Biomedical Research 2017; 28 (22): 10048-10052 *ISSN 0970-938X*
www.biomedres.info

Influences analysis of lenalidomide on multiple myeloma, inflammatory factor, regulatory T cell and T-lymphocyte subsets.

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Abstract

Objective: To explore influences of lenalidomide on multiple myeloma, inflammatory factor, regulatory T cell and T-lymphocyte subsets.

Methods: 176 Multiple Myeloma (MM) patients in our hospital from April, 2013 to April, 2013 were divided into the observation group and the control group. The control group was given VAD treatment. The observation group was given lenalidomide and dexamethasone. One month was one course. The treatment gives 5 courses. M protein, β2-microglobulin level, HGB, BMPC in serum before and after treatment were detected and analysed and given effective rate statistics for patients according to indexes above. Changes of immune function relevant inflammatory factor of IL-6, TNF-α concentration, regulatory T cell (Tregs), T-lymphocyte subsets, $CD4^+$T cell, $CD8^+$T cell, Natural Killer cell (NK) percentage *in vivo* before and after treatment in two groups were detected and analysed.

Results: After 5 courses' analysis, the effective rate in the observation group was 79.5% (70/88) which higher than 68.2% in the control group (60/88). Before treatment, there is not significant differences (P>0.05) in M protein, β2-microglobulin, bone marrow plasma cell and haemoglobin of both groups. However, after the treatment, the M protein, β2-microglobulin, bone marrow plasma cell and haemoglobin are (15.29 ± 5.29 g/L), (8.03 ± 0.039 mg/L), (6.09 ± 0.099%) and (99.21 ± 9.21 g/L), respectively, in the observation group, but (20.37 ± 0.37 g/L), (10.04 ± 0.04 mg/L), (13.09 ± 3.09%) and (80.67 ± 0.67 g/L), respectively, in the control group. M protein, β2-microglobulin level, BMPC all decreased greatly after treatment in the observation group, the increased level of HGB all higher than the control group, and there were significant differences in data between two groups after treatment (P<0.05). before treatment, IL-6, tumor necrosis factor, regulatory T cell percentage and T-lymphocyte subsets ($CD4^+$T cell, $CD8^+$T cell, NK) of both groups are not different significantly (P>0.05), after treatment, the testing results of the observation group are (1.3 ± 0.3 pg/ml), (0.8 ± 0.8 pg/ml), (1.09 ±0.09l %), (43.89 ± 3.89%), (27.48 ± 7.48%) and (13.98 ± 3.98%), respectively, while the results of the control group are (2.3 ± 0.3 sets pg/ml), (1.5 ± 0.5 m pg/ml), (2.21 ± 0.21l%), (37.67 ± 7.67%), (34.88 ± 4.88%) and (16.47 ± 6.47%), respectively. The decreased level of two inflammatory factors of IL-6, concentration of tumor necrosis factor, regulatory T cell percentage, $CD8^+$T cell in T-lymphocyte subsets, NK percentage *in vivo* in the observation group all higher than the control group, the increased level of $CD4^+$T cell percentage and CD8+T cell percentage all higher than the control group, and there were significant differences in data between two groups after treatment (P<0.05).

Conclusion: After lenalidomide in treating MM, the clinical effects are well. Immune function improves better, it shows the clinical value of lenalidomide is high.

Keywords: Lenalidomide, MM, Inflammatory factor, Regulatory T cell, T-lymphocyte sets.

Accepted on October 20, 2017

Introduction

MM is a kind of malignant tumor in blood system caused by dysplasia of plasma cell in bone marrow, which belongs to lymph tumor of B lymph cell [1]. In addition, because M protein and monoclonal immunoglobulin in serum or urine of MM patients higher than the healthy group [2,3], at the same time, plasmocyte in bone marrow increased abnormally [4].

