Original Article

The prognostic role of p-53 protein Immunohistochemical expression in multiple myeloma

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Abstract:

Background: Several factors render multiple myeloma (MM) an interesting subject for study by researchers. These include marked progress in understanding the molecular biology of normal and neoplastic plasma cells and recent advances in molecular genetics techniques. Among molecular markers, p-53 cancer suppressor gene have been widely studied.

Aim: is to correlate p-53 protein expression in multiple myeloma, as examined by immunohistochemical method, with some pathological and clinical parameters (Clinical stage and cytological grade).

Patients and methods: This is a retrospective study; whereby archival paraffin-embedded BM tissue blocks along with the clinical and hematological records of fifty patients (29 males and 21 females), with multiple myeloma and twenty controls were obtained from the Department of Hematology of the Medical City Teaching Laboratories in the period from April 2012 to April 2014. P-53 was studied by immunohistochemical staining.

Results: There was a significant positive linear correlation between increasing scores of p-53 and advancing clinical stages of disease. There was a significant positive linear correlation between increasing scores of p-53 and decreasing maturity of plasma cells.

Conclusions: Multiple myeloma patients with advanced clinical stages and immature plasma cell morphology had high p-53 scores. Since the above-mentioned factors are known independent prognostic factors, p-53 expression can readily be adopted to identify myeloma patients with an adverse prognosis.

Key words: Multiple myeloma; P-53; IHC.

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Introduction:

P53 gene, located on chromosome 17 band p13.1, is frequently mutated in a wide variety of human tumors, including multiple myeloma. It encodes a 53-kD phosphoprotein that is normally present in the nuclei of the cells. [1-3,] The p53 tumor suppressor protein is a transcription factor that is involved in the cell cycle arrest and induction of apoptosis in genetically damaged cells. Structural alterations and point mutations of the p53 tumor suppressor gene occur in variable frequencies in MM; they have been associated with poor survival and nonresponse to therapy, suggesting that p-53 may play a role in the clinical course of the disease.[4]

Patients and methods:

This is a retrospective study; whereby archival paraffin-embedded bone marrow (BM) blocks along with the clinical and hematological records of fifty patients with multiple myeloma (MM) were obtained from the Department of Hematology of the Medical City Teaching Laboratories in the period from April 2012 to April 2014. The study include 29 males and 21 females, with a mean age of 60.24 (± 5.44) years. The patients were newly diagnosed and did not receive prior treatment. The BM biopsies were performed at diagnosis. All relevant clinical and laboratory data available for all patients were reviewed, and the peripheral blood and marrow aspirate smears (stained with Leishman stain) were re-examined carefully. BM biopsy sections stained with H&E were also re-examined. All patients were selected according to the WHO diagnostic criteria, [5] that include the followings:

Symptomatic myeloma: M-protein in serum or urine, Bone marrow clonal plasma cells increased, related organ or tissue impairment (e.g. hypercalcemia, renal insufficiency, anemia, bone lesions, hyperviscosity and recurrent infection).

Histological grade of the disease was based on morphological characteristics of the dominant cell population: ●Grade-I: mature. ●Grade-II: intermediate. ●Grade-III: plasmablastic. [6]

According to the degree of bone marrow infiltration, patients were classified into two groups: ●Group-I: BM plasma cells less than 50%. ●Group-II: BM plasma cells more than 50%. [6]

The Salmon-Durie staging system [7] was used in this study for clinical staging of the patients.

