Study of Kappa to Lambda ratio in Multiple Myeloma as a predictor of outcome and prognostic factor in Egyptian patients

Mohammed Osman Azazzi [1], Mohamed Mahmoud Moussa [1], Hany Mohamed Hegab [1], Ibrahem Abd El hak El-Saied

1 Professor of Clinical Haematology, Internal Medicine, Faculty of Medicine, Ain Shams University

***Corresponding author:** Ibrahem Abd El hak el saied, **E-mail:** hema0106878820@gmail.com

ABSTRACT

Background: Multiple myeloma (MM) is heterogeneous malignant neoplasm that occurs mainly in patients over 50 years of age .Detection of monoclonal free light chains (FLCs) is important for diagnosis and monitoring of monoclonal gammopathies.

Aim of the Work: this prospective study was done to correlate between K:L ratio and other parameters used in diagnosis for patients with Multiple Myeloma in Egyptian patients.

Patients and Methods: twenty eight Egyptian patients with newly diagnosed MM who attend the hematology unit of Ain Shams university hospital and Sheikh Zayed specialized hospital.

Results: The study included 28 patients 14 males with percent of 50%and 14 females with percent of 50% .Age: mean=52.46±9.63, Range 28-74. There is statistical significance between K:L ratio and cytogenetics with p-value 0.027. The initial level of K;L ratio affect the response to chemotherapy being higher in poor response patients .

Conclusion: K;L ratio is highly sensitive and highly specific test in diagnosis and follow up of MM patients and its use as routine test may aid in the prognostic stratification of newly diagnosed MM patients together with other prognostic parameters .

Keywords: K;L ratio , FLCs , SPEP, Immunofixation, cytogenetics ,osteolytic lesions , anaemia

INTRODUCTION

Multiple myeloma (MM) is heterogeneous malignant neoplasm that occurs mainly in patients over 50 years of age 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[(1)]..

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 discovered incidentally, is common, acute renal failure with a bland urinalysis or rarely the nephrotic syndrome due to concurrent primary amyloidosis [(2)].

In Egypt the data are limited to determine the actual incidence rate but there is study done To determine the clinical and laboratory characteristics and survival of diagnosed Egyptian multiple myeloma patients admitted to the Haemato-Oncology Department between 2000 and 2010. Records of all patients in whom multiple myeloma was diagnosed at the Kasr Al Aini Hospital between 2000 and 2010 were included in this retrospective study. The mean age of patients was 58.5 years (range, 27-80 years). Fifty-nine percent were males. The majority of patients (73 %) had an immunoglobulin G monoclonal band and 70 % were Kappa chain-positive. Mean overall survival was 37.5 months (range, 1-84 months). [(3)]

Detection of monoclonal free light chains (FLCs) is important for diagnosis and monitoring of monoclonal gammopathies. Although FLCs are especially important in light-chain diseases, such as light-chain myeloma, primary systemic (AL) amyloidosis, and light-chain deposition disease, FLC abnormalities occur in as many as 97% of plasma cell disorders**.** **(4).**

Monoclonal immunoglobulin free light chains are important tumor markers often present in serum and urine of patients with monoclonal gammopathies. Serum kappa (κ) and lambda (λ) FLC assays measure total polyclonal and monoclonal FLC in serum and the calculated κ/λ FLC ratio is a surrogate measure of clonality. **(5)**.

AIM OF THE WORK

To study the correlation between Kappa to Lambda ratio and other prognostic parameters used in diagnosis of Multiple Myeloma as age, sex, Plasma cell count in bone marrow,B2 macroglobulin, SPEP,IF and cytogenetic and its impact on the outcome after induction

PATIENTS AND METHODS

This study is a prospective study conducted on twenty eight Egyptian patients with newly diagnosed MM who attend the hematology unit of Ain Shams university hospital and Sheikh Zayed specialized hospital.

***Inclusion criteria:**

1-Patients with newly diagnosed MM.

2-Age above 25.

3-All are free of any IHD, DM, Hepatitis B or Hepatitis C at time of diagnosis

55% >Fair cardiac muscle condition with EF 4-

***Exclusion criteria:**

*Patients previously diagnosed as MM and received treatment before.

*Patients with other solid tumors.

All patients will be subjected to:

1-Full history taking and clinical examination for medications and infections or systemic diseases.

2-Thorough physical examination.

