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# Plasma levels of norepinephrine and expression levels of ß2-adrenergic receptor gene correlate with the incidence of acute graft-versus-host disease

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## Abstract

**Background:** Acute graft-versus-host disease is a major complication in allogeneic hematopoietic stem-cell transplantation. Epinephrine and norepinephrine are stress hormones which affect many cells, including immune cells through interaction with adrenergic receptors, mainly β2-adrenergic receptor. The immunomodulatory effects of epinephrine, norepinephrine, and signaling of the adrenergic receptor have been shown to decrease the probability of the acute graft-versus-host disease in animal models. The aim of our study was to investigate the possible correlations between the serum levels of epinephrine and norepinephrine and also leukocytic expression levels of β2-adrenergic receptor with the incidence of acute graft-versus-host disease in patients undergoing allogeneic hematopoietic stem-cell transplantation.

**Methods:** In this study, the plasma levels of epinephrine and norepinephrine and the leukocytic expression of β2-adrenergic receptor gene were measured and compared in allogeneic hematopoietic stem-cell transplantation patients with and without acute graft-versus-host disease. Data were analyzed and illustrated using SPSS 19 and GraphPad Prism 6. The student T-test, Pearson, and Spearman's tests were performed and $p<0.05$ was considered as significant.

**Results:** We showed that the plasma levels of norepinephrine and the relative amount of the mRNA of β2-adrenergic receptor at 7 and 21 days after allogeneic hematopoietic stem-cell transplantation were significantly lower in patients with acute graft-versus-host disease than recipients without acute graft-versus-host disease. There were also negative correlations between the plasma levels of norepinephrine, leukocytic levels of the mRNA of β2-adrenergic receptor, and the incidence of acute graft-versus-host disease.

**Conclusion:** Our results suggest that stress hormones and their receptor might have a role in preventing acute graft-versus-host disease and could be promising factors in controlling the outcome of allogeneic hematopoietic stem-cell transplantation.

**Keywords:** Norepinephrine, Epinephrine, β2-adrenergic receptor, Acute graft-versus-host disease

***Conflicts of Interest:*** *None declared*
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***↑What is "already known" in this topic:***
The previous findings indicate the beneficial effects of norepinephrine and signaling of ß2-adrenergic receptor on the modulation of the immune system and resistance to acute graft-versus-host disease in animal models. However, no investigation has been conducted to confirm this association in humans so far.

***→What this article adds:***
We showed that the plasma levels of norepinephrine and leukocytic expression of ß2-adrenergic receptor gene negatively correlates with the incidence of acute graft-versus-host disease, suggesting that this axis might have a role in the prevention of acute graft-versus-host disease and could be promising factors in controlling the outcome of allogeneic hematopoietic stem-cell transplantation.

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## Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) was a revolution in the treatment of hematologic malignancies. Despite the benefits of allo-HSCT, acute graft-versus-host disease (aGvHD) is of a major post-transplantation complication accounts for the majority of mortalities in patients (1). The main contributors involved in the pathogenesis of GvHD are antigen-presenting cells (APCs) and T lymphocytes, whose removal before transplantation leads to a reduction in the beneficial effects of graft-versus-tumor (GvT) (2). Researchers are looking for ways to enhance the recipient's immune system and control the aGvHD. Studies in recent decades have shown the reciprocal interaction of the nervous and the immune systems (3, 4). Several studies reported the effects of neurotransmitters on various immune cells, including APCs and lymphocytes (5-7). Two important hormones of the sympathetic nervous system (SNS), which affect the function of the immune system are epinephrine and norepinephrine. These hormones act through adrenergic receptors present on many cell types, including immune cells. The association between high serum norepinephrine levels, β-adrenergic receptor signaling, APC and T lymphocyte activation has been reported in various conditions including transplantation (7-9) .In the conditions of tumor or infectious disease in which immune suppression exacerbates the disease condition (10-12), studies have shown that neurotransmitters, particularly epinephrine and norepinephrine, through adrenergic receptor signaling in immune cells can modulate the anti-tumor functions of the immune system (13). In other circumstances such as GvHD in which a regulated immune system is desirable, the neurologic-mediated immune-modulation is beneficial to mitigate the consequences and control the disease (8). Mouse models have shown that cold stress due to increased serum norepinephrine levels and increased signaling of β2-adrenergic receptors (β2-AR) can enhance the host immune system leading to resistance to acute GvHD. The GvHD-resistance was eliminated in β2-AR$^{-/-}$ mice and mice treated with β2-AR blockers (8). These studies indicate the beneficial effects of norepinephrine and β2-AR signaling on the immune system and resistance to aGvHD in animal models. However, no investigation has been conducted to confirm this association in humans so far. In this study, serum epinephrine and norepinephrine levels, expression of β2-adrenergic receptor and their associations with the incidence of aGvHD in patients undergoing allo-HSCT were investigated which might open a new window for the role of short-term stress in patients undergoing hematopoietic stem cell transplantation.

