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Original Article

Correlation analysis between regulatory T cells and the degree of depression in acute leukemia patients

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Abstract: Malignant tumor patients are often complicated with depressive disorder, which may affect tumor progression and threatens life quality or span. This study aimed to investigate the effect of depression on immune function and survival quality of acute leukemia patients. A total of 200 acute leukemia patients were recruited. Those had depression based on scale. Other non-depressive patients were recruited in the control group. Clinical information of patients was collected, and depressive condition was evaluated by Hamilton depression scale (24) and self-evaluative depression scale. General survey of health status (SF-36) was used to evaluate life quality. Count and ratio of regulatory T (Treg) cells and Th17 cells in peripheral blood were quantified by flow cytometry. ELISA was used to measure serum secretion level of IL-6, IL-17, IL-10 and TGF-β1. Correlation analysis was performed between two groups. Among 200 acute leukemia patients, 46 fitted diagnostic criteria of depressive disorder. 46 patients were randomly collected from non-depressive patients, and education level was significantly different between two groups ($P<0.05$). Compared to control group, depressive patients had significantly elevated peripheral Treg and Th17 levels, with decreased Treg/Th17 ratio ($P<0.05$). Depression was significantly correlated with Treg ($r=0.533$, $P=0.013$), Th17 ($r=0.316$, $P=0.028$) and Th17/Treg ($r=0.397$, $P=0.010$). Depression patents had higher serum IL-6, IL-17, IL-10 and TGF-β1 levels, and lower score in SF-36 scale ($P<0.05$). Depression significantly suppressed immune function of acute leukemia, and decreased life quality of patients.

Keywords: Acute leukemia, depression, immune function, Th17

Introduction

Depressive disorder is one common psychiatric disorder, and is mainly presented as depressive like mental status, body depression, mental depression, sleep disorder and fatigue. Epidemic survey showed that malignant tumor patients were more susceptible for depression. Previous study showed that nearly 50% cancer patients may develop mental disorder, among those 70% manifest depression and anxiety. Depressive disorder can compromise patient's life quality, and shortens survival time of cancer patients by 20%~30% [1, 2]. With the transition of biomedical research to biology-psychology-social medical mode, the treatment target for malignant tumors has become focusing life quality and life survival, which allow the survival with tumor loading, from the solely target of radical curing. Therefore, the early identification of depressive disorder with timely intervention, is of critical importance for mental health of patients. However, due to the lack of psychiatric knowledge and intervention, tumor related depression is often neglected in clinics [3].

Regulatory T cell (Treg) and helper T cell 17 (Th17) are two types of CD4+ lymphocytes different from Th1 and Th2 cells. As one immune modulatory cells, Treg has significantly potentiated function in malignant tumors including leukemia and liver cancer, as it can secret inflammatory factors such as IL-10 to suppress reactive inhibition on body immune response against tumors, leading to tumor proliferation, onset, development and metastasis, and is closely correlated with prognosis [4]. Th17 cell is one important component of inflammatory response, and can facilitate inflammatory response via secreting pro-inflammatory factors such as IL-17, and also plays important roles in tumor occurrence and development [5]. Tumor animal model has demonstrated that TGF-β

could induce Treg cell differentiation, whilst IL-6 enhancement inhibited Treg differentiation but induced Th17 differentiation [6]. These two types of cells are inter-connected in the sense of differentiation, and antagonize each other function. Animal study showed that depression was correlated with Treg cells [6]. However, no clinical study has been performed regarding Treg in tumor related depression. Therefore, this study aimed to investigate the correlation between depressive condition of acute leukemia patients and Treg cells, and to elucidate its effects on patient life quality, in order to reveal the correlation between acute leukemia complicated with depression and Treg cells, plus potential mechanisms.

Materials and methods

General information

A total of 200 acute leukemia patients who received treatment in Guizhou Provincial Hospital and Tumor hospital from October 2014 to March 2015 were recruited, including 106 males and 94 females. A cohort study was performed. According to Hamilton depression scale and self-evaluating depression scale, those acute leukemia patients with depression were recruited in the disease group. A control group was randomly collected from the whole cohort of acute leukemia patients, which aged between 14 and 70 years (average age =56.83±3.74 years).

The study protocol was approved by the Research Ethics Committee of Guizhou Provincial People's Hospital, and all patients gave their informed consent before study commencement.

Inclusive and exclusive criteria

Inclusive criteria: (1) Aging between 14 and 70 years; (2) Fitting diagnostic criteria of acute leukemia (NCCN); (3) No dysfunction of major organs such as heart, lung or brain: (4) Willing to attend this study. Exclusive criteria: (1) Aging younger than 14 or older than 70 years; (2) Transplant recipient within 6 months.

