PyrexJournals

Pyrex Journal of Clinical Pathology and Forensic Medicine
Vol 1 (2) pp. 009-016 November, 2015
http://www.pyrexjournals.org/pjcpfm

Original Research Article

Haematological Features and Serum Protein Pattern on Electrophoresis of Multiple Myeloma in Sudanese Patients

I.O.M Khair[1], EM Abdelrahman[2], EA abdelgadir[3], SE Abdalla[3*], IM osman[4], A.A.K Humeida[3], MM Eltahir[5] and AA Salman[3]

[1]Internal Medicine Specialist
[2]Khartoum College of Medical Sciences, RICK, Khartoum, Sudan.
[3]Al Neelain University Faculty of Medicine Pathology Department, Sudan.
[4]Al Zaiem Alazhari University Faculty of Medicine Pathology Department, Sudan.
[5]University of Science and Technology, Faculty of Medicine, Pathology Department, Sudan.

Accepted 30th October, 2015

Multiple myeloma is a haematological malignancy characterized by neoplastic proliferation of a single clone of plasma cells producing a monoclonal immunoglobulin. This clone of plasma cells proliferates in the bone marrow and often results in extensive skeletal destruction with osteolytic lesions, osteopenia, and/or pathologic fractures. Most patients with MM present with signs or symptoms related to the infiltration of plasma cells into the bone marrow, kidney damage or other organs from excess light chains. The aim of this study is to determine the haematological features of Multiple Myeloma in Sudanese patients.

Methods: This is a descriptive cross-sectional hospital based study, was conducted in Radiotherapy and Isotpes Centre Khartoum (RICK), 49 patients with multiple myeloma were included in the study. Hb, TWBCs, ESR and platelets count were done using an automated haematological analyzer (Sysmex). Peripheral blood films were done and bone marrow biopsies were performed to all participants.

Results: showed that Hb levels of less than 11g/dl was found in 87% in females, and less than 13 g/dl was found in all males. While 13% showed normal haemoglobin. Leucopenia was found in 18.4% of patients while 4.1% have leucocytosis. 16.3% of patients have thrombocytopeania and 10.2% have thrombocytosis. Most of the patients (48.98%) have an ESR of 100 mm/hour or more.

Conclusion: Anaemia was found in all male patients and most of the female ones. Other haematological finding shows leucopenia, leucocytosis, thrombocytopenia, and thrombocytosis. Almost all patients showed have plasma cells in bone marrow biopsy. High ESR and high serum protein were common. Most of the patients showed a monoclonal band (M band) on SPEP. Normal serum protein electrophoresis was found in a few patients.

Key words: Myeloma, plasma cell, serum protein, bone marrow, anaemia.

INTRODUCTION

Multiple myeloma (MM)

This is characterized by the neoplastic proliferation of a single clone of plasma cells producing a monoclonal immunoglobulin. This clone of plasma cells proliferates in the bone marrow and often results in extensive skeletal destruction with osteolytic lesions, osteopenia, and/or pathologic fractures. The diagnosis of multiple myeloma is often suspected because of one or more of some clinical presentations as; bone pain with lytic lesions discovered on routine skeletal films, an increased total serum protein concentration and/or the presence of a monoclonal protein in the urine or serum, systemic signs or symptoms suggestive of malignancy, such as unexplained anemia. Hypercalcemia, which is either symptomatic or

Corresponding Author: sanaseed@hotmail.com

discovered incidentally, is common, acute renal failure with a bland urinalysis or rarely the nephrotic syndrome due to concurrent primary amyloidosis [1]. It is important to distinguish multiple myeloma both from other causes of the clinical presentations above and from other plasma cell dyscrasias for the purposes of prognosis and treatment. It is also important to evaluate patients suspected of having multiple myeloma in a timely fashion since a major delay in diagnosis has been associated with a negative impact on the disease course [1].

Etiology

The precise etiology of MM has not yet been established. Roles have been suggested for a variety of factors, including genetic causes, environmental or occupational causes, radiation, chronic inflammation, and infection [2].

Pathological Features

The presence of a monoclonal (M) protein in the serum or urine is a major criterion for the diagnosis of MM. The vast majority (97 percent) of patients with MM will have an M-protein produced and secreted by the malignant plasma cells, which can be detected by protein electrophoresis of the serum (SPEP) and/or of an aliquot of urine (UPEP) from a 24-hour collection combined with immunofixation of the serum and urine [3]. The M-protein usually presents as a single narrow peak, like a church spire, in the gamma, beta, or alpha-2 region of the densitometer tracing or as a dense, discrete band on the agarose gel figure (1). Infrequently, two M proteins are present (biclonal gammopathy) figure 2(1).