## Methods

### Patients

Fifteen patients (median age of 38 years old) diagnosed with acute myeloid leukemia and candidates for allo-HSCT were randomly selected from the HSCT and Cell Therapy Center at Taleghani Hospital of Tehran, Iran. All patients received full 6/6 human leukocyte antigen (HLA) matched grafts. At days -7, 0, +7, and + 21 (day of transplantation was considered as day 0), 5 mL of peripheral blood samples were collected in EDTA containing tube and the plasma samples were isolated and preserved at -80°C. Patients were followed-up for 100 days after transplantation to determine the presence or absence of aGvHD based on the patients' clinical manifestations and NIH criteria (14). The demographic and clinical data of patients are shown in Table 1. All candidates have given informed consent, and the study received ethics approval by the university ethics committee.

### Epinephrine and norepinephrine quantification by ELISA

The plasma levels of epinephrine and norepinephrine were measured using quantitative competitive enzyme-linked immunosorbent assay ELISA following the manufacturer's protocol (ELISA Fast Track, LDN, Nordhorn, Germany). Briefly, epinephrine and norepinephrine were extracted using a cis-diol-specific affinity gel, then acylated and enzymatically converted. The antigen was bound to the microtiter plate solid phase and competed with the standards, controls and patients' plasma in binding to the antibody. The solid phase-bound antibody was detected by an anti-rabbit peroxidase conjugate. The sensitivity of kits for epinephrine and norepinephrine were 0.018 and 0.093 ng/ml, respectively. The specificity of both ELISA kits for their specific analytes was 100% and for other related analytes such as dopamine, metanephrine, normetanephrine, tyramine ant etc. were less than 0.2%.

### RNA extraction and cDNA synthesis

Buffy coats were isolated by centrifugation of 3 ml of anticoagulated whole blood and washed with phosphate buffer saline (PBS). Total RNA extraction was performed using Trizol reagent (Invitrogen, Carlsbad, CA). One mL Trizol was added to 200 µl of buffy coat and after 5 min incubation at room temperature and centrifugation at 1000 g for 15 min at 4°C, 200 µl cold Chloroform was added, and the sample was centrifuged for 15 min at 1000 g and 4°C. 500 µl of isopropanol was added to isolated supernatant and incubated for an extra 15 min at 4°C followed by the same centrifugation protocol. The pellet was re-suspended in 0.5 ml of 75% ethanol and then left out to dry. The recovered RNA was eluted in diethyl pyrocarbonate (DEPC) water. The quantity and quality of extracted RNA were evaluated by the NanoDrop spectrophotometer using an optical density (OD) ratio of 260nm/280nm. A ratio of 1.8 to 2 is acceptable. The integrity of each RNA extracted samples was verified by running on (1% w/v) RNase-free



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agarose gel and visualization of SYBR Safe stain (Invitrogen, CA) under ultra-violet light. The reverse transcription of extracted RNA into first-strand complementary DNA (cDNA) was performed using RevertAid First Strand cDNA Synthesis Kit (thermo-scientific, Lithuania), according to the manufacturer protocol. The positive control was ABL RNA (40 ng/µl).

