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The Changes of the Levels of Tumor Necrosis Factor α, Interferon γ and Vascular Endothelial Growth Factor in the Sera of Children with Acquired Aplastic Anemia after Blood Transfusion

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Abstract

Objective: To discuss how blood transfusion affects the progression of acquired aplastic anemia by assaying the levels of TNF-α, IFN-γ and VEGF in the sera of children with acquired aplastic anemia both before and after blood transfusion.

Methods: In the study, the sera of 15 children with acquired aplastic anemia were collected both before and one or two days after blood transfusion. And during the study period the sera of 12 normal children who had body check-ups in our hospital were also collected. The levels of TNF-α, IFN-γ and VEGF in the sera of children were assayed by the method of ELISA with the corresponding kits.

Results: The levels of TNF-α and IFN-γ in the sera of children with acquired aplastic anemia were 2.29 ± 0.36 ng/L and 21.30 ± 9.12 ng/L respectively. After blood transfusion they were 1.27 ± 0.12 ng/L and 15.33 ± 4.45 ng/L respectively. The levels of TNF-α and IFN-γ in the sera of children with acquired aplastic anemia after blood transfusion were lower than these before blood transfusion. But they were higher than those in the sera of normal children (TNF-α 0.85 ± 0.04 ng/L, IFN-γ 10.91 ± 4.67 ng/L) respectively. The level of VEGF in the sera of patients with acquired aplastic anemia was 50.07 ± 12.83 Pg/L and it was 80.02 ± 11.04 Pg/L after blood transfusion. The level of VEGF in the sera of patients with acquired aplastic anemia after blood transfusion was higher than this before blood transfusion, and both of them were lower than that in the sera of normal children (100.77 ± 36.83 Pg/L). There were significant differences between them ($p<0.05$).

Conclusions: Blood transfusion can decrease the levels of TNF-α and IFN-γ and increase the level of VEGF in the sera of children with acquired aplastic anemia, which may affect the progression of the disease.

Keywords: Acquired aplastic anemia; TNF-α; IFN-γ; VEGF; Blood transfusion

Abbreviations

AA: Aplastic Anemia; HLA: Human Lymphocyte Antigen; CFU: Colony Forming Unit; GM: Granulocyte Macrophage; TNF-α: Tumor Necrosis Factor A; IFN-γ: Interferon γ; VEGF: Vascular Endothelial Growth Factor; RBC: Red Blood Cell; Hb: Counts/Haemoglobin; WBC: White Blood Cell; PLT: Platelet; ELISA: Enzyme Linked Immunosorbent Assay; Wt: Weight; Kg: Kilogram; HSCT: Hematopoietic Stem Cell Transplant; CTLs: Cytotoxic T Cells; TSCM: Memory Stem T Cells; HSC: Hematopoietic Stem Cell

Introduction

Acquired aplastic anemia (AA) is a bone marrow failure syndrome with an incidence of 2/1,000,000 in Western countries and 4-6/1,000,000 in Asia [1,2]. The pathophysiology of acquired AA involves immune-mediated destruction of hematopoietic stem cells causing pancytopenia and empty bone marrow [1,3]. Patients with acquired AA often suffer from such complications as bleeding, infection and anemia. Each complication is life-threatening to the patient. Therefore, patients with acquired AA often need blood transfusion to alleviate the suffering. However, blood transfusion can bring some side-effects which may affect the progression of the disease. It is suspected that many of the immunologic abnormalities in acquired AA may be related to blood transfusions [4,5]. Singer et al described preferential inhibition of human lymphocyte antigen (HLA)-matched normal colony forming unit-granulocyte macrophage (CFU-GM) by lymphocytes derived from transfused, as opposed to untransfused AA patients [6,7]. Torok-Storb et al observed lymphocyte-medicated inhibition in 12 of 34 transfused but in none of eight untransfused AA patients [8]. In another experiment, Torok-Storb et al observed that the effect of lymphocytes on littermate marrow was significantly altered after blood transfusion: transfusion-sensitized cells either failed to increase or decreased in vitro erythroid colony growth [9]. In order to find out how blood transfusion affect the progression of the disease, the cell factors such as tumor necrosis factor α (TNF-α), interferon γ (IFN-γ) and vascular endothelial growth factor (VEGF) in the sera of patients with acquired AA both before and after

