Decreased T530-pSIRT1 Expression in CD45- Cells After Red Grape Administration

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ABSTRACT

T530-pSIRT1 is one of the biomarkers that shows senescence activity. CD45- cells are the phenotype for late Endothelial Progenitor Cells (EPCs) expressing endothelial antigens. Resveratrol in red grapes is thought to be able to increase T530-pSIRT1 expression and improve endothelial quality. This study aimed to determine the change in T530-pSIRT1 expression by CD45- cells after the administration of red grapes. This study had a quasi-experimental pre-test-post-test one-group design. Research subjects were taken using consecutive sampling methods in the adult population aged 45-55 years. Expression of T530-pSIRT1 was analyzed from the number of CD45- cells and Mean Fluorescence Index (MFI) of CD45- cells using the immune flow cytometry method. Statistical analysis used GraphPad version 9.2.0. The number of research subjects was 17 people with a mean age of 47.3 years and 52.9% were female. There was a significant decrease in the number of CD45- cells (p=0.02) and a significant decrease in T530-pSIRT1 expression indicated by MFI CD45- cells, which were significant (p = < 0.0001). Decrease in T530-pSIRT1 expression in CD45- cells is thought to be caused by several factors that cannot be controlled during the study subject's consumption of red grapes such as diet, exercise, mental stress, and rest periods. Further research is needed to determine the appropriate dose and timing of red grape consumption to increase SIRT1 levels. Consumption of red grapes decreased expression of T530-pSIRT1, which could be caused by the dose and time of consumption of red grapes and the lifestyle of the research subjects that could not be controlled.

Keywords: T530-pSIRT1, CD45- cells, red grape, resveratrol

INTRODUCTION

Sirtuins (SIRTs) belong to the family of nicotinamide adenine dinucleotide (NAD+)-dependent protein deacetylases, which act by removing acetyl groups from several target proteins, such as histones, transcription factors, and cytoplasmic proteins.¹ SIRT1 is widely recognized as an important epigenetic regulator that is involved in many biological processes, including metabolism, maintenance of genome stability, reprogramming, aging, and tumorigenesis.² Utani et al. reported that phosphorylation of human SIRT1 deacetylase on Threonine 530 (T530-pSIRT1) mediates DNA synthesis. SIRT1 phosphorylation is a marker of SIRT1 activation that can prevent DNA damage and ensure genome stability.³ SIRT1 expression is markedly decreased in aging vascular tissue. SIRT1 deficiency in Endothelial Cells (EC), Vascular Smooth Muscle Cells (VSMC), and monocytes/macrophages will accelerate vascular aging, which will contribute to increased inflammation, oxidative stress, and endothelial dysfunction.⁴ In a study conducted by Rawal et al., the inhibition of SIRT1 showed an increase in senescence and a decrease in the proliferative ability of Endothelial Progenitor Cells (EPC). Endothelial Progenitor Cell (EPC) is a cell with angiogenic potential, the latter is known as an option for cellular-based therapy to induce intimal repair.⁵ SIRT1 itself has a protective effect against endothelial dysfunction by preventing stress-induced premature senescence in endothelial cells.⁶ CD45 regulates receptor signaling by direct interaction with components of the receptor complex or by dephosphorylation and activation of various Src Family Kinases (SFKs). CD45 activity is critical for efficient immune response, as its deficiency results in Severe Combined Immunodeficiency (SCID) in mice and humans.⁷ CD45- cells is the phenotype for late EPC. Late EPC is derived from Peripheral Blood Mononuclear Cells (PBMC) attached, cultured in endothelial media for 6-21 days.⁸ Unlike early EPC, late EPC does not show hematopoietic and monocyte markers but expresses endothelial antigens only. In addition, late EPCs produce “endothelial colony-forming cells” (ECFCs) in-vitro, and, for this, they are considered legitimate EPCs and are also called...
Resveratrol is a natural polyphenolic compound, including stilbenoid polyphenols, which have two phenol rings interconnected by an ethylene bridge. Resveratrol (synthesized by plants) is a defense mechanism against bacteria or fungi. Resveratrol itself in humans can function as an antioxidant, and anti-cancer.