### Relative quantitative real-time PCR

The mRNA expression levels of the β2-adrenergic receptor were relatively quantified by quantitative-PCR (qPCR) using RealQ Plus Master Mix Green + ROX (Ampliqon, Odense, Denmark) on Step One Real-Time PCR System thermocycler (Applied Biosystems). gene-specific primers for β2 adrenergic receptor were used for real-time-PCR reaction (forward: 5′-TTCTTGCTGGCA CCCAATGGAAG-3′, reverse 5′-CCAGTGAAGTGA TGAAGTTGGTG-3′) (13) The ABL gene was selected as the reference gene (forward: 5'-TGTGATTATAGC CTAAGACCCGGAG-3', reverse 5'-CAGCGGCCAGTA GCATCTGACTT-3) (15). The amount of 0.5 mM of primers and 2 µl of template cDNA were added to 10 µl of Master mix and topped-up to 20 µl with nuclease-free water. The amplification setting was: 5 min at 95° C followed by 40 cycles of 94° C for 30s, 58° C for 30s, and 72° C for 15s. Melting analysis was conducted on the samples to ensure amplification specificity. The results of the melting curve were analyzed as cycle threshold (Ct). The mean Ct values were normalized to ABL expression levels (ΔCt). $2^{-\Delta\Delta CT}$ method was used to evaluate the relative mRNA expression levels of the target gene (16).

### Statistical Analysis

All experiments were performed in duplicate for each sample. The data were analyzed and illustrated using SPSS version 19 and GraphPad Prism version 6. The normality of the data distribution was evaluated by the Kolmogorov–Smirnov test. Using the normal distribution of data, parametric analyses (student T-test and Pearson) were carried out. The Spearman's test was performed to study the correlations of epinephrine, norepinephrine, and gene expression with aGvHD status and also, the Pearson tests were used to determin e correlations between epinephrine, norepinephrine and gene expression levels. Moreover, the factor association of Eta-squared was determined to evaluate the perfect association. P-value < 0.05 was considered as significant.

## Results

### Plasma levels of norepinephrine and epinephrine

Five patients out of 15 recipients (33.3%) developed aGvHD (Table 1). The patients' plasma levels of norepinephrine and epinephrine at days -7, 0, +7, and + 21 were quantitated using ELISA. The plasma levels of norepinephrine were higher in the non-aGvHD group compared to the aGvHD group (Fig. 1 A). The norepinephrine levels were significantly higher at days +7 ($p<0.001$) and +21 ($p<0.001$). No significant differences were observed at any time point in the plasma levels of epinephrine between the two groups. As it is shown in Figure 1 B, the plasma levels of epinephrine in aGvHD group was slightly higher before allo-HSCT (53 pg/mL in aGvHD groups and 42.5 pg/mL in

