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Evaluation of Correlation between Serum Free Light-Chain Assay and Bone Marrow Study in Multiple Myeloma Patients

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ABSTRACT

Background: Multiple myeloma is a hematologic malignancy manifested by the secretion of abnormal immunoglobulin. Different methods have been described for diagnosis and patient response to management. Serum free light-chain assay is recently approved in the diagnosis of multiple myeloma patients.
This study aimed to evaluate the diagnostic accuracy of serum free light-chain assay and its agreement to bone marrow findings.

Materials and Methods: Forty-six patients with the diagnosis of multiple myeloma were enrolled in the study. The patients were grouped into newly diagnosed cases (22 patients,47.8%) and known cases who were under treatment (24 patients,52.2%). Bone marrow study was done and percentage and clonal status of plasma cells were evaluated by a combination of immunohistochemistry and flow cytometry. Free light-chain assay was done in all patients and sensitivity, specificity, positive predictive value, and negative predictive value were analyzed.

Results: Thirty of 46 patients showed monoclonal plasma cell infiltration and 16 patients showed polyclonal plasma cell infiltration based on bone marrow findings. An abnormal κ/λ ratio was seen in 15(68.18%) of new cases and 16(66.6%) of known cases. Sensitivity, specificity, PPV and NPV for κ/λ ratio were 72.73%, 46.15%, 71%, and 50%, respectively.

Conclusion: In conclusion, due to high false positive and false negative results, the presence of an abnormal serum FLC ratio was not equal to the presence of monoclonal gammopathy, and observation of a normal ratio does not exclude the presence of monoclonal gammopathy.

Key word: Multiple myeloma; Serum free light-chain; Bone marrow

INTRODUCTION

Multiple myeloma (MM) is a hematologic malignancy that is caused by the clonal proliferation of malignant plasma cells with subsequent production of monoclonal immunoglobulin[1-3]. This monoclonal protein may be an intact immunoglobulin , or the heavy or light chain, only. Measurement of this protein in serum or urine has been in corporates in clinical guidelines as a diagnostic tool[5].

Different methods have been described in this regard including serum protein electrophoresis (SPEP), urine protein electrophoresis (UPEP), and immunofixation electrophoresis (IFE). Among these test, the IFE technique is considered the gold standard for confirming the presence of these proteins and to distinguish light and heavy chains in

MM[6]. Another recently standardized test is the serum free light chain (sFLC) assay[2]. The hypothesis of checking serum free light chain as a diagnostic test in MM is that when there is excess production of a monoclonal protein in patients with a plasma cell neoplasm, the resultant serum κ/λ ratio will be abnormal, so it will be regarded as a sign of clonality[7]. Minimal residual disease (MRD) detection becomes essential to assess the efficacy of treatment. Nowadays, multiparametric flowcytometry (MFC)is the most used method for MRD presence in multiple myeloma patients[4,8,9]. But, it has been stated that the monitoring of individual light chain levels and the κ⁄λ ratio can be used to see if therapy is working. According to guidelines, a >50% decrease in light chain is considered" partial response". A >90% decrease in light chain levels is regarded as " very good partial response" and "complete response " is defined as normalization of individual light chain and serum κ⁄λ ratio[10]. So in this study, we want to evaluate the effectiveness of serum free light chain assay in diagnosis and follow up the MM patients.

MATERIALS AND METHODS

Forty-six patients with the diagnosis of multiple myeloma that referred to Faghihi Hospital, affiliated to Shiraz University of Medical Sciences, Shiraz, Iran for bone marrow study were selected. The patients were categorized into two groups; newly diagnosed and under-treatment patients. After taking informed consent, the patients' demographic data including age and sex and the results of blood urea nitrogen and creatinine for determination of renal function were recorded. The study was approved by ethics committee of Shiraz University of Medical Sciences.

Bone marrow aspiration and biopsy were performed according to the standard method. The bone marrow aspiration was stained by Wright-Giemsa method and bone marrow biopsy was decalcified by EDTA and embedded in paraffin blocks and stained by hematoxylin and eosin. Bone marrow aspiration and biopsies were reviewed by a hematopathologist and the clonality status were evaluated by using Immunohistochemistry (IHC) for CD 138, kappa and lambda and also flowcytometry for CD138, CD56, CD19, CD38, CD117, and cytoplasmic kappa and cytoplasmic lambda. The typical immunophenotype of myeloma cells includes expression of CD56, CD117, CD138 and CD38, lack of CD19, and monotypic cytoplasmic immunoglobulin expression.

