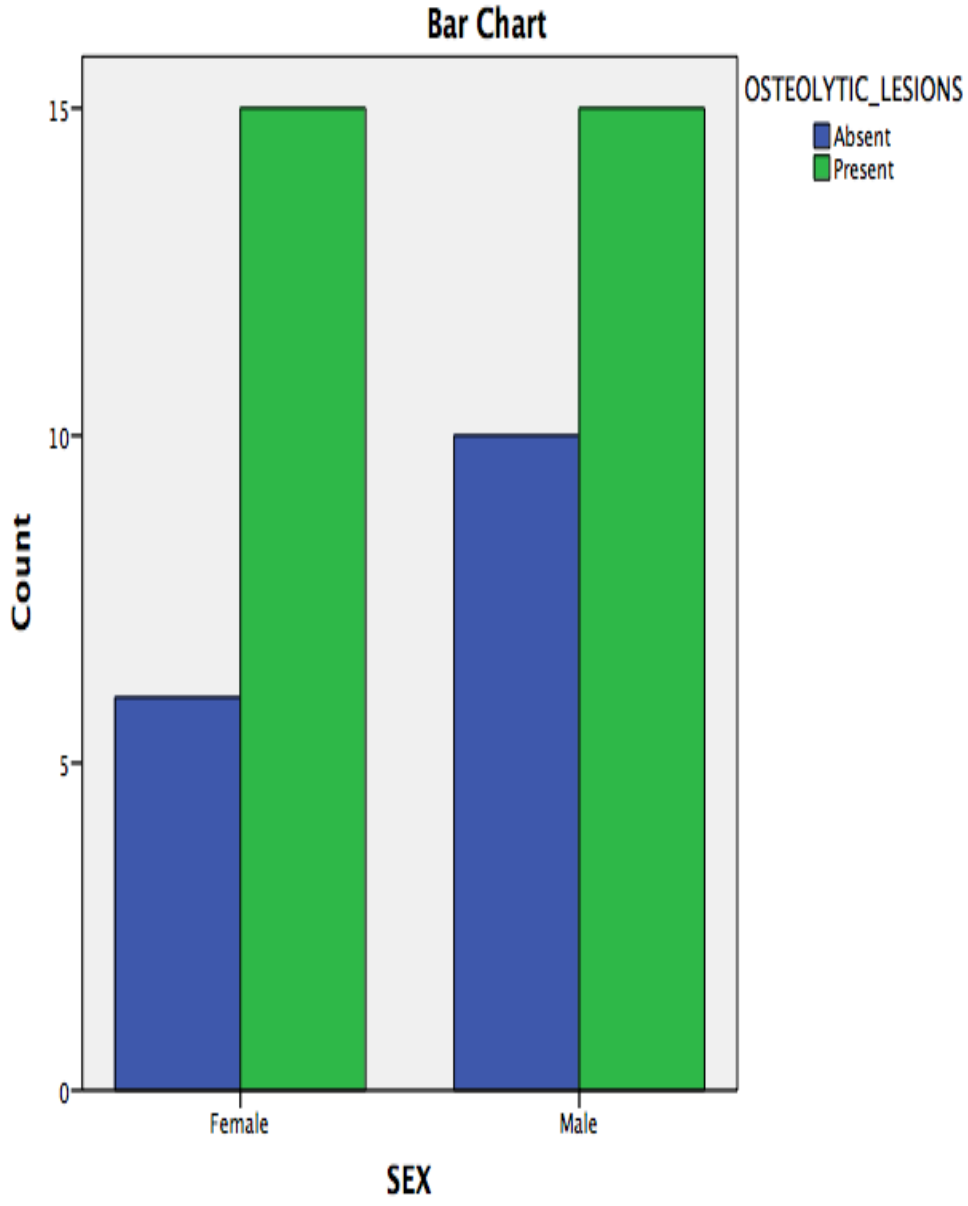


Figure 3G: Distribution of osteolytic lesions between males and females.

Bone pain vs. pathological fractures

5(10.9%) had both bone pain and pathological fractures, 16 (34.8%) bone pain without pathological fractures and 7 (15.2%) had pathological fractures without bone pain. Prevalence of bone pain in individuals with pathological factures was statistically insignificant with a p-value of 1.000 (Figure 3H).

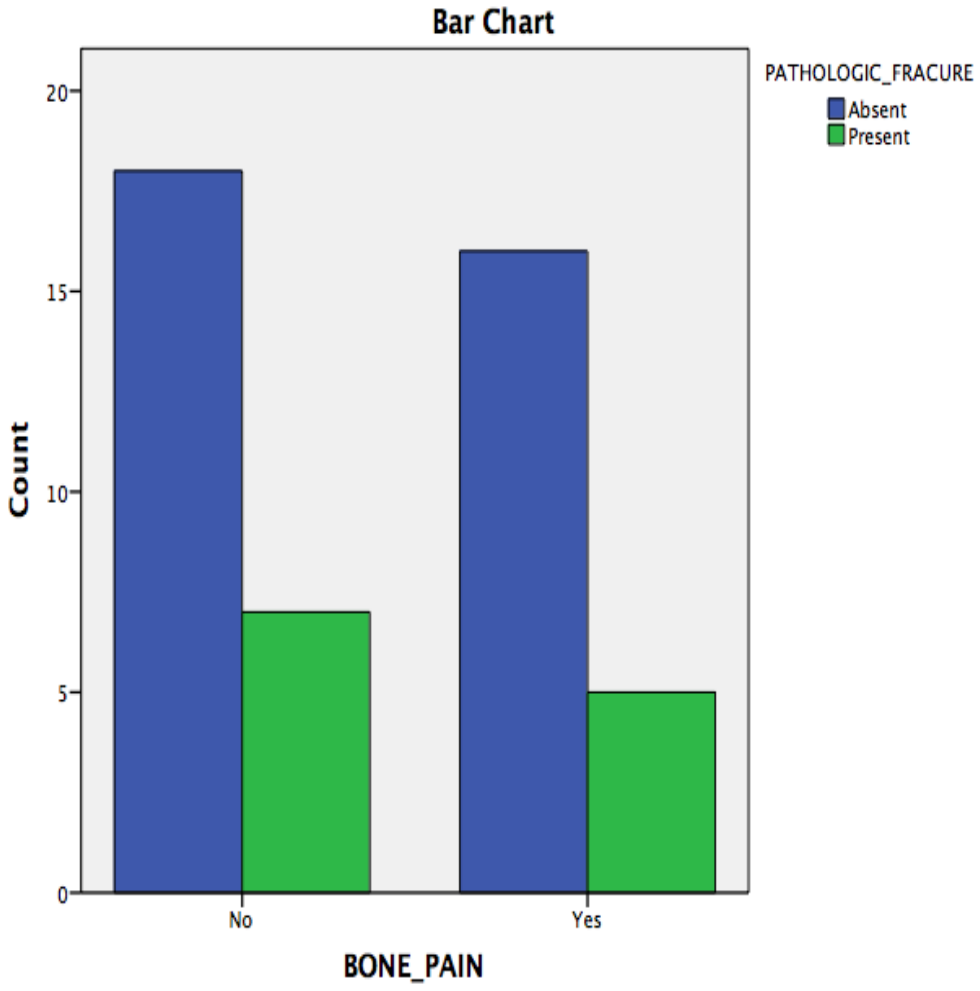


Figure 3H: Distribution of bone pain in individuals with pathological fractures: There were more individuals with bone pain without pathological fractures.

Bone pain vs. osteolytic lesions

16(34.8%) had bone pain and osteolytic lesions, 5 (10.9%) only had bone pain without osteolytic lesions and14 (30.4%) had osteolytic lesions without bone pain. Prevalence of bone pains in individuals with osteolytic lesions were statistically insignificant with a p-value of 0.217 (Figure 3I).