*Table 1.* Demographic and clinical data of patients

| Patient No. | Sex | Age | Diagnosis | GvHD status | Conditioning regimen | Donor | CD3 (×10⁶/kg) | CD34 (×10⁶/kg) | GvHD prophylaxis |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Female | 46 | AML | NO | Busulfan + Fludarabine | Brother | 410 | 4 | Methotrexate + Cyclosporine A |
| 2 | Male | 31 | AML | NO | Busulfan + Fludarabine | Brother | 310 | 5.8 | Methotrexate + Cyclosporine A |
| 3 | Male | 50 | AML | NO | Busulfan + Fludarabine | Brother | 300 | 3.8 | Methotrexate + Cyclosporine A |
| 4 | Male | 25 | AML | NO | Busulfan + Fludarabine | Sister | 151 | 4.9 | Methotrexate + Cyclosporine A |
| 5 | Female | 29 | AML | NO | Busulfan + Fludarabine | Brother | 265 | 3.2 | Methotrexate + Cyclosporine A |
| 6 | Male | 47 | AML | NO | Busulfan + Fludarabine | Sister | 360 | 3.5 | Methotrexate + Cyclosporine A |
| 7 | Male | 24 | AML | NO | Busulfan + Fludarabine | Brother | 273 | 2.1 | Methotrexate + Cyclosporine A |
| 8 | Male | 23 | AML | NO | Busulfan + Fludarabine | Brother | 273 | 3.5 | Methotrexate + Cyclosporine A |
| 9 | Male | 28 | AML | NO | Busulfan + Fludarabine | Brother | 390 | 6.9 | Methotrexate + Cyclosporine A |
| 10 | Male | 31 | AML | NO | Busulfan + Fludarabine | Sister | 350 | 6 | Methotrexate + Cyclosporine A |
| 11 | Female | 41 | AML | Acute | Busulfan + Fludarabine | Brother | 330 | 2.37 | Methotrexate + Cyclosporine A |
| 12 | Male | 25 | AML | Acute | Busulfan + Fludarabine | Brother | 435 | 5.5 | Methotrexate + Cyclosporine A |
| 13 | Female | 17 | AML | Acute | Busulfan + Fludarabine | Sister | 365 | 2.8 | Methotrexate + Cyclosporine A |
| 14 | Male | 30 | AML | Acute | Busulfan + Fludarabine | Sister | 230 | 5.8 | Methotrexate + Cyclosporine A |
| 15 | Female | 27 | AML | Acute | Busulfan + Fludarabine | Sister | 335 | 4.5 | Methotrexate + Cyclosporine A |

AML, acute myeloid leukemia; GvHD, graft-versus-host disease

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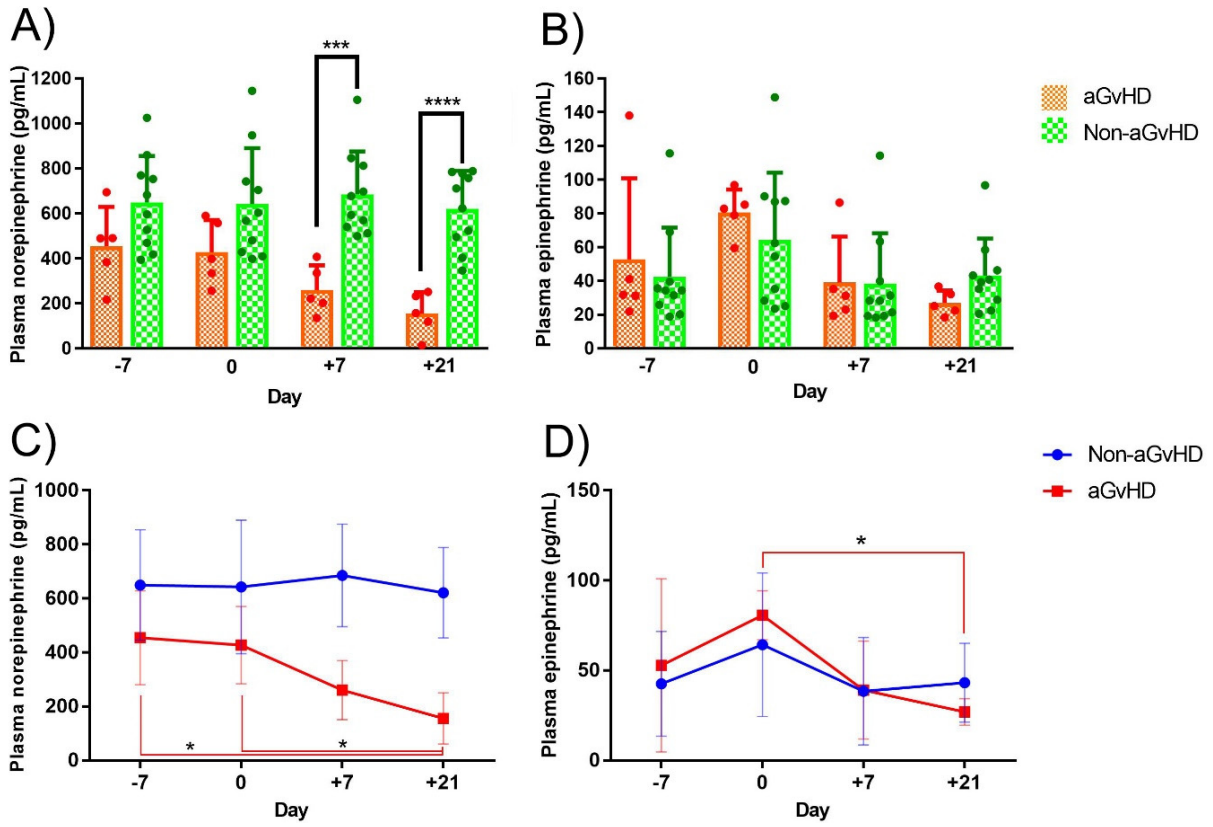


