

⇒ Original Article



Age-Related Changes in the T Helper 17 and Regulatory T Cell-Related Cytokines During Cardiac Surgery

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Background: The age-associated dysregulated immune response may partly contribute to the greater mortality and morbidity in elderly patients undergoing cardiopulmonary bypass (CPB). The aim of the present study was to first investigate the possible relation between age and levels of interleukin (IL)-17 and IL-35 in CPB patients.

Methods: In general, 90 patients undergoing CPB from February 2018 to March 2019 in Jorjani Heart Center, Bandar Abbas, were enrolled in this cross-sectional study. IL-35 and IL-17A were measured before and 12 hours after CPB using enzyme-linked immunosorbent assay. The relation of age and the mentioned cytokines was assessed in patients <70 and ≥70 years old.

Results: IL-17A levels were slightly increased (25.7 ± 8.7 ng/mL versus 20.9 ± 6.3 ng/mL), while IL-35 levels represented a slight decrease (20.6 ± 10 ng/mL versus 23.2 ± 11.9 ng/mL) in CPB patients ≥70 years in comparison with those below 70 years ($P > 0.05$). There was a positive correlation between IL-17 and age ($r = 0.4$, $P = 0.002$) in patients older than 70 years. Eventually, IL-35 was negatively associated with age ($r = -0.2$, $P = 0.03$) in ≥70 years patients.

Conclusion: These data suggested that the age-related elevation of IL-17A levels, as well as a decline in the IL-35 serum concentration, contributing to the imbalanced pro- and anti-inflammatory response, may play a crucial role in the stronger adverse post-CPB outcomes in the elderly.

Keywords: IL-17, IL-35, Cardiopulmonary bypass, Age, Inflammation

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Background

Cardiopulmonary bypass (CPB) is often accompanied by adverse postoperative outcomes and is responsible for increased morbidity and mortality in elderly patients (1-3). Previous studies have shown that increasing patient age is an important risk factor that worsens the post-CPB outcomes (4). Recent data have confirmed the critical role of the immune response in adverse *postoperative* complications. The studies demonstrated that CPB triggers systemic inflammatory response syndrome (SIRS) via different mechanisms, including ischemia/reperfusion injury, surgical trauma, changes in body temperature, and anesthesia (5). Postoperative SIRS is characterized by an elevated release of inflammatory cytokines such as interleukin (IL)-1, IL-6, and IL-8 (6) and is associated with serious postoperative complications, including myocardial, renal and neurologic dysfunction, and multiple organ failures (7).

Although the mechanisms responsible for the worsened clinical outcomes in cardiac surgery elders are poorly characterized, there is a growing appreciation for the loss of immune steady-state with increasing age. There is consensus that aging is associated with a progressive trend toward an imbalance between pro- and anti-

inflammatory cytokines, called inflamm-aging (8). The role of excessive inflammatory mediators such as IL-6, acute phase proteins, stress hormones, *reactive oxygen species*, and coagulation factors in aging-related diseases (8,9) such as osteoporosis, atherosclerosis, diabetes, and Alzheimer's disease (8) have been reported in some studies. Apparently, the dysregulation of the homeostasis of cytokines and their signaling disturbance leads to disproportionate inflammation, which is a characteristic feature of both aging and aging-related maladies (10). T helper 17 (Th17) and CD4+ CD25+ Foxp3+ regulatory T (Treg) cells are the two subgroups of CD4+ T lymphocytes, which play an important role in immunological homeostasis (11,12). The imbalance of Th17 and Treg cells has been proposed to contribute to the pathogenesis of inflammatory diseases with increasing age (13).

Th17 cells, having the capability to produce pro-inflammatory cytokines, including IL-17A, IL-17F, IL-21, and IL-22, perform critical functions in autoimmunity (14,15). IL-17A is a pro-inflammatory cytokine and plays an important role in the development of several inflammatory diseases (14,16), including psoriasis (17), asthma (18), and rheumatoid arthritis (19).

On the other hand, Treg cells produce anti-inflammatory

cytokines, including IL-10 and IL-35, modulate the function of effector T cells, and prevent autoimmunity (20,21). IL-35 is a newly discovered immunosuppressive cytokine that not only suppresses effector T cell activity (22) but also limits the differentiation and functions of Th17 cells (23).

Objectives

This study aimed to shed further light on the mechanisms underlying the poorer outcomes of elderly patients who underwent cardiac surgical procedures and to investigate whether the Th17/Treg imbalance occurred during CPB. Accordingly, the possible relation between age and levels of the IL-17A and, IL-35 in patients undergoing cardiac surgery was assessed in this study.

Materials and Methods

Characteristics of Study Participants

The participants of this cross-sectional study included 90 patients who were candidates for CPB graft surgery in Bandar Abbas Jorjani Heart Center from February 2018 to March 2019. Patients with any kind of immunologic or infectious disease, left ventricular dysfunction, distal artery function disorders or valve disease, and major organ dysfunction were excluded from the investigation, and patients who died during or after the surgery were not enrolled in the study.