3-Laboratory investigation including:

-Complete blood count.

-Kidney and liver functions.

-Bone marrow aspiration.

-Serum protein electrophoresis.

-Immunofixation.

-Serum B2 macroglobulin.

-Serum free light chain Kappa; Lambda ratio.

-Serum calcium.

-Cytogenetics (Karyotyping and/or FISH on peripheral blood or bone marrow aspirate for :

-Deletion of 17p, t(4;14), t(11;14) hyperdiploidy, deletion of chromosome 13.

4-Radiological investigation including: MRI or plain X-ray to detect osteolytic lesions.

The Kappa; Lambda ratio will be correlated with the known MM diagnostic and prognostic parameters and with the course of the disease and outcome before and after induction of treatment by VCD (Velcade, cyclophosphamide, dexamethasone) or VTD(Velcade, thalidomide, dexamethasone) or VAD protocol

Technique of measuring Kappa and Lambda FLC in our study

SFLC immunoassays utilize antibodies that have high specificity and affinity. For assays detecting homogeneous antigens, monoclonal antibodies (Mabs) are very successful [(6)].

International guidelines for SFLC measurement are based on results obtained with Freelite, and the data discussed in this review has been obtained using these polyclonal assays by Freelite Human Kappa and Lambda Free kit used for Siemens BN ProSpec.

Statistical analysis:

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS)

Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.P-value $\leq$0.05 was considered significant. P-value $\leq$0.001 was considered as highly significant. P-value >0.05 was considered insignificant

RESULTS

They are 28 patients 14 males with percent of 50% and 14 females with 50%. Age: mean=52.46±9.63 Rang from 28-74 years

Table (1): Demographic data distribution of the study group.

Demographic Data	Total (N=28)
Sex	
Male	14 (50.0%)
Female	14 (50.0%)
Age (years)	
<50	10 (35.7%)
≥50	18 (64.3%)
Range [Mean±SD]	28-74[52.46±9.63]

Table (2):: Diagnostic test for multiple myeloma descriptive of the study group

Diagnostic test for multiple myloma	Total (N=28)
Plasma cell % in bone marrow	6-90[39.71±26.12]
Immunofixation	
IgA	7 (25.0%)
IgG	18 (64.3%)
IgM	1 (3.6%)
KLC	2 (7.1%)
SPEP	
IgA	5 (17.9%)
IgG	20 (71.4%)
IgM	1 (3.6%)
Normal	2 (7.1%)
B2 (mg/L) category	
<2.4 Normal	4 (14.3%)
>2.4 Abnormal	24 (85.7%)
Range [Mean±SD]	1.5-18.745[6.98±4.80]
kappa level (mg/L)	1.6-229[59.38±68.27]
Lambda level (mg/L)	0.26-191[19.83±41.21]
K:L ratio	0.08-47[12.09±11.98]
Cytogenetics	
delation 13	6 (21.4%)
t(11:14)	2 (7.1%)
t(4: 14)	4 (14.3%)
Normal	16 (57.1%)
Osteolytic Lesions	
Found	18 (64.3%)
Not Found	10 (35.7%)

NB: The patients had M band elevation in SPEP was confirmed and sub grouped by results of immunofixation

Table (3); Disease status post induction, auto BMT (after 1st line) and auto BMT distribution of the study group

Parameters	Total (N=28)
Disease Status Post Induction	
Stringent complete response	7 (25.0%)
Complete response	10 (35.7%)
Partial response	1 (3.6%)
Refractory	10 (35.7%)
Auto BMT	
Done	21 (75%)
Not done	7 (25%)
Auto BMT (after 1st line)	
Done	17 (60.7%)
Done after 2nd line	2 (7.1%)
Done after 3ed line	2 (7.1%)
Not done	7 (25.0%)

Table (4): Relation between K:L ratio and cytogenetics,osteolytic lesions , disease status post induction and auto BMT

cytogenetics	K:L ratio		T-test	p-value
	Mean	±SD		
t(4: 14)	19.56	20.27	F=5.211	0.027*
delation 13	16.20	10.88		
t(11:14)	6.09	8.50		
Normal	9.43	9.90		
Osteolytic Lesions			3.937	0.016*
Found	14.40	13.70		
Not Found	7.93	6.81		
Disease Status Post Induction			F=3.562	0.029*
Stringent complete response	2.43	2.57		
Complete response	12.06	11.58		
Partial response	7.80	0.00		
Refractory	19.32	12.63		