For free light chain assay, 5 cc blood clot sample was taken and after serum separation, the test was performed immunologically by a turbidometric assay method (FREELITE, The Binding Site, Birmingham, United Kingdom). Expected values of serum free light assay were as follow based on kit reference values:

Adult serum	Mean concentration(mg/L)	95 percentile range (mg/L)
Free kappa	8.36	3.30 – 19.40
Free lambda	13.43	5.71 – 26.30
	Mean	Total range
Kappa/Lambda	0.63	0.26 – 1.65

It has been stated that in patients with decreased glomerular filtration rate and Cr>1.3, the reference κ/λ ratio should increase to 0.37-1.73 mg/dl.

Statistical analysis was performed using statistical software SPSS version 20. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of serum free light chain assay were calculated. P-value less than 0.05 were regarded as significant.

RESULTS

The present study consists of 46 patients, 37 (80.4%) males, and 9 (19.6%) females. The age ranged from 37 to 85 years with a mean ± SD of 60.30 ± 10.53. The patients were grouped into newly diagnosed ones (22 patients,47.8%) and known cases who were under treatment (24 patients,52.2%).

Blood urea nitrogen (BUN) and creatinine (Cr) available for 42 patients (91.3%).

Mean ± SD of BUN and Cr was 21.08±15.89 and 1.31±1.01, respectively.

The renal function was compared in newly diagnosed and known patients. BUN didn't show significant difference between two groups but Cr ≥ 1.3 was seen in 4 (14.04%) patients who were in known cases group and 9 (42.8%) patients in the new case group (p-value:0.02) which was statistically significant (Table 1).

Regarding the percentage of plasma cells in bone marrow aspiration and biopsy, we categorized the patient in 4 groups: (the results were based on bone marrow study, flow cytometry and/or IHC).

- Group 1: patients with 0 - 5% plasma cells in bone marrow aspiration and biopsy
- Group 2: patients with 6 - 15 % plasma cells in bone marrow aspiration and biopsy
- Group 3: patients with 16 - 50 % plasma cells in bone marrow aspiration and biopsy
- Group 4: patients with > 50% plasma cells in bone marrow aspiration and biopsy

16 (34.7%) patients had 0-5% plasma cells in bone marrow, 9 (19.5%) cases had 5-15% plasma cells in bone marrow,15 (32.6%) cases had 16-50% plasma cells in bone marrow and 6 (13.04%) cases showed > 50% plasma cells in bone marrow.

In known cases, 16 (66.6%) cases had 0-5% plasma cells in bone marrow, 4 (16.6%) cases showed 5-15% plasma cells in bone marrow,4(16.6%) cases had 16-50% plasma cells and none had > 50% plasma cells in the bone marrow.

In new cases patients 0 (00/0%) cases had 0-5% plasma cells in bone marrow, 5 (22.7%) cases had 5-15% plasma cells in bone marrow,11 (50 %) cases had 16-50% plasma cells in bone marrow and 6 (27.2%) cases had > 50% plasma cells in bone marrow. The difference between two groups regarding plasma cell percentage were statistically significant (P-value : < 0.001) (Table 2).

IHC was done for 27 (58.6%) patients (12 known cases and for 15 new cases) and Flow cytometry was done for 22 (47.8%) patients. IHC study on known case-patient showed monoclonal plasma cells in 8(66.6%) but 4(33.3%) of them showed polyclonal plasma cells infiltration and in new case group, all the patients showed monoclonal plasma cell infiltration.

Serum free light chain assay was performed in all patients. The mean and SD for kappa and lambda and κ/λ ratio were 183.05 ± 361.33, 39.99 ± 79.53, 20.83 ± 53.92, respectively. (Table 3)

Among the 46 patients with multiple myeloma (known cases and new cases), 36 (78.2%) patients showed increased serum FLC concentration, 14(38.8%) patients had high lambda chain and 32(88.8%) cases had high kappa chain. 31 (67.3%) patients showed abnormal κ/λ ratio.

Kappa free light chain showed high level in 16 (66.7%) of known cases in 16 (72.7 %) of new case-patients and Lambda free light chain showed high level in 5(20.8 %) of the known case in 9 (40.9 %) of new case-patients.