I. High tumor mass (stage III) (>1.2 x 1012 myeloma cells/ m2).

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II. Low tumor mass (stage I) (<0.6 x 1012 myeloma cells/ m2).
III. Intermediate tumor mass (stage II) (0.6–1.2 x 1012 myeloma cells/m2).
Paraffin-embedded BM blocks of twenty control individuals (14 males and 6 females), with a mean age of 60.9 (± 6.4) years, (age and sex matched), along with their hematological reports were also collected. All the control BMs were negative for infiltrative lesions and were obtained from patients with anemia. All paraffin-embedded bone marrow blocks (patients and control) were subjected to immunohistochemical staining for p-53 antigens in the histopathology department of the Medical City Teaching Laboratories. Immunohistochemistry: Serial 5-µm sections of the diagnostic BM biopsy were cut, deparaffinized and heat-induced antigen retrieved. The myeloma cells were immunostained for p-53, DO-7 (DAKO, Glostrup, Denmark), at a 1:50 dilution using immunoperoxide reagents and Diaminobenzidin-tetrahydrochloride (DAB) (BioGenex, laboratories, San Ramon, CA). A paraffin biopsy of squamous cell carcinoma of the skin specimen served as a positive immunohistochemistry control. For the negative control, all reagents were added except the diluted primary antibody. The percentage of p53 immunostained nuclei was evaluated microscopically under ×400 and ×1000 magnification (the percentage of p-53 positive plasma cells was determined by counting 500 plasma cells in different visuals fields). Any myeloma cell nuclear p53 immunoreactivity was considered positive. Cases were considered positive if p53 stained 10% or more of the myeloma cell nuclei. [8, 9]
Statistical analysis: was performed with the SPSS 20 statistical software program. Univariate data were summarized using standard descriptive statistics. Associations between categorical variables were assessed via cross tabulation and chi-square. ANOVA and t-test were used to compare means of continuous variables. Spearman correlation was used to measure the association between two continuous variables or when at least one variable was ordered. Exact tests were used to calculate the p value. In all statistical analyses, a p value < 0.05 was considered to indicate statistically significant difference.

Results:

Fifty patients with multiple myeloma, 29 males and 21 females were included in this study. The Ages incidence ranged between 47 and 69 years with mean age of (60.24 ± 5.44) years with an M:F ratio of 1.4:1. (All twenty control BMB were negative for p-53).Based on the IHC staining pattern, the 50 patients were subdivided into two groups: 1. P-53-positive [n = 11 (22 %)] (Fgure-1), and 2. P-53-negative [n = 39 (78 %)].

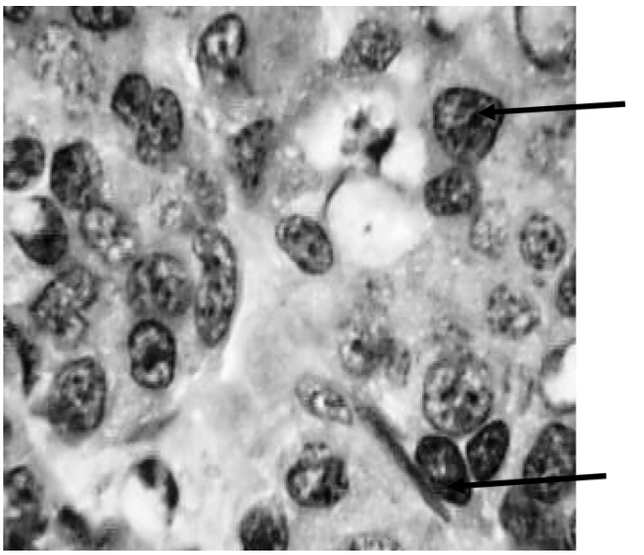


Figure-1: P-53 positive IHC nuclear staining on a BM biopsy of MM (arrows), (×1000).

There were no significant differences in age and laboratory parameters between the two groups as shown in table-1.

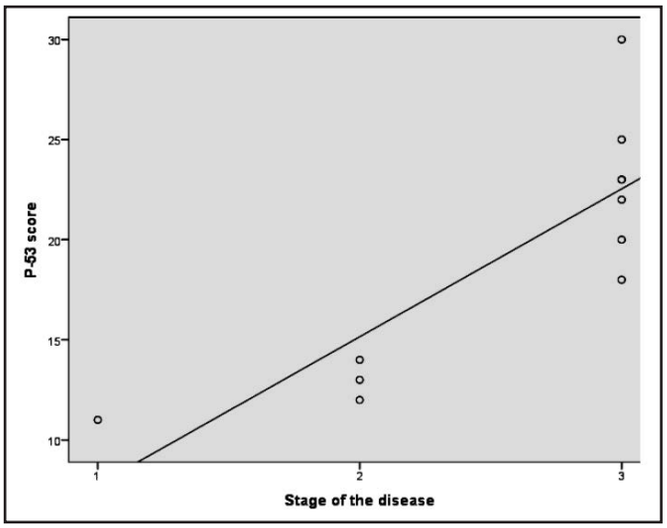
Table-1. Patient's characteristics according to p-53 expression in MM patients