The malignant plasma cells can produce immunoglobulin heavy chains plus light chains, light chains alone, or neither with the following frequencies on serum immunofixation: IgG 52%, IgA 21%, Kappa or lambda light chain only (Bence Jones) 16%, IgD 2%, Biclonal 2%, IgM 0.5% and 6.5% are negative. Kappa is the predominant light chain Isotype compared with lambda, by a factor of 2 to 1 with the exception that lambda light chains are more common in IgD myeloma figure (3) [5].

Haematological Features

Anemia

A normocytic, normochromic anemia (hemoglobin ≤12 g/dL) is present in 73 percent at diagnosis and in 97 percent at some time during the course of the disease. This anemia can be related to bone marrow replacement, kidney damage, and/or can be due to dilution in the case of a large M-protein. Anemia commonly results in complaints of fatigue and pallor seen on physical examination. A larger than expected fraction of patients may have megaloblastic anemia due to either folate or vitamin B12 deficiency [6].

ESR and serum viscosity

Monoclonal protein can increase the serum viscosity and erythrocyte sedimentation rate (ESR). The ESR is >20 mm/h in 84 percent, and >100 mm/h in one-third of patients with MM [6].

Peripheral smear

The most frequent findings on peripheral smear are rouleaux formation (>50 percent), leukopenia (20 percent), and thrombocytopenia (5 percent). Rouleaux formation is the phenomenon when red cells take on the appearance of a stack of coins in diluted suspensions of blood and is seen in patients with elevated serum protein levels. A leukoerythroblastic reaction is uncommonly seen [3].

Monoclonal plasma cells are rarely seen in the peripheral smear in patients with myeloma; a detectable absolute peripheral blood plasma cell count ≥100 cells/microL (≥0.1 x 109/L) is found in approximately 10 percent [7]. Plasma cell leukemia, a rare, yet aggressive form of MM characterized by high levels of plasma cells circulating in the peripheral blood should be considered whenever circulating plasma cells are readily detected on conventional complete blood count evaluation. Circulating monoclonal plasma cells can be detected using a slide-based immunofluorescence assay, a two-color immunoassay technique (ELISPOT), or flow cytometry by gating on CD38+/CD45- cells. Using these sensitive techniques, circulating monoclonal plasma cells can be identified in the majority of patients with MM; the absolute percentage depends upon the sensitivity of the test used

Bone marrow examination

A bone marrow aspirate and biopsy are a key component in the diagnosis of MM. The bone marrow of the vast majority of patients contains 10 percent or more clonal plasma cells. However, due to patchy bone marrow involvement, bone marrow aspirate and biopsy may show less than 10 percent plasma cells in approximately 4 percent of patients. A diagnosis of MM can be made in patients with less than 10 percent clonal plasma cells on biopsy if other diagnostic criteria are fulfilled and after histopathologic confirmation of a soft tissue or bony plasmacytoma [8]. Since bone marrow involvement may be more focal than diffuse, some patients may require bone marrow aspirate/biopsy from several different sites or a guided biopsy of a focal lesion diagnosed by either MRI or PET/CT (integrated positron emission tomography and computed tomography) scan in order to establish the diagnosis [8].

Morphology

The morphological features of plasma cells can differ depending upon their maturity and, at times, they may be morphologically indistinguishable from myeloblasts. Mature plasma cells are oval with abundant basophilic cytoplasm. The nucleus is round and eccentrically located with a marked perinuclear hof, or cytoplasmic clearing. The nucleus contains "clock-face" or "spoke wheel" chromatin without nucleoli. Immature plasma cells have dispersed nuclear chromatin, prominent nucleoli and a high nuclear to cytoplasmic ratio. The cytoplasm of myeloma cells may contain condensed or crystallized cytoplasmic immunoglobulin resulting in the following unusual findings, which are not limited to MM, multiple pale bluish-white, grape-like accumulations (eg, Mott cells, Morula cells), Cherry-red refractive round bodies (eg, Russell bodies), Vermilion staining glycogen-rich IgA (eg, Flame cells), Overstuffed fibrils (eg, Gaucher-like cells, thesaurocytes) and Crystalline rods [9].