blood transfusion as well as in normal children were assayed. TNF-α, IFN-γ and VEGF are important cell factors which may participate in the immune reaction or angiogenesis. All of them are involved in the pathogenesis of acquired AA [10,11]. Therefore, by assaying the levels of TNF-α, IFN-γ and VEGF, we tried to discuss the side-effects brought by blood transfusion to the patients.

Methods

Study samples

The diagnoses of acquired AA children were made based on pancytopenia and bone marrow puncture, and by exclusion of an underlying disease. From October 1st, 2014 to April 1st, 2018, the data of the children with acquired AA who visited Zibo Central Hospital were collected. All of them were under 13 years old. During the study period, the data of 12 normal children who had body check-ups in our hospital were also collected. The genders, ages, weights of the children with acquired AA as well as normal children were collected. The red blood cell (RBC) counts, hemoglobins (Hbs), white blood cell (WBC) counts, and platelets (PLTs) in acquired AA children were also collected both before and after blood transfusion. The study was approved by the ethics committee of Zibo Central Hospital. Written informed consent was obtained from all the parents of the children.

The assay of TNF-α, IFN-γ and VEGF

The peripheral bloods from children with acquired AA both before and one or two days after blood transfusion were collected. The peripheral bloods from 12 normal children were also collected. The levels of TNF-α, IFN-γ and VEGF were assayed by enzyme linked immunosorbent assay (ELISA) according to the instruction of the kits on Bio-Rad Model 450 Microplate Reader. All the kits of the three factors were produced by French Diaclone Company.

Statistical analysis

The categorical data were expressed as cases and χ2 used to compare them. The measured data were expressed as x ± s and group t-test was used to compare them. Paired t-test was used to compare the levels of TNF-α, IFN-γ and VEGF before blood transfusion and those after blood transfusion. Statistical significance was indicated by a P value of 0.05. All analysis was performed with the use of Stata software, version 12.1.

Results

Study population

From October 1st, 2014 to April 1st, 2018, there were 15 patients with acquired AA who visited Zibo Central hospital. Three patients were boys and the rest were girls. The ages ranged from 3.3 to 9.5 years old. The weights (Wts) of the patients were 14~42 kilogram (Kg). And there were also 12 normal children including 5 boys and 7 girls who had body check-ups during the study period. These children aged from 3.5 to 8.6 years old. The Wts of the children were 16~40 Kg. There were no significant differences between the two groups in ages, genders and Wts (see Table 1).

	Acquired AA patient	Normal children	Analysis
Age (years)	6.56 ± 1.87	5.3 ± 3.06	T=0.26, P>0.05
Gender (M:F)	03:12	02:08	χ^2=0, P>0.05
Weight (kg)	26.8 ± 9.21	23.25 ± 13.5	T=0.78, P>0.05

Table 1: The comparison between acquired AA patients and normal children in age, gender and weight.

Clinical data

The genders, ages, Wts as well as WBCs, RBCs, Hbs, PLTs were collected from the acquired AA children both before and one or two days after blood transfusion. All of them were transfused with 2 U RBCs. The clinical data were in Table 2 as follows.

