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Original Article

Allogeneic Red Blood Cell Transfusion Following the Total Hip Arthroplasty Triggers the Immunomodulatory Responses

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Background and Aims: Total hip arthroplasty (THA) is a common orthopedic surgery. The main focus in the research of THA is immune responses in these patients following the blood transfusion.Therefore, in this study, we evaluated the immune modulation indexes such as T cell amplification, Tcell surface markers and also production of cytokines in the patients undergoing THA surgery following blood transfusion.

Materials and Methods: A total of 30 patients referred to Imam Hussein Hospital for THA surgery were included in the study between May 2015 to May 2016. Transfusions of 500 ml allogeneic red blood were done during and immediately after recovery of THA operation. Along with pre- and post-operation, immune modulatory effects of transfusion, including proliferation of T lymphocyte, secretion of T cell cytokines such as interferon gamma (IFN-γ), Interleukin(IL)-4 and expression of T lymphocyte surface markers such as human leukocyte antigen D related (HLA-DR) and CD25 were evaluated.

Results: Blood transfusion induced the expression of CD25 and HLA-DR markers in the THA patients. Blood transfusion also increased the secretion of IL-4 in post-operative stages in the THA patients. Results showed the increased IL-4 levels after blood transfusion. Whereas the levels of secretion in INF- γ were decreased after blood transfusion.

Conclusions: Allogeneic blood transfusion in the patients with the THA induced a cascade of immune and inflammatory responses, including increasing in T cell amplification and change in the secretion of interleukins that causes a modulation of immune responses. Further investigations should be performed to verify this hypothesis.

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Introduction

Total hip arthroplasty (THA) is a prevalence and successful orthopedic surgical procedure performed in the recent decade. Regarding to the increasing rate of THA frequency, it can be predicted that its occurrence will reach to 137% in the 2030 [1, 2]. Blood loss is commonly occurred along with THA operation and therefore in this surgery the risk of transfusion of allogeneic red blood cell (RBC) is higher than 30 percentages [3, 4]. However, transfusion of allogeneic blood is commonly associated with several risks and complications such as allergic reactions, transmission of infectious agents and immune modulatory effects [5, 6]. These responses are related to the foreign antigens in both the soluble and the cell-associated forms. The presence of these elements in the circulation can cause alloimmunization and down regulation of the immune responses. The regulation of immune responses has generally been referred to as transfusion-associated immunomodulation (TRIM). It has been reported that TRIM has been associated with some immunological signs such as decreased helper to suppressor Tcell ratio; decreased NK cell function; defective antigen presentation; and reduction in cell mediated immunity [7, 8]. Previously, it has been revealed that allogeneic transfusion is associated with increasing in lymphomas rate diagnosed during 5 to 10 years of post-transfusion [9]. Since lymphomas are the most important elements of acquired defects of the immune system, these results confirm the hypothesis that transfusion causes a mild to moderate deficiency of immune functions [10]. Recent studies suggest that altered cytokine regulation may contribute to transfusion-associated immunomodulation [10, 11]. Allogeneic transfusion seems to drive the immune system toward a T helper (Th)2 response and away from a Th1 response [12, 13], providing a hypothetical mechanism for transfusion-induced immunomodulatory effects. The TRIM has been suggested as possible mechanisms for clinical effects such as allograft rejection [14], increased rates of cancer recurrence [15, 16], increased prevalence of bacterial infections, reduced rates of spontaneous abortion [17], improvement in some diseases such as Crohn's disease and rheumatoid arthritis [14], rapid progression of chronic viral infections, and transfusion-related multiple organ dysfunction syndrome [18]. There is limited information about the immunomodulation responses of allogeneic RBC transfusion following the THA surgery [19]. Therefore, in this study, we surveyed the impacts of allogeneic RBC transfusion following the THA surgery on the indicators of immunomodulation, including T cells proliferation, some biomarkers expression and also the profile of cytokine production.