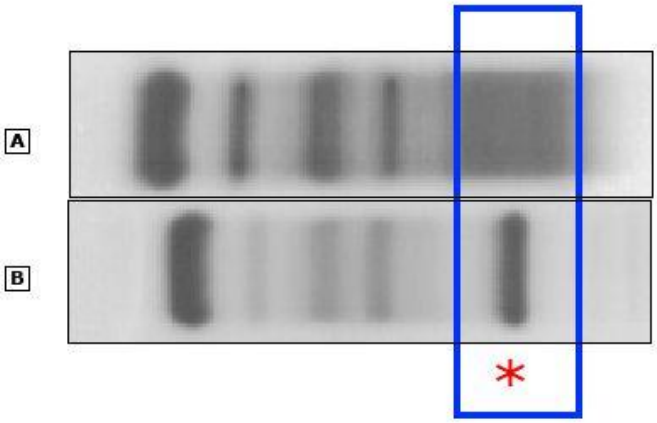

Figure 1: Immunoglobulins in multiple myeloma 25 [4]

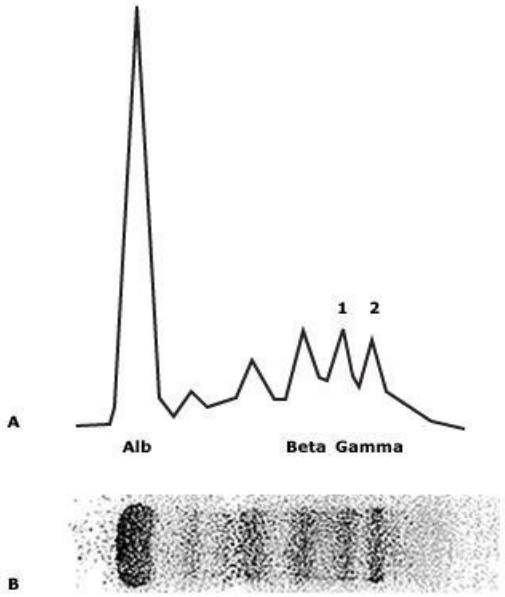

Figure 2: Biclonal gammopathy [4]

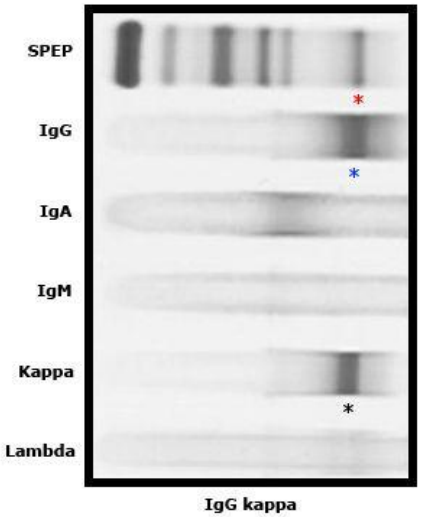

Figure 3: Monoclonal gammopathy on immunofixation [4]

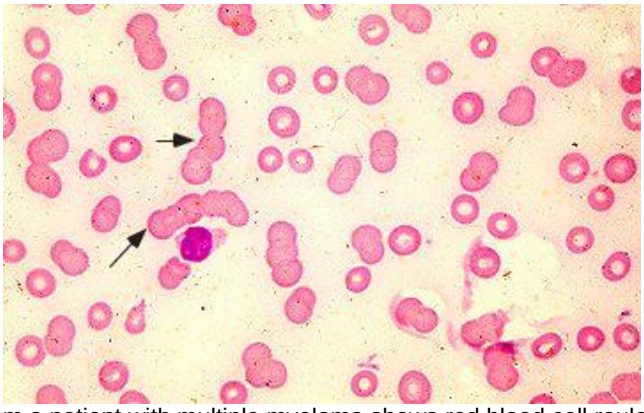

Figure 4: Peripheral blood smear from a patient with multiple myeloma shows red blood cell rouleaux (arrows), giving the appearance of stacked coins [7]

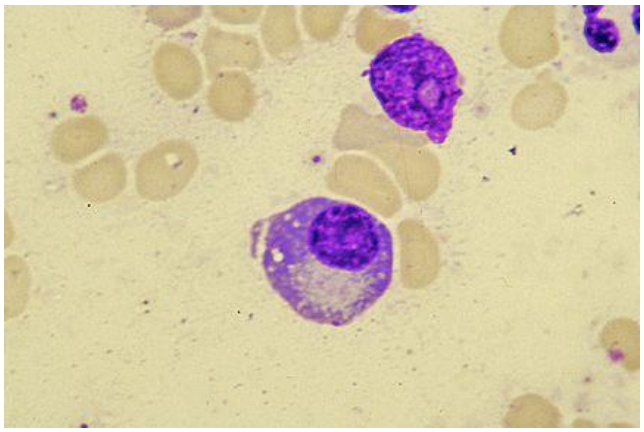

Figure 5: Smear of the peripheral blood demonstrates a circulating plasma cell [7]