Immunohistochemical result for P-53			p
Patient's characteristics	Positive (N = 11) Mean ± SD	Negative (N = 39) Mean ± SD	
Age	61.36 ± 4.3	59.92 ± 5.74	N.S
Plasma cell % (BM)	62.73 ± 25.7	47.85 ± 18.5	N.S
Hb (gm/ dL)	8.81 ± 1.35	9.48 ± 1.7	N.S
Platelet count (× 10^9/L)	221.27 ± 92.07	226.01 ± 82.23	N.S
Total WBC (×10^9/L)	7.27 ±1.93	7.17 ± 2.53	N.S
Serum calcium (mg/dL)	10.5 ± 1.55	10.49 ± 2.125	N.S
Serum creatinine (mg/dL)	1.96 ± 0.53	1.84 ± 0.721	N.S
Total serum protein (gm/dL)	9.24 ± 2.15	9.46 ± 1.66	N.S

There was no significant association between p-53 expression and clinical stages of disease (Table-2).
There was a significant positive linear correlation between increasing scores of p-53 (p-53 positive cases) and advancing clinical stages of disease (p = 0.001, figure-2).

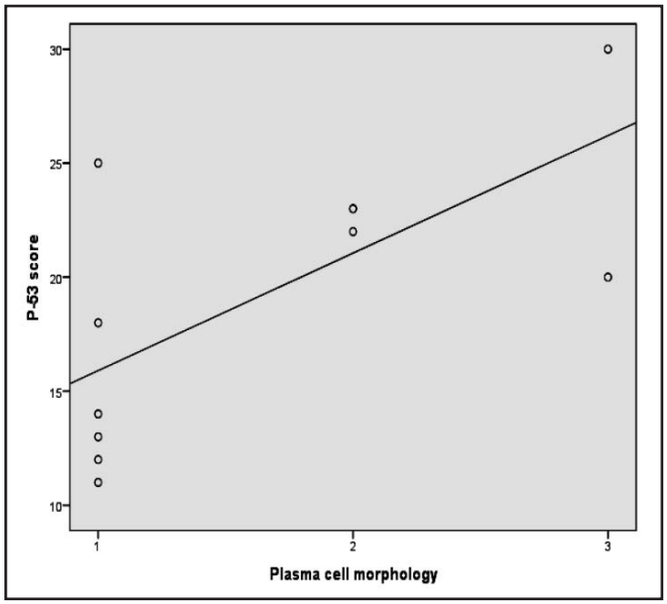
Table-2. Associations between p-53 expression and pathological characteristics of MM patients.

Immunohistochemical result for P-53				p
Patient's characteristics		Positive (N = 11)	Negative (N = 39)	
Stage of the disease	Stage-I	1/11 (9.1%)	5/39 (12.8%)	N.S
	Stage-II	3/11 (27.3%)	10/39 (25.6%)	
	Stage-III	7/11 (63.6%)	24/39 (61.5%)	
Plasma cell % (BM)	Less than 50 %	5/11 (45.5%)	26/39 (66.7%)	N.S
	More than 50 %	6/11 (54.5%)	13/39 (33.3%)	
Plasma cell morphology	Mature	6/11 (54.5%)	20/39 (51.3%)	N.S
	Intermediate	3/11 (27.3%)	15/39 (38.5%)	
	Immature	18.2%))2/11	4/39 (10.3%)	
BM infiltration pattern	Diffuse	7/11 (63.6%)	25/39 (64.1%)	N.S
	Non-diffuse	4/11 (36.4%)	14/39 (35.9%)	



r = 0.855. p = 0.001
Figure-2. Correlation between P-53 score and clinical stages of MM.

