

TNF- α and TGF- β level after intraoperative allogeneic red blood cell transfusion in orthopedic operation patients

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Background/aim: Blood transfusion is associated with immunosuppression, referred to as transfusion-related immunomodulation (TRIM). In this study, for the first time, changes in the concentration of TGF- β and TNF- α were measured postoperatively in orthopedic patients with intraoperative allogeneic red blood cell transfusion. Considering the use of packed cell units with different ages, it is possible to suggest the more appropriate product for clinical applications.

Materials and methods: Two groups of 35 orthopedic surgery patients (with or without transfusion as case and control groups, respectively) were involved. Serum levels of TNF- α and TGF- β were measured by ELISA.

Results: The data suggested significant differences in age ($P = 0.0001$), lowered hemoglobin ($P = 0.003$), and hematocrit ($P = 0.003$) between the control and case groups. Pre- and postoperation levels of TNF- α and TGF- β were not significantly different, but the results showed significant increases in levels of both cytokines after the operation ($P = 0.0001$) in both groups.

Conclusion: Increased levels of TNF- α and TGF- β are probably related to surgery and packed cell transfusion, respectively. Further studies using more packed cell units or other blood products and assessment of more cytokines are needed to have better understanding about this issue.

Key words: Blood transfusion, orthopedic surgery, TNF- α , TGF- β

1. Introduction

Healthy blood is a lifesaver. Many people require blood and its products globally in everyday life. One of every 3 people requires transfusion of blood and its related products in his/her life. Currently, despite the salient developments in medical sciences an alternative for blood has not been yet invented and only donated blood can save people from dying. The absence of an appropriate alternative for blood, limitations in storage time of blood and its products, and continual need for transfusion have made blood donation a special event (1).

Blood transfusion is defined as transient transplantation of cells and a vast amount of antigens, which obeys organ transplantation rules, via vascular injection. Similar to every other treatment, this event can cause beneficial or harmful outcomes in patients. There is no doubt about the effectiveness of blood transfusion and similar to every

other treatment its advantages and disadvantages have been determined (2). The major obstacles and side effects of allogeneic transfusion are of immunologic nature. One type of the delayed transfusion reactions is transfusion-related immunomodulation (TRIM). Some of the possible clinical outcomes of TRIM are postoperative infection and recurrence of cancer and, on the other hand, lower graft rejection in kidney transplant recipients (3,4).

TRIM has an important effect on the world's public health (5). Therefore, studies with efficient and clear results in the field of blood transfusion in various patients who experience difficult operations are required.

There is a lack of literature data about the impact of blood transfusion on the serum levels of immunosuppressive TGF- β and proinflammatory TNF- α cytokines, although these cytokines are important mediators in the immune system. In this study, for the first time, changes in the

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concentration of cytokines TGF- β and TNF- α were directly measured postoperatively in orthopedic patients with intraoperative allogeneic red blood cell transfusion.

Here we aim to investigate new TRIM-related strategies and take a step towards clarifying the mechanisms underlying transfusion-related immunosuppression. In the present study, considering the use of packed cell units with different ages, it is possible to suggest a more appropriate product for clinical applications, for instance, in order to reduce the risk of kidney rejection, postoperative infections, or cancer relapse (6–8). These items will result in providence in health and care costs via shortening the hospitalization time and aiding the better outcomes of patients.

2. Materials and methods

2.1. Subjects

Seventy orthopedic operation patients were involved in this study. The study was approved by the local research ethics committee and informed consent was obtained from all patients. Patients' specifications and possible involved factors were recorded. The inclusion and exclusion criteria are summarized in Table 1.

The median age of patients was 51 years (range 20–65). Among them, 34 (51.4%) patients were men and 36 (48.6%) were women. All patients were divided into two groups: the control group consisted of 35 patients who did not receive any blood product during the operation and the case group comprised 35 patients who received blood in the form of packed cells in an amount relative to their blood loss during the operation.

Peripheral venous blood was obtained from each patient 15 min before surgery and serum was separated. The same procedure was carried out immediately after the operation. The samples were frozen and stored at -70°C until assayed.