Hamilton depressive scale (24 items)

Normal: total score <8; Possible depression: total score between 8 and 20; Definite depression: total score between 20 and 35; Severe depression: total score >35 [7].

Self-evaluating depressive scale

Raw score equals the summation of all 20 items. Standard score it the integral part of 1.25 fold of raw score. The upper limit of total raw score is 41, and total standard score was 53. Disease severity = cumulative score/80. Results deduction: no depression: <0.5; minor to mild depression: 0.5-0.9; moderate to severe depression: 0.6-0.9; severe depression: >0.7 [8].

Peripheral Treg assay

Fasted venous blood samples were collected from all subjects in the morning. 2 mL blood samples were collected in EDTA anti-coagulation tubes and were stored at 4°C. Flow cytometry was used to quantify CD4+CD25+Foxp3+ T cell sub-population. In brief, 100 μL peripheral blood was mixed with 5 μL FITC-CD4 and PE-CD25 flow cytometry antibody. Controlled antibody with identical phenotype was used as the control and for compensation. After 4°C dark incubation for 30 min, PBS containing 2% FBS was used for twice rinsing, followed by 4% paraformaldehyde fixation at 4°C for 30 min. Cells were treated in PBS containing 0.1% Triton X-100, and was incubated with 5 μL APC-Foxp3 for 20 min dark incubation at room temperature. Cells were then washed twice in PBS containing 2% FBS by centrifugation, and were re-suspended in 0.5 mL PBS containing 2% FBS. Flow cytometry was immediately performed for quantifying CD4+CD25+Foxp3+ T cell ratio.

Peripheral blood Th17 cell assay

All subjects were collected for venous blood, which was kept in EDTA anti-coagulation tubes for 4°C storage. Th17 cell sub-population level was measured by flow cytometry. In brief, 100 μL peripheral blood samples were fixed in buffer for 20 min, and the supernatant was discarded after centrifugation. Permeable treatment was performed for 20 min. After centrifugation and discarding supernatant, cells were washed in permeable buffer by centrifugation, and were re-suspended in 100 μL permeable buffer. 20 μL Alexa Fluor 647 mouse IgG1, k isotype control/CD3 FITC/CD4 PE or

Table 1. Clinical information of two groups of patients

		Depression (N=46)	Control (N=46)	t/χ^2	*P*
Age		56.84±4.23	57.96±3.95	1.313	0.096
Gender	M	27	24	0.396	0.529
	F	19	22		
Treating cost (in 10 000)		10.45±2.81	9.84±2.63	1.075	0.143
Education level	Junior below	12	23	6.513	0.039
	Middle school	26	20		
	College and above	8	3		

Table 2. Peripheral Treg, Th17 and Treg/Th17 ratio between two groups of patients (mean ± SD, %)

	Treg (%)	Th17 (%)	Treg/Th17 (%)
Depression	6.31±0.52	0.23±0.05	27.43±5.83
Control	5.83±0.46	0.12±0.03	48.58±9.27
T	4.689	12.795	13.099
P	<0.001	<0.001	<0.001

IL-17 Alexa Fluor 647/CD3 FIT/CD4 PE was added for 30 min dark incubation at room temperature. After twice rinsing in permeable buffer by centrifugation, cells were re-suspended in staining buffer for online testing.

Serum IL-6, IL-17, IL-10 and TGF-β expression and secretory level

All subjects were collected for 2~4 mL fasted venous blood samples (EDTA anti-coagulation) and were stored at 4°C. ELISA was performed following the manual instruction of ELISA kits (Meixuan Biotech, China). In brief, 10 μL serum and 40 μL sample dilution buffer were added into the plate with antibody coating. Standard curve was installed. The plate was incubated at 37°C for 60 min, followed by 5 times of washing (2 min each). 50 μL buffer A and buffer B was added, followed by 37°C incubation for 15 min. 50 μL quenching buffer was added for measurement at 450 nm wavelength.

Life quality evaluation

Brief scale of health survey (SF-36) was used to evaluate life quality of patients, including eight dimensions: physiological function (PF), role of physiology (RP), body pain (BP), general health condition (GH), vigor (VT), social function (SF), mental role (RE) and mental health (MH). The former four items belong to physiological healthy filed, whilst the latter four belongs to mental health, as higher score indicates better life quality.

Statistical analysis

All data were input into SPSS 13.0 software for statistical processing. Enumeration data were presented ± standard deviation (SD). Student t-test or analysis of variance (ANOVA) was performed for test. Enumeration data were shown as percentage of number, and were analyzed by chi-square test. Pearson analysis was used to reveal correlation between indexes. A statistical significance was defined when $P<0.05$.

