Observational study of the correlation between peripheral Treg cell senescence and serum total cholesterol level

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Ethics Committee

Tianjin Medical University General hospital

Protocol Abstract

Title	Observational study of the correlation between peripheral Treg cell senescence and serum total cholesterol level
Ethics Committee (Grant Number)	The ethics committee of Tianjin Medical University General Hospital (IRB2022-YX-244-01)
Principal Investigator	Ping Lei (Ph.D.)
Sponsor	Tianjin Medical University General Hospital
Fundings	Natural Science Foundation of China (Grant No. 82071394, 82072166)
Execute Time	12/01/2022-03/31/2023
Recruiting Time	01/01/2023-03/31/2023
Objective	Clarify the correlation between human peripheral Treg cell senescence and serum total cholesterol level
Study Type	Observational
Time Perspective	Cross-Sectional
Enrollment	200 participants
Biospecimen	Description: Whole blood and serum Retention: Samples with DNA
Sampling Method	Probability sample
Condition	Hypercholesterolemia
Inclusion Criteria	1) Age $\geqslant$ 30 and $\leqslant$ 60 years old with independent behavior ability. 2) The participants need to fully understand the purpose and content of the study, and voluntarily participate in the study and sign the informed consent.
Exclusion Criteria	1) Pregnant or lactating women. 2) Acute and severe diseases in the last 3 months, including but not limited to, acute myocardial infarction, acute cerebral infarction, cerebral hemorrhage, circulatory failure, respiratory failure (internal diseases), trauma requiring hospitalization, or undergoes surgery under general anesthesia (surgical diseases).

	3) History of severe diseases, including but not limited to, tumors, serious hematological diseases, serious cardiopulmonary diseases (interventional therapy for coronary artery disease, atrial fibrillation, chronic obstructive pulmonary disease, etc.), renal failure, liver failure, old stroke with serious sequelae. 4) Have participated in clinical trials in the past 3 months. 5) The investigator considers that not appropriate for inclusion.
Outcome Measures	Treg cell senescence evaluated by the beta-galactosidase (SA-betagal) activity test.
Reference standard	Protocols to detect senescence-associated beta-galactosidase (SA-betagal) activity, a biomarker of senescent cells in culture and in vivo. Nat Protoc 4, 1798-1806, doi:10.1038/nprot.2009.191 (2009)
Statistical Analysis	All statistical analyses were performed using SPSS Statistics Version 27.0 (IBM, Armonk, NY, USA). A two-tailed p value of less than 0.05 was considered to be statistically significant.

1. Research Background and Objectives

Hypercholesterolemia is a risk factor for the occurrence and development of atherosclerosis. Recent animal studies have found that increased serum cholesterol level is associated with peripheral Treg cell senescence, but clinical evidence is still lacking. The purpose of this study is to analyze the correlation between human peripheral Treg cell senescence and serum total cholesterol level using clinical blood samples, thus laying a foundation for the establishment of novel therapeutic strategies for atherosclerosis based on the regulation of Treg cell senescence.

2. Study design

2.1 Overall design

This research is an observational cross-sectional study.

2.2 Estimated number of subjects

Estimated by statistical analysis, a total of 200 subjects were randomly selected from the population receiving physical examination in the Cadre Physical examination Center of Tianjin Medical University General Hospital from January 2023 to March 2023.

3. Inclusion, Exclusion and Withdrawal Criteria

3.1 Inclusion Criteria

1) Age $\geqslant$ 30 and $\leqslant$ 60 years old with independent behavior ability.

2) The participants need to fully understand the purpose and content of the study, and voluntarily participate in the study and sign the informed consent.

3.2 Exclusion Criteria

1) Pregnant or lactating women.

2) Acute and severe diseases in the last 3 months, including but not limited to, acute myocardial infarction, acute cerebral infarction, cerebral hemorrhage, circulatory failure, respiratory failure (internal diseases), trauma requiring hospitalization, or undergoes surgery under general anesthesia (surgical diseases).