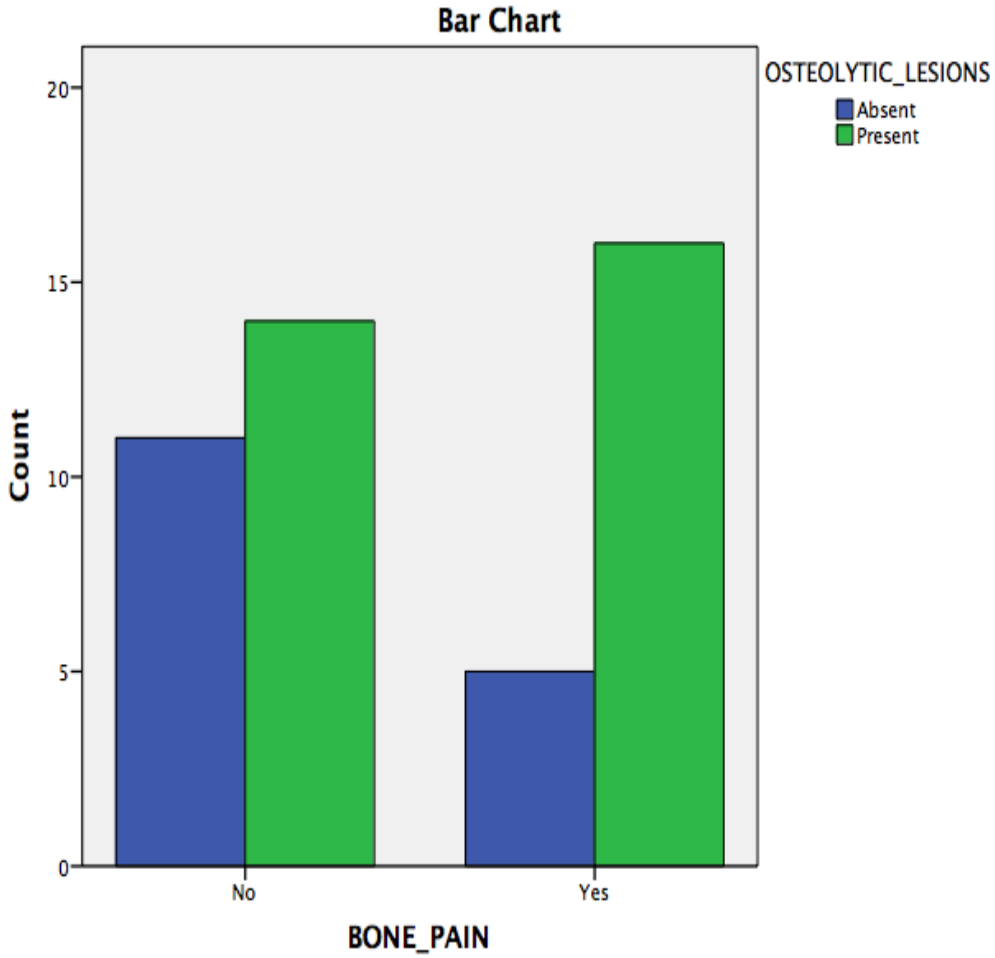


Figure 3I: Distribution of bone pain in individuals with osteolytic lesions: There were more individuals with osteolytic lesions without bone pains.

Osteolytic lesions vs. Pathological fractures

3(6.5%) had both pathological fractures and osteolytic lesions, 27(58.7%) had osteolytic lesions but without pathological fractures and 9(19.6%) had pathological fractures without osteolytic lesions. Prevalence of pathological fractures in individuals with osteolytic lesions was statistically significant with a p-value of 0.001 (Figure 3 J).

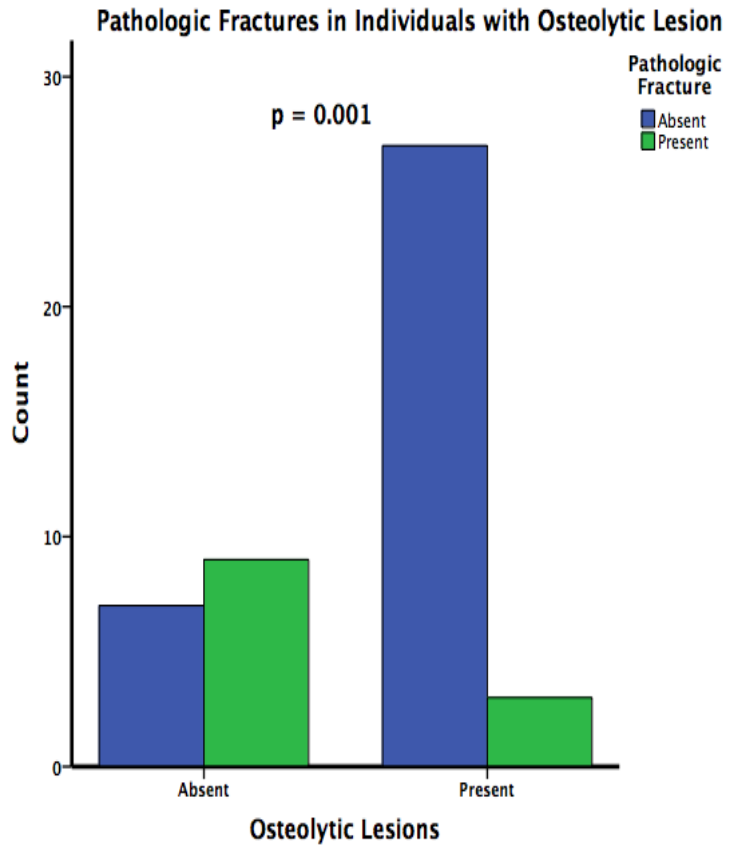


Figure 3J: Distribution of pathological fractures in individuals with osteolytic lesions: There were more individuals with osteolytic lesions but without pathological fractures.

6.4 Laboratory Characteristics

The mean value (which is a sum of a collection of numbers divided by the number of all observations) of laboratory tests done were calculated and it was observed that MM participants had some normal values of haematocrit, white blood cells, platelets, calcium and urea (Table.2). They also had abnormally high levels of creatinine and lactate dehydrogenase and low levels of haemoglobin and albumin compared to the normal reference ranges (Table. 2).

Table 2: Mean Concentrations of Laboratory Tests

Variables	**Mean concentrations**	**Reference values**
Haemoglobin	11.4 ± 3.83 g/Dl	12 g/dL - 18 g/dL
Haematocrit	41 ± 14.99%	34.9-50%
White Blood Cell Count	4.8 ± 1.52× 10^9 /L	3.5-10.5 × 10^9 /L
Red Blood Cell Count	4 ± 1.48× 10^9 /L	3.9-5.7 × 10^9 /L
Platelets	221.7 ± 93.9× 10^9 /L	150-450 × 10^9 /L
Calcium	2.5 ± 0.21 mmol/L	2.10 - 2.55 mmol/L
Lactate dehydrogenase	229.1 ± 95.3 U/L	115 - 211 U/L
Urea	7.9 ± 6.31 mmol/L	2.5 - 8.0 mmol/L
Creatinine	125.1 ± 64.1 µmol/L	50 - 110 µmol/L
Albumin	17.3 ± 5.78 g/dL	35 - 50 g/dL

Table 2: HCT, WBC, RBC, platelets, calcium and urea values were normal, Hb and albumin values were low, and creatinine and LDH were high.

Chapter 4

Discussion

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

There are no incidence figures of MM in Zambia. Similarly, there is paucity of data from other developing countries. It is not clear if MM is more common in the developing countries. To better understand the biological and clinical characteristics, and the pathogenesis of MM at the University Teaching Hospital, we evaluated the clinical and radiological features at presentation, determined the laboratory characteristics and determined the protein isotype of MM seen.

Clinical features

Multiple myeloma is characterized by one or more of the following features which are denoted by the acronym CRAB (Hypercalcemia, renal disease, anaemia and bone abnormalities including osteolytic lesions, pathological fractures, backache and osteopenia) (Nau and Lewis, 2008).The results of this study showed back pain, bone pain, epistaxis, fatigue and chest pain as the clinical features, anaemia, hypercalcemia and renal impairment as laboratory features, pathological fractures and osteolytic lesions as radiological features of MM at presentation.

The most frequent and common features in order of occurrence were osteolytic lesions, back ache, bone pain, anaemia, pathological fractures, chest pain and fatigue.

Females had a higher proportion of fatigue than men with (P= 0.036). This finding is similar to the findings of the study done in the USA by (Coleman *et al.,* 2012) in which patients diagnosed with MM had fatigue and it was more in females than males. This finding could be attributed to anaemia that is caused by the myeloma cells infiltrating the bone marrow and thus not making enough red blood cells. Fatigue is a problem in the MM population as many of the patients are usually older individuals who have issues that are straining their physical, mental and functional capacity.

The osteolytic lesions in this study were seen in 65% of patients at presentation and this is similar to the findings of a study done by Kyle *et al* in 2003 in the USA who found that out of 1027 patients diagnosed with MM, osteolytic lesions were found in 66% of patients. Osteolytic lesions usually occur in late stages when the disease has progressed. The presence of osteolytic

lesions is a hall mark of MM. These lesions are as a result of increased bone resorption due to increased levels of IL-6 which activates osteoclasts. These lesions represent uncoupling between osteolytic and osteoblastic activities (Casciato and Territo, 2009).

Another study done by Bataile *et al* in 2003 also found that almost all MM patients develop osteolytic lesions. These lesions result in associated abnormalities such as pathological fractures, bone pain, backache and hypercalcemia.

Bone and back pain are common presenting features in MM. Majority of patients in this study had bone and back pains and this is similar to the findings of a study done in Nigeria by Talamo *et al* in 2010. Two thirds of patients complain of bone pain frequently located in the back, long bones, skull and pelvis (Chen *et al.,* 2012). This could be due to osteolytic lesions, bone metastases and associated pathological fractures.