S No.	Gender	Age (years)	Wt (kg)	Before blood transfusion				After blood transfusion			
				WBC (× 10^9/L)	RBC (× 10^{12}/L)	Hb (g/L)	Plt (× 10^9/L)	WBC (× 10^9/L)	RBC (× 10^{12}/L)	Hb (g/L)	Plt (× 10^9/L)
1	M	3.3	15	2.5	1.2	40	38	2.4	2.2	74	36
2	F	3.5	14	1.9	1.1	37	40	2.4	1.9	81	42
3	F	4.9	18	2.5	0.9	35	31	3.2	1.8	68	43
4	F	5.8	19	2.8	2.1	49	56	3.5	2.5	82	53
5	F	7	25	1.8	1.5	40	31	2.1	2.3	71	42
6	F	6.9	23	0.8	1.3	31	42	1.8	2	59	45
7	M	8.4	30	1.4	1.4	39	40	1.8	1.9	70	40
8	F	6.3	24	2.9	1.6	45	30	3.8	2.4	73	42
9	F	9.5	42	1.5	2.1	49	50	2.5	2.9	73	45
10	F	9.1	40	0.9	1.8	40	64	1.7	2.4	65	46

11	F	6.8	32	1.2	1.7	45	53	2.1	2.8	68	60
12	M	7.2	38	3.1	2.2	48	39	3.5	2.8	70	42
13	F	8.3	37	2.5	1.1	45	38	2.9	1.9	68	45
14	F	6.7	25	3.2	1.8	38	45	4	2.5	75	41
15	F	4.7	20	2.6	1.6	39	43	3.5	2.3	76	38

Table 2: The clinical data of acquired AA patients.

Levels of TNF-α, IFN-γ and VEGF

The levels of TNF-α, IFN-γ and VEGF in acquired AA patients were assayed by ELSIA both before and after blood transfusion. The levels of TNF-α and IFN-γ in acquired AA patients after blood transfusion were lower than these before blood transfusion. But all of them were higher than those in normal children. There were significant differences between the groups (P<0.05). The levels of VEGF in acquired AA patients after blood transfusion were higher than these before blood transfusion. But they were lower than those in normal children. There were significant differences between the groups (P<0.01). The results were in Tables 3 and 4 and Figure 1 as follows.

S No.	Gender	Age	Wt	Before blood transfusion			after blood transfusion		
				TNF-α (ng/L)	IFN-γ (ng/L)	VEGF (pg/L)	TNF-α (ng/L)	IFN-γ (ng/L)	VEGF (pg/L)
1	M	3	15	2.2	21.2	51.2	1.3	16.2	80.2
2	F	4	14	1.4	22.9	48.5	0.9	15.1	74.3
3	F	5	18	1.8	24.8	59.1	1.1	17.6	84.6
4	F	6	19	2.5	25.5	49.8	1.8	14.2	79.4
5	F	7	25	1.5	19.6	50.6	0.8	13.4	77.5
6	F	7	23	1.6	17.8	49.2	1.3	13.2	76.3
7	M	8	30	1.9	26.4	46.7	1.4	18.9	82.1
8	F	6	24	2.9	23.2	50.8	1.6	17.6	75.9
9	F	10	42	2.8	17.3	47.3	1.1	12.5	78.8
10	F	9	40	2.7	18.9	48.6	1.5	15.3	77.9
11	F	7	32	1.7	19.1	48.2	0.8	14.3	79.8
12	M	7	38	2.4	21.6	45.6	1.3	14.8	84.7
13	F	8	37	3.1	24.1	49.1	1.6	19.2	81.4
14	F	7	25	3.2	17.9	49.4	1.9	14.2	82.7
15	F	5	20	1.6	19.2	56.9	0.9	13.5	84.7
Note: Paired-t TNF-α=2.24, P<0.05; Paired-t IFN-γ=3.14, P<0.05; Paired-t VEGF=7.79, P<0.05									

Table 3: The levels of TNF-α, IFN-γ and VEGF in acquired AA patients.

Infactors	Acquired AA patient	Normal children	Analysis
TNF-α(ng/L)	2.29 ± 0.36	0.85 ± 0.04	T=12.51, p<0.01
TNF-α(ng/L)	1.27 ± 0.12		T=10.62, p<0.01
IFN-γ(ng/L)	21.30 ± 9.12	10.91 ± 4.67	T=3.31, p<0.01
IFN-γ(ng/L)	15.33 ± 4.45		T=2.39, p<0.05
VEGF(Pg/L)	50.07 ± 12.83	100.77 ± 36.83	T=4.94, p<0.01
VEGF(Pg/L)	80.02 ± 11.04		T=2.07, p<0.05

Table 4: The comparison of TNF-α, IFN-γ and VEGF between acquired AA patients and normal children.