Therefore, WHO regards MM as plasmacytoma or plasma cell myeloma. During treatment, some remaining micro-focuses easily cause reoccurrence of the disease and affect the treatment speed [5]. In clinical treatment, this study adopts VAD or other treatment programs [6] at present, but it cannot guarantee whether drugs prolong lifetime of patients and improve survival quality of patients. Enalidomide (Len) is one of new type for treating MM at present. Oral-administration drug inhibits proliferation of tumor cell and tumor cell induction death through regulating immune system, changing micro-environmental of tumor, stimulating corresponding immune cell production of anti-tumor cell factors [7]. At present, relevant studies have proved the effect of lenalidomide in treating multiple myeloma [8-10], but there are a few studies in regulating the mechanism of the immune system. This study analyses the treatment effects of Len, and explores its influences on inflammatory factors *in vivo*, regulatory T cell, T-lymphocyte subsets of patients to analyse its treatment safety, and to improve the mechanism of the patient's immune system so as to find out theoretical references for a new and effective drug of multiple myeloma.

Materials and Methods

Study objects

176 patients in our hospital from April, 2013 to April, 2017 were selected and divided into the control group and the observation group randomly, of which, there were 43 male patients, 45 female patients in the control group. The age was from 45 to 68 y old. The average age was 52.9 ± 3.4 y old, of which, there were 73 initial treatment patients and 15 recurrence treatment patients. Patients were divided into different stages according to Durie-Salmon Staging and International Staging System [11] of patients above, which included 27 cases in I stage, 35 cases in II stage, 26 cases in III stage. There were 45 male cases and 43 female cases in the observation group. The age was from 47 to 67 y old. The average age was 53.1 ± 4.7 y old, of which, there were 76 initial treatment patients and 12 recurrence treatment patients, 23 cases in I stage, 39 cases in II stage and 26 cases in III stage. This study has been approved by the Medical Ethics Commission (our hospital) with the approval file number of ().

Inclusive criteria: According to the China Multiple Bone Marrow Diagnosis Guide [12] in 2015, this study selects patients randomly, who diagnosed as MM by bone marrow cytology, bone marrow biopsy, M protein level in serum and urine etc. The age was from 45 to 70 y old, they can survive at least half a year, and sign relevant contract note.

Exclusive criteria: Patients who had severe heart, liver and kidney diseases; patients who cannot follow this programs; pregnant or lactation period patients; patients who given treatment for other diseases at the same time; patients who had unclear treatment effects.

Treatment methods

Patients in the control group: from the first day to the fourth day, patients were given 0.5 mg vincristine, 10 mg adriamycin, 40 mg dexamethasone in Changchun by 250 ml normal saline every day. Once one day. It was given the above treatment about every five days. One month was one course.

Patients in the observation group: from the first day to the fourth day, patients were given 40 mg dexamethasone. Once one day. It was given the above treatment about every five days. One month was one course. According to the actual conditions of patients, they were given another 50 mg/d Len every week. Patients in two groups give effects evaluation after 5 courses' treatment.

Clinical detection indexes and effects evaluation

Patients were given M protein, β2-microglobulin level, HGB, BMPC detection and records before treatment in two groups, inflammatory factors *in vivo* of IL-6, TNF-α concentration, regulatory T cell and T-lymphocyte subsets given detection and records. After five courses' treatment, patients give relevant index detection before treatment repeatedly. This study compared and analysed the influences of changes of relevant indexes and Len before and after treatment in the same group and after treatment and between two groups on curative effects of patients and their immune system.

The effects of Len on treatment of patients can be divided into four categories: (1) CR: M protein decreased to 0 in serum. β2-microglobulin level decreased to the normal range. HGB increased. BMPC was equal to or less than 5%; (2) PR: M protein in serum decreased to 50% and even more low, β2-microglobulin level decreased, HGB increased. BMPC was equal to or less than 50%; (3) progress (I) M protein in serum decreased to 75% and even more low, β2-microglobulin level decreased, HGB increased. BMPC was equal to or less than 75%; (4) NR: M protein in serum, β2-microglobulin level, BMPC increased, HGB had no changes.