*Fig. 1.* Plasma levels of norepinephrine and epinephrine in aGvHD and Non-aGvHD groups. The blood samples were taken at days -7, 0 (The day of allo-HSCT), +7, and +21 and the plasma levels of NE and E were measured using ELISA. Data shown in bar-chart for norepinephrine (A) and epinephrine (B) with mean and standard deviation. Independent student t-test was used to compare the two groups. Dots represent individuals in each group. The trends of mean plasma levels of norepinephrine (C), epinephrine (D) were displayed in aGvHD and Non-aGvHD groups across four days of sampling. The significant differences are shown in figures by the stars on lines with the same color of the analyzed group. In both (C) and (D) graphs there were only significant differences in aGvHD group (red line). *p<0.05, ***p<0.001, ****p<0.0001. aGvHD. Acute Graft-versus-Host Disease; pg/mL. picogram per milliliter.

Non-aGvHD group). After transplantation, the plasma levels of epinephrine in aGvHD patients gradually decreased and fell below the levels of epinephrine in Non-aGvHD group on day +21 (27 pg/mL in aGvHD groups and 43 pg/mL in Non-aGvHD group). The plasma levels of norepinephrine in Non-aGvHD patients showed a steady-state trend with the mean rang of about 620 to 680 pg/mL while patients who developed aGvHD had a decreasing trend in which the mean plasma levels at day +21 (≈155 pg/mL) were significantly lower than those of day -7 and day 0 (≈455 pg/mL) (Fig. 1 C). Plasma epinephrine levels had fluctuations across all time-points in both groups. Specifically, at day 0 it increased in both groups and had a significant difference compared to the day +21. The fluctuation range of epinephrine in aGvHD group was approximately 28-70 pg/mL and in Non-aGvHD group was about 39-64 pg/mL (Fig. 1 D). There were significant differences in norepinephrine levels in aGvHD group at the day +21 compared to day -7 (p=0.013) and the day 0 (p=0.026) (Fig. 1 C). There was also a significant difference in the levels of epinephrine in the aGvHD group between day 0 and +21 (p=0.041) (Fig. 1 D).