12 (75%) patients of group 1, 7 (77.8%) cases of group 2, 9 (60%) patients of group 3 and 4 (66.7%) of group 4 showed high free kappa level. And for lambda free light chain: 1 (6.2%) of group 1, 5 (55.6 %) patients of group 2, 6 (40 %) of cases of group 3 and 2 (33.3 %) patients of group 4 showed high level.

Evaluation of κ/λ ratio in 4 groups of patients was as follows: 10 (62.5%) cases of group 1 had abnormal κ/λ ratio, 6 (66.6%) cases of group 2, 10 (66.6%) cases of group 3 and 6 (100%) cases of group 4 had abnormal κ/λ ratio (Table 4). over all abnormal κ/λ ratio was seen in 15(68.18%) of new cases patient and 16(66.6%) in known cases patients.

In this study, the final diagnosis and percentage of monotypic plasma cells were based on bone marrow biopsy and aspiration and the result of immunophenotyping (whether flow cytometry, IHC or both). So out of 46 patients, we had 30 patients who showed monoclonal plasma cell infiltration and 16 patients showed polyclonal plasma cell infiltration.

According to serum free light assay, 23 patients showed an abnormal kappa/lambda ratio (true positive), 7 patients showed normal ratio in spite of monoclonal plasma cell in bone marrow (false negative), 7 patients showed normal ratio and polyclonal plasma cell infiltration (true negative and 9 patients showed abnormal kappa/lambda ratio in spite of polyclonal plasma cell in bone marrow (false positive).

So, Sensitivity and specificity, PPV and NPV and kappa correlation for the free light chain was determined by comparing their result with bone marrow findings. For kappa free light chain sensitivity and specificity, PPV and NPV was 48.5% and 92.3 %, 91.4% and 51.2% respectively.

For lambda free light chain sensitivity and specificity ,PPV and NPV was 21.2 % and 100 %, 100% and 18.53% ,respectively.

Sensitivity and specificity, PPV and NPV for κ/λ ratio were determined by comparing their result with bone marrow findings, was as follows 72.73%,

46.15%, 71%, and 50%, respectively. The kappa correlation between two methods was 0.21which showed a fair agreement (Table 5).
So, in 30 (65.2%) cases, κ /λ ratio and the result of bone marrow biopsy was concordant but in 16 (34.7%) cases, kappa/lambda ratio was discordant with the result of bone marrow.

Table 1. Mean of lab data in two study groups (new cases and known cases)

Lab data	Mean		P
	Known case	New case	
BUN	28.3	25.7	0.07
Cr	1.01	1.6	0.02

Table 2. Percentage of plasma cells in known cases patients and known cases patients

B BM / %PC M / %PC	Total	Known case	Knew case
0-5%	16 (34.7%)	16 (66.6%)	0 (0.00%)
6-15%	9 (19.5%)	4 (16.6%)	5 (22.7%)
16-50%	15 (32.6%)	4 (16.6%)	11 (50%)
>50%	6 (13.04%)	0 (0.00%)	6 (27.2%)

Table 3. Mean and SD for kappa and lambda and κ/λ in MM patients

Free light chain	Reference interval	Mean	SD
Kappa (mg/L)	3.30 – 19.40	183.05	361.33
Lambda (mg/L)	5.71 - 26.30	39.99	79.53
κ/λ (mg/L)	0.26 - 1.65	20.83	53.92

Table 4. Percentage of normal and abnormal κ/λ ratio in 4 groups

BM / % PC	Normal κ/λ ratio	Abnormal κ/λ ratio
0-5%	6 (38.5%)	10 62.5%
6-15%	3 (33.4%)	6 (66.6%)
16-50%	5 (33.4%)	10 (66.6%)
> 50%	0 (00.0%)	6 (100%)

Table 5. Sensitivity and specificity of free light chain assay

Free light chain	Sensitivity	Specificity
Abnormal kappa	48.5%	92.3%
Abnormal lambda	21.2%	100%
/λ	72.73%	46.15%

DISCUSSION

Multiple myeloma is account for 10% of all hematological malignancies and 1 % of all malignancies. Multiple myeloma is diagnosed by abnormal proliferation of plasma cells with subsequent production of a monoclonal immunoglobulin or free light chains [3]. The mainstay of the diagnosis and monitoring of these disorders is the evaluation of an abnormal monoclonal immunoglobulin, the M component, in the serum and/or urine, by means of electrophoresis and immunofixation[11].