Resveratrol in red grapes is thought to be able to activate SIRT1. Recent studies have shown that phosphorylation is an important regulatory mechanism underlying the control of SIRT1 activity. The role of resveratrol as an antioxidant is expected to prevent premature aging through SIRT1 activation. This is in line with research by Wu et al. that SIRT1 levels increased when given resveratrol. Based on this fact, in this study, a test will be carried out to determine changes in T530-pSIRT1 levels expressed by CD45- cells after the consumption of red grapes.

**METHOD**

The design of this study was a quasi-experimental pre-test post-test one-group design, which was carried out in Dr. Saiful Anwar General Hospital, Malang, Indonesia. The sample in this study was taken using the consecutive sampling method by taking research subjects who meet the criteria for sample acceptance sequentially until the desired sample size is met. In this study, there were 17 research subjects. This research has been approved by the Health Research Ethics Committee, Faculty of Medicine, Brawijaya University Malang (Ethical Eligibility Statement No. 178/EC/KEPK/06/2021).

The inclusion criteria for this study were males or females aged 45 to 55 years, who liked and had no problem-consuming red grapes, had no history of chronic disease and were declared physically healthy. Meanwhile, the exclusion criteria were having a history of chronic diseases such as hypertension or diabetes mellitus.

Blood samples were taken by venipuncture with EDTA anticoagulant vacutainer tube and vacutainer serum separator tube. A complete blood count was performed on the same day when the sample was taken. The type of antibody used in this study consisted of primary and secondary antibodies. The primary antibody used was SIRT-1 [pT530] antibody (JJ206-6) and the secondary antibody used was Goat anti-rabbit IgG H&L (Alexa Fluor 488) (Abcam, Cat. no: Ab150077).

The research subjects were taken pre-test blood samples before being given red grapes and then consumed red grapes for 3 weeks at a dose of 7.5 g/kg body weight per person then after 3 weeks the post-test blood sample was taken.

T530-pSIRT1 levels of research subjects were analyzed using Beckman Coulter, Inc. Flow cytometry. Navios EX Flow Cytometer. The software used was FlowJo version 10.6.2, which came in an on-board version and an off-line version that could be used for file analysis on workstations separate from the computer. Quality control was carried out before analyzing the research sample data.

Subject demographic data such as height, weight, Body Mass Index (BMI), age, and gender were taken during the pre-test. Blood pressure was measured before taking the pre-test and post-test blood samples.

Statistical analysis used GraphPad Prism version 9.2.0.

**RESULTS AND DISCUSSIONS**

The number of research subjects was 17 people, which can be seen in Table 1 with a mean age of 47.3 and 52.9% females. The average BMI of the research subjects was 20.47 kg/m². The hemoglobin, leukocyte, and platelet levels of the research subjects were still within normal limits.

<table>
<thead>
<tr>
<th></th>
<th>all (n=17) mean (SD)</th>
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<tbody>
<tr>
<td>Age, years</td>
<td>47.3 (3.9)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>8 (47.1)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>9 (52.9)</td>
</tr>
<tr>
<td>BMI</td>
<td>20.47 (2.48)</td>
</tr>
<tr>
<td>Complete blood</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.72 (1.58)</td>
</tr>
<tr>
<td>Leukocytes (x10^3/µL)</td>
<td>8.173 (2.49)</td>
</tr>
<tr>
<td>Platelets (x10^3/µL)</td>
<td>337.8 (75.24)</td>
</tr>
</tbody>
</table>

Flow cytometry analysis used a gating strategy to determine the expression of T530-pSIRT1 in a mononuclear cell population with a CD45- cells phenotype (see Figure 1).

The percentage of CD45- cells were not different in the pre-test and post-test data, but the absolute number of CD45- cells were significantly decreased in the post-test data compared to the pre-test data (Figure 2A-B). In addition, a decrease in T530-pSIRT1 expression was found in CD45- cells, as indicated by the shift in the MFI of the pSIRT1 marker on the histogram scale (Fig. 2C-D).

