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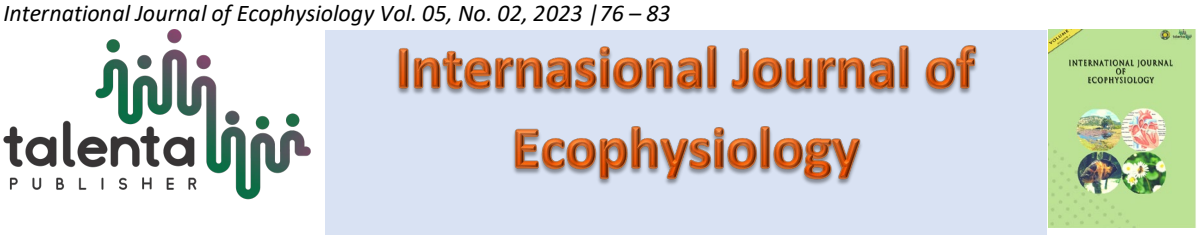

The Relationship Between Polymorphism (Rs2273773) in The SIRT1 Gene and Plasma SIRT1 Levels, and Frailty in The Elderly

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Abstract. Frailty is a physical aging syndrome characterized by health vulnerability. Diagnosis is established incorrectly based on three or more of the five criteria: weakness, decreased walking speed, fatigue, decreased physical activity, and weight loss. Sirtuin 1 plays a role in weakness, particularly in the elderly, acting as a protective factor during weakness, and its activation could provide a novel therapeutic approach. However, research in this area is still very limited. A cross-sectional method with observation or measurement of study subjects was employed in this research, involving a total of 118 elderly subjects selected through non-probability sampling. Pearson correlation test indicates a relationship between the polymorphism genotype (rs2273773) in the SIRT1 gene and the frailty scale (p-value < 0.05), while no relationship was found between plasma SIRT1 levels and the frailty scale (p-value > 0.05). Furthermore, the F-test reveals a significant simultaneous relationship between the polymorphism genotype (rs2273773) and plasma SIRT1 levels with frailty (p-value < 0.05).

Keywords: Frailty, Sirt1, Polymorphism (rs2273773), Elderly, Physical Aging

1. Introduction

One in every five elderly individuals in Indonesia faces a range of serious problems, including frailty, functional dependency, malnutrition risk, depression, history of falls, prior hospitalization, and polypharmacy, all of which are associated with the condition of frailty[1]. To comprehend the root causes of aging related to the accumulation of genetic mutations, in-depth research is needed to unravel mechanisms capable of reversing the aging process and identifying frailty-related biomarkers[2]. The identification of frailty-related biomarkers is of utmost importance to deepen the understanding of this disorder and to assist in early diagnosis, appropriate interventions, and frailty management [3].

Physical weakness increases with age, affecting 4-59% of elderly individuals in the community and being more common in women. Its prevalence is influenced by chronic conditions such as depression, nutrition, socioeconomic status, and education [4]. SIRT1 plays a crucial role in longevity through calorie restriction, being high in young individuals [5]. Studies in India have

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shown a decline in plasma SIRT1 with age related to weakness and cognitive impairment [6]. found that frail elderly men have lower plasma SIRT1 levels, which are associated with nutritional status and body composition [7].

Genetic polymorphism SNP rs2273773 in the SIRT1 gene is associated with changes in metabolism, body fat, blood pressure, oxidative stress, and dyslipidemia in the elderly [8],[9],[10]. Chronic diseases such as cardiovascular diseases, asthma, depression, hypertension, metabolic syndrome are also linked to this SNP [9],[10], [11],[12]. Previous studies have indicated its association with metabolism and nutritional status, but its link to frailty has not been established, necessitating further research.

2. Frailty

Frailty syndrome associated with aging diminishes physical capability and health vulnerability. Symptoms include weakness, fatigue, medical complexity, and medical procedure intolerance. Appropriate management enhances recovery in vulnerable patients [13]. Frailty syndrome slows movement, induces fatigue, and causes weight loss. Timely intervention is crucial to mitigate adverse effects on the elderly by detecting and monitoring biomarkers related to complex weakness. However, some biomarkers associated with aging have limitations as disease predictors [1].