Cardiopulmonary Bypass Technique

Medication with cardiac drugs such as antihypertensives continued until the day of the operation. Eight hours before the surgery, patients received 1 mg of alprazolam orally as pre-anesthetic medication. Anesthesia was induced with fentanyl (5 µg/kg), midazolam (0.05 mg/g), etomidate (2 mg/kg), and cisatracurium (0.2 mg/kg). The anticoagulant (heparin, 300-400 U/kg) was used, and the activated clotting time was maintained greater than 450 seconds. The applied prime solutions of the pump included 1000 mL of lactated Ringer's solution and 500 mL of Voluven colloid solution. The hematocrit level was maintained at 26 ± 2 . The pulsatile arterial pressure of 50-70 mm Hg and the pump flow of 2.2-2.4 were maintained. The body temperature during the surgery was cooled down to 30 °C, and the times of surgery and cross-clamp were recorded finally.

Sample Collection and Cytokine Measurements

The serum was obtained from blood samples before and 12 hours post-operation. The blood sample was coagulated at room temperature and then centrifuged at 2500 g for 10 minutes at 4°C (Sigma, USA). Both cytokines (IL-17A and IL-35) were measured using enzyme-linked immunosorbent assay (ZellBio, Germany), and the concentration was calculated based on the linear standard curve developed from provided standard solutions. The

intra- and inter-assay variations were <10%, and the lower limit of detection of IL-17A was <2.3 pg/mL.

Statistical Analyses

The analysis was performed using SPSS statistical software, version 20.0. (SPSS Inc., Chicago, IL, USA). Quantitative variables were compared using Student's *t* test for 2 groups and one-way analysis of variance for more than 2 groups. Equal non-parametric tests were used for non-normally distributed variables. Data were presented as the mean \pm standard deviation (SD). Categorical variables were compared using the chi-square test and presented as percentiles. Correlation analyses were performed, and Pearson or Spearman correlation coefficient was reported, and $P < 0.05$ was considered statistically significant. The sample size was calculated considering the precision/absolute error of 5% and a type 1 error of 5%, and the SD of variables was taken from previous studies.

Results

Characteristics of the Study Population

In this cross-sectional study, a total of 90 patients undergoing CPB surgery were enrolled, consisting of 51 males and 39 females with mean ages of 58.6 ± 11 and 61.5 ± 11.2 years, respectively. Patients were divided into two groups based on their age and patients who were below 70 years old ($n = 59$) compared to those who were 70 years old or higher ($n = 31$). Based on the data in Table 1, demographic and operation variables were not statistically different between the two groups.

Comparing Cytokines in Different Age Groups of CPB Patients

The serum levels of IL-17A and IL-35 were compared between CPB patients over or below 70 years old before and 12 hours after the operation. Based on the results (Table 2), IL-17A levels were non-significantly increased (25.7 ± 8.7 ng/mL versus 20.9 ± 6.3 ng/mL), whereas IL-35 levels were non-significantly decreased (20.6 ± 10 ng/mL versus 23.2 ± 11.9 ng/mL) in patients ≥ 70 years old in comparison with <70 years old subjects ($P > 0.05$).

Association of Age and Serum Cytokines in Different Age Groups

The correlations of IL-35 and IL-17A levels with age were evaluated in CPB patients higher and below 70 years old before and 12 hours post-operation (Table 3). Regarding the assessment of IL-35 concentrations in patients <70 years old, there was no correlation between the IL-35 level and age before and after the operation. However, post-operative IL-35 serum levels were negatively correlated with age ($r = -0.2$, $P = 0.03$) in patients ≥ 70 years old. With regard to IL-17A concentrations in patients <70 years old, there was no correlation between the levels of IL-17A and age before and after the operation, but

Table 1. Demographic and Operation Variables of Patients Bellow and Higher Than 70 Years Old

Variables	Age <70 Years (n=59)	Age ≥70 Years (n=31)	P Value
Gender (%)			
Male (n=51)	55.6	57.1	0.8
Female (n=39)	44.4	42.9	0.9
BMI (kg/m ²), Mean±SD	24±4.3	24.9±3.7	0.5
Systolic blood pressure (mm Hg), Mean±SD	126.6±31.8	131.0±22.5	0.8
Diastolic blood pressure (mm Hg), Mean±SD	74.8±15.5	70.2±10.1	0.5
Cross-clamp time, Mean±SD	81.8±33.3	74.8±10.7	0.4
Bypass time, Mean±SD	129.6±37.5	127.3±21.2	0.8
One graft (%)	24	22	0.8
Two grafts (%)	30	37	0.5
More than two grafts (%)	46	41	0.6
Ejection fraction (%)	47.7	40.3	0.5
Diabetes (%)	50.5	58.4	0.5
Hypertension (%)	43.1	54.8	0.3
Smoking (%)	25.6	12.5	0.4

Note. BMI: Body mass index; SD: Standard deviation.