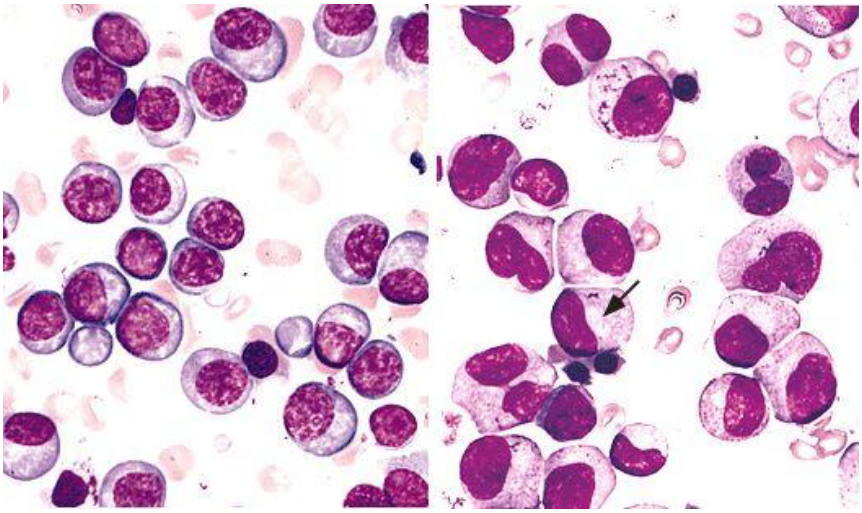

Figure 6: Bone marrow aspirate smears from patients with multiple myeloma, showed mature-appearing plasma cells with eccentrically placed nuclei and prominent Golgi zones [12]

Immunophenotype

Immunohistochemical staining, immunofluorescent studies, and flowcytometry detect either kappa or lambda light chains, but not both, in the cytoplasm of bone marrow plasma cells in patients with myeloma; surface immunoglobulin is absent. The normal kappa/lambda ratio in the bone marrow is 2:1. A ratio of more than 4:1 or less than 1:2 is considered to meet the definition of kappa or lambda monoclonality, respectively. This finding distinguishes the monoclonal gammopathies from reactive plasmacytosis due to autoimmune diseases, metastatic carcinoma, chronic liver disease, acquired immunodeficiency syndrome (AIDS), or chronic infection, in which the plasma cells show reactivity for both light chain types and the kappa/lambda ratio is within the normal range. A CD138 stain can identify plasma cells and aid in the accurate determination of percentage involved [10].