3. Sirtuin

Sirtuin is an anti-aging therapy target with deacylase and/or ADP ribosyltransferase protein activities, regulating aging and related diseases. SIRT1 combats aging, obesity, and diseases by repairing proteins. The levels of SIRT1 protein are associated with cardiovascular diseases in the elderly, indicating a significant role in aging-related disease therapy. SIRT1 also influences frailty in the elderly and can be enhanced through nutritional status for health interventions. SIRT6, along with SIRT1, enhances health and physical activity, reducing frailty in old age [14], [6], [15].

4. *Polimorfisme Nukleotida Tunggal* (SNP) SIRT1

Single Nucleotide Polymorphism (SNP) refers to a DNA variation at a single nucleotide within the genome, impacting the amino acid of a protein and medical genetic research [16]. The SIRT-1 gene (chromosome 10q21.3) is associated with the SNP rs2273773 C > T and silent mutations in exon 5. In relation to chronic diseases and age, rs2273773 is linked to different phenotypes and increases in the elderly [9]. Sirtuins (SIRT1, SIRT3) decline is correlated with frailty but not significantly in SK Hep1 cells [17], [6].

5. Method

This research employed a cross-sectional research design to investigate the association between the SIRT1 gene polymorphism (rs2273773) and plasma SIRT1 levels concerning frailty in the elderly at the University of North Sumatra Hospital. The research subjects were selected using the Consecutive sampling technique based on predetermined criteria suitable for this research

design. The sample size was calculated using the formula as follows: [18]

$$n = \frac{Z_{1-\alpha/2}^{2} p(1-p)}{d^2}$$

Infromation:

n = Number of subjects

Z1-α/2 = Type 1 error (p<0.05) = 1.96

p = Frailty proportion in previous research = 18.7%

d = Absolute precision (0.05)

n =

n = 118 *1,96 x 0,187 (1-0,187)*

6. Result

6.1 Subject Characteristics Based on the Frailty Scale

Table 1. Distribution based on the frailty scale of subjects.

Characteristic	n	%
Robust	40	33,8%
Pre-frail	54	45,7%
Frail	24	20,3%
Total	**118**	**100%**

Table 1 shows the dominant characteristic of subjects based on frailty is the pre-frail with a total of 54 individuals (45.7%), robust with 40 individuals (33.8%), and frail with 24 individuals (20.3%).

6.2 Electrophoresis Analysis

Electrophoresis Separates DNA Based on Size and Charge, Presented in Inverse Representation

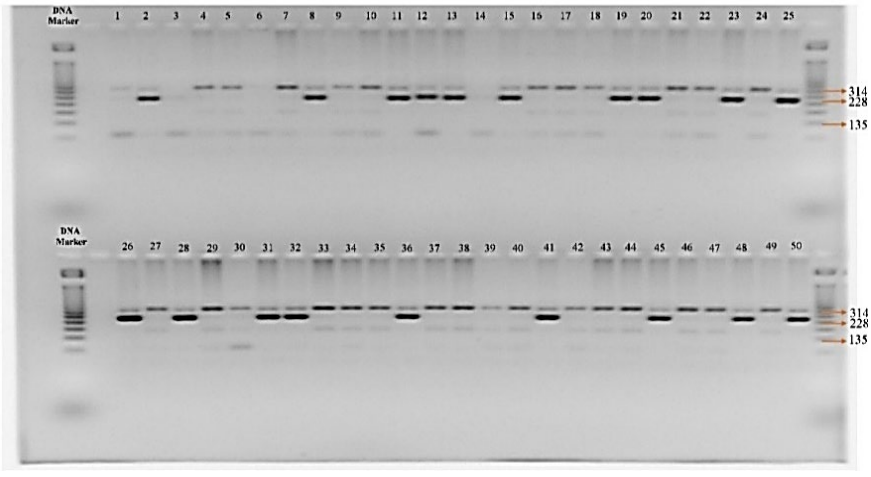

Figure 1. Electrophoresis Results of PCR Products for SIRT1 Gene Polymorphism rs2273773 in Subjects 1-50bp