Pathological fractures are also present in about 30% of patients at diagnosis. In this study, pathological fractures were seen in 26% of patients which is slightly lower than the ones seen in other studies. Salawu *et al* observed pathological fractures in 44% of MM. This study also found that pathological fractures in individuals with osteolytic lesions were statistically significant with (P=0.001). This should be a red signal for clinicians to quickly investigate for MM in patients presenting with both pathological and osteolytic lesions.

Anaemia was seen in 30% of patients in this study. This is similar to the findings of a study in Nigeria done by Fasola *et al* in 2012. Anaemia is caused by ineffective erythropoiesis as a result of myeloma cells infiltrating the bone marrow. These findings are also at variance with the findings of Kyle *et al* in the USA in 2008 who recorded anaemia in 70% of patients. This variance could be because of a small sample size in our study. Anaemia may promote tumor hypoxia which is thought to impart resistance to irradiation and some chemotherapeutic agents and to give rise to malignant progression (Ludwig *et al.,* 2002). Therefore, adequate iron supplies are necessary to support increased erythropoiesis.

Chest pain was seen in 26% of patients. Many patients with MM develop chest infections caused by bacteria. This increased susceptibility of infections is as a result of hypogammaglobulinemia, granulocytopenia and low cell mediated immunity. The common pathogens implicated in these infections are the gram positive organisms e.g. *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae* (Augustson *et al.,* 2005).

The findings of this study are at variance with the findings of a study done in Taiwan by Shang-yi *et al* who found plasmacytoma and extra medullary myeloma as the clinical features of MM. This variance could be attributed to the late diagnosis of MM.

Protein isotype of Multiple myeloma

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 (Kyle and Rajkumar 2004).

Another study done by Kyle *et al* in 2009 in the USA in the diagnostic criteria of MM 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%.

Our findings are not in agreement with the findings of the study by Kyle *et al* in the USA in 2003 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. Our study showed a dominance of IgG MM over IgA MM.

The clinical features of IgG and IgA MM which are bone pain, anaemia and renal failure are said to be similar to those of IgE and light chain MM. In a study done by Macro *et al* in 2008, it was reported that the mean age at diagnosis of IgG and IgA was 62 years with a slight predominance of male patients. 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 done by Hobbs *et al* in 1969 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.

Management of IgG and IgA is similar to that of other isotypes (Dimopoulous *et al.,* 2011). Nevertheless, the monitoring of disease response to treatment of IgG and IgA MM may be different because of less excess antigens (Talamo *et al.,* 2010).

IgG and IgA MM has been reported to have a better prognosis than other types of MM (Reece *et al.,* 2010). However, these findings are in contrast to the findings of Gertz *et al* in 2012 who reported that the survival of IgG and IgA MM are not different from IgD, IgE, IgM and light chain MM.

Relapse with the rising levels of free light chain and no change in paraprotein occurs in 5% of IgG and 15% of IgA MM (Mead and Drayson 2009).

A study done by Merlini et al in 1980 showed that in MM, the prognostic variables depend on the types of paraprotein produced. They found that serum creatinine, calcium and percentage of bone marrow plasma cells predicted prognosis for IgG and Bence Jones MM patients whereas Hb and calcium together with the level of monoclonal protein were predictive for IgA MM.

Laboratory characteristics

In this study, the mean values of laboratory tests done were calculated and it was observed that the values for HCT, WBC, RBC, 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 (Salawu *et al.,* 2005). 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 (Kumar *et al.,* 2001). Anaemia may promote tumor hypoxia which is thought to impart resistance to irradiation and some chemotherapeutic agents and to cause malignant progression (IMWG, 2003).

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 done by Cheng *et al* who found low albumin levels in MM patients. The lower albumin concentration among patients may be due to a homeostatic mechanism controlling the plasma oncotic pressure causing changes in serum albumin (Kyle et *al.,* 2008). 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 (Cheng *et al.,* 2012).

It was also observed that LDH concentrations (229 IU/L) were higher compared to the normal reference ranges of 115 - 211 IU/L, and these findings are similar to the findings of a study done in Turkey(Teke *et al.,* 2013) 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 with Beta 2 microglobulin (Dimopoulous *et al.,* 1991). 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 refer to the increased level of tumor or relapse (Sanal *et al.,* 1996).

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 toxic effects of light chains to renal structures (Cohen *et al.,* 2009). Patients presenting with renal failure have an early death rate (Augustson *et al.,* 2005). It is therefore important to prevent renal failure (Clark *et al.,* 2005) as this will improve survival (Knudsen *et al.,* 2000).

7.1 Conclusion

This study showed that IgG and IgA were the common isotype protein of MM in blood of patients presenting at UTH. The predominant light chain of the IgG and IgA was Kappa. It was further shown that osteolytic lesions which are a radiological feature of MM were the most frequent feature in patients presenting with MM at UTH, Lusaka Zambia.

Findings of this study suggest that patients who are in their fifth decade complaining of chronic backache and bone pain should quickly be investigated for MM by SPEP, IFE, UPEP and serum free light.

7.2 Implications and Recommendations

The major problem of MM is that it is not curable and it has a poor prognosis and causes end organ damage (anaemia, renal insufficiency, hypercalcemia and bone disease).The early diagnosis of both new and relapsed MM enables early intervention and prevention of the damage caused by the myeloma cells (Drayson *et al.,* 2006).

Serum protein electrophoresis and immunofixation are simple and important diagnostic tools to diagnose and characterize MM which could help in diagnosis and checking response to treatment (Rajkumar *et al.,* 2009). Therefore, the findings of this study also suggests that all international myeloma working group diagnostic criteria should be used to diagnose MM and the new staging system based on serum albumin and Beta-2 microglobulin. These diagnostic criteria will help in the proper budgeting for MM management.

7.3 Limitations/ Weaknesses and Assumptions

- The study was limited to MM patients who came in for their routine follow up conveniently thus having a small sample size.
- Some record files were not completely filed in.
- The study had a participant who did not show a light chain on immunofixation thus, urine protein electrophoresis and serum free light chain tests would have provided light chain detection.
- The study did not provide any data pertaining to incidence and prevalence of MM in Zambia.
- The study did not provide any data pertaining to staging, a follow up programme could have provided morbidity and mortality data for our participants.
- Staging of MM analysis would have been an important tool for determining the extent of disease.
- The study did not have a follow up programme to assess the prognostic significance of phenotypic markers of MM. A follow up would have provided the response to treatment of the patients involved.

7.4 Future Directions

- With respect to the above considerations, more supportive and definitive investigations are required. Follow up studies may be warranted to determine the stage at presentation of MM patients.

8.0 References

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9.0 Appendices

Appendix 1: Table 3: Multiple myeloma individuals Raw Data on clinical presentations