2.2. ELISA

Levels of cytokines were determined by a sandwich enzyme-linked immunosorbent assay (ELISA) method. A commercially available ELISA kit (Boster biological technology- China) was used according to the manufacturer's instructions to quantify the concentrations of tumor necrosis factor-alpha (TNF- α) and transforming

growth factor- β 1 (TGF- β). Briefly, standards and samples were added to a microplate precoated with an antibody against each cytokine. After incubation and removal of the unbound substances, an enzyme-conjugated secondary antibody specific for each cytokine was added. After a second wash, a substrate solution was added and a yellow color developed in proportion to the amount of the bound cytokine in the first step. The color development was stopped by adding the stop solution, and optical density at 450 nm was measured using the microplate reader (HumaReader HS, Germany). All measurements were performed in triplicate.

2.3. Statistical analysis.

Statistical comparisons were made using ANOVA and $P < 0.05$ was considered significant. The results are expressed as mean \pm SD.

3. Results

The case group had significantly older age and longer duration of operation ($P = 0.0001$) as well as lower hemoglobin and hematocrit ($P = 0.003$) when compared to the control group (as shown in Table 2).

However, there was no significant difference between the two groups in terms of the basal and postoperation state of TGF- β and TNF- α ($P > 0.05$) (as shown in Tables 3 and 4 and Figure 1).

Furthermore, the results demonstrated a significant increase in both TGF- β and TNF- α in cases and controls ($P = 0.0001$), but there was no significant difference between the two groups in terms of changes in TGF- β or TNF- α ($P = 0.77$, $P = 0.73$, respectively) (as shown in Figure 2).

The multivariate analysis did not reveal any impact of age ($P = 0.59$) or sex ($P = 0.57$) on the change in the cytokines. Meanwhile, there was no relationship between the product age and cytokine status ($P = 0.59$, 0.73 for TGF- β and TNF- α , respectively) (data not shown).

4. Discussion

Allogeneic blood transfusion is associated with postoperative complications due to the effects, referred to as TRIM. For the first time, the study by Opelz in the 1970s revealed improved renal allograft survival in previously transfused patients compared to nontransfused patients (9).

Table1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Age 40–60 years Hemoglobin concentration > 100 g/L Hematocrit $> 30\%$ Weight > 60 kg	Active infection Active inflammatory disease Previous blood transfusion Taking immunosuppressive drugs or on chronic anticoagulation

Table 2. Laboratory parameters of peripheral blood.

Variables	Analysis		
	n	Control	Case
Blood sugar (mg/dL)	35	101.71 ± 20.36	111.69 ± 34.90
Hb (g/dL)	35	14.06 ± 1.49	12.81 ± 1.83
Hct (%)	35	42.65 ± 3.99	39.14 ± 6.36

Control: nontransfused patient group; Case: transfused patient group

Table 3. Serum levels of TGFβ and TNFα in the control group.

Concentration of cytokines	Analysis		
	n	1st	2nd
TGF-β (pg/mL)	35	612.47 ± 162	1373.54 ± 447
TNF-α (pg/mL)	35	90.14 ± 36	120.67 ± 46

1st analysis: before operation; 2nd analysis: after operation

Table 4. Serum levels of TGFβ and TNFα in the case group.

Concentration of cytokines	Analysis		
	n	1st	2nd
TGF-β (pg/mL)	35	634.37 ± 120	1415.39 ± 143.72
TNF-α (pg/mL)	35	77.66 ± 33	± 48

1st analysis: before operation; 2nd analysis: after operation

Some of the possible adverse clinical effects of TRIM include increased risk of cancer recurrence, postoperative infection, and short-term mortality. However, the clinical significance of TRIM remains controversial (10). The precise mechanisms of TRIM are still not well understood although presumed to be mediated by allogeneic leukocytes and its products including the HLA peptides and cytokines (11).

