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Original Article

Levels of interleukin-16 in peripheral blood of 52 patients with multiple myeloma and its clinical significance

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Abstract: Purpose: To explore the role of serum interleukin-16 (IL-16) in the occurrence of multiple myeloma (MM) and after the success chemotherapy and its clinical significance. Methods: 52 cases of MM patients, 30 cases of AML patients and 30 healthy volunteers from Jan. 2011 to Jan. 2015 were collected in this study. There was 39 MM patients received chemotherapy. Among those, 24 patients received VAD regimen chemotherapy and 15 patients received BD regimen chemotherapy. Serum IL-16, cystatin C (Cys-C), lactate dehydrogenase (LDH) and levels of β2-microglobulin (β2-MG) were detected before and after the therapy of MM patients. And those results were compared to that of patients with acute myelogenous leukemia (AML) and normal people respectively. Results: The levels of serum IL-16, Cys-C, LDH and β2-MG in MM group were remarkably higher than that of normal control. It was of statistical significance of this difference ($P<0.05$). Levels of serum IL-16, Cys-C and LDH of MM patients who received therapy were all lower than that of patients before therapy. The serum IL-16 and β2-MG of 52 patients by preliminary diagnosis were analyzed through Pearson correlation analysis before they received therapy. The results showed that there was positive correlation between levels of IL-16 and β2-MG ($r=0.782$, $P<0.01$). Conclusions: A high serum IL-16 level detected in newly diagnosed MM patients and its correlation with known factors of disease activity as well as the decrease of IL-16 after chemotherapy suggest that IL-16 may be implicated and a potential therapeutic target for MM.

Keywords: Interleukin-16, multiple myeloma, clinical significance

Introduction

Multiple myeloma (MM) which makes up 10% of hematological malignancy is a malignant disease caused by abnormal proliferation of bone marrow plasmacyte [1]. IL-16 is an important immunomodulatory factor and has close relation with the occurrence and development of several cancers [2, 3]. For the purpose of exploring the role of IL-16 in the occurrence of MM and its clinical significance, serum IL-16, cystatin C (Cys-C), serum lactate dehydrogenase (LDH) and levels of β2-microglobulin (β2-MG) were detected before and after the therapy of MM patients. And those results were compared to that of patients with acute myelogenous leukemia (AML) and normal people respectively.

Materials and methods

Characteristic of patients

52 cases of MM patients and 30 cases of AML patients recorded in Department of Hematology, Affiliated Hospital of Jinggangshan University from Jan. 2011 to Jan. 2015 were collected in this study. Among 52 cases of MM patients (MM group) by preliminary diagnosis, there were 28 male cases and 24 female cases. The median age of group MM was 58 (25-85). The staging of International Staging System (ISS) was as below: 10 cases in period I, 14 cases in period II, and 28 cases in period III; 26 cases of type IgG, 15 cases of type IgA and 11 cases of light chain type. There were 39 patients received chemotherapy. Among those, 24

Table 1. Comparison of levels of IL-6, Cys-C, LDH and β2-MG in MM, AML and Control groups before treatment (mean ± SD)

Groups	Cases (n)	IL-16 (ng/L)	Cys-C (mg/L)	LDH (U/L)	β2-MG (mg/L)
MM	52	172.35±21.45*	2.94±0.68*,#	230.65±44.91*,#	9.17±1.02*
AML	30	199.33±34.67*	0.91±0.13	490.01±79.24	/
Control	30	88.23±13.81	0.74±0.09	110.33±13.24	1.14±0.37

Note: Compared to the control group, *P<0.05; Compared to AML group, #P<0.05; /: No examination.

Table 2. Comparison of serum levels of IL-16, Cys-C and LDH in MM patients with different ISS staging

ISS staging	Cases (n)	IL-16 (ng/L)	Cys-C (mg/L)	LDH (U/L)
I	10	128.24±11.60	1.34±0.31	171.55±22.32
II	14	160.11±25.48*	1.95±0.89	210.45±35.41
III	28	199.67±46.81*,#	3.13±0.92	235.32±49.32*

Note: ISS: International Staging System; compared to Stage II, *P<0.05; compared to Stage I, #P<0.05.