Materials and Methods

Patients and settings

Patients with osteoarthritis undergoing THA in Imam Hussein Hospital (IHH) were included in the study between May 2015 and May 2016. It is of note that the patients with autologous

blood transfusion, age less than 18 years, history of liver disease, coagulation disorder, and autoimmune disease or those were under treatments with immune suppressive drugs were excluded from the study. All patients were given informed consent before inclusion in the research. This study was conducted in accordance with the "Declaration of Helsinki for Human Research" and the protocol was accepted by the University Ethics Committee at High Institute for Research and Education for Transfusion Medicine (IR.TMI.REC.1393.7).

The isolation of peripheral blood mono-nuclear cells (PBMCs)

Transfusion of 500 ml red blood cell (RBC) was done during and immediately after recovery of THA operation. Ten milliliter of whole blood samples were collected in Ethylenediaminetetra acetic acid plastic tubes, one hour before the operation and three days after the operation in the groups of RBC transfusion and none transfusions. Blood was diluted with an equal amount of 1X phosphate buffered saline (PBS). Subsequently, PBMCs were isolated by ficoll (Ficoll-Hypaque 1.077, cedar lane, Canada) density gradient centrifugation.

T cell isolation

For magnetic labeling, the suspensions of 10^7 PBMCs were centrifuged at 300×g for 10 minutes. Pellets were re-suspended in 40 µL of buffer (0.05% sodium azide). In the next step, 10 µL of Pan Tcell biotin-antibody cocktail was added to the suspension and after incubation in the refrigerator (2-8°C) for 5 minutes, 30 µL of buffer was again added. 20 µL of pan T cell micro bead cocktail were added and incubated for 10 minutes in the refrigerator (2-8°C), and proceeded to magnetic separation with magnetic particle concentrator (Invitrogen, USA) following the manufacturer's instructions (Miltenyi Biotec, Order no. 130-096-535). Flow cytometry (Partec cyflow) with FITC-conjugated anti CD3 (BD, USA), FITC-conjugated mouse anti-human CD25 (BD, USA) and PE-conjugated mouse anti human leukocyte antigen D related (HLA-DR) (BD, USA) were used to survey the expression of CD25 and HLA-DR surface markers on T cells of all the studied groups.

T cell proliferation assay

Firstly, 2×10^5 of isolated T cells were cultured in the Roswell Park Memorial Institute (RPMI) medium included 10% fetal bovine serum (FBS) for 72 hours, and the proliferation of the cells were evaluated by WST-1 Cell Proliferation Assay Kit (Cayman, USA) and K562 (Pasteur Institute, Iran) as the control cells. This assay was based on the enzymatic cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases present in the viable cells. Briefly, 10^7 cells of the culture media were added into the wells of a culture plate and 10 µl of the reconstituted WST-1 mixture was added to each well. After mixing, cells were incubated at 37°C in a CO_2 incubator for four hours. Finally, the absorbance of the wells was measured using a micro plate reader at the wavelength of 450 nm.

Cytokine production

For the measurement of cytokines in the supernatants of the cultured T cells, the quantity of Interleukin (IL)-4 and interferon gamma

(IFN-γ) was measured by the enzyme-linked immunosorbent assay (ELISA) Kit (Invitrogen, USA) using Anthos 2020 ELISA reader (Austria) in accordance with the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using the statistical package SPSS (SPSS, Chicago, IL, USA). The mean values (±SD) for IFN-γ and IL-4 Student's t-tests were calculated. P-values of ≤0.05 were used to show the significant differences.

Results

Out of 30 patients included in this study, one group comprised of 15 patients who were transfused with 2 to 4 units of RBC. Expression of CD25 and HLA-DR in the pre and post operation groups shows that this biomarker was significantly increased after THA surgery (Table 1. and Fig. 1). As it was shown, the THA surgery increased the expression of mentioned biomarkers near to two folds in comparison with the pre-operative groups. Results also indicated that transfusion of allogeneic red blood cells in the post operation status increased the expression of CD25 and HLA-DR, although these changes were not significant (Table 2).