Allogeneic RBC transfusion can be associated with the variations in cytokines. Dzik et al. presupposed that blood transfusion can stimulate the synthesis of the immunosuppressive cytokine TGF-β. Consequently concentrations of TGF-β and TNF-α in peripheral blood of orthopedic surgery patients after RBC transfusion were analyzed (12).

Changes in Th1 to Th2 ratio and inclination to Th2, and induction of tolerance are some debated issues in allogeneic blood transfusion. Increases in IL-4 and IL10 and reductions in IL-2, IL-12, and IFN-γ secretion have been observed in

posttransfusion experimental models in vitro. Changes in cytokines are detectable 3 days after transfusion and at least for 2 weeks (13,14). In addition to the mentioned cytokines, secretion of TGF-β and prostaglandin E2 has also been brought up after transfusion. Both of these factors contribute to the inhibition of cell-mediated immunity (CMI) and strong Th2 response to a specific antigen and may lead to an increase in TGF-β and in turn nonspecific attenuation of immune response (15).

Bleeding-related immunomodulation is mostly covered by operation-related immunomodulation effects. A significant increase in TGF-β in blood has been observed in experimental models 24 h after bleeding. This is accompanied by proliferative response to concanavalin A and antigen presentation by spleen macrophages. Reduction in spleen T cells proliferation occurs with an increase in IL-4 and IL-10. Administration of ibuprofen as an inhibitor of cyclooxygenase pathway or anti-TGF-β antibodies impedes the production of these two cytokines (16,17).

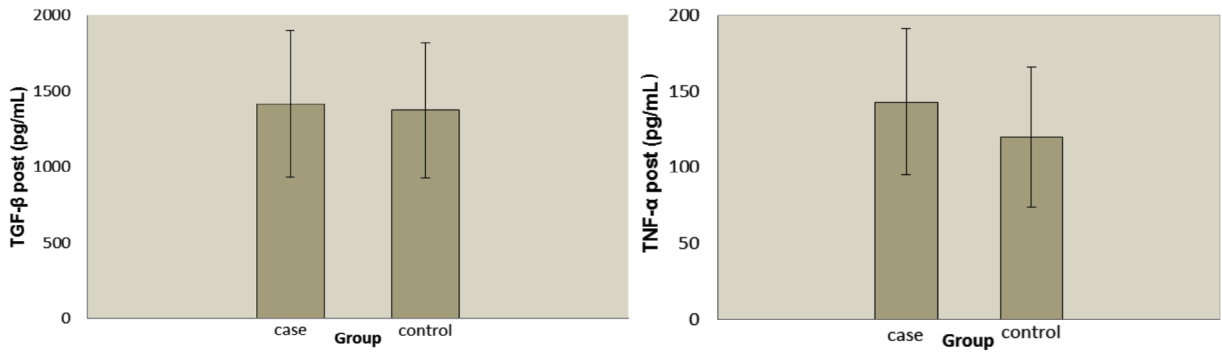


Figure 1. Concentrations of TGF-β and TNF-α in the case and control groups.