RR=(CR+PR+I)/cases × 100%. It followed EBMT/ABMTR [11].

Statistical methods

Data was given SPSS 18.0 to do statistical analysis. Experimental data represented by $\bar{x} \pm s$ and percentage. This study used t-test to do data analysis. When $P<0.05$, there were statistical differences.

Results

Comparison analysis of M protein, β2-microglobulin level, HGB, BMPC of patients in two groups

As seen in Table 1, M protein, β2-microglobulin level, BMPC decreased before and after treatment of patients in two groups, HGB increased, and there were significant differences in indexes above before and after treatment ($P<0.05$). At the same

time, the degrees of above indexes decrease in the observation group higher than the control group, and after treatment, there were significant differences of indexes between the observation group and the control group (P<0.05).

Table 1. *Comparative analysis of M protein/β2-microglobulin/hemoglobin/BMPC between two groups.*

Detection indexes	The control group		The observation group	
	Before treatment	After treatment	Before treatment	After treatment
M protein (g/L)	65.28 ± 4.01	20.37 ± 3.12*	67.36 ± 3.48	15.29 ± 2.47*#
β2-microglobulin (mg/L)	15.31 ± 1.34	10.04 ± 1.24*	16.09 ± 1.72	8.03 ± 1.02*#
HGB (g/L)	66.28 ± 3.41	80.67 ± 5.68*	59.07 ± 3.91	99.21 ± 8.03*#
BMPC (%)	53.38 ± 4.07	13.09 ± 2.17*	58.21 ± 3.09	6.09 ± 1.04*#

Compared between pre-and post-treatment, *P <0.05. Compared between two groups, #P<0.05.

Effects results statistics of patients

This study count treatment effects of patients in two groups according to M protein, β2-microglobulin level, HGB, BMPC, of which, there were 6 CR cases, 37 PR cases, 17 I cases, 28 NR cases, and the RR was 68.2% (60/88) in the control group. The RR was 68.2% (60/88).There were 10 CR cases, 43 PR cases, 17 I cases, 18 NR cases. The RR was 79.5% (70/88). RR in the observation group higher than the control group, and there were significant differences in two groups (P<0.05).

Analysis of inflammatory factor concentration of patients in two groups

As seen in Table 2, compared with before treatment, IL-6 *in vivo* and TNF-α concentration decreased of patients in two groups, and there were significant differences in inflammatory factor before and after treatment (P<0.05). At the same time, the decreased level of IL-6 *in vivo* and TNF-α concentration *in vivo* after treatment of patients in the observation group higher than the control group, and there were significant differences in data between two groups (P<0.05).

Table 2. *Analysis of in-vivo inflammatory factor concentration between two groups.*

Detection indexes	THE control group		The observation group	
	Before treatment	After treatment	Before treatment	After treatment
(IL-6) (pg/ml)	7.9 ± 0.4	2.3 ± 0.2*	8.1 ± 0.3	1.3 ± 0.2*#
(TNF-α) (pg/ml)	4.1 ± 0.3	1.5 ± 0.1*	3.7 ± 0.2	0.8 ± 0.1*#

Compared between pre-and post-treatment, *P<0.05. Compared between two groups, #P<0.05.

Analysis of regulatory T cell and T-lymphocyte subsets percentage changes of patients in vivo in two groups

As seen in Table 3, compared with before treatment, regulatory T cell of patients in two groups decreased. $CD8^+T$ in T-lymphocyte subsets, NK decreased. But $CD4^+T$, ratio of $CD4^+T$ and $CD8^+T$ increased, and there were significant differences before and after treatment in data between two groups (P<0.05). In addition, the decreased level of Tregs, CD8+T and NK percentage after treatment of patients in the observation group higher than the control group obviously. The increased level of $CD4^+T$, ratio of $CD4^+T$ and $CD8^+T$ higher than the control group.