### *Expression levels of β2-adrenergic receptor gene*

The mRNA expression levels of the β2-adrenergic receptor were relatively quantified using real-time PCR and normalized to the expression levels of the ABL gene. Results have been illustrated as the relative expression of the β2-AR gene in two groups in Figure 2 A. β2-AR expression was significantly higher in Non-aGvHD patients on days +7 and +21 compared with the aGvHD group (p<0.001). To examine the extent to which the expression of β2-AR is higher in Non-aGvHD group, a ratio was developed such that the expression level of β2-AR in each group was divided by the expression levels of β2-AR in the aGvHD group. This way the expression level of β2-AR in aGvHD group was always 1 and the relative expressions of β2-AR in Non-aGvHD group was calculated and illustrated in Figure 2 A. The fold changes of β2-AR expression in Non-aGvHD group was shown as a trend across all time points in Figure 2 B. The expression levels of the β2-AR in Non-aGvHD group were 1.37, 1.91, 4.35 and 5.55-fold higher than aGvHD patients in four sampling days, respectively. The relative expression of the β2-AR gene in fold change mode showed a gradual increase in Non-aGvHD group. Besides, the inter-day comparisons of β2-AR relative expres-



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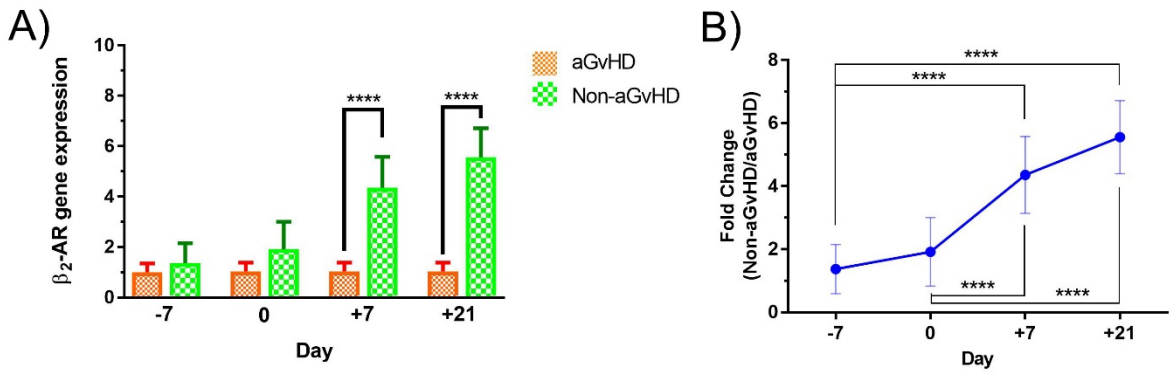


*Fig. 2.* Gene expression of β2-AR in aGvHD and Non-aGvHD groups. Blood samples were collected at -7, 0 (The day of allo-HSCT), +7, and +21. Total RNA was extracted from buffy coats and synthesized cDNA was used as a template to evaluate the mRNA expression of β2-AR. The graph (A) illustrated the relative expression of the β2-AR gene. The expression levels of β2-AR in both groups are divided by the expression levels of β2-AR in aGvHD group. The error bar represents standard deviation. Independent student t-test was used to compare the two groups. Also, the fold change of β2-AR gene expression by dividing the expression levels of β2-AR in Non-aGvHD group by the expression levels of β2-AR in aGvHD group were shown as a trend across four time-points in graph (B). The significant inter-days differences were shown by stars. ****p<0.0001. aGvHD. Acute Graft-versus-Host Disease; β2-AR. β2-Adrenergic Receptor;

sion in Non-aGvHD group showed that there were significant differences between the day +7 and +21 compared to the days -7 and 0 (p<0.001) (Fig. 2 B).

### *Correlations between plasma levels of norepinephrine, epinephrine, and ß2-AR gene expression with aGvHD incidence*

To study the correlations of the plasma levels of norepinephrine, epinephrine, and β2-AR gene expression with aGvHD incidence, the Spearman's test was used and the Pearson test was performed to determine the correlation of parametric variables including plasma levels of norepinephrine, epinephrine, and expression levels of β2-AR gene together. The factor association of Eta-squared was determined to evaluate the perfect association. Only significant correlations were illustrated in Table 2 with the related P-value and correlation coefficient. There were negative correlations between the levels of norepinephrine, β2-AR gene expression and the incidence of aGvHD on days +7 and +21. The epinephrine levels have no correlation with other measured components, except for the β2-AR gene expression on day +21 and some inter-days correlations of epinephrine levels. Some of the inter-days correlations in norepinephrine and also β2-AR gene expression levels are significant. In addition, there are several positive correlations between norepinephrine and the β2-AR expression (Table 2).