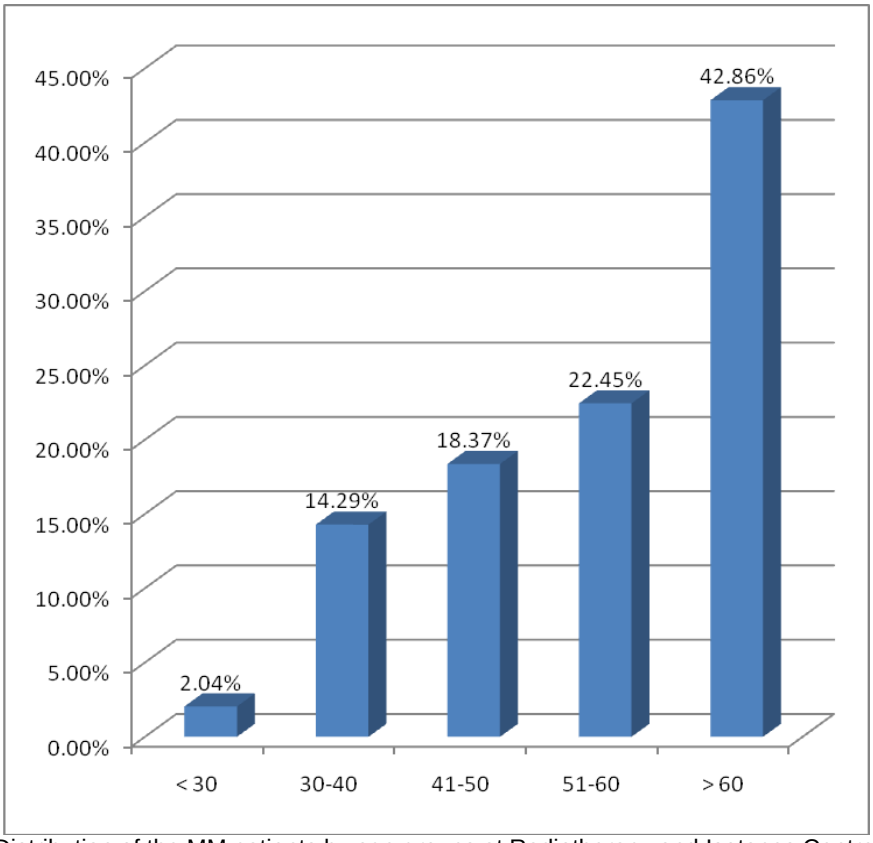

Figure 7: Distribution of the MM patients by age groups at Radiotherapy and Isotopes Centre Khartoum

Table 1: Haemoglobin level in females with Multiple Myeloma in (RICK)

Hb/gdl	Frequency	Percent
Low	20	87.0
Normal	3	13.0
Total	23	100.0

Table 2: TWBCs in Patients with Multiple Myeloma in (RICK)

TWBCs/cumm	Frequency	Percent
Low	9	18.4
Normal	38	77.6
High	2	4.1
Total	49	100.0

Table 3: Frequency and Percentage of Platelets count in study population

Platelets count $\times 10^9$	Frequency	Percent
Low	8	16.3
Normal	36	73.5
High	5	10.2
Total	49	100.0

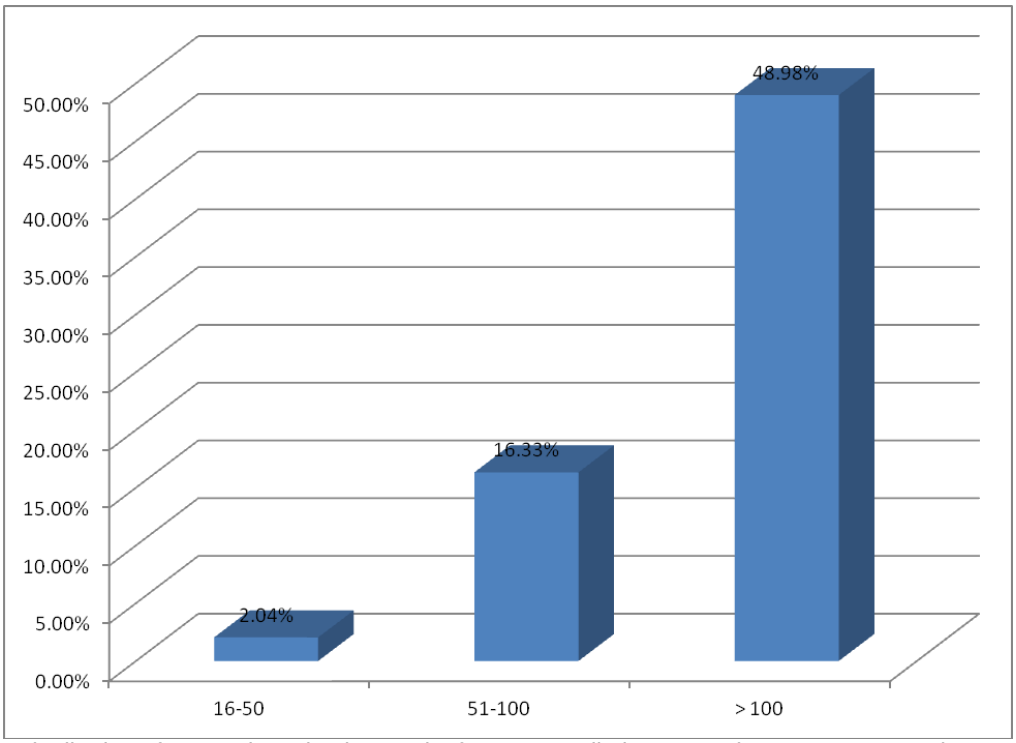

Figure 8: Distribution of MM patients by the result of ESR at Radiotherapy and Isotopes Centre Khartoum (RICK)