Table 3. *Comparison of Tregs/$CD4^+T$/ $CD8^+T$ /NK between two groups.*

Detection indexes	The control group		The observation group	
	Before treatment	After treatment	Before treatment	After treatment
Tregs	4.31 ± 1.01	2.21 ± 0.66*	4.47 ± 0.91	1.09 ± 0.22*#
$CD4^+T$ cell	26.37 ± 1.97	37.67 ± 2.67*	27.23 ± 2.11	43.89 ± 3.22*#
$CD8^+T$ cell	43.29 ± 2.38	34.88 ± 3.06*	41.38 ± 4.31	27.48 ± 2.64*#
$CD4^+/CD8^+$	0.61 ± 0.03	1.08 ± 0.07*	0.66 ± 0.09	1.60 ± 0.22*#
NK	22.44 ± 3.78	16.47 ± 2.01*	23.78 ± 4.03	13.98 ± 1.09*#

Compared between pre-and post-treatment, *P<0.05. Compared between two groups, #P<0.05.

Discussion

According to data statistics, MM accounts for 1% in malignant group around the world [13], which only next to NHL of malignant lymph tumor [14,15], it accounts for 10% in blood system cancer [16], and mainly in middle and old age group. In our country, the population of aging is increasing gradually. The number of MM people increases at the same time. In

clinic, the main treatment methods for MM are BMT, chemotherapy, VAD etc. Traditional chemotherapy treatment will damage human immune system greatly. And the cost of BMT is high, so it is not easily to be generalized [17]. Therefore, MM treatment faces with high recurrence rate, fatality rate, high cost. During treatment, it influences physical and mental health of patients greatly [18]. Therefore, an effective treatment method for MM is very important.

Len is a kind of new-type immune regulatory drugs in recent years, it is the derivative of thalidomide, which inhibits MM proliferation and induces death by inducing immune function upgrade of patients *in vivo*. It has subtle influences in health of patients and slight adverse reaction, which improves clinical effects of patients greatly [19].

In this study, comparing with routine VAD treatment method, the RR reaches to 79.5% through combination of Len and dexamethasone by MM patients, which is obviously higher than the VAD treatment group and identical to the research results of Yang et al. [20] M protein, β2-microglobulin level decrease, HGB increase, and BMPC decreases. Therefore, comparing with VAD, Len has more obvious effects in treating MM.

In foreign studies [21,22], it has been found that IL-6 and TNF-α level in MM group higher than healthy people. The higher the incidence rate, higher IL-6 and TNF-α level. Inflammatory factor has relative high relevance with MM deterioration. Through this study, this study finds that inflammatory factor decrease *in vivo* of patients after Len treatment, which shows that the condition of patients relieve, at the same time, can reduce inflammation and fever rate, finally lowering bodily injury by MM and improve physical health of patients indirectly.

Immune cell in MM patients *in vivo* for resisting tumor is T cell, of which, the function of regulatory T cell is to regulate body immune response and maintain immune stable. $CD4^+$T cell is assistant T cell. The main function of $CD8^+$T cell is to poison tumor cell. NK cell is used to induce tumor death. In some relevant studies find that regulatory T cell and T-lymphocyte group *in vivo* of MM patients have abnormal function [21,22]. In the studies of Gao [23] find that $CD8^+$T percentage in healthy people lower than MM group, $CD4^+$T/$CD8^+$T percentage higher than MM group. Bernal et al. [24] and Jurisic et al. [25] find that NK cell percentage increase and activity decrease in MM group comparing with healthy people. From this study, it is found that $CD4^+$T cell increases and Tregs, $CD8^+$T, NK decreases of patients *in vivo* after Len treatment, changes of these indexes keep correspondence with healthy people, which shows that Len for MM has obvious effects. Len has treatment effects through promoting immune of patients themselves and keeping immune balance of patients of patient.

From studies above, we can conclude that Len and dexamethasone for MM can improve RR of treatment effectively, survival quality of patients, improve immune system, have subtle influences in physical and mental health of patients, which have better clinical significance. At the same time, Len can be used to oral-administration. Patients can be given treatment without hospital stay treatment, so which provide new direction for MM patients in later stage.

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