Results

General information of patients

Depressive level was evaluated among 200 acute leukemia patients, and found 46 of them fitted the diagnostic criteria of depressive disorder. Among those patients there were 19, 17 and 10 cases belonging to mild, moderate and severe depression, respectively. Another cohort of 46 acute leukemia patients without depression were randomly sampled as the control group. No significant difference existed in age, gender or treating cost between two groups, and depression patients showed lower education level than non-depression group ($P<0.05$, **Table 1**).

Effects of depression on patient immune function

Compared to control group, depressive disorder patients had significantly elevated peripheral Treg and Th17 levels, whilst Treg/Th17 ratio was decreased, with statistical significance ($P<0.05$, **Table 2**). Representative figures were shown in **Figure 1A** and **1B**.

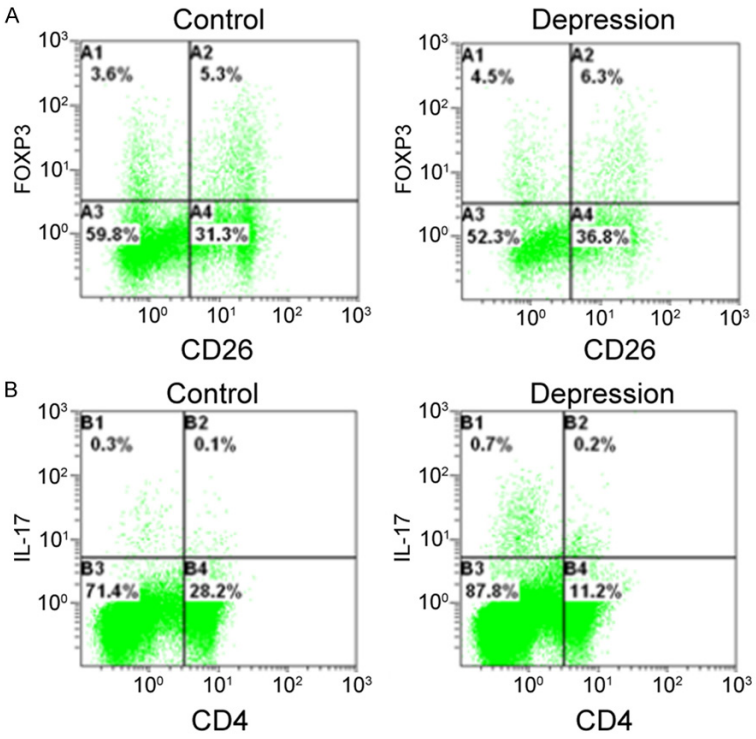

Figure 1. Flow cytometry for Treg and Th17 flow cytometry in peripheral blood samples. A: Flow cytometry for peripheral Treg cell ratio; B: Flow cytometry for peripheral Th17 cell ratio.

Table 3. Correlation analysis between peripheral Treg, Th17 and Th17/Treg and depression

	Treg	Th17	Treg/Th17
r	0.533	0.316	0.397
P	0.013	0.028	0.010

Correlation analysis between depression and patient immune function

Person correlation analysis was performed and found significant correlation between depression and peripheral Treg (r=0.553, P=0.013), Th17 (r=0.316, P=0.028) and Th17/Treg ratio (r=0.397, P=0.010), as shown in **Table 3**.

Comparison of serum Treg and Th17 cell related cytokines in patients

ELISA was used to test serum IL-10, TGF-β1, IL-6 and IL-17 expression levels and found significantly elevated cytokine expression level in depression group (P<0.05, **Table 4**).

Effects of depression on patient life quality

Compared to control group, depression patients had significantly lower score in SF-36 scale (P<0.05, **Table 5**).

Discussion

Currently tumor has become a major threaten for public health in the sense of both mental and tumor occurrence. Malignant tumor can has adverse effects on patient mental and psychological features, and adverse mental factors can aggravate disease condition and progression of patients, and exert negative effects on treatment efficacy and rehabilitation. Depression has become a major complication in cancer patients, and is frequently accompanied with anxiety, or secondary to anxiety disorder. With elevated incidence of malignant tumor, epidemiology survey showed yearly increasing rate of depressive disorder in tumor patients. Multiple studies have confirmed the prevalence of mental disorder in cancer patients, and higher than 45% of anxiety and depression rate in cancer patients, with similar functional status as normal people [9, 10]. Mental disorders such as anxiety or depression can aggravate patient's body disease, and affect treatment efficacy. Acute mental disorder can affect the motor regulation of central nervous system (CNS) on various organs such as gastric-stomach, cardiovascular systems and endocrine system, thus aggravating disease condition. Chronic depressive disorder also suppress patient immune function, and facilitate tumor progression and recurrence [11, 12]. Therefore, timely mental intervention is necessary for tumor patients.