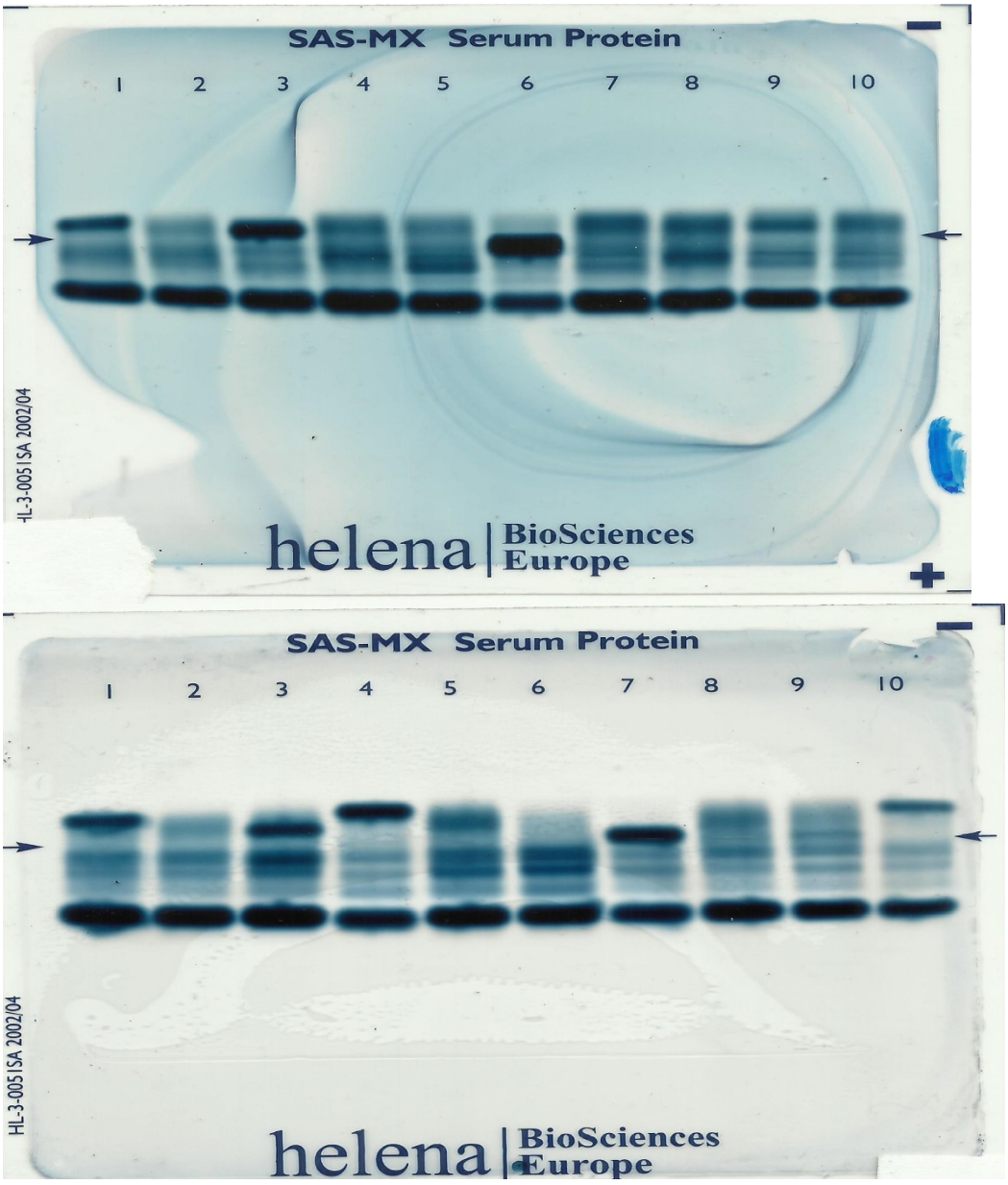
No#	AGE	SEX	CLINICAL FEATURES							
			Anaemia	Bone pain	Backache	Hypercalceamia	Epistaxis	Renal dysfunction	Fatigue	Chestpain
001	64	F	1	1	0	0	0	0	1	1
002	63	M	0	1	1	0	0	0	1	1
003	48	M	0	1	1	0	0	0	0	0
004	58	F	1	1	0	0	0	1	0	0
005	35	F	0	0	1	0	0	0	1	1
006	58	F	0	0	0	0	0	0	1	0
007	61	M	1	0	0	0	0	0	0	1
008	50	M	0	1	1	1	0	0	0	0
009	42	M	0	1	1	0	0	0	0	0
010	50	F	1	1	0	0	0	0	0	0
011	33	F	0	1	0	0	0	0	0	0
012	58	F	1	1	0	0	0	0	1	0
013	61	F	1	0	1	0	0	0	0	0
014	63	M	0	1	1	0	0	0	0	0
015	56	M	0	1	1	0	0	0	0	0
016	48	M	1	0	0	0	1	1	0	0
017	55	M	1	0	0	1	0	0	0	0
018	32	F	0	0	1	0	0	0	1	0
019	59	M	1	0	0	0	0	0	0	0
020	48	M	0	1	0	0	0	0	0	0
021	35	M	0	1	0	0	1	0	0	1
022	59	M	0	1	0	0	0	0	0	0
023	52	F	0	0	1	0	0	0	0	0
024	37	M	0	0	1	0	0	0	0	0
025	50	M	0	0	1	1		0	0	1
026	35	M	0	0	1	0	0	0	0	0
027	50	M	0	0	1	1	0	0	0	0
028	58	M	0	1	1	0	0	0	0	0
029	70	M	0	0	1	0	0	0	0	0
030	81	F	0	0	1	0	0	0	0	0
031	73	F	0	1	1	0	0	0	0	1
032	79	M	1	0	1	0	0	1	0	0
033	64	F	1	0	1	0	0	0	1	0
034	51	M	0	0	1	0	0	0	0	1
035	47	F	0	1	0	0	0	0	0	0
036	50	M	0	0	1	0	0	0	0	0
037	68	F	0	1	0	0	0	0	0	0
038	50	M	1	0	1	1	0	0	0	1
039	52	M	0	0	1	0	0	0	0	0
040	33	F	0	1	0	0	0	0	0	0
041	57	F	0	0	0	0	0	0	0	1
042	46	F	0	0	1	0	0	0	0	1
043	63	F	0	0	1	0	0	0	0	0
044	50	F	1	0	1	0	0	0	0	0
045	42	M	0	1	0	0	0	0	0	0
046	42	F	1	1	0	0	0	0	0	1
047										

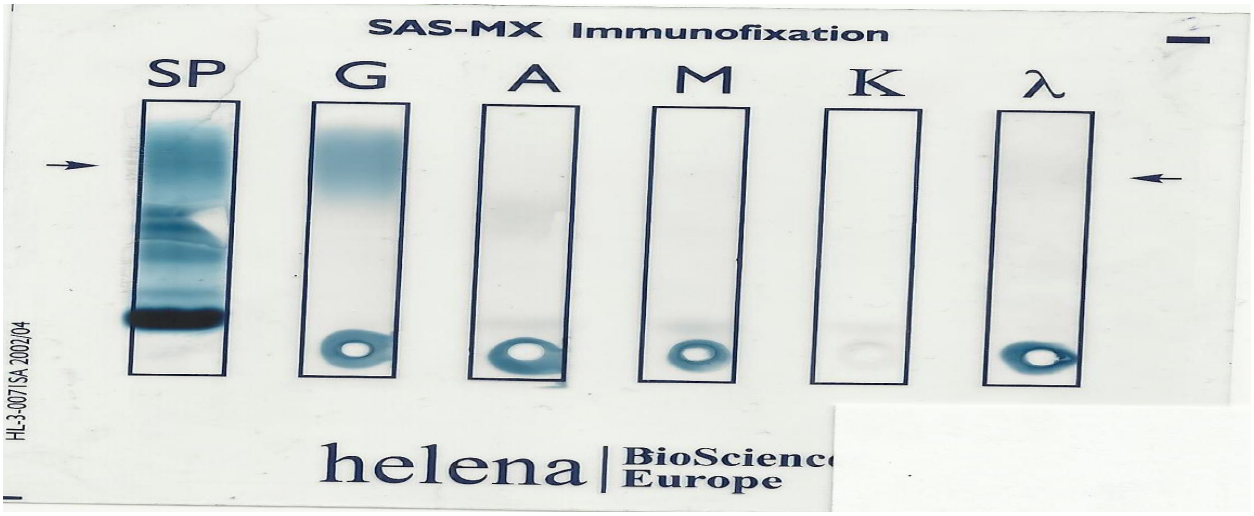
NOTE: 0=ABSENT, 1=PRESENT

Appendix 2: Table 4: Multiple myeloma individuals Raw Data on tests done

Results	g/dl	mmol/l	U/L	mmol/l	µmol/L	g/dl	10^9/L	10^9/L	%	10^9/L
Ig type	HB	Calcium	Lactate d	Urea	Creatinine	Albumin	WBC	RBC	HCT	Platelets
IgA Lamda	7.3	2.58	201	4.6	137	32	2.08	1.89	22	144
IgG	13.8	2.48	196	6.1	100	41	3.77	5.1	47.7	185
IgG Kappa	13.9	2.27	295	7.6	125	32	7.21	4.38	48.5	210
IgA Kappa	4.2	3.15	101	20.6	204	19	3.78	1.48	16.1	118
IgG Kappa	12.6	2.42	96	3.3	73	41	3.55	3.9	42	250
IgG Kappa	12.4	2.35	435	4.4	108	39	3.75	4	44.3	289
IgG Kappa	12.1	2.31	155	2.8	77	36	3.33	4.18	43.2	233
IgG Kappa	17.6	2.33	159	2.8	75	40	5.02	5.64	58.2	183
IgG Kappa	13.8	2.45	148	3.6	87	38	5.99	6.88	72.5	215
IgG Lamda	6.7	2.21	347	23.5	302	28	3.98	2.33	23.3	44
IgG Kappa	12.7	2.45	266	9.1	140	36	4.75	4.5	44.6	432
IgG Kappa	8.4	2.54	280	14.7	211	35	5.42	2.9	30.2	314
IgG Kappa	5.7	2.32	350	7.4	81	33	7.51	2.35	22.3	166
IgA Kappa	14.1	2.57	164	3.8	83	36	5.04	4.7	47.8	185
IgG Kappa	15.9	2.4	243	4.7	73	42	7.04	5.52	53	357