Table (5): Relation between disease status post induction and IF, SPEP ,B2, CG and K: L ratio

Parameters	Disease Status Post Induction				x2/t#	p-value
	Stringent complete response (N=7)	Complete response (N=10)	Partial response (N=1)	Refractory (N=10)		
Immunofixation						
IgA	1 (14.3%)	4 (40.0%)	1 (100.0%)	1 (10.0%)		
IgG	5 (71.4%)	6 (60.0%)	0 (0.0%)	7 (70.0%)	11.749	0.038*
IgM	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
KLC	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (20.0%)		
SPEP						
IgA	1 (14.3%)	2 (20.0%)	1 (100.0%)	1 (10.0%)		
IgG	5 (71.4%)	7 (70.0%)	0 (0.0%)	8 (80.0%)	8.820	0.014*
IgM	1 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
Normal	0 (0.0%)	1 (10.0%)	0 (0.0%)	1 (10.0%)		
B2 (mg/L)						
<2.4 Normal B2	2 (28.6%)	0 (0.0%)	1 (100.0%)	1 (10.0%)	8.983	0.030*
>2.4 Abnormal	5 (71.4%)	10 (100.0%)	0 (0.0%)	9 (90.0%)		
Cytogenitics						
delation 13	0 (0.0%)	3 (30.0%)	0 (0.0%)	3 (30.0%)		
t(11:14)	1 (14.3%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	8.204	0.043*
t(4: 14)	1 (14.3%)	0 (0.0%)	0 (0.0%)	3 (30.0%)		
Normal	5 (71.4%)	6 (60.0%)	1 (100.0%)	4 (40.0%)		
K:L ratio	2.43±2.57	12.06±11.58	7.80± 0	19.32±12.63	3.562#	0.029*

This table shows statistically significant relation between disease status post induction with IF, IgA patients are mostly responder (85.71%) 6 patients of 7 while IgG are less responder (61.1%) 11 patients of 18, SPEP show outcome near to the results of IF, B2, cytogenetics t(4;14) 3patients of 4 are refractory and K:L ratio mean was (2.43)in patients on sCR and (19.32) in refractory patients **.**

NB: The patients had M band elevation in SPEP was confirmed and sub grouped by results of immunofixation

Table (6): Correlation between plasma cell % in bone marrow and Hb, K:L ratio

	Plasma cell % in bone marrow	
	r	p-value
HB	-0.461	0.013*
K:L ratio	0.389	0.019*

positive correlation was found between HB level , K:L ratio and percentage of plasma cell in bone marrow

Table (7): Relation between osteolytic lesions and SPEP , CG

	Osteolytic Lesions		t/x2#	p-value
	Found (N=18)	Not Found (N=10)		
SPEP			7.194	0.024*
IgA	5 (27.8%)	0 (0.0%)		
IgG	11 (61.1%)	9 (90.0%)		
IgM	1 (5.6%)	0 (0.0%)		
Normal	1 (5.6%)	1 (10.0%)		
Cytogenitics			8.770	0.018*
deletion 13	5 (27.8%)	1 (10.0%)		
t(11:14)	1 (5.6%)	1 (10.0%)		
t(4: 14)	4 (22.2%)	0 (0.0%)		
Normal	8 (44.4%)	8 (80.0%)		

This table shows statistically significant relation between osteolytic lesions with SPEP and cytogenetics.

Highest percentage of osteolytic lesion was found in patients with IgA myeloma detected by SPEP(5 patients of 5), also patients with t(4;14) all had osteolytic lesions ,patients with del 13 chromosome 5 of 6 patients had osteolytic lesions, While normal cytogenetics appear not affecting presence of oteolytic lesions (16 patient with normal CG , 8 had osteolytic lesions and 8 were free of osteolytic lesions)

NB: The patients had M band elevation in SPEP was confirmed and sub grouped by results of immunofixation

DISCUSSION

This study aimed to detect the correlation between disturbed Kappa to Lambda ratio and other prognostic parameters used in diagnosis of Multiple Myeloma as age, sex, Plasma cell count in bone marrow , B2 macroglobulin, SPEP,IF and cytogenetic and it`s impact on the outcome after induction.