Table 1. Comparison of CD25 and HLA-DR expression in pre and post pperation status

Biomarker	Position	Number of patients	Mean± SD (%)	P-Value
CD25	Pre operation	10	6.76 ±1.4	0.001
	Post operation	10	15.98 ±4.92	
HLA-DR	Pre operation	10	9.68 ± 4.10	0.001
	Post operation	10	17.36 ± 6.01	

Table 2. Effects of transfusion on CD25 and HLA-DR expression in the post operation patients

Biomarker	Position	Number of patients	Mean (rank)	P-Value
CD25	Transfused	7	6.00	0.51
	Non-Transfused	3	4.33	
HLA-DR	Transfused	7	6.14	0.38
	Non-Transfused	3	4.00	

Comparison of T cells amplification in transfused and non-transfused patients showed that transfusion of allogeneic red blood cells decreases the amplification rate of the T cells in both the pre and post operation of THA (Table 3). As shown in table 3, these differences between the transfused and non-transfused groups were significant ($p<0.01$).

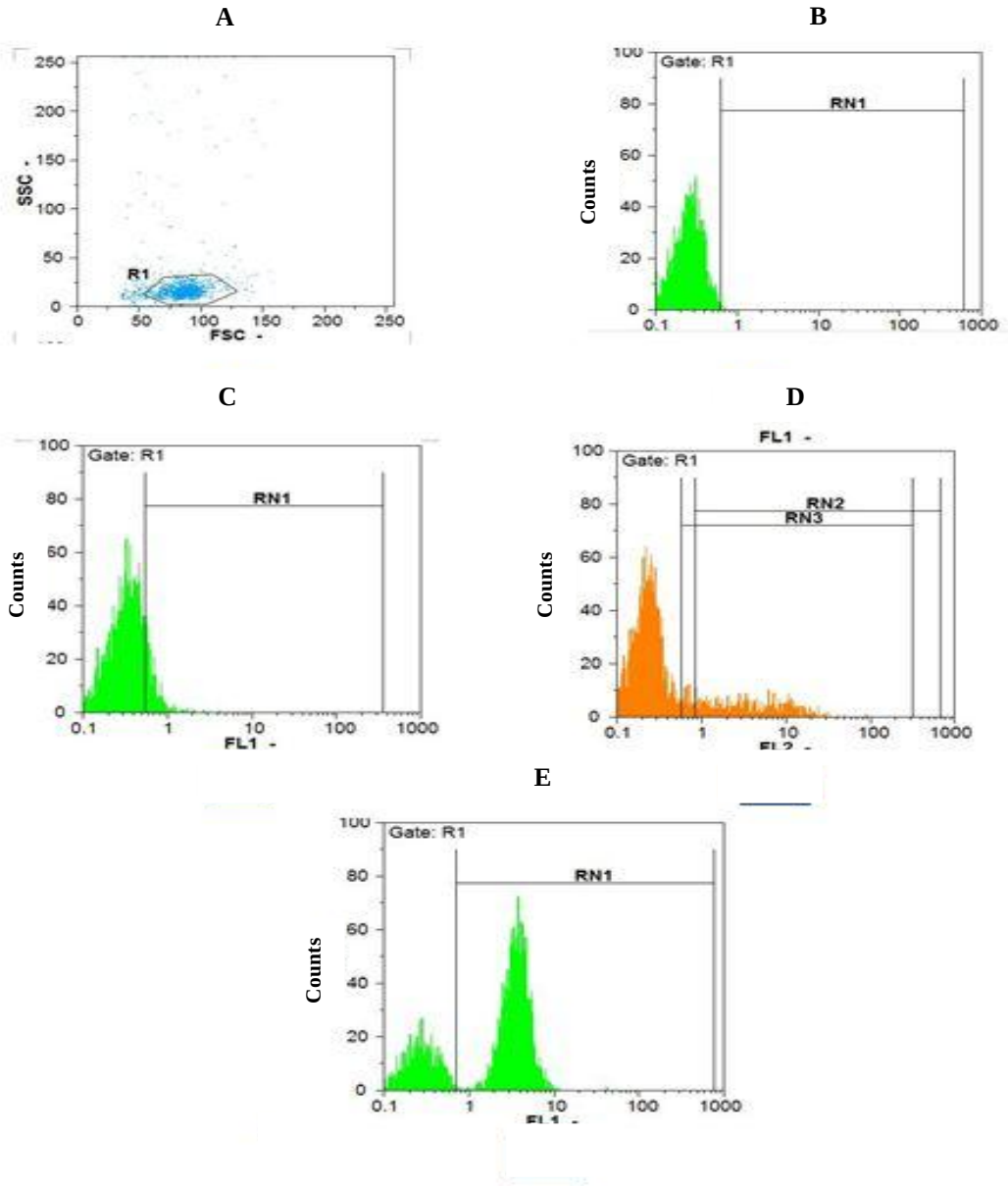

Fig. 1. Flow cytometry plot. The expression analysis of CD3, CD25 and HLA-DR on the surface of T cells of THA patients after operation and blood transfusion. **A**: Gating of the isolated cell population; **B**: Isotype control; **C**: The level of CD25 Expression on the isolated cells; **D**: The level of HLA-DR Expression on the isolated cells; E: The level of CD3 Expression on the isolated cells.

Table 3. Comparison of the T cells amplification in transfused and non-transfused patients

Patients	Number of patients	Transfusion status	Mean± SD	P-Value
Pre-operation	15	Transfused	0.22±0.040	0.001
		Non-Transfused	0.265±0.030	
Post-operation	15	Transfused	0.467±0.073	0.001
		Non-Transfused	0.334±0.032	