Appendix3: Serum protein and Immunofixation figures




SAS-MX Immunofixation
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HL-3-007 ISA 2002/04
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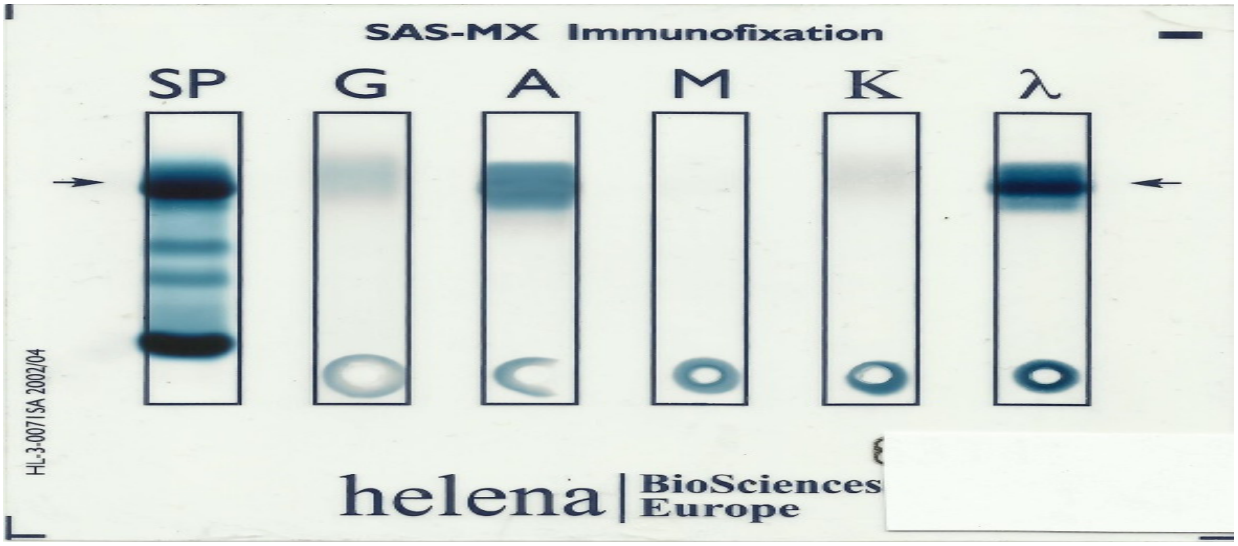
SAS-MX Immunofixation
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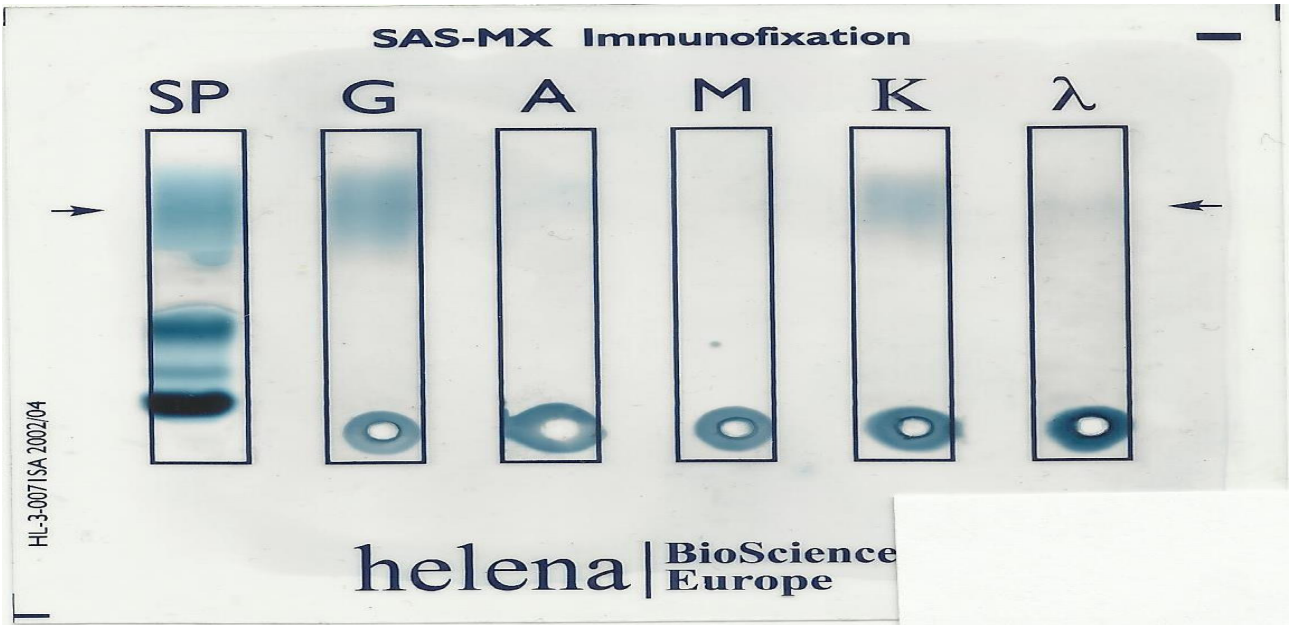