There were no significant differences between the pre-test and post-test study subjects blood
pressures (systolic blood pressure, \(p=0.8767\); diastolic blood pressure, \(p=0.0596\)). Likewise, the results of the hemoglobin (\(p=0.4738\)), leukocytes (\(p=0.0570\)), and platelets (\(p=0.1383\)) examinations did not produce a significant difference. Significant differences were seen in the total cholesterol (\(p=0.0046\)), LDL (\(p=0.0259\)), HDL (\(p=0.0384\)), and triglycerides (\(p=0.0450\)) examinations. The fasting blood glucose was also not significantly different (\(p=0.0686\)) (see Table 2).

This study showed a decrease in T530-pSIRT1 levels in CD45- cells after consumption of red grapes containing resveratrol, which could be caused by many factors. Factors that could not be controlled

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**Table 2. Supporting data for pre-test and post-test**

<table>
<thead>
<tr>
<th></th>
<th>Pre-Test (n=17)</th>
<th>Post-test (n=17)</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>135.6 (21.5)</td>
<td>135.1 (22.9)</td>
<td>0.8767</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>84.5 (16.0)</td>
<td>90.0 (13.7)</td>
<td>0.0596</td>
</tr>
<tr>
<td><strong>Complete blood count</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.72 (1.58)</td>
<td>14.01 (2.19)</td>
<td>0.4738</td>
</tr>
<tr>
<td>Leukocytes (x10^3/µL)</td>
<td>8.173 (2.49)</td>
<td>7.183 (1.89)</td>
<td>0.0570</td>
</tr>
<tr>
<td>Platelets (x10^3/µL)</td>
<td>337.8 (75.24)</td>
<td>317.7 (68.97)</td>
<td>0.1383</td>
</tr>
<tr>
<td><strong>Lipid profile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>170.0 (24.98)</td>
<td>193.3 (29.15)</td>
<td>0.0046</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>131.4 (54.76)</td>
<td>25.2116.7 (25.80)</td>
<td>0.0259</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>47.47 (13.2)</td>
<td>45.00 (10.33)</td>
<td>0.0384</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>155.71 (77.91)</td>
<td>202.59 (119.86)</td>
<td>0.0450</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>91.18 (15.21)</td>
<td>94.41 (15.36)</td>
<td>0.0686</td>
</tr>
</tbody>
</table>

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**Figure 1.** Flow cytometry gating strategy

Gating strategy to determine mononuclear cell population in FSC-A plots /SSC-A. Then proceed with gating to determine the population of CD45- cells. The intensity of T530-pSIRT1 expression was determined using Mean Fluorescence Intensity (MFI) analysis on the histogram scale of the CD45- cell population.

**Figure 2.** Data Analysis of Pretest and Posttest levels of T530-pSIRT1 in CD45- cells

The percentage of CD45- cells expressing T530-pSIRT1 (A). The absolute number of CD45- cells expressing T530-pSIRT1 (B). MFI T530-pSIRT1 on CD45- cells (C). Representative histogram scale of one of the subjects showing the intensity of T530-pSIRT expression on CD45- cells pre-test (red curve) and post-test (blue curve) (D).
during the 3 weeks of the patient's red grape diet; include exercise, food consumed, exposure to cigarettes, and rest.

Research conducted by Utani et al. reported that phosphorylation of human SIRT1 deacetylase on Threonine S30 (T30-pSIRT1) modulates DNA synthesis. T30-pSIRT1 is the active form of SIRT1. CD45- cells is the phenotype for late EPC. Late EPC does not show hematopoietic and monocyte markers but expresses endothelial antigens only. Thus, decreased expression of T30-pSIRT1 in CD45-cells will cause endothelial damage and accelerate vascular aging.