Table 3. Comparison of serum levels of IL-16, Cys-C and LDH in MM patients before and after treatment

Groups	Cases (n)	IL-16 (ng/L)	Cys-C (mg/L)	LDH (U/L)
Before treatment	39	161.35±45.12	2.47±0.64	220.35±36.41
After treatment	39	104.35±31.12	1.12±0.29	138.64±30.15

Note: Levels of serum IL-16, Cys-C and LDH of MM patients who received therapy were all lower than that of patients before therapy (All P<0.05).

patients received VAD regimen chemotherapy: vincristine 0.5 mg/d from the first to forth day; adriamycin 10 mg/d from the first to forth day; dexamethasone 40 mg/d from the first to forth, ninth to twelfth and seventeenth to twentieth day with a 28-day treatment period. 15 patients received BD regimen chemotherapy: bortezomib 1.3 mg/m^2 at the first time, forth, eighth and eleventh day, dexamethasone 20 mg/d, at the first, second, forth, fifth, eighth, ninth, eleventh and twelfth day with a 21-day treatment period. Symptomatic and supportive treatment was received at the meantime. There were 30 cases of AML patients by preliminary diagnosis without treatment (AML group) among which there were 19 male cases and 11 female cases and the median age was 51 (22-59). There were 30 healthy volunteers (normal control) among which there were 18 men and 12 women and the median age was 54 (35-66). This research has been approved by Ethics Committee of our hospital. Written informed consents were obtained from all patients and normal subjects.

Specimen collection and preparation

2 ml of limosis vein blood (heparin anticoagulant) was collected from MM patients before chemotherapy and after 4-6 courses of treatment of chemotherapy respectively. Then serum was extracted and reserved in EP tube at the temperature of -80°C after limosis vein blood was centrifuged at the speed of 2000 r/min for 10 minutes (centrifugal radius was 8 cm). Operating steps followed the kit instructions. The methods of collection and preparation of specimen from AML patients (before therapy) and normal controls were the same.

Examination methods

Serum IL-6 was detected by ELISA (kit from Shanghai Sangon Biological Engineering co., Ltd.); serum β2-MG was detected by latex turbidimetry (kit from American Applied Biosystems Company); Cys-C was detected by immunoturbidimetry (kit from Ningbo Meikang Biotechnology co., Ltd.); and LDH was detected by velocity method (kit from Shanghai Fosun Long March Medicine and Science co., Ltd.). Automatic enzyme mark of model 680 was the product of American Bio-Rad Company. 3 wells were set for each experiment.

Statistical analysis

Quantitative data were presented as mean ± standard deviation (SD). The paired student' t test and unpaired student' t test was performed to compared data within group and between the groups. Pear man correlation coefficients

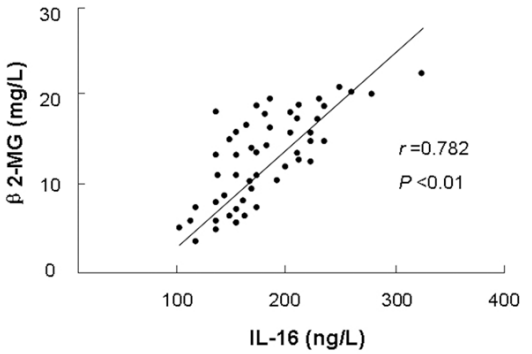

Figure 1. Relationship between serum IL-16 and β2-MG in MM patients before treatment.

(r) were employed to analyze the correlation between serum IL-16 and β2-MG of MM patients. All statistical analyses were performed using SPSS 19.0 for Windows (SPSS, Inc., Chicago, IL, USA). A *P*-value <0.05 was considered statistically significant.

Results

The comparison of the levels of serum IL-16, Cys-C, LDH and β2-MG between MM group (before therapy) and two controls

As shown in **Table 1**, the levels of serum IL-16, Cys-C, LDH and β2-MG in MM group were remarkably higher than that of normal control. It was of statistical significance of this difference (P<0.05). The difference of over-expression of IL-16 in MM group and AML group represented no statistical significance (P>0.05).

The comparison of levels of IL-16, Cys-C and LDH among patients in MM group with different ISS staging

As shown in **Table 2**, the higher levels of IL-16, Cys-C were observed in MM patients of Stage III than that of patients of Stage I and II (P<0.05) and the level of serum LDH was higher than that of patients of Stage I. It was of statistical significance of this difference (P<0.05), while the difference of levels of IL-16, Cys-C and LDH between patients of Stage I and II reflected no statistical significance (P>0.05).

The comparison of levels of serum IL-16, Cys-C and LDH of MM patients before and after therapy

There was 39 MM patients received chemotherapy among the total 52 MM patients. Levels of serum IL-16, Cys-C and LDH of MM patients who received therapy were all lower than that of patients before therapy. It was of statistical significance of this difference (All P<0.05, **Table 3**).

The correlation between serum IL-16 and β2-MG of MM patients

The serum IL-16 and β2-MG of 52 patients by preliminary diagnosis were analyzed through Pearson before they received therapy. The results showed that there was positive correlation between levels of IL-16 and β2-MG (r=0.782, P<0.01, **Figure 1**).