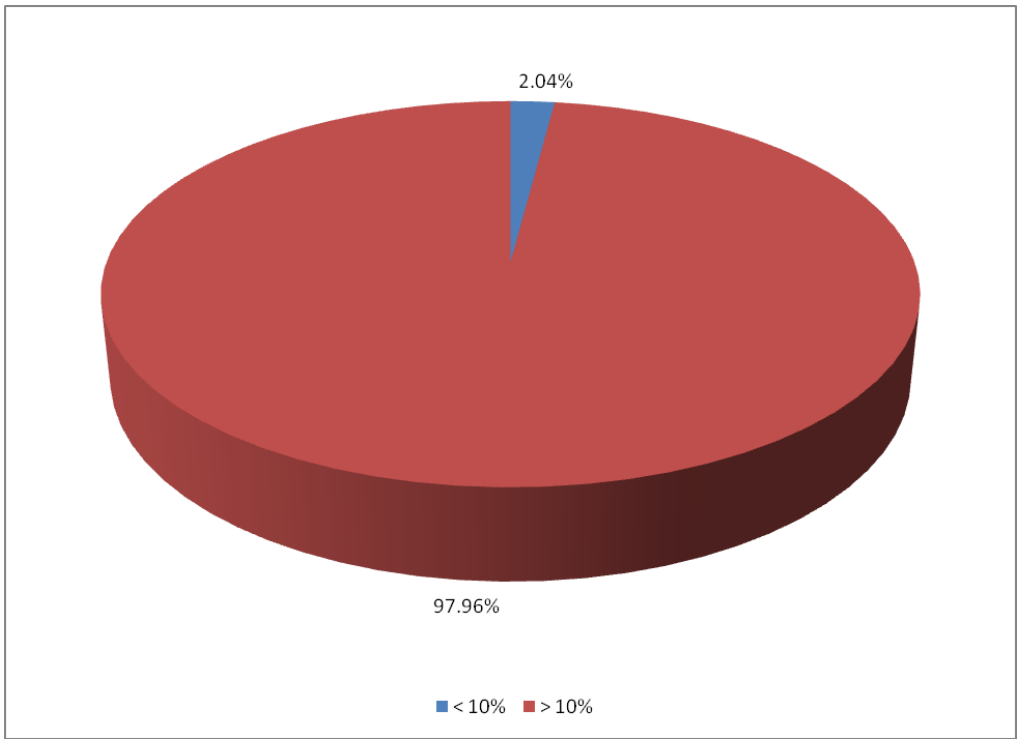

Figure 9: Distribution MM patients by the percentage of plasma cells on bone marrow biopsy at Radiotherapy and Isotopes Centre Khartoum (RICK)

Much like normal plasma cells, myeloma cells express CD79a, VS38c, CD138, and CD38. In contrast to normal plasma cells, myeloma cells infrequently express CD19. Approximately 70 percent of myeloma cells will express CD56, which is typically negative in normal plasma cells and in plasma cell leukemia