Several recent studies have evaluated the important role of serum free light chain measurements for the diagnosis, management and follow up of patients with multiple myeloma[12]. Recently, the International myeloma working group has been adding serum FLC ratio (involved/uninvolved) greater than or equal to 100 to multiple myeloma diagnostic criteria. This update means that asymptomatic patients, without evidence of related end-organ damage (CRAB criteria), can be diagnosed with MM if they have serum FLC ratio of more than 100 in addition to 10% bone marrow plasma cells or having plasmacytoma[13].

Traditionally in patients under treatment, quantitation of bone marrow plasma cells on trephine biopsies (with a combination of hemotoxylin and eosin stains and immunohistochemistry) allows for accurate response assessment even in patients with negative serum and urine immunofixation. Originally, a complete response was defined as bone marrow with less than 5% plasma cells, irrespective of their clonal nature. The definition was further refined to stringent complete response, by the addition of the sFLC assay plus immunohistochemical clonal assessment on the trephine biopsy [13].

In this study, we measured serum free light chain in 46 MM patients in different stages of disease (new cases or under treatment). We observed abnormal serum free light chain in 78.2% of patients. The abnormal free chain was predominantly kappa (88.8%) versus lambda chain which was present in excess amount in 38.8% patients and about 67.3% of our patients showed abnormal kappa/lambda ratio. Our results were in concordance with previous studies.

Sthaneshwar et al (2009) studied free light chain in 45 multiple myeloma patients and they stated that 77% of their patients showed higher kappa free light chain, and 22% had higher lambda free light chain[14]. Also in 2017 Kubicki et al, compared two different tests for analysis of serum free light chain in multiple myeloma patients, overall in most of the patients a high level of kappa FLC was seen compared to the level of lambda free light chain[15].

According to our results, kappa free light chain was high in (66.7 %) of known cases and (72.7 %) of new patients and Lambda free light chain was in excess in (20.8 %) of known cases and in (40.9 %) of new patients and as it was expected the free light chain was increased in new patients more commonly than known cases who are under treatment.

In this study, the final diagnosis of multiple myeloma and percentage of monotypic plasma cells was based on bone marrow biopsy and aspiration and the result of immunophenotyping (whether flow cytometry, IHC or both). So out of 46 patients, we had 30 patients who showed monoclonal plasma cell infiltration and 16 patients showed either polyclonal plasma cell infiltration or plasma cell count of less than 5% in which the IHC was not performed.

According to serum free light assay, 23 patients showed an abnormal kappa/lambda ratio (true positive), 7 patients showed normal ratio in spite of monoclonal plasma cell in bone marrow (false negative), 7 patients showed normal ratio and polyclonal plasma cell infiltration (true negative) and 9 patients showed abnormal kappa/lambda ratio in spite of polyclonal plasma cell in bone marrow (false positive).

Proposed causes of false-positive results include polyclonal hypergammaglobulinemia, infection, inflammation and connective tissue disease such as SLE, chronic kidney disease[14,16,17]. In normal conditions, the κ/λ ratio is about 2/1. FLCs have a serum half-life of 2–6 h as they are rapidly cleared by the glomeruli and metabolized in the proximal tubules [11].

In patients with renal failure, decrease clearing of sFLC occurs leading to an increase in sFLC half-life

and their serum level. In additopn, FLC ratio can also change[18]. It has been stated that in patients with decreased glomerular filtration rate and Cr>1.3, the κ/λ ratio should increase to 0.37-1.73 mg/dl [18,19].

Gurmukh Singh in 2016 stated that Abnormal kappa/lambda ratio was reported in 36.4% of in the absence of monoclonal gammopathy. When the renal κ⁄λ ratio was used, the rate of abnormal κ⁄λ ratio was 30.1%. In contrast, in patients with monoclonal gammopathy, the usual κ⁄λ ratio was abnormal in 54.8% of the patients (26). He claimed that because (1) an abnormal κ⁄λratio is not diagnostic of monoclonal gammopathy, (2) a normal κ⁄λ ratio does not exclude monoclonal gammopathy, (3) there is a high false-positive rate for abnormal κ⁄λ ratio in samples without monoclonal gammopathy, and (4) a high rate of false-negative results for κ⁄λ ratio is noted in samples from patients with monoclonal gammopathy, the usefulness of serum-free light chain assay, in routine clinical testing, is questionable [20].

Also, analytical issues may lead to false-positive results because of polymerization and aggregation of light chains [21].