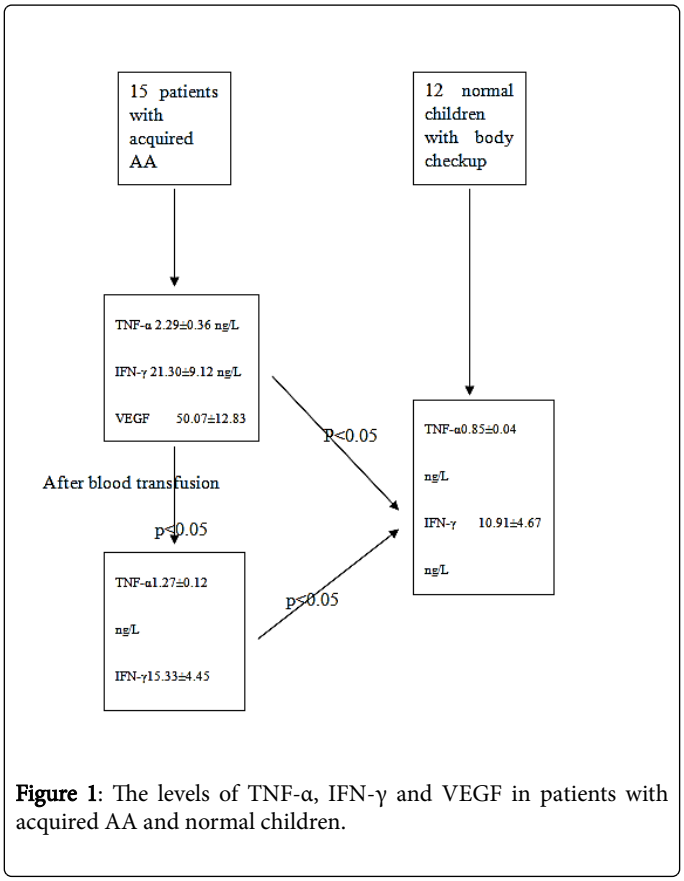

Figure 1: The levels of TNF-α, IFN-γ and VEGF in patients with acquired AA and normal children.

Discussion

AA is a serious disease which poses great challenge to the public health of people in the world. It includes acquired AA and inherited AA. Most patients are acquired AA. One report suggested that 95% patients were acquired AA while only 5% were inherited AA [12]. The incidence of acquired AA varied in different regions. It is reported that the prevalence of acquired AA in Europe was 2/1,000,000 while in Asia it was twice or more. For example, the prevalence of acquired AA was 5.16/1,000,000 in Korea. At present, acquired AA has been considered immune-mediated disorder internationally [1]. The exposure to viral infection, toxins, drug side effects, autoimmune diseases, and prior radiochemotherapy may be important precipitating factors. The major treatments for acquired AA currently consist of either hematopoietic stem cell transplant (HSCT) or immunosuppressive therapy with antithymocyte globulin and cyclosporine. However, not every patient can be cured. And some patients can't pay for the expensive fee of the treatment in developing countries. Therefore they need frequent blood transfusions during late stage of the disease. Then how blood transfusion affects the progression of the disease? This research tried to discuss the question by analyzing the changes of the levels of TNF-α, IFN-γ and VEGF in the sera of patients with acquired AA after blood transfusion.

TNF-α is a cellular factor which is secreted by activated macrophage cells and its over-expression is toxic to cells [13]. In acquired AA patients, it was negatively relative to WBCs, PLTs, Hb in peripheral blood and myeloid, erythroid proportion, megakaryocyte counts in bone marrow [14,15]. This research suggested that the level of TNF-α in acquired AA patient was higher than that in normal children, which was similar to the previous report [16]. The proofs above showed that TNF-α was a negative regulator and damage factor in patients with acquired AA. This study showed that blood transfusion could decrease the level of TNF-α in patients with acquired AA, which suggested blood transfusion may delay the progression of the disease.