Of 28 patients there was 14 male and 14 female this data can be attributed to the random selection and small sample size.(50% males and 50% females). Our result is on contrary to the documented incidence in Egypt according to Cancer Incidence in Egypt: Results of the National Population-Based Cancer Registry Program [7] .There was 516 newly diagnosed cases of MM all over the country including 316 males (61.24%)and 200 female(38.75%) so the male to female ratio there in Egypt is 1.58. And with the worldwide ratio of 1.5 male: female.

The median age was 52.5 years range from 28 to74 years old. this result also is comparable to the results of Multiple myeloma in a descriptive study of 217 Egyptian patients done by ***El Husseiny .N, et al (2013)*** [3]**.**

which show median age of 58.5 years old ranging from 28 to 80 years old.

The FLC ratio was outside of the reference interval (range: 0.26-1.65) in 92.85 % of patients in agreement with **Snozek . et al (2008)** [8]**.** Who found that 95.1% of patients had abnormal K: L ratio. Regarding the Ig isotype, distribution of myelomas has been known for a long time with about 55 % of IgG myelomas, 25 % of IgA myelomas [9]**.**

In our study IgG subtype represented about 71.4 % of cases and IgA subtype (25.0%) by immunofixation**.**

As regard disease status post induction when correlated with IF, IgA patients are mostly responder (85.71%) 6 patients of 7 while IgG are less responder (61.1%) 11 patients of 18, cytogenetics t(4;14) 3patients of 4 are refractory and K:L ratio mean was (2.43)in patients who achived sCR and (19.32) in refractory patients

As regard cytogenetics 57.1% had normal cytogenetics this is in agreement with the previous study by **. *Debes-Marun. C et al, (2003)*** [10]. Who reported that in most of cases (50-70%), the karyotype reveals normal metaphases that originate from the myeloid elements by conventional cytogenetics

In our study t(4;14) was found in 14.3 % of our studied patients which is very near to the results reported by Myungshin Kim, et al. (2013) [11]..Abnormal karyotypes were detected in 42.3% (55/130) of the patients (in our study 42.9%). A 14q32 rearrangement was detected in 29.2% (N=38) of the patients (in our study 21.4%), and these most commonly included t(11;14), which was followed by t(4;14) and t(14;16) (16.2%, 11.5%, and 0.8%, respectively) in contrary to our study which showed that t(4;14) first then t(11;14) (14.3% and 7.1% respectively) del(13q) was a common genetic abnormality, and it had an incidence of 26.9% (35/130) in the previously mentioned study (Myungshin study) and in our study it present21.4 % of cases (6 patients of 28).

A high FLC ratio in the study by ***Kumar*** was associated with high plasma cell burden (*P*=0.007) (in our study P= 0.019) abnormal karyotypes (*P*=0.020 and *P*=0.020, respectively),

. High FLC ratio was associated with 14q32 rearrangements (*P*=0.047) in our study there was significant relation between K:L ratio and cytogentics but the study design not allow us to separate the significance for each abnormality but the P=0.027***.Kumar, etal.,2010*** [12]**.** have hypothesized that 14q32 rearrangements lead to the unbalanced production of light chains and

more extreme abnormalities of FLC. They have demonstrated that an abnormal FLC ratio are frequently detected in patients with 14q32 rearrangements and are associated with poor prognosis, especially in patients with t(14;16). We found that the FLC ratios were higher in patients with 14q32 rearrangements. In addition, the FLC ratio was associated with 1q+ and plasma cell burden. These findings support the idea that an increase in the FLC is due to the cumulative effect of several events, such as genetic aberrations, including 14q32 rearrangements and 1q+ and plasma cell proliferative activity, rather than any single abnormality. In our study we have the same net result nearly with high FCL in correlation to t(4;14) with mean K;L ratio 19.56. The patients with cytogenetic abnormality had lower outcome only 50% response to treatment on comparison to 68.75 % response either go to sCR or CR in patients with normal cytogenetics

There was 21 patient underwent ASCT either after CR1 CR2 or CR3 and 7 patients had not underwent ASCT ,3 patients of those 7 had t(4;14) with percentage (42.9 %) of refractory patients

Negative correlation was found between B2 microglobin and percentage of plasma cells on bone marrow, while positive correlation was found between HB level , K:L ratio and percentage of plasma cell in bone marrow