Secretion of two cytokines (IL-4 and INF-γ) was also surveyed in the pre and post operation groups that were transfused with allogeneic red blood cells. Results showed that transfusion decreased the IL-4 concentration in comparison to non-transfused patients in the pre-operation groups. As shown in table 4, the mean concentrations of IL-4 in the non-transfused and transfused groups were 5.02±0.60 and 5.62±0.63 pg/ml, respectively. After THA surgery, these patterns were different. Results showed that following the THA transfusion triggered the secretion of IL-4 in comparison to the non-transfused groups. These changes were significant (p=0.001) (Table 4). Regarding the INF-γ, transfusion decreased the concentration of INF-γ. As shown in table 4, the concentrations of INF-Y in the transfused and non-transfused group were 0.15±0.03 and 0.168±0.036, respectively (Table 4). However, the differences were not significant (p=0.32).

Table 4. Effects of transfusion in the concentration of IL-4 and INF-γ in pre and post operation patients.

Patients		IL-4			INF-γ		
		Transfusion status			Transfusion status		
		Transfused	Non-transfused	P-value	Transfused	Non-transfused	P-value
Pre-operation	15	5.02±0.60	5.62±0.63	0.001	0.17±0.05	0.2±0.60	0.1
Post-operation	15	7.63±0.59	6.38±0.53	0.001	0.15±0.03	0.168±0.036	0.32

Data are presented as Mean±SD; IL-4=Interleukin-4; INF-γ= Interferon gamma

Discussion

In this study, we observed that the lymphocyte proliferation was significantly higher in the transfused patients following THA surgery compared to one day before surgeryor than in non-transfused groups. This is in contrast with the results of the previous report of Yu et al. [20]. Unlike the results of our study, he and coworkers showed that the lymphocyte proliferation rate that was promoted by RBC was significantly reduced after surgery.