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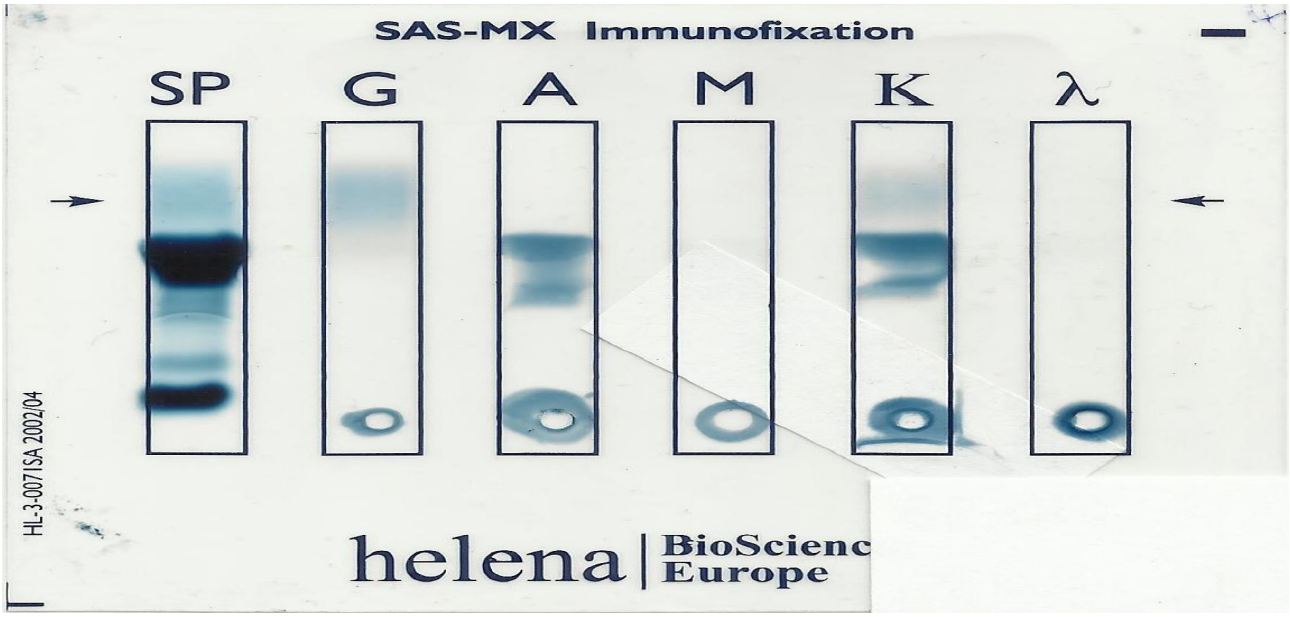
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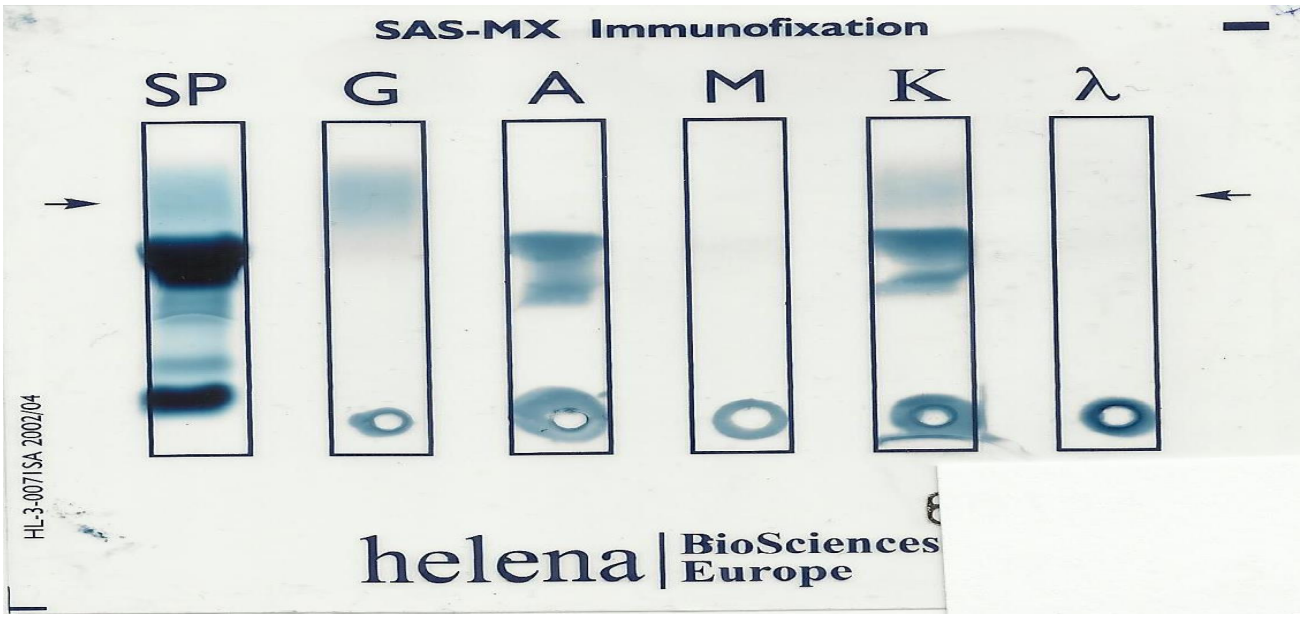
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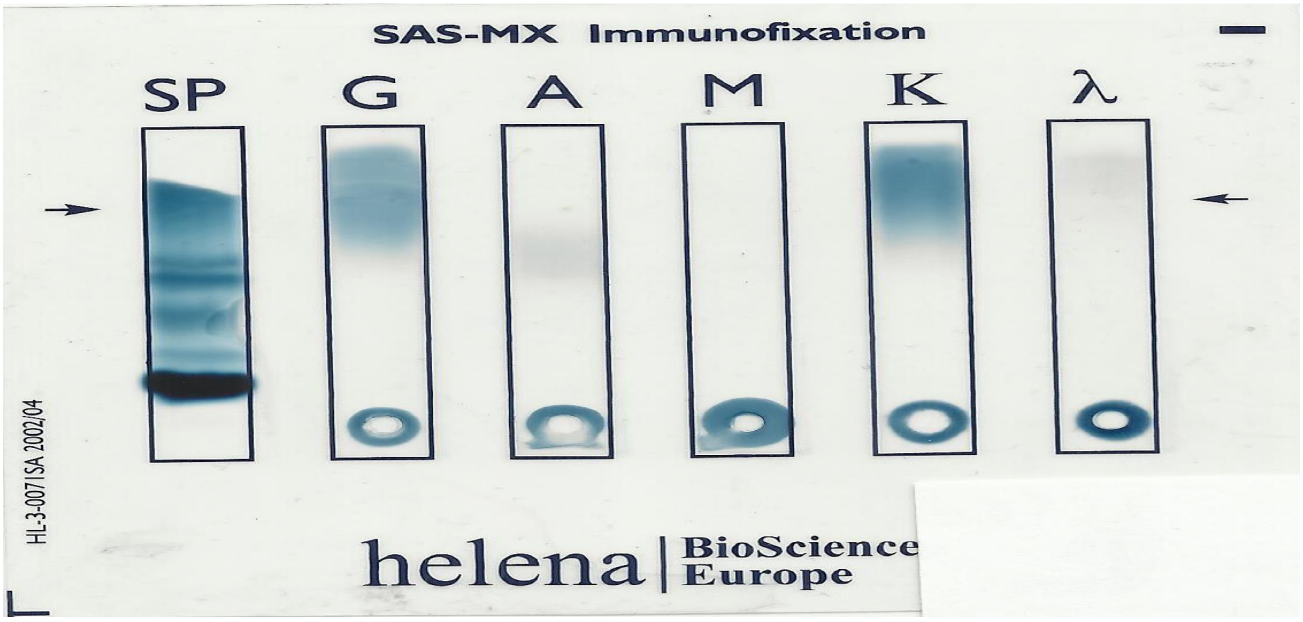
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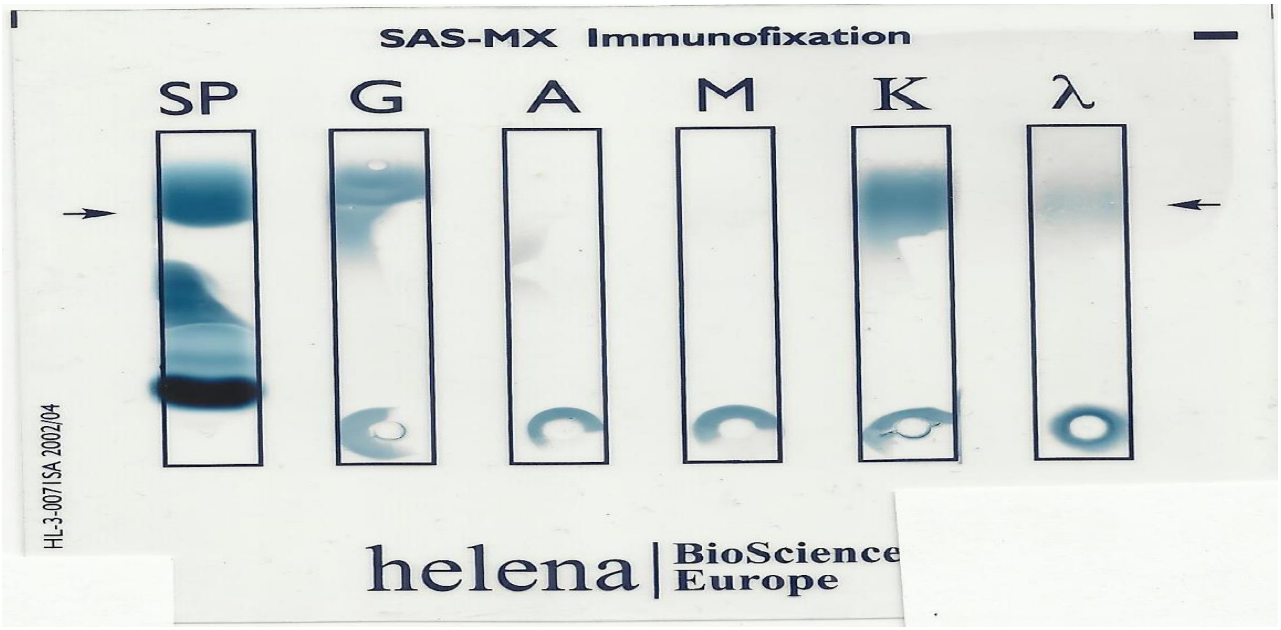