## Discussion

In this study, for the first time, we have shown that patients with higher norepinephrine and β2-AR expression are less susceptible to the development of aGvHD. This finding is in agreement with the animal study showing increased levels of norepinephrine due to mild cold stress can reduce GvHD incidence (8). Using β2-AR agonist and antagonist and also genetic deletion, Leigh et al. found that

*Table 2.* The correlations between plasma levels of norepinephrine, epinephrine, β2-AR gene expression and aGvHD incidence

| | | Epinephrine | | Norepinephrine | | | β2-AR relative expression | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Day | | +7 | +21 | 0 | +7 | +21 | -7 | 0 | +7 | +21 |
| aGvHD incidence | | | | | -0.786 | -0.845 | | | -0.63 | -0.686 |
| Epinephrine | 0 | +0.749 (.001) | +0.541 (.037) | | | | | | | |
| | +7 | | +0.806 (.0001) | | | | | | | |
| | +21 | | | | | | | | | +0.616 (.015) |
| Norepinephrine | -7 | | | +0.894 (.0001) | +0.701 (.004) | +0.605 (.017) | +0.741 (.001) | +0.566 (.028) | +0.755 (.001) | +0.609 (.016) |
| | 0 | | | | +0.768 (.001) | +0.599 (.018) | +0.696 (.004) | +0.658 (.008) | +0.71 (.003) | |
| | +7 | | | | | +0.891 (.0001) | | | +0.684 (.005) | +0.785 (.001) |
| | +21 | | | | | | | | +0.74 (.002) | +0.826 (.0001) |
| β2-AR relative expression | -7 | | | | | | | | +0.556 (.031) | |
| | +7 | | | | | | | | | +0.596 (.019) |

Only significant correlations were illustrated. The positive and negative marks (+,-) represent positive and negative correlation. The numbers in cells are correlation coefficients and the numbers in parentheses are P-values. aGvHD, acute graft-versus-host disease; β2-AR, β2-adrenergic receptor.



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this resistance to GvHD is due to the immune-modulation through β2-AR signaling (8). One of the most important limitations of our study is that the β2-AR signaling and also the expression of β2-AR protein were not assessed in this study. The expression of β2-AR mRNA cannot exactly reflect the protein level of the β2-AR and in the case of measuring the protein expression, the finding would be more reliable. However, the expression of β2-AR gene and plasma levels of norepinephrine confirm the previous animal model studies. The plasma epinephrine level has no significant differences between aGvHD and Non-aGvHD patients, which perhaps indicates the greater importance of norepinephrine in neuro-immune interactions. Various studies on the role of the nervous system in progression and metastasis of different cancers have reported the elevated levels of norepinephrine but not epinephrine in their experiments (13, 17, 18). High production of norepinephrine by macrophages and sympathetic nerves in the tumor microenvironment was brought up as a possible justification in these reports (19, 20). However, due to the fluctuating trend of plasma epinephrine across sampling time-points and the small sample size of our study, it is hard to completely rule out the contribution of epinephrine to the development of aGvHD.