IFN-γ is another important cellular factor which is secreted by CD8+ cytotoxic T cells (CTLs) and memory stem T cells (TSCM) [17,18]. IFN-γ suppresses hematopoietic stem cells (HSCs) self-renewal and multilineage differentiation, thus impairing normal hematopoiesis [19-23], particularly in synergy with tumor necrosis [24-27]. IFN-γ plays important role in the HSC microenviroment or niche [28]. After effective treatment the level of IFN-γ in acquired AA patients would decrease [29]. Jichun Chen et al reported that IFN-γ was deleterious because it served as a critical component of an aberrant immune response that prepared hematopoietic cells for destruction [30]. Therefore, IFN-γ was linked to the pathogenesis of acquired AA [24]. The level of IFN-γ in acquired AA patients was higher than that in normal children, which was similar to the previous report [16]. In this research, blood transfusion decreased the level of IFN-γ. It suggested that blood transfusion can also delay the progression of the disease by decreasing the level of IFN-γ.

Bone marrow microenvironment consists of endosteal cells, macrophages, fat cells, fibroblasts, and microvascular endothelial cells. An abnormal growth and function of such microenvironmental cells in the bone marrow may contribute to the development of bone marrow aplasia in patients with acquired AA [31,32]. A central regulator of bone marrow microenvironment is VEGF [33-38]. Within the hematopoietic system, VEGF is considered to be primarily produced in megakaryocytes and immature myeloid cells [39,40]. VEGF has been implicated in the control of growth and differentiation of most primitive hematopoietic progenitors in mice. Notably, VEGF has been described as an autocrine growth regulator of murine hematopoietic stem cells. VEGF-deficient mice exhibit reduced bone marrow function and reduced angiogenesis [41]. It was reported that patients with acquired AA exhibited decreased VEGF levels and reduced angiogenesis compared to normal bone marrow [10]. In the early stage of acquired AA, the improvement of VEGF can stop the progress of the disease and be beneficial to hematopoietic angiogenesis [42,43]. Blood transfusion could increase the level of VEGF in patients with acquired AA, which suggested that blood transfusion may delay the progression of the disease.

Why the level of TNF-α and IFN-γ decreased and VEGF increased after blood transfusion? There may be two reasons. On the one hand, the changes of the three cell factors may be brought by blood transfusion. On the other hand, the changes may be an intrinsic feature of the disease and be unrelated to blood transfusion. W Hinterberger et al. reported that the changes of TNF-α and IFN-γ were intrinsic features of the disease and were unrelated to blood transfusion [44]. However there have been no reports on whether the levels of VEGF are related to blood transfusion or not.

However, our study has several limitations. First, our conclusion was an inference. As TNF-α and IFN-γ were negative regulators and VEGF was positive regulator. We inferred that blood transfusion may delay the progression of the disease by analyzing the changes of the three factors. Second, bone marrow detection was a gold standard for the patients with acquired AA. But all the patients in our study were in the late stage of the disease, the parents of them refused to have bone marrow detections. Third, as acquired AA was a rare disease, the number of the patients was small.

Conclusion

In a summary, blood transfusion could decrease the level of TNF-α and IFN-γ and increase the level of VEGF, which suggested that blood transfusion may delay the progression of the disease.

Author Contributions

Li Ying, Zhang Haixia, Yan Guangxing and Xu Feng collected the data and did the experiment. Jia Zhiyi, Wang Fengyu, Zhang Yu, Xue Yuanyuan Wang Lingzi, Chao Chunxia, Bi Fangjie, and Ma Liji all contributed essential data for the analysis. Li Yuyun and Zhang Xiaoyue wrote the paper and designed the study.

Conflict of Interest

The authors have no conflicts of interest relevant to this article to disclose.

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