Recent study showed that depressive disorder can lead to immune dysfunction in body cells [13]. In the assay of immune function on depressive animal models, levels of CD4+, CD4+/CD8+ and NK cells were all significantly depressed, leading to compromised survival quality of experimental animals [14, 15]. Treg and Th17 are two types of CD4+ lymphocytes different from Th1 and Th2 [16]. Treg is one T cell sub-population with immune suppressing effects, and plays crucial roles in maintaining body immune tolerance and immune response

Table 4. Serum Treg and Th17 related cytokine levels

	IL-10 (pg/mL)	TGF-β1 (pg/mL)	IL-6 (pg/mL)	IL-17 (pg/mL)
Depression	88.56±6.84	108.54±8.41	682.45±34.28	134.83±12.65
Control	47.83±4.36	61.24±7.63	214.56±11.27	110.86±10.41
t	34.056	28.215	87.942	9.924
P	<0.001	<0.001	<0.001	<0.001

Table 5. SF-36 scale score comparison between two groups (Mean ± SD, score)

	Control	Depression	t	P
PF	71.25±23.06	66.17±22.84	2.315	0.011
RP	55.48±24.63	33.23±18.51	4.898	<0.001
BP	73.65±16.84	58.96±15.38	4.369	<0.001
GH	67.18±15.86	52.93±10.62	5.064	<0.001
VT	64.87±14.62	41.07±13.94	7.991	<0.001
SF	76.25±12.43	54.38±12.17	8.527	<0.001
RE	51.09±17.74	30.69±15.38	5.893	<0.001
MH	74.13±15.52	58.65±13.30	5.137	<0.001

[17]. Various studies showed enhanced Treg level in tumor patients, and the secretion of suppressive factors such as IL-10 to down-regulate reactive suppression of body immune system targeting tumors, thus playing a crucial role in tumor immune escape [18, 19]. Th17 cells are important components of inflammatory response, and secrete pro-inflammatory factors such as IL-17 to facilitate inflammation, and is important in tumor occurrence and progression. Animal study showed the correlation between depression and Treg. However, no clinical study has been performed targeting Treg in tumor related depression. Therefore, this study selected Treg, Th17 and related cytokines IL-10, TGF-β1, IL-6 and IL-17 to evaluate immune functions, thus investigating the effects of tumor related depression on immune function.

Study results showed significantly elevated peripheral Treg and Th17 ratio in acute leukemia patients with depressive disorder compared to those without depression, whilst Treg/Th17 ratio was decreased, indicating that depression could cause depressed immune function, and enhanced tumor immune escape, as similar with previous reports [20, 21]. Further analysis of depression and immune function showed significant correlation between depression and peripheral Treg, Th17 and Th17/Treg, further proving that depression could weaken immune function of leukemia patients. Moreover, depressive patients had significantly elevated peripheral IL-10, TGFβ1, IL-6 and IL-17 expression levels than control group, indicating that Treg and Th17 could exert their functions via enhancing downstream cytokine secretion. Previous study separated and cultured breast cancer cells from patients, and found decreased defense potency of killer cells targeting tumors in those patients during depressive episode [22]. Clinical study also found improvement of immune function in tumor patients after anti-depression therapy [22]. However, the mechanism underlying the weakened immune function by depression is inconclusive. Previous study showed that the constitutive activation of hypothalamus-pituitary-adrenal gland axis may inhibit T cell activation or function, thus weakening patient immune function and resistance [23, 24]. Moreover, under chronic inflammation and malignant tumor status, MDSC can be abundantly proliferated to inhibit T cell immune response. During this process, depression dysfunction can amplify inflammatory response and further accelerate MDSC cell proliferation, further enlarging immune escape of tumor cells, thus accelerating tumor occurrence and progression [25, 26]. Previous study believed that antibody produced by tumor basic protein (TBP) can interfere the binding between 5-HT and receptor, lowering its activity, compromising immune function and elevating the risk of depression [27, 28]. Depression can directly affect patient's life quality, and this study also found lower life quality of depressive patients.

Certain weakness also existed in this study. Firstly, due to relatively small sample size, only 46 depression patients were included, and further studies are required to collect more patients. In addition, no stratified analysis was performed based on depression condition due to limited sample size, and only preliminary results were obtained. Follow-up study was required for more analysis.

Conclusion

Strengthening of psychological intervention on tumor patients, and the encouragement of

healthy mental status can improve self-immune functions of patients, facilitate post-treatment recover, elongate survival time and improve life quality.

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Disclosure of conflict of interest

None.

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