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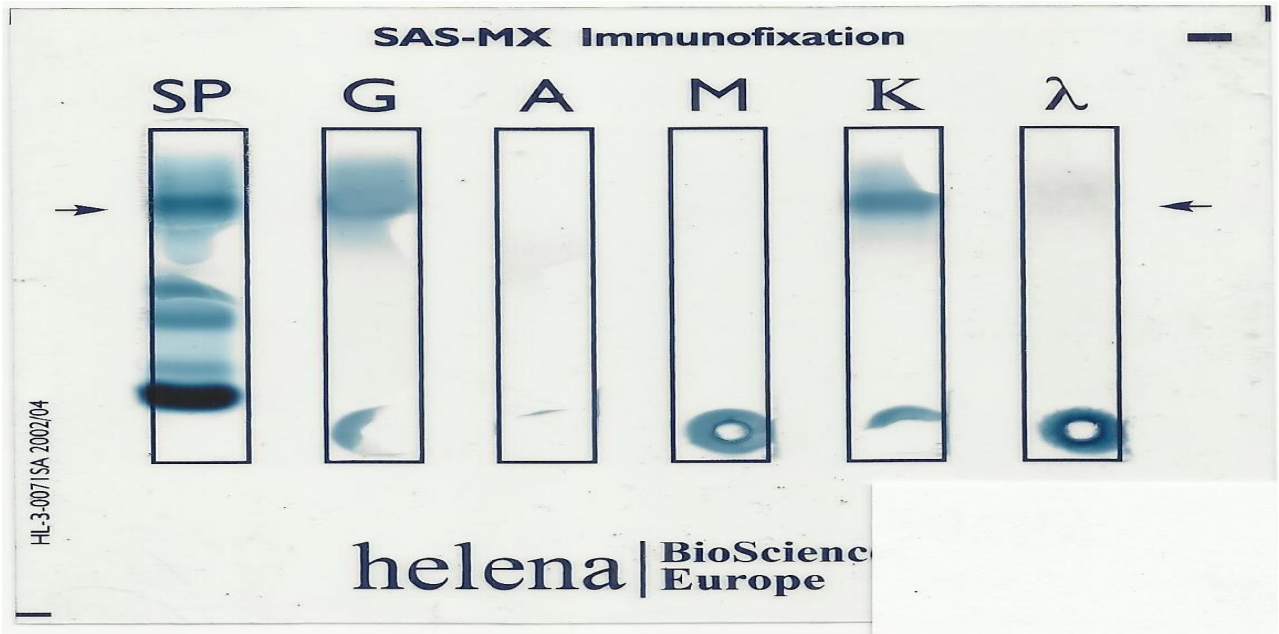
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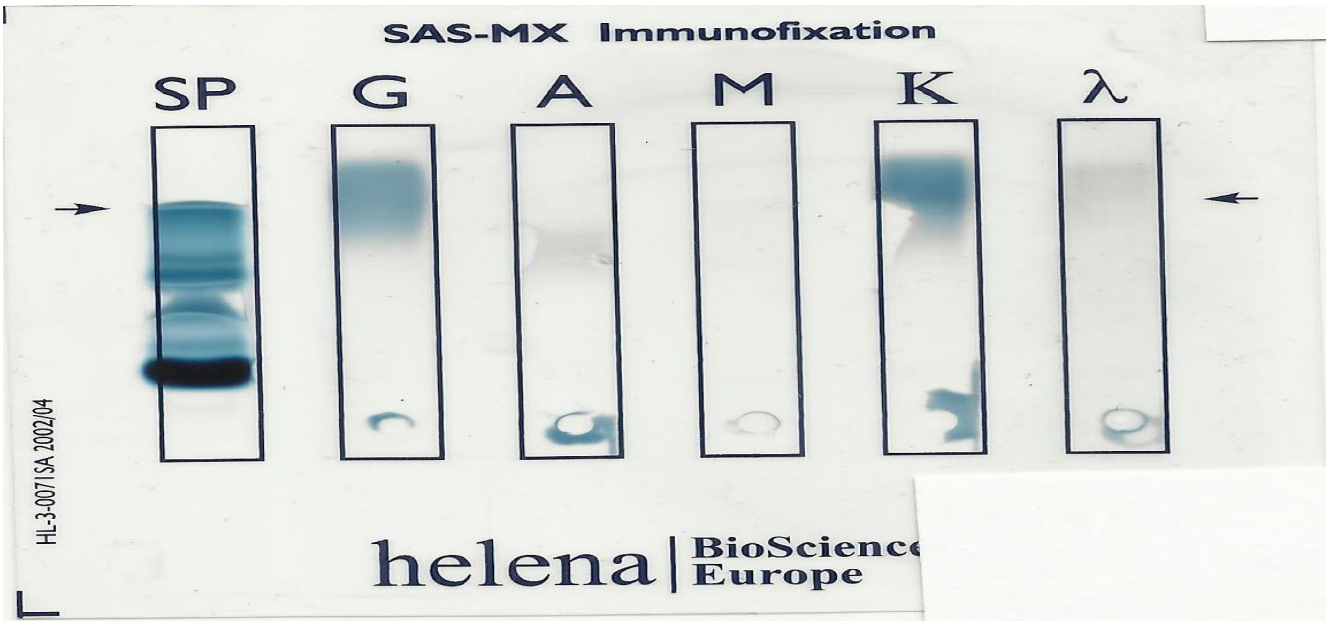
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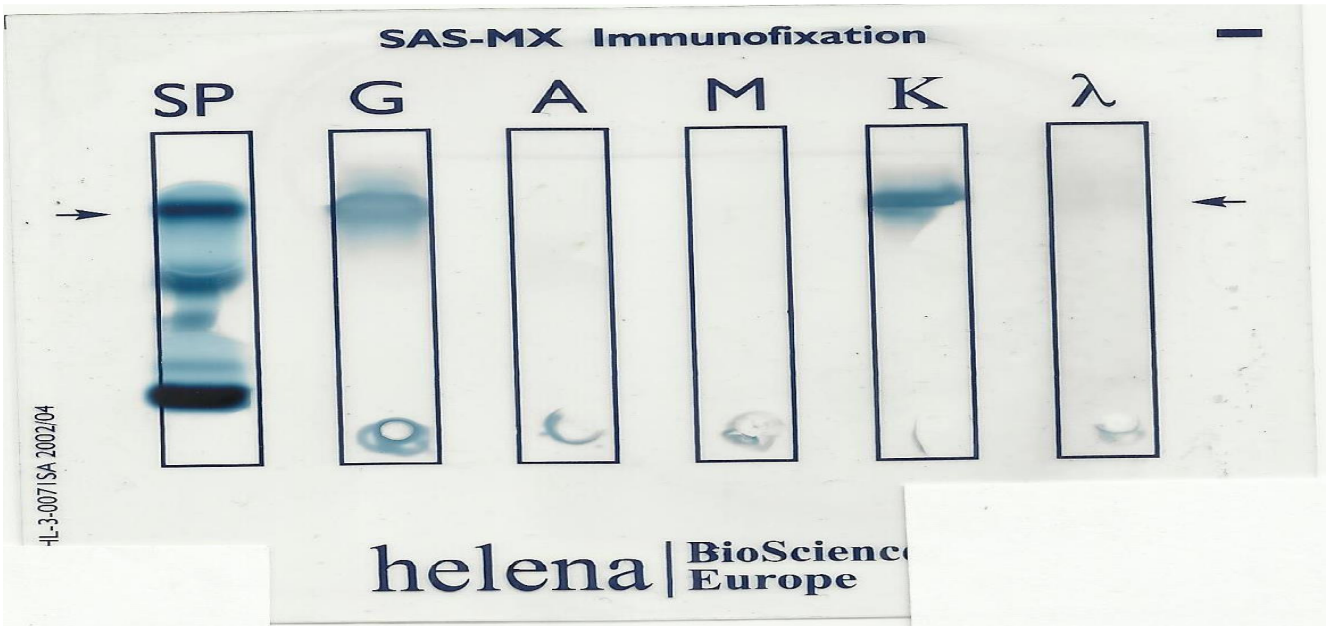
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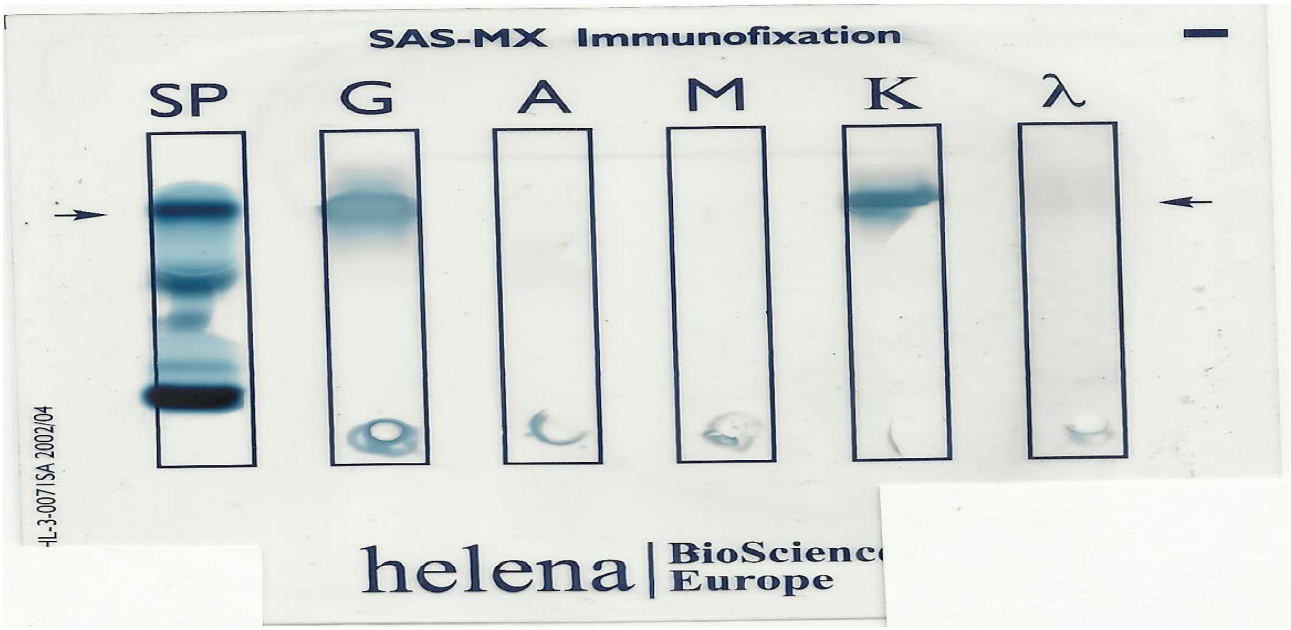