Discussion

The pathogenesis of MM was complex. The change of the level of cytokines took effect in its occurrence and development [4]. Cytokines such as IL-6, IL-16 and TNF-α could be the autocrine or paracrine of both MM cells and marrow stroma cells, forming mutual response effect on the basis of cell factors so as to ultimately influence the proliferation, survival and migration of MM cells [5]. IL-16 was nonspecific multi-functional control agent, combined with T cell, eosinophilic granulocyte, dendrite cell and monocyte through receptor CD4 [6, 7] or CD9 [8]. CD4(+) cells were recruited to inflammatory site by chemo taxis in order to provoke immune response. Meanwhile, IL-6, a kind of chemoattractants, could stimulate the peripheral mononuclear cells of CD14(+) CD4(+) to secrete proinflammatory cytokine IL-1β, IL-6, IL-15 and TNF-α to participate in the proof procedure of immune diseases [9].

This research has found that the over-expressed IL-16 in MM patients would be declined by chemotherapy. ELISA was applied to measure the levels of serum IL-16 in MM group and normal control. The results showed that compared with normal control, the level of serum IL-16 of MM patients by preliminary diagnosis was significantly increased before therapy and it was positive correlation with clinical staging. Alexandra is et al. [10] have obtained the same results which indicated that the level of serum IL-16 reflected the severity of diseases, which could be regarded as one of clinical staging gists. The over-expression of IL-16 was marked in a variety of solid tumors in some researches [11-13]. In this study, it was also observed that the level of serum IL-16 of MM patients was decreased

dramatically after therapy. Researches have stated that tumor burden could be reduced by chemotherapy medicine. However, there was a gap that whether the decreased level of serum IL-16 accounted for MM cells decreasing which induced the decrease of IL-16 so that bone marrow microenvironment was changed and clinical symptom was improved. This study has also found that the variation trend of the level of IL-16 was in accordance with the levels of serum LDG and Cys-C, which indicates that IL-16 like LDH and Cys-C could be the auxiliary index to reflect the tumor burden and prognostic of MM patients.

Alexandrakis et al. [14] have reported that the levels of cell factors of MM patients such as serum IL-6R and angiogenine were increased significantly. And the increase degree was parallel to clinical staging and severity of diseases. It indicated that the level of IL-6 was concerned with MM progression and the severity. The research verified that the levels of serum IL-6 and IL-16 of MM patients were both higher than that of normal people and there was positive correlation between them [10]. Presumably, IL-16 could be likely to take effect in the occurrence of MM just as IL-6.

There was no evidence to prove that IL-16 could induce apoptosis directly. However, bacteria and viruses could ultimately cause apoptosis through combining with receptor CD4 [15, 16]. Oken et al. [17] have also verified that the total number of T lymphocytes and the ratio of CD4(+) T cells were lower than that of normal people, and the increase of CD8(+) T cells expressed by HLA-DR could lead the decrease of the CD4/CD8 ratio. Moreover, the decrease degree was consistent with the decrease degree of patients' survival time. In MM, the decrease of CD4(+) T cells could be mediated by activated CD8(+) T by IL-16 so as to induce immunoregulating disorder [18]. The reports by Atanackovic et al. [19] showed that overexpressed IL-16 was observed in MM cell lines and MM cells from marrow of MM patients than that of normal people, which concluded that IL-16 could the autocrine of MM cells. In addition, the gene silence of IL-16 was caused by transfecting the intracellular IL-16 siRNA in MM cell line EJM and KMS-12-BM, and then it was found that the degree of MM cell proliferation was decreased by 80% compared to the former condition. Additionally, applied the monoclonal antibody of IL-16 could achieve the same result. Therefore, IL-16 was an important cell factor which could promote the MM cell proliferation and disease development.

Previous studies have verified that β2-MG could reflect the activity of MM cell proliferation and it could be regarded as independent prognostic factor of MM [20]. In this study, it was found that the level of serum IL-16 of MM patients was considerably higher than that of normal control and there was positive correlation with the level of β2-MG. It indicated that IL-16 was likely to be a significant prognostic evaluation index of MM just as β2-MG.

In conclusion, a high serum IL-16 level detected in newly diagnosed MM patients and its correlation with known factors of disease activity as well as the decrease of IL-16 after chemotherapy suggest that IL-16 may be implicated and a potential therapeutic target for MM.

Disclosure of conflict of interest

None.

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References

[1] Kyle RA, Rajkumar SV. Multiple myeloma. Blood 2008; 111: 2962-2972.

[2] Richmond J, Tuzova M, Cruikshank W, Center D. Regulation of cellular processes by interleukin-16 in homeostasis and cancer. J Cell Physiol 2014; 229: 139-147.

[3] Mahindra A, Anderson KC. Role of interleukin 16 in multiple myeloma pathogenesis: a potential novel therapeutic target? J Natl Cancer Inst 2012; 104: 964-965.

[4] Preston SL, Alison MR, Forbes SJ, Direkze NC, Poulsom R, Wright NA. The new stem cell biology: something for everyone. Mol Pathol 2003; 56: 86-96.