Table 2. Comparing Serum Cytokine Levels Between Patients Bellow and Higher Than 70 Years Old Before and 12 Hours Post-operation

Variables	Time of Sampling	Age <70 Years (n=59)	Age ≥70 Years (n=31)	P Value
IL-35 (ng/mL)	Before CPB	8.7±4	6.8±2.2	0.1
	After CPB	23.2±11.9	20.6±10	0.5
IL-17 (ng/mL)	Before CPB	16.2±7.3	15.9±6	0.9
	After CPB	20.9±6.3	25.7±8.7	0.09

Note. CPB: Cardiopulmonary bypass; IL: Interleukin.

Table 3. Correlation Analyses of IL-17 and IL-35 With Age in Patients Undergoing Cardiopulmonary Bypass Surgery Before and After Operation

Variables	Time of Sample Collection	Age <70 Years (n=59)	
		R value	P Value
IL-35 (ng/mL)	Before CPB	-0.1	0.3
	After CPB	-0.2	0.03
IL-17 (ng/mL)	Before CPB	0.1	0.2
	After CPB	0.4	0.002

Note. IL: Interleukin; CPB: Cardiopulmonary bypass.

in patients ≥70 years old, the IL-17A serum levels were positively correlated with age only after the operation ($r=0.4$, $P=0.002$).

Discussion

The novel finding of the present study is the age-related changes in the post-CPB concentration of IL-17A and IL-35 in the elderly. The patients who were aged ≥70 showed a positive correlation between age and IL-17A levels while a negative correlation between age and IL-35 circulating concentrations after cardiac

surgery. Several studies reported that increased levels of pro-inflammatory cytokines play critical roles in the development and progression of age-related diseases (24). Considering that IL-17A, as a pro-inflammatory cytokine, plays an important role in the development of several inflammatory diseases (14,16), our findings regarding the age-related increase in IL-17A concentrations during CPB are consistent with previous reports, showing that elderly CPB patients generate strong proinflammatory cytokine responses. Howell et al found a positive correlation between IL-6 production and age during cardiac surgery (4). Wei et al measured IL-6 and IL-8 in CPB patients and concluded that IL-6 and IL-8 levels increased in patients aged >70. They confirmed the disturbance in pro- and anti-inflammatory cytokine response in older patients (25). Ouyang et al isolated CD4+T cells from the peripheral blood of healthy aged humans and stimulated them with phorbol myristate acetate and ionomycin. They found that the mRNA levels of T helper 17-related cytokines, IL-17A, IL-17E, and IL-22, as well as the transcription factor retinoic acid-receptor-related orphan receptor (ROR) C2, were significantly elevated in the aged CD4+T cell in comparison with a healthy young population (26). Interestingly, previous studies indicated that Th17 immune responses contribute to the development of inflammatory disorders such as colitis (26) and prostate cancer (27) in the elderly. Although the clinical relevance of the elevated levels of IL-17 in post-CPB SIRS in the elderly is still unknown, the most probable explanation is the effects of IL-17 on the production of other inflammatory mediators such as serum amyloid A, C-reactive protein, IL-8, IL-6, tumor necrosis factor- α , and the acute phase reactants (28), as well as the mobilization and recruitment of the immune cells, especially neutrophils, during CPB (29-31). Our finding regarding lower IL-35 levels in old patients is in line with those of Wei et al about the lower levels of another Treg-related anti-inflammatory cytokine (IL-10) in old subjects (25). Spencer et al confirmed the age-associated dysregulation in the production of IL-10 (32). Recent studies provided evidence that the decreased levels of IL-35 were correlated with the aggravation of sarcoidosis (33), systemic lupus erythematosus (34) asthma (35), and Graves' disease (36). Overall, although the limitations of our study, including small sample size and the retrospective design, must be placed in perspectives, our results confirmed the intrinsic bias toward a Th17 immune response in the aging process (26). There is growing evidence that an imbalance between Th17 and Treg cells and their related cytokines such as IL-17A and IL-35 have a profound effect on the development of inflammatory diseases such as autoimmune diseases and cancer (37), and it can be assumed that the post-operative age-related changing in IL-17 and IL-35 levels can intensify the imbalance between the pro- and anti-inflammatory cytokines in the elderly and generate

a strong pro-inflammatory cytokine profile, which is accompanied by other risk factors such as myocardial infarction, diabetes mellitus, and hypertension, can result in poorer outcomes in elders with cardiac surgery.

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Authors' Contribution

Conceptualization: NN and MR, Data curation: MR, Formal analysis: MR, Funding acquisition: MR and HM, Investigation: MR and HM, Methodology: NN and MR, Project administration: NN and MR, Resources: HM, Supervision: MR and NN, validation: MR and NN, Visualization: NN and MR, Writing-original draft: NN and MR, Writing-review and editing: NN, MR and HM.

Competing Interests

The authors declare that they have no competing interests.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The Ethics Committee of the Hormozgan University of Medical Sciences confirmed the study (With reference number HUMS.REC.1395.81).

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