The level of norepinephrine in subjects without aGvHD showed a high steady-state trend, while in aGvHD patients, it had a decreasing trend. This study showed the expression of the β2-AR gene in Non-aGvHD subjects at days +7 and +21 was higher than those of aGvHD patients. β2-AR gene expression had a gradual increase in Non-aGvHD individuals, and there were statistically significant differences across all sampling points, indicating that the β2-AR gene expression might be playing an immunomodulatory role in Non-aGvHD patients. Interestingly, both of the significant differences in norepinephrine levels and β2-AR gene expression between the two groups are at day +7 and +21 which suggests this period as a crucial time to follow-up the adrenergic status of patients. Surprisingly, while the norepinephrine level is generally higher in Non-aGvHD patients than aGvHD patients, there is no further increase in the norepinephrine level whilst the expression of β2-AR increased across all sampling time-points. This finding, which might be because of the small sample size, complicates the interactive correlation between these two components. It may be that increasing expression of β2-AR in leukocytes could enhance the response to the high invariant levels of norepinephrine through increasing the signaling. Past studies clearly define the necessity of β2-AR signaling along with the high norepinephrine levels in immune modulation. It was shown that by increasing the levels of norepinephrine, the phosphorylation of the β2-AR significantly increased (13) and this effect was dependent on signaling of host-derived β2-AR (8). Our analysis confirmed the positive correlation and probable reciprocal interaction between the norepinephrine and the expression of its receptor. As it is indicated in the results, the plasma levels of norepinephrine and the relative expression of the β2-AR gene at days +7 and +21 are negatively correlated with the incidence of aGvHD. Noteworthy, the use of chemotherapy and immunosuppressive drugs such as cyclosporine A has been reported to affect the levels of stress hormones including norepinephrine (21, 22). To diminish the confounding effects of these drugs, patients using the same chemotherapeutic and immunosuppressive drugs were included in the study.

Many reported biomarkers cannot predict the outcome of the allo-HSCT before transplantation. This study shows, however, norepinephrine and β2-AR could potentially be predictive biomarkers. Further delineation requires larger sample size studies with more sampling time-points and also the assessment of the other adrenergic receptors which is an ongoing project in our lab.

In future studies, the roles of norepinephrine and β2-AR signaling can be further investigated in each category of GvHD such as skin-, gastrointestinal-, and liver-GvHD. It is also necessary to follow up with the patients to assess the role of β2-AR signaling in the possible development of chronic GvHD and GvHD relapse. This may help elucidate the relationship between β2-AR signaling and stress hormones and the development of GvHD. The probable mechanisms by which the adrenergic axis could affect the outcome of GvHD is the modulation of immune cells function, specially APCs and T cells (2, 23, 24). In the context of the tumor, Bucsek and his coworkers (25) have shown that the suppressing effect of adrenergic signaling on the anti-tumor response is mainly CD8+ T cell-mediated and this suppression is reversible using a pan β-blocker, propranolol. Interestingly, this benefit of propranolol was lost in CD8+ T cell-depleted mice (25).

Regarding the major role of APCs and CD8+-T cells in the pathogenesis of aGvHD (26, 27) and the expression of β2-AR on both activated and memory CD8+-T cells, we hypothesize that the impairment of APCs and CD8+ T cells might be a possible mechanism by which the norepinephrine and β2-AR signaling affect the outcome of aGvHD. This requires further investigation. The other question which should be addressed in the future is the role of β2-AR signaling on the trafficking of immune cells to lymph nodes and non-lymphoid organs, which could be a trigger for GvHD onset. Norepinephrine and adrenergic signaling were shown to be essential mediators in the trafficking of T cells through chemokines and adhesion molecules (28).

## Conclusion

In conclusion, further insight into the possible role of catecholamines in the development of aGvHD can be gained by the cautious use of β-blockers in allo-HSCT patients. The role of other immune-modulatory neurotransmitters in aGvHD needs a closer examination. Note the beneficial effects of GvT and the probability of losing GvT by suppressing GvHD should not be forgotten, and so it is imperative to practice caution when considering the prevention of GvHD.

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ical Sciences, Tabriz, Iran. The study protocol received approval from the ethics committee of Tabriz University of Medical Science with the code of "IR.TBZMED.REC.1396.709".

### *Conflict of Interests*

The authors declare that they have no competing interests.

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