In another study by Singh G that overall, serum FLC κ/ λ ratio was falsely negative in nearly 27% of the cases in which a monoclonal Ig was detectable by electrophoretic methods. The false-negative rate was higher for lesions with lambda light chains (31.2%) than those with kappa light chains, (23.7%). Among different plasma cell disorders, MGUS showed the highest false-negative rate (55%) [22].

In this study, the diagnosis was based on bone marrow plasma cell percentage and clonality, which was determined, by flow cytometry and or IHC and plasma cell count in bone marrow smears. Complete hematological response was considered in patients with less than 5% plasma cells in marrow after treatment although the kappa/lambda ratio was abnormal in some of them. We categorized them as false-positive results of serum FLC but it may be a true finding because the involvement of bone marrow may have a patchy distribution especially in patients under treatment. The other possibility is the presence of extramedullary disease which results in the abnormal section of FLCs. So correlation with other tests such as serum immunofixation electrophoresis and imaging modalities such as PET CT scan is recommended.

Sensitivity and specificity, PPV and NPV and kappa correlation for the free light chain was determined by comparing their result with bone marrow findings. For kappa free light chain sensitivity and specificity, PPV and NPV was 48.5% and 92.3 %, 91.4% and 51.2% respectively. For lambda free light chain sensitivity and specificity, PPV and NPV was 21.2 % and 100 %, 100% and 18.53 %, respectively.

Sensitivity and specificity, PPV and NPV For κ⁄λ ratio were determined by comparing their result with bone marrow findings, was as follows 72.73%, 46.15%, 71%, and 50%, respectively.

The kappa correlation between the two methods was 0.21which showed a fair agreement. So, In 30 (65.2%) cases, κ /λ ratio and the result of bone marrow biopsy was concordant but in 16 (34.7%) cases, kappa/lambda ratio was discordant with the result of bone marrow. Also according to data, we detected abnormal κ/λ ratio in 15 (68.18%) of new patients and 16 (66.6%) of known cases.

Yang et al compared two methods of FLC assays and clinical sensitivity was determined for each method. Based on the clinical diagnosis, the sensitivity of the κ/λ For the Freelite assay (which is used in our study) was 83.8% [17].

Singhal S et al evaluated the relationship between serum FLC and serum immunofixation electrophoresis and reported a sensitivity and specificity of 66% and 69%, respectively for FLC [23].

Jurczyszyn et al in 2015 reported that more than 90% of patients with multiple myeloma show abnormal κ⁄λ ratio and reported Immunofixation together with the serum FLC assay has a sensitivity of 93.8% and a specificity of 96.8% in patients examined for a monoclonal gammopathy [24].

Sthaneshwar et al. evaluated the serum free light chain ratio in 45 newly diagnosed multiple myeloma patients and reported the sensitivity and specificity of 86% and 100 %, respectively [14].

Mohammad R. Nowrousian et al. in 2005 showed abnormal FLC in 54% of serum samples compared with 25% of urine tests. They reported serum FLC assays are significantly more sensitive for detecting

monoclonal FLC than urine IFE analysis, for monitoring disease course and response to treatment [16].

Bradwell et al. in 2003 demonstrated that patients with light chain multiple myeloma had abnormal concentrations of the serum FLC and abnormal κ/λ ratios at the time of diagnosis [25].

It has been proposed that the measurement of serum FLCs may have a role in assessing the residual disease. In addition to the patients with light chain myeloma and nonsecretory disease, Changes in free light chain levels has been reported to be useful for tracking the disease status in almost all people with myeloma. Due to the short half-life of FLCs (2–6 h, compared with 21 days for intact immunoglobulin G), the assay can be useful potentially for earlier monitoring of the efficacy of a given therapeutic program [26].

The free light chain assay can help in the detection and monitoring of myelomas by quantifying monoclonal protein in multiple disease settings. The goal is to have the individual light chain numbers and ratio come back within the normal reference range by treatment (stringent complete response). A decrease in light chain levels of 50%of more is considered the partial response. A decrease in light chain levels of> 90% is a very good partial response[10].

Kumar et al. in 2010 report that a decreased in serum FLC level correlates to response to treatment[10].

CONCLUSION

In conclusion, due to high false positive and false negative results, the presence of an abnormal serum FLC ratio does not equate to the presence of a monoclonal gammopathy, and observation of a normal ratio does not exclude the presence of a monoclonal gammopathy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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