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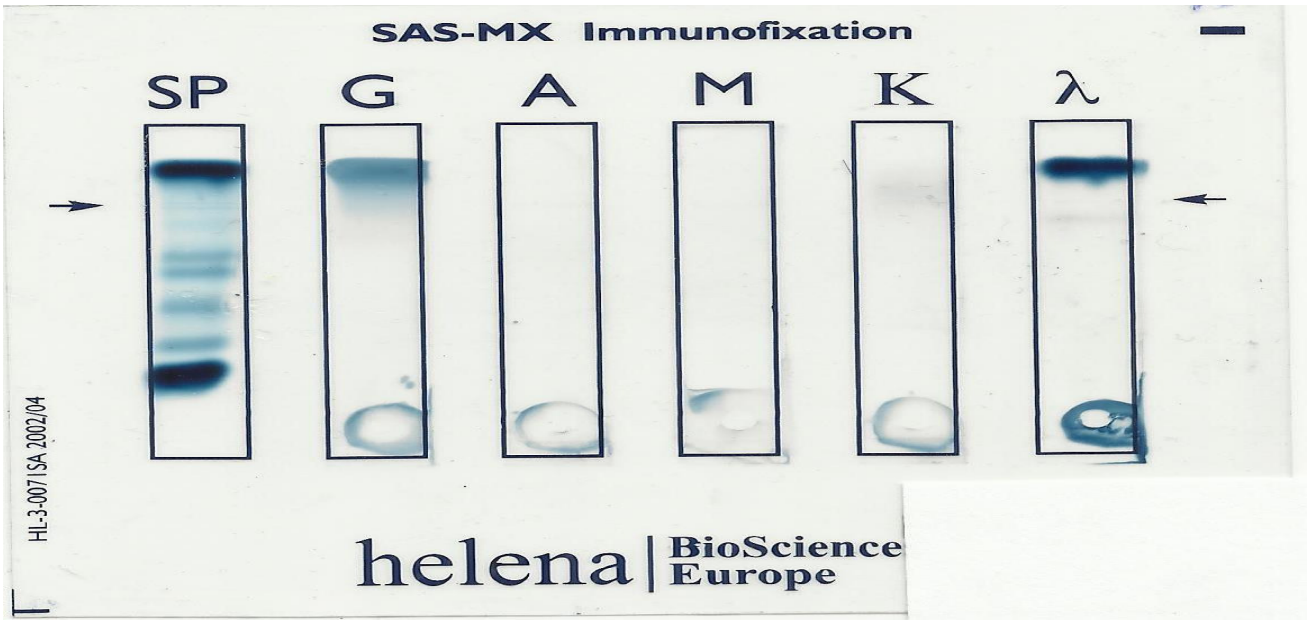
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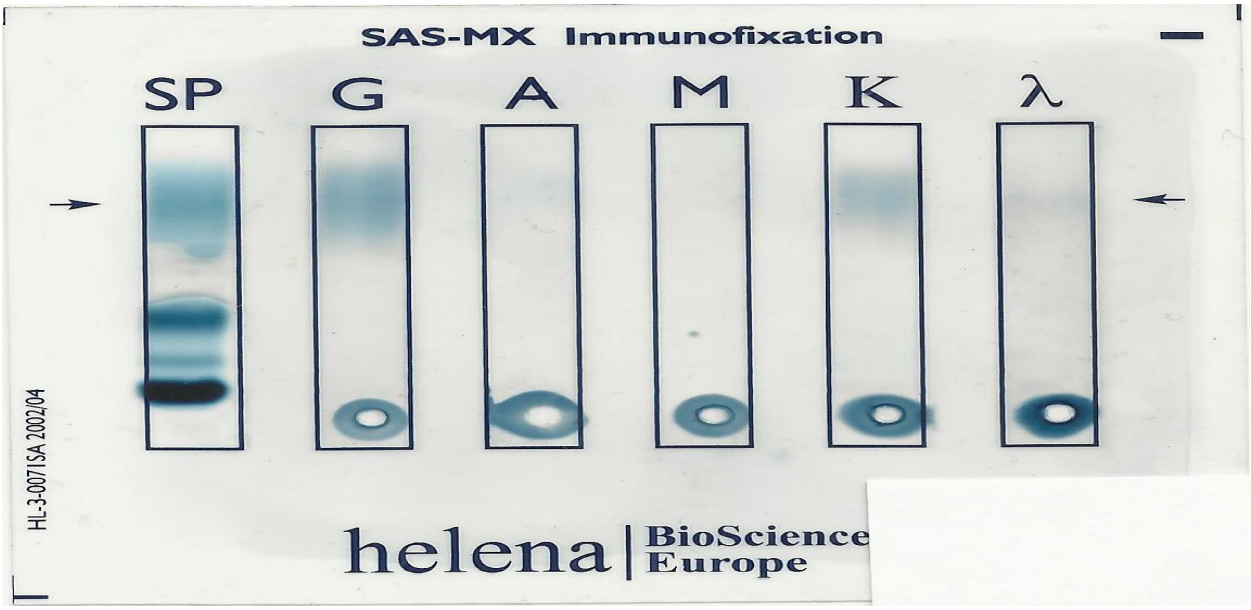
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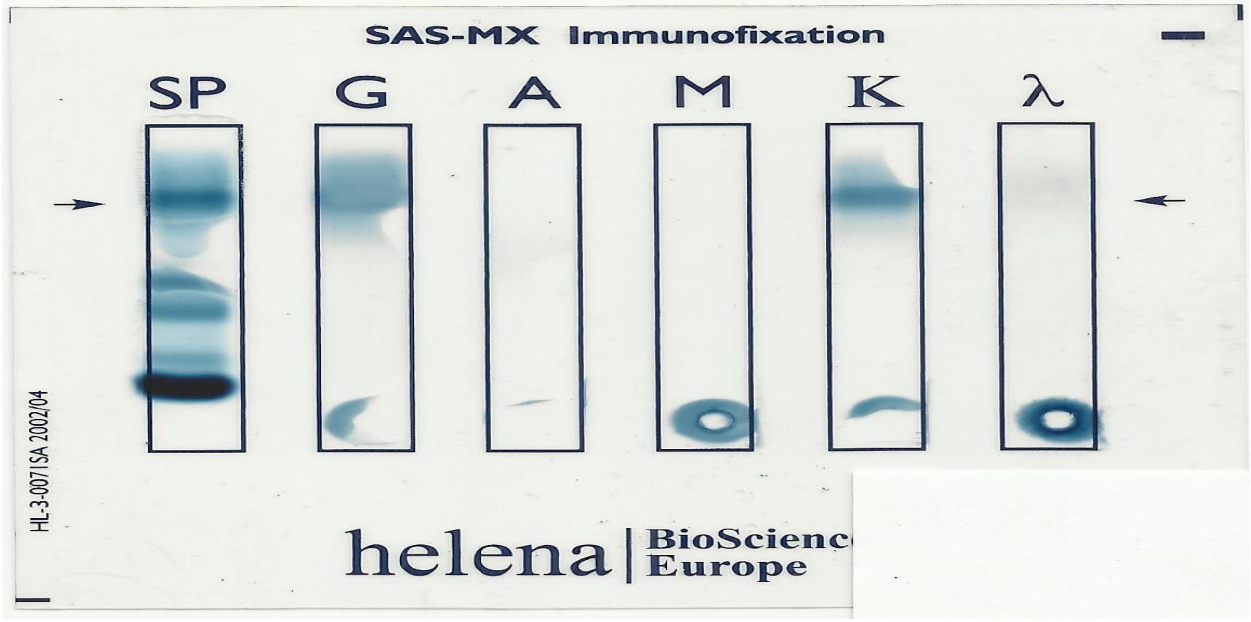
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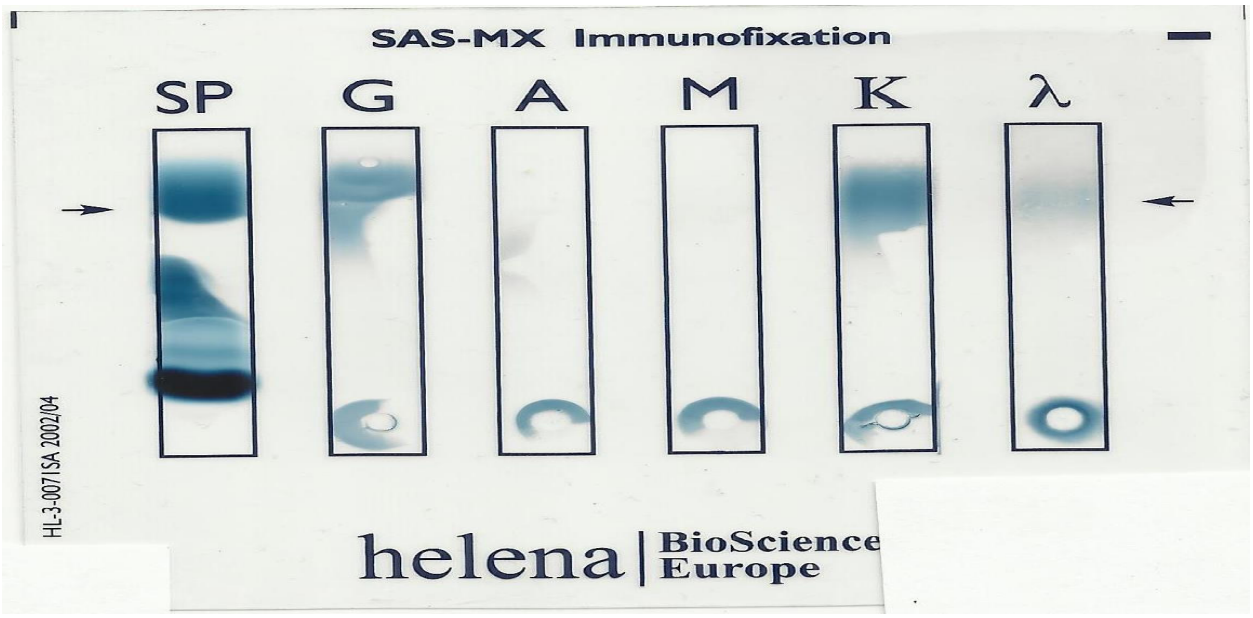
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