There was no significant association between p-53 expression and plasma cell percent in the bone marrow (table-2). There was no significant association between p-53 expression and plasma cell morphology (table-2), however there was a significant positive linear correlation between increasing scores of p-53 (P-53 positive cases) and decreasing maturity of plasma cells (p = 0.04, figure-3).



r = 0.625. p = 0.04
Figure-3. Correlation between p-53 score and plasma cell morphology.
No significant association found between p-53 expression and the pattern of bone marrow infiltration (Table-2).

Discussion:

Multiple Myeloma occurs with variable frequencies throughout the world, with the highest rates in the USA black populations and least in Japan and some Eastern and European countries. [10-12] The national prevalence rate constitutes about 0.75% of all cancers registered, [13] a figure, which is comparable to figures, reported from International countries. [10, 11, 14] Immunohistochemistry has provided an objective method for assessing P-53 that was shown to be a significant prognostic indicator of various malignancies including multiple myeloma. [1, 3, 4]

In this study, the mean age of 60.24 years is similar to previous national and international reports, which ranged between 60 and 61 years. [8, 9, 15, 16] The male/female ratio of 1.4:1 in this study is similar to other previous national and international studies. [8, 9, 15, 16] The frequency of p-53 protein expression detected by immunohistochemistry in myeloma patients in this study was 22%, which was in accordance with other studies that varies from 8-39%. [17-19] No significant differences were found in age and laboratory parameters between p-53-positive and p-53-negative patients (Table-1). Other studies reported similar results. [17-20] The coexistence of p-53-positive and -negative cells within the same tumor population supports the hypothesis that p-53 dysregulation can be a late event in the progression of the disease. Although the p-53 alteration

may occur early in the course of the disease, as shown by the p-53 positivity in a proportion of patients in stage-I and II of the disease; the highest frequency p-53-positive cells, has been observed in stage-III (Table-2). These findings are in agreement with previous studies, which have shown a strong correlation between p-53 mutations and progression in hematologic malignancies including MM. [9, 17, 18, 20]

This study revealed that p-53 score is significantly correlated with the clinical stage of the disease (Figure-2), and thus it is an important prognostic variable in patients with MM. These data concur with those reported by other workers, in their analyses of p-53 expression in patients with MM. These investigators reported that significantly larger proportion of their p-53-positive patients are associated with advanced clinical stage of the disease than patients in early clinical stages. They also showed that the score of p-53 was strongly correlated with advanced disease, progressive disease, refractoriness to therapy and reduced survival. [17-20] There was no significant association between p-53 expression and plasma cell percent in the BM (Table-2), this result is in accordance with the results of previous studies. [19, 20]There was no significant association between p-53 expression and plasma cell morphology (Table-2). This result is in accordance with the results of a previous international study [21]; however, there was a significant positive linear correlation between increasing scores of p-53 and decreasing maturity of plasma cells (figure-3). Our findings are similar to the result reported by other workers. [22] This finding suggests that impairment of the p-53 growth control pathway is associated with tumor progression in MM. [19, 20, 22] This study showed no significant association found between p-53 expression and the pattern of BM infiltration (Table-2). Our findings are similar to the results reported by other workers. [20-22] Regarding the thirty-nine p-53-negative MM cases, the IHC procedure cannot exclude p-53 gene deletion in MM cells. This deletion can be confirmed by the application of FISH technique on chromosomal preparations. The absence of a positive fluorescent signals confirm gene deletion. [20-22]

Conclusions:

Multiple myeloma patients with advanced clinical stages and immature plasma cell morphology had high p-53 scores. Since the above-mentioned factors are known independent prognostic factors, p-53 expression can readily be adopted to identify myeloma patients with an adverse prognosis who may be a candidate for risk -adopted therapies.

Author contribution:

Muna Abdulmueen Abdullah:
- Study conception.
- Acquisition of data.
- Interpretation of data.

Abdulkareem Mohammad Jaafar:
- Study design.
- Data analysis.
- Drafting of manuscript.
- Critical revision.

Adel Rabeea ALsaadawi:
- Interpretation of data.
- Drafting of manuscript.

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