Expression of major histocompatibility complex II is an essential element in the immune response and it is a prerequisite for the effective antigen presentation [21]. Reduced levels of HLA-DR expression following the surgery have been found [21]. Also, Handy et al. [22] declared that surgery operations cause a reduction in the expression of HLA-DR biomarker. Unlike previous reports, the results of our study showed that the expression of CD25 and HLA-DR on the lymphocyte membrane was increased after the surgery compared with before the surgery and transfused than non-transfused group. This is in contrast with the previous reports [20]. Yu et al. [20] declared that the expression of RBC membrane markers; CD35, CD58 and CD59 were significantly decreased in the postoperative phase compared to pre-operative levels. In line with us, Baumgartner [23], and

also Cata [24], showed that the transfusions of RBC increased the expression of CD25. Moreover, Sun et al. reported that blood transfusion in cancerous patients causes increased levels of CD25 expression, which was in agreement with our results. These increased expressions improved the antigenic presentation and diagnosis by T cells [25]. On the other hand, the overexpression of CD25 inhibited T cells amplification and activation of IL-2 signaling pathways and consequent modulation of immune responses [26]. T cells comprise CD3/CD4 helper/inducer and CD3/CD8 cytotoxic/suppressor lymphocytes with two distinct functions: Th1 lymphocytes produce IFN-γ and IL-2, whereas Th2 are producers of IL-4 and IL-10 [12, 27]. The balances between the two types of Tcells are crucial for the immune regulation. Therefore, disturbance in the levels of each of the T cell types causes on the effects of different diseases. It has been proposed that cytokine regulation is one of the mechanisms in the transfusion-related immunomodulation [28-30]. In the present study, we have surveyed the influence of allogeneic red cell transfusion following the THA surgery on the production of IFN- γ and IL-4 as a functional index of immunologic responses. IL-4 induces the differentiation of B lymphocytes, cytotoxic activity of T lymphocytes and acts as inhibitory elements for the production of pro-inflammatory cytokines [31]. Increased IL-4 production took place in the patients who received transfusion following THA surgery. In line with the results of this study, Pandey et al. [32] reported that in the patients who received allogeneic transfusion, a remarkable increase in both IL-4 and IL-10 was seen as compared to those patients who received either no transfusion or autologous transfusion. Moreover, increasing the IL-4 transcripts in the graft and spleen using a murine heart allograft model has been reported [33, 34]. No significant change in the levels of Th1 pathway cytokine (IFN-γ) was seen in the patients who received transfusion following THA surgery. In contrast, Bordin et al., [35] reported that the transfused patients with allogeneic RBCs following the hip replacement surgery had decreased levels of IFN-γ and tumor necrosis factor (TNF)-α. IFN-γ induces the expression of MHC class-II molecules, and consequently stimulating a specific immune response. Furthermore, it induces the activation of monocytes and macrophages and their production of inflammatory cytokines such as TNF-α and IL-1 [36, 37]. In consistence with the overall results of this study, it has been proposed that the anti-inflammatory cytokines such as IL-4, IL-10 and IL-13 serve to down-regulate synthesis of pro-inflammatory cytokines IL-1, IL-6, TNF-α and IFN-γ [20, 30, 38]. Interestingly IL-4 induce bone formation and other anti-inflammatory cytokines do not induce bone [39]. Higher levels (6-7 pg/ml) of this cytokine found in our study can be a clue to better improvements in the patients with THA surgeries. Our study had some limitations such as low size of samples. Further research is needed for evaluation of other immunological indexes such as other cytokines in THA and also in transfused patients in order to get complete insights about the immunological modulation in THA surgery.

Conclusion

Patient blood management and utilization of transfusion therapy based on local guidelines in THA surgery can be the effective ways to reduce the patient's complications related to transfusion-associated immunomodulation. Optimizing the patients' conditions before the surgery among the elective patients can be an effective way in improving outcomes of the treatment in the patients who undergo orthopedic surgery.

Conflict of Interest

There are no conflicts of interest or financial involvement with this manuscript as confirmed by all authors.

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