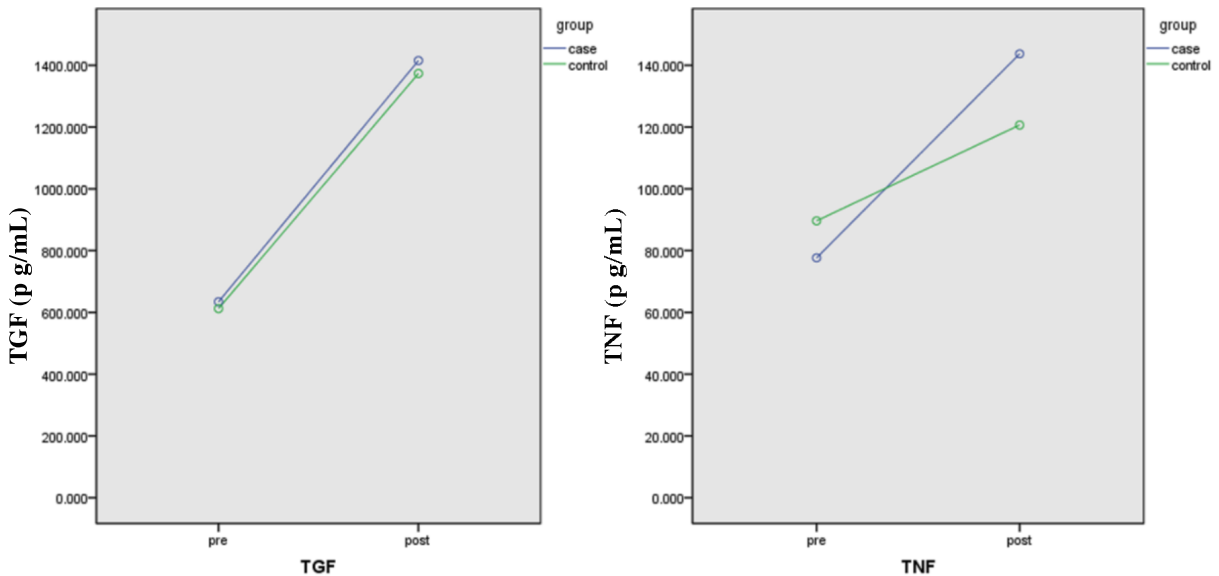


Figure 2. Basal and postoperation concentrations of TGF-β and TNF-α in the case (blue) and control (green) groups.

Considering the fact that surgery as an invasive and inflammatory process and blood transfusion as an immunomodulatory factor may affect the profile of cytokines, it is interesting to observe the quality of the changes in TNF-α and TGF-β. Therefore, the serum levels of TNF-α and TGF-β were analyzed in this study.

Previously Sablowski et al. performed a similar study in cardiovascular operation patients. They assessed plasma levels of IL-10 and TGF-β in 30 patients who had experienced elective coronary artery bypass surgery in order to determine levels of immunosuppressive cytokine secretion during cardiopulmonary bypass (CPB). The results suggested that plasma levels of IL-10 and TGF-β significantly increase after CPB and reach their maximum during skin closure. They also showed that IL-10 and TGF-β levels are reduced 6 h after the operation. Considering these findings, they stated that both cytokines are major immunoregulatory factors and have negative

effects on T cell-mediated immunologic response. The significantly increased levels of IL-10 and TGF-β at the end of CPB suggest that they may be important factors in dysregulation of the immune system following CPB (18).

In the same study by Sitniakowsky et al., the effect of red blood cell transfusion during cardiac surgery on TGF-β and TNF-α gene expression was investigated. Their results showed that these patients have increased gene expression after surgery and blood transfusion (19).

Gulhan et al. determined the cytokine changes after transfusion in patients with thalassemia major and reported that TGF-β rates were decreased after blood transfusion (20).

In Biedler et al.'s study, TNF-α and IL-10, which were selected as prototypical proinflammatory and antiinflammatory cytokines, respectively, were assessed. They suggested that blood transfusion may in part insert its immunosuppressive effects through induction

of Th2-derived production of IL-10. Their investigation demonstrated that leukocytes are not responsible per se, since fresh allogeneic blood with normal numbers of leukocytes had no significant effect on IL-10 or TNF- α secretion (21).

A similar study was conducted by Milasiene et al. on colorectal cancer patients to determine the changes in TGF- β and TNF- α levels after blood transfusion. The results showed increases in these two cytokines (22).

Furthermore, the study by Miki et al. in the same patients demonstrated that the plasma level of IL-6 is elevated in transfused patients (23).

The design of the present study entails a few problems that might limit the validity of our results. First, transfused

patients presented with lower preoperative hemoglobin and hematocrit levels, as well as longer surgery duration. Second, due to the high cost of ELISA kits, we limited our study to only two cytokines.

Considering the results of previous reports, increased levels of TNF- α and TGF- β are probably related to surgery and packed cell transfusion, respectively. The data of our study corroborate the findings of other investigators, who showed that allogeneic RBC transfusion might exert immunomodulatory effects. Further studies using more packed cell units or other blood products and assessment of more cytokines are needed to have better understanding about this issue.

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