Also the presence of osteolytic lesion was correlated to disturbed FLC ratio as it was found in patients with disturbed ratio with mean K:L ratio of 14.40 ±SD 13.70 this median ratio is lower in patients who did not have osteolytic lesion (mean 7.93 ±SD 6.81) with p value= 0.016.Also Highest percentage of osteolytic lesion was found in patients with IgA myeloma detected by SPEP and confirmed to subtype IgA(5 patients of 5), also patients with t(4;14) all had osteolytic lesions 4 patients of overall study group ,patients with del 13 chromosome 5 of 6 patients had osteolytic lesions, While normal cytogenetics appear not affecting presence of oteolytic lesions (16 patient with normal cytogenetics , 8 had osteolytic lesions and 8 were free of osteolytic lesions)

60.07% were subjected to ASCT after 1st Line and over all patients post 2nd and 3ed line who are responded and had ASCT was 75% this can show high statistical significance of disease status post induction and achieving an ASCT with p value = 0.004. The interpartetion of this result is in agreement with ***(Raoudha Mansouri, et at., 2016)*** [13]**.** Where is Overall, 141 patients (70%) (of 202 patients) underwent ASCT, 121 of whom had evidence of chemo-sensitive MM at completion of induction

CONCLUSION

K;L ratio is highly sensitive and highly specific test in diagnoses and follow up in MM patients and its use as routine test may improve early diagnosis of disease and also early relapse without ignoring other parameters assessment. The Test now is standered of care in developed countries and we hope to use it in Egypt as routine investigation for myeloma patients as soon as possible

REFERENCES

1. ***Kyle. RA and Rajkumar. SV. (2004):*** **Multiple Myeloma**. N Engl J Med.; 351:1860-1873

2. ***Kyrtsonis. M et al. (2007):***British journal of hematology Volume137, Issue3 May Pages 240-243

3 ***El Husseiny .N, Neemat Kasem ,Hamdy. A. El Azeeim & Mervat.W. Mattar (2013):*** a descriptive study of 217 Egyptian patients Ann Hematol DOI 10.1007/s00277-013-1849-3.

4. ***Anderson. K.C, Alsina. M, Bensinger.W, et al. (2011):*** NCCN Clinical practice guidelines in oncology for multiple myeloma J Natl Compr Canc Netw, 9, pp. 1146-1183

5. ***Dispenzieri.A, Kyle. RA, Merlini. G, et al. (2009):*** International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders Leukemia, 23, pp. 215-224

6 ***Campbell. J.M, Cobbold.M, Wang.Y, et al. (2013):*** Development of a highly-sensitive multiplex assay using monoclonal antibodies for the simultaneous measurement of kappa and lambda immunoglobulin free light chains in serum and urine J Immunol Methods, 391, pp. 1-13

7. ***Kamel. H et al. (2014):*** Cancer Incidence in Egypt: Results of the National Population-Based Cancer Registry Program. September 2014 Hindawi Publishing Corporation Journal of Cancer Epidemiology Volume, pp 9-11

8. **Snozek. CLH, Katzmann.JA, Kyle.RA, Dispenzieri.A, Larson.D, Therneau. TM, et al. (2008):** Prognostic value of the serum free light chain ratio in newly diagnosed myeloma: proposed incorporation into the International Staging System. Leukemia; 22: 1933-1937

9. ***Morea . P, et al blood (2015):*** **126:4191;**

10. ***Debes-Marun. C, Dewald. G, Bryant. S, Picken. E, Santana-Dávila. S, González-Paz. N, Winkler. J. M, Kyle. R, et al., (2003):*** Chromosome abnormalities clustering and its implications for pathogenesis and prognosis in myeloma. Leukemia (Baltimore), 17: 427-436

11. ***Myungshin Kim, et al. (2013):*** in study carry the name of Impact of Genetic Abnormalities on the Prognoses and Clinical Parameters of Patients with Multiple Myeloma; Revised March 18, 2013; Accepted May 16, p.p 248-250.

12. ***Kumar.S, Zhang.L, Dispenzieri A, Van Wier.S, Katzmann.JA, Snyder.M, et al. (2010)*****:** Relationship between elevated immunoglobulin free light chain and the presence of IgH translocations in multiple myeloma. Leukemia;24:1498-1505.

13 ***Raoudha Mansouri, et al (2016):*** (Depth of Response to ≥VGPR at Completion of Induction Influences Outcome Post ASCT, on Behalf of the Tunisian Myeloma Study Group blood volume 12.