[5] Hideshima T, Mitsiades C, Ikeda H, Chauhan D, Raje N, Gorgun G, Hideshima H, Munshi NC, Richardson PG, Carrasco DR, Anderson KC. A

proto-oncogene BCL6 is up-regulated in the bone marrow microenvironment in multiple myeloma cells. Blood 2010; 115: 3772-3775.
[6] Cruikshank W, Kornfeld H, Berman J, Chupp G, Keane J, Center D. Biological activity of interleukin-16. Nature 1996; 382: 501-502.
[7] Cruikshank WW, Kornfeld H, Center DM. Interleukin-16. J Leukoc Biol 2000; 67: 757-766.
[8] Qi JC, Wang J, Mandadi S, Tanaka K, Roufogalis BD, Madigan MC, Lai K, Yan F, Chong BH, Stevens RL, Krilis SA. Human and mouse mast cells use the tetraspanin CD9 as an alternate interleukin-16 receptor. Blood 2006; 107: 135-142.
[9] Mathy NL, Scheuer W, Lanzendörfer M, Honold K, Ambrosius D, Norley S, Kurth R. Interleukin-16 stimulates the expression and production of pro-inflammatory cytokines by human monocytes. Immunology 2000; 100: 63-69.
[10] Alexandrakis MG, Passam FH, Kyriakou DS, Christophoridou AV, Perisinakis K, Hatzivasili A, Foudoulakis A, Castanas E. Serum level of interleukin-16 in multiple myeloma patients and its relationship to disease activity. Am J Hematol 2004; 75: 101-106.
[11] Richmond J, Tuzova M, Parks A, Adams N, Martin E, Tawa M, Morrison L, Chaney K, Kupper TS, Curiel-Lewandrowski C, Cruikshank W. Interleukin-16 as a marker of Sézary syndrome onset and stage. J Clin Immunol 2011; 31: 39-50.
[12] Yellapa A, Bahr JM, Bitterman P, Abramowicz JS, Edassery SL, Penumatsa K, Basu S, Rotmensch J, Barua A. Association of interleukin 16 with the development of ovarian tumor and tumor-associated neoangiogenesis in laying hen model of spontaneous ovarian cancer. Int J Gynecol Cancer 2012; 22: 199-207.
[13] Compérat E, Rouprêt M, Drouin SJ, Camparo P, Bitker MO, Houlgatte A, Cancel-Tassin G, Cussenot O. Tissue expression of IL16 in prostate cancer and its association with recurrence after radical prostatectomy. Prostate 2010; 70: 1622-1627.
[14] Alexandrakis MG, Passam FH, Boula A, Christophoridou A, Aloizos G, Roussou P, Kyriakou DS. Relationship between circulating serum soluble interleukin-6 receptor and the angiogenic cytokines basic fibroblast growth factor and vascular endothelial growth factor in multiple myeloma. Ann Hematol 2003; 82: 19-23.
[15] Newell MK, Haughn LJ, Maroun CR, Julius MH. Death of mature T cells by separate ligation of CD4 and the T-cell receptor for antigen. Nature 1990; 347: 286-289.
[16] Sekigawa I, Koshino K, Hishikawa T, Kaneko H, Takasaki Y, Hashimoto H, Hirose S, Inagaki Y, Yamamoto N. Inhibitory effect of the immunosuppressant FK506 on apoptotic cell death induced by HIV-1 gp120. J Clin Immunol 1995; 15: 312-317.
[17] Oken MM, Kay NE. T-cell subpopulations in multiple myeloma: correlation with clinical disease status. Br J Haematol 1981; 49: 629-634.
[18] Koike M, Sekigawa I, Okada M, Matsumoto M, Iida N, Hashimoto H, Oshimi K. Relationship between CD4(+)/CD8(+) T cell ratio and T cell activation in multiple myeloma: reference to IL-16. Leuk Res 2002; 26: 705-711.
[19] Atanackovic D, Hildebrandt Y, Templin J, Cao Y, Keller C, Panse J, Meyer S, Reinhard H, Bartels K, Lajmi N, Sezer O, Zander AR, Marx AH, Uhlig R, Zustin J, Bokemeyer C, Kröger N. Role of interleukin 16 in multiple myeloma. J Natl Cancer Inst 2012; 104: 1005-1020.
[20] Greipp PR, San Miguel J, Durie BG, Crowley JJ, Barlogie B, Bladé J, Boccadoro M, Child JA, Avet-Loiseau H, Kyle RA, Lahuerta JJ, Ludwig H, Morgan G,Powles R, Shimizu K, Shustik C, Sonneveld P, Tosi P, Turesson I, Westin J. International staging system for multiple myeloma. J Clin Oncol 2005; 23: 3412-20.