According to Vargas-Ortiz et al. levels of activated SIRT1 after exercise can increase the phosphorylation of AMP-Activated Protein Kinase (AMPK). Increased levels of SIRT1 can also increase levels of peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) and mitochondrial biogenesis. Therefore, physical activity can affect SIRT1 levels in study subjects during the treatment period. In addition, a sedentary lifestyle is also a root cause of many problems and exercise has a holistic effect on health through mobilization, proliferation, differentiation, function, and survival.

The diet also affects SIRT1 levels. Consequences of consuming a high-fat diet, reduce SIRT1 and PGC-1α levels. Low fruit and vegetable consumption can also affect SIRT1 levels, as in a study conducted by Iside et al., which shows that these natural polyphenols and non-polyphenolic substances can affect SIRT1 activation. Consumption of milk and its dairy products can also increase SIRT1 activation in key target tissues such as muscle and adipose tissue. So an unbalanced diet during treatment can also affect SIRT1 levels.

Another factor that can affect SIRT1 levels is insufficient rest time. According to a study in rats conducted by Zuo et al., SIRT1 expression in the rat's hippocampus was greatly decreased after exposure to lack of sleep. Therefore, rest is also an important factor influencing the results of the study although further research should be done to see the effect of lack of sleep on SIRT1 expression in humans.

Mental stress can also be one of the factors that cannot be controlled in this study because it is proven that SIRT1 activity in the dentate gyrus decreases in chronic stress.

SIRT1 is a positive regulator for the Liver X Receptor (LXR). Liver X receptor is a cell nucleus receptor that has a major role in cholesterol metabolism. When activated, LXR induces a series of genes involved in the efflux, absorption, transport, and excretion of cholesterol. SIRT1 deacetylates LXR, resulting in cholesterol efflux from cells. Liver X receptor activation is beneficial because it inhibits intestinal absorption of cholesterol, enhances reverse cholesterol transport, and exerts a strong anti-inflammatory effect. Reverse cholesterol transport is a mechanism, in which the body removes excess cholesterol from peripheral tissues and sends it to the liver, where it is either redistributed to other tissues or excreted from the body by the gallbladder. Previous studies have shown that SIRT1 is a key regulator of lipid metabolism. SIRT1 can regulate lipid metabolism by deacetylating certain proteins through deacetylating activity. SIRT1 may be a new therapeutic target for the prevention of diseases associated with lipid metabolism disorders. The deterioration of the lipid profile in this study may be due to the inappropriate and unbalanced dose of resveratrol with the fat intake consumed by the study subjects during the consumption of the red grape diet.

The resveratrol in red grapes is found in concentrations ranging from 1.5 to 7.3 g/g. The resveratrol content in grapes is determined by three factors, namely cultivar, disease exposure, and geographic origin. Research conducted by Yoshino et al. in females with normal BMI for 12 weeks with the administration of 75 mg/day resveratrol supplementation did not improve metabolic function in terms of insulin sensitivity and lipid profile in that study.

Limitations of this study were a very small sample size, a very short time-consuming red grapes, and minim knowledge about red grape species in Indonesia. In the future should try to evaluate this study with a bigger sample size and evaluate the contents of red grapes species in Indonesia, so, could determine the appropriate dose of resveratrol in the future. Therefore, further research should be carried out to determine the appropriate dose of resveratrol to improve metabolic function. Further research is also needed to determine the right time to consume red grapes containing resveratrol in order to achieve improved metabolic function, the type of red grapes used, and the method of cultivation, specifically red grapes in Indonesia.

CONCLUSIONS AND SUGGESTIONS

Consumption of red grapes, which contains resveratrol, which can activate T530-pSIRT1, actually...
decreases SIRT1 activity but this decrease is correlated with changes in lipid profile in this study. This can be caused by various factors such as the level of compliance of the research subjects in the consumption of red grapes and the lifestyle of the research subjects. Therefore, further research should be carried out to support this hypothesis but with a larger scale of research subjects and monitoring of lifestyle and level of compliance of subjects in red grape consumption.

Acknowledgements

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