3) History of severe diseases, including but not limited to, tumors, serious hematological diseases, serious cardiopulmonary diseases (interventional therapy for coronary artery disease, atrial fibrillation, chronic obstructive pulmonary disease, etc.), renal failure, liver failure, old stroke with serious sequelae.

4) Have participated in clinical trials in the past 3 months.

5) The investigator considers that not appropriate for inclusion.

3.3 Withdrawal criteria

1) The subject decides to withdraw from the study.

2) The doctor or the researcher asks the subject to withdraw from the study (e.g. poor compliance).

4. Study procedure

Researchers initiate the study according to the inclusion/exclusion criteria. After confirming the subject has signed the informed consent, doctors will record the past medical history and medication (especially hypolipidemic drugs) in the past 3 months. Thereafter, an additional 6 ml of venous blood will be drawn during the routine blood tests for examinations of Treg cell senescence.

5. Evaluation Parameters

Treg cell senescence will be evaluated by the beta-galactosidase (SA-betagal) activity test.

6. Withdrawal of the subject

The subject has the right to withdraw from this trial at any time for any reason. The researcher should have a thorough understanding and a record of the reasons for withdrawal. Doctors and researchers also have the right to stop the subject from continuing to participate in the trial at any time during the research. Besides, subjects with early withdrawal should not be replaced by others.

As too many subjects withdrawing from the trial will lead to unreliable results, unnecessary withdrawal should be avoided. The falling rate of the trial should be controlled within 20%.

7. Statistical analysis

1. Pearson correlation coefficient was used to evaluate the correlation between the number of Treg cells in peripheral blood and serum total cholesterol level.

2. Taking the serum total cholesterol level of 5.17 mmol/L as the cut-off value, the subjects were divided into normal cholesterol level group and hypercholesterolemia group. Subsequently, the Propensity Score Matching (PSM) method was used to match the subjects according to gender, age and past medical history, and the Student's t-test was performed to compare the difference of Treg cell senescence between the two groups.

3. The serum total cholesterol level was stratified (e.g., trisection or quartering), and the difference of Treg cell senescence among all groups was compared using one-way ANOVA followed by LSD post-hoc test.

8. Trial Management

8.1 Ethics

To obtain the approval documents of the clinical trial, researchers should submit the trial protocol and a copy of the research documents including the informed consent to the ethics committee.

The approval documents from the ethics committee should be accompanied by the name list of ethics committee members and their respective responsibilities. These documents will be delivered to the researchers in written form before the start of the study.

Any safety-related issues must be promptly reported to the ethics committee, which includes revision on the trial protocol and modification on the subject information page. The end or early termination of the trial should also be reported.

8.2 Informed consent and data protection agreement

It is the responsibility of the researchers to explain the objectives, study procedures, benefits and potential risks of the trial for each subject. The researchers should receive the informed consent signed by the subject before starting the trial, and keep it properly. The subject should also permit researchers and the clinical trial management agency to check his or her original data. Thus, the reliability of the research findings could be ensured.

The personal information of each subject, including name, gender, age, home address and telephone number, should be collected in detail. And the researcher/doctor should give the subject his or her contact information, so that the subject can contact with him or her when needed. This is also helpful for the research and medical care.

8.3 Subjects privacy

The researchers should protect the privacy of the subject. All research documents can only be identified using the subject's number instead of name or physical examination number. In addition, the grouped table that records the correspondence between the subject's name and number can only be kept by the researcher, and could not be submitted to any institution or individual.

8.4 Modification of the trial protocol

The trial protocol is approved by the ethics committee before starting the trial. During the research, any proposed modification on the trial protocol should be reported to the principal investigator, and then be submitted to the ethics committee for review. The clinical research can only be resumed after the re-approval of the ethics committee.

8.5 Original records certification

Researchers should ensure that the subject's privacy is protected when collecting and organizing data. In addition, the data manager is authorized to review the original records in order to confirm the accuracy of the original data.

8.6 Documents on file

According to relevant laws and regulations, researchers should keep the original records properly for at least 5 years from the end of the study.

8.7 Quality control

The clinical trial management agency has the authority to review the study progress, in order to ensure that the trial is carried out as predetermined and that the research data be veritably recorded.