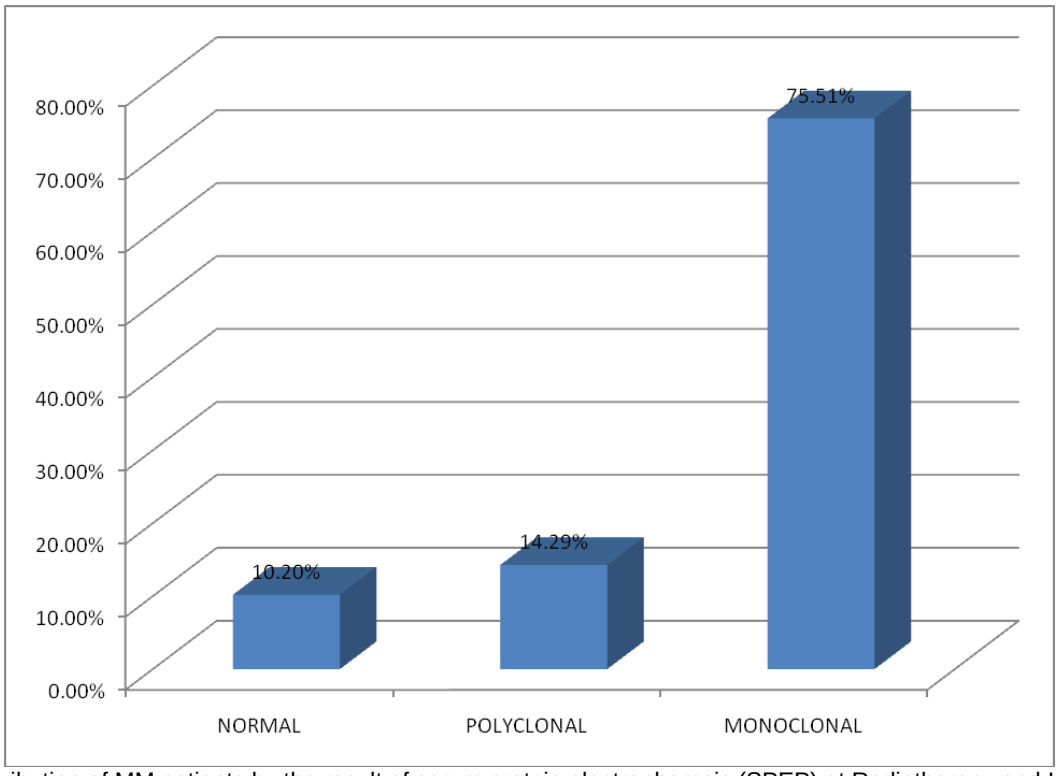

Figure 10: Distribution of MM patients by the result of serum protein electrophoresis (SPEP) at Radiotherapy and Isotopes Centre Khartoum (RICK)

Cytogenetics

There is no single cytogenetic abnormality that is typical or diagnostic of MM. The majority of myeloma tumors have genetic abnormalities that can be detected with sensitive molecular genetics techniques, such as interphase fluorescent in situ hybridization (FISH). In contrast, only 20 to 30 percent of patients will have cytogenetic abnormalities detected in bone marrow plasma cells by conventional karyotyping, due to a low number of metaphases in myeloma cells in such specimens [11].

OBJECTIVES

The main objectives of this study are to determine the haematological parameters and serum protein pattern in patients with multiple myeloma in Sudanese patients attending Radio Isotope Center in Khartoum

METHODS

This is a prospective, descriptive cross-sectional hospital based study, was conducted in Radiotherapy and Isotpes Centre Khartoum (RICK), 49 patients with multiple myeloma seen in RICK from January 2010 to February 2013 were included in the study. Data was collecting used combined interview questionnaires and observation check list. Blood samples were collected for the following investigations; Hb, TWBCs, ESR and platelets count were done using an automated haematological analyzer (Sysmex). Peripheral blood films were done and bone marrow biopsies were performed. Ethical consents were obtained from all participants, and ethical clearance was obtained from (RICK).

RESULTS

There were 49 patients diagnosed with multiple myeloma at national institute of oncology and radiotherapy during the 3 years of the study, 26 (53.1%) were males and 23 (46.9%) were females. Male to female ratio is 1.1:1. The frequency of multiple myeloma increases with age, most of the patients included in the study were older than 40 years (83.68%) and the disease is rare below the age of 40(16.33%) the mean age at presentation was 57 years, figure (7). Anaemia (Hb less than 11g/dl in females, and less than 13 g/dl in males) was found in all male patients and 87% of the female as in table (1), while 13% showed normal haemoglobin. Leucopenia was found in 18.4% of patients while 4.1% have leucocytosis, table (2). 16.3% of patients have thrombocytopeania, and 10.2% have thrombocytosis, table (3).

Most of the patients (48.98%) have ESR of 100 mm/hour or more, 16.33% have ESR less than 100 but more than 50, while only 2.04% have ESR of less than 50 but no one has normal ESR, seen in figure (8), the remaining 32.65% are missing system.

Almost all patients (97.96%) have plasma cells of 10% or more on bone marrow biopsy only 2.04% of patients have less than 10%, figure (9). In this study most of the patients (79.6%) showed high total serum protein level and (75.5%) have hypoalbuminaemia. 37 patients (75.51%) showed a monoclonal band (M band), 36 of them in Gamma region and one in α1 antitrypsin region. Polyclonal band was found in 7patients (14.29%), 3 in Gamma region, 3 in gamma and α2, and one patient in α1, α2 and Gamma regions. Normal serum protein electrophoresis were found in 5 patients (10.2%), figure (10). Biclonal band was not fond in any of the patients under study.

DISCUSSION

In this study there were 49 patients diagnosed with multiple myeloma in Radiotherapy and Isotopes centre Khartoum (RICK) during the 3 years of the study, with male to female ratio as; male: female ratio is 1.1:1. Most of the patients included in this study were older than 40 years and the mean age at presentation was 57 years. Most of the patients were from central and west of Sudan, while from the east of Sudan is less represented in the study population. This almost goes with A review of 869 cases of multiple myeloma seen at the Mayo Clinic which revealed that 98% of patients were 40 years of age or older and that 61% of them were males [13]. In the present study bone pain and anaemia are the commonest presenting symptoms, this goes with the Mayo clinic study which showed that bone pain and anaemia were the commonest presenting symptoms in (62%), (68%) respectively [13]. Anaemia was found in all male patients and most of the female ones. Leucopenia was found in some of the patients while few have leucocytosis. Some of the patients have thrombocytopenia, and others have thrombocytosis. Almost all patients have plasma cells in bone marrow biopsy. ESR of 100 mm/hour or more was detected in almost 50% of the patients, few have ESR less than 100 but more than 50, and very few of them were less than 50 but no one has normal ESR.

In this study most of the patients showed high total serum protein level, and almost all of them have hypoalbuminaemia. Most patients showed a monoclonal band (M band) on SPEP, some of them in Gamma region and one in α1 antitrypsin region. Polyclonal band was found in few patients, seen in Gamma region, gamma and α2, and in α1, α2 and Gamma regions. Normal serum protein electrophoresis was found in few patients. Biclonal band was not found in any of the patients under study. This goes with the Mayo clinic study where Serum protein electrophoresis showed a spike in 76%, hypogammaglobulinemia in 9%, and minor or no abnormalities in 15%, and a globulin spike was seen 75% of the urinary electrophoretic patterns. Immunoelectrophoresis of the serum revealed a monoclonal heavy chain in 83% and a monoclonal light chain in the serum in 8% (Bence Jones proteinemia). Three patients had no monoclonal protein in the serum or the urine ("nonsecretory"). Amyloidosis was found in 7% of the patients [13].

CONCLUSION

This study concluded that most of the common age group in multiple myeloma is more than 40 years old with predominance of male patients. Anaemia was found to be common in all male patients and most of the female ones. Leucopenia, leucocytosis, thrombocytopenia, and thrombocytosis were common haematological features. Plasma cells of (10%) or more on bone marrow biopsy was a constant finding. Half of the patients present with high ESR and high serum protein. Most of them showed a monoclonal band (M band) in Gamma region and α1 antitrypsin region. Polyclonal band was found in Gamma region, gamma and α2, and in α1, α2 and Gamma regions. Normal serum protein electrophoresis was found in few patients.

REFERENCES

[1] Multiple myeloma: causes and consequences of delay in diagnosis.Kariyawasan CC, Hughes DA, Jayatillake MM, Mehta AB QJM. 2007;100 (10):635.

[2] Multiple myeloma: Karen Seiter, MD, Professor, Department of Internal Medicine, Division of Oncology/Hematology, New York Medical College

[3] Kyle RA, Gertz MA, Witzig TE, et al. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin Proc 2003; 78:21.

[4] Plasma cell disorders. In: Cecil textbook of medicine, 22nd ed, Goldman, L, Ausiello, DA (Eds), WB Saunders, Philadelphia 2004. p.1184. Copyright © 2004 Elsevier.

[5] Bladé J, Lust JA, Kyle RA. Immunoglobulin D multiple myeloma: presenting features, response to therapy, and survival in a series of 53 cases. J Clin Oncol 1994; 12:2398.

[6] Baz R, Alemany C, Green R, Hussein MA. Prevalence of vitamin B12 deficiency in patients with plasma cell dyscrasias: a retrospective review. Cancer 2004; 101:790.

[7] Anderson, SC, PhD. Anderson's Atlas of Hematology. Wolters Kluwer Health/Lippincott. 2003 Lippincott Williams & Wilkins.

[8] Huang SY, Yao M, Tang JL, et al. Epidemiology of multiple myeloma in Taiwan: increasing incidence for the past 25 years and higher prevalence of extramedullary myeloma in patients younger than 55 years. Cancer 2007; 110:896.

[9] Swerdlow SH, Campo E, Harris NL, et al. World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues, IARC Press, Lyon 2008.

[10] Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. CA Cancer J Clin 2009; 59:225.

[11] Fonseca R, Bergsagel PL, Drach J, et al. International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. Leukemia 2009; 23:2210.

[12] Brunning, RD, McKenna, RW. Tumors of the bone marrow, Atlas of tumor pathology (electronic fascicle), Third series, fascicle 9, 1994, Washington, DC. Armed Forces Institute of Pathology.

[13] Multiple myeloma: review of 869 cases. Mayo Clinic Proceedings, Journal Article, Research Support, U.S. Gov't, P.H.S. Mayo Clinic 1975, 50(1):29-40