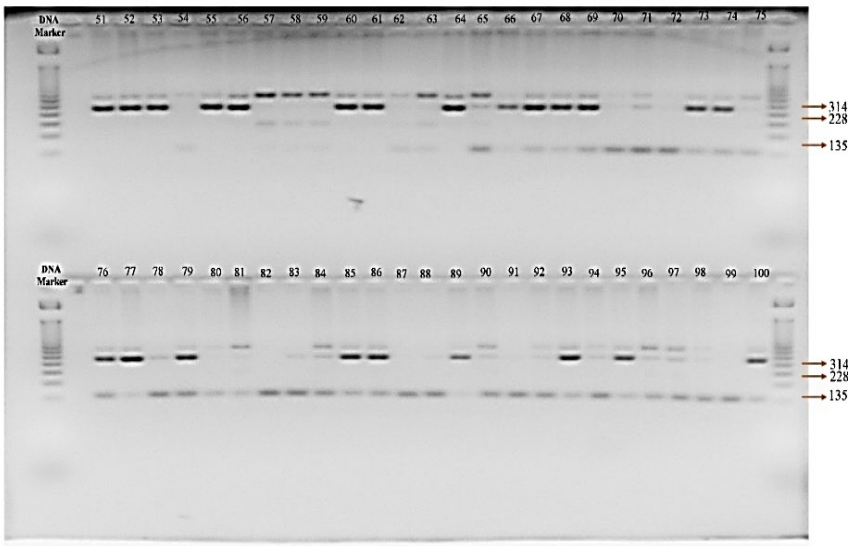

Figure 2. Electrophoresis results of PCR products of SIRT1 gene polymorphism rs2273773 in subjects 51-100bp

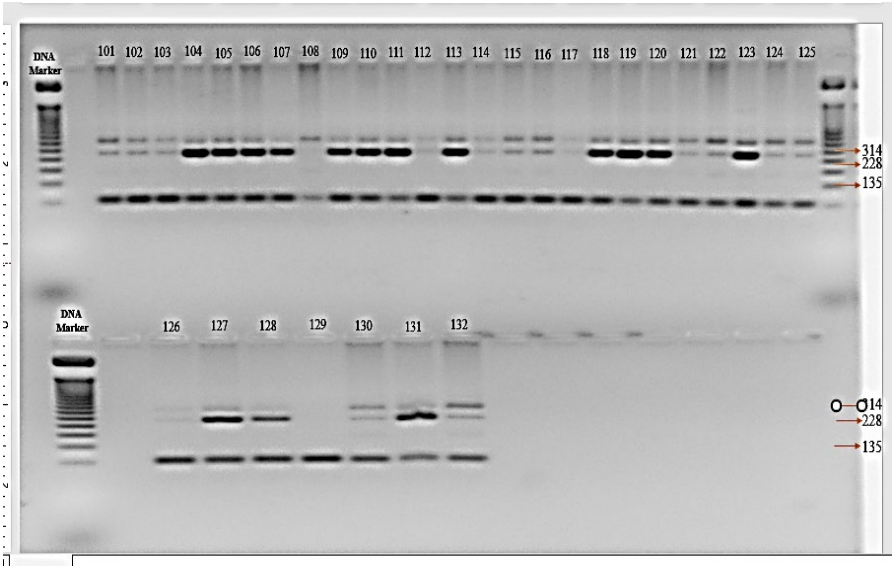

Figure 3. Electrophoresis Results of PCR Products for SIRT1 Gene Polymorphism rs2273773 in Subjects, Ranging from 101 to 118 base pairs.

6.3 Genotype Distribution and Allelic Polymorphism of rs2273773 in the Elderly

Table 2. Genotype Distribution and Allelic Polymorphism of rs2273773 in the Elderly

Genotipe	**N**
CC	86
CT	28
TT	4
Total	**118**
Alel	**N**
C	389
T	13
Total	**402**

In accordance with Table 2, the frailty scale exhibits a maximum value of 86, out of a total of 118. Furthermore, the distribution of the rs2273773 polymorphism alleles based on the frailty scale

reveals that the highest value is associated with allele C, totaling 389 and allele T is observed 13 times, summing up to a total of 402.

6.4 *Hardy-Winberg Equilibrium (HWE)*

Table 3. Hardy-Weinberg Equilibrium (HWE) of SIRT1 Gene Polymorphism (rs2273773)

	Genotipe			Alel		
	CC	**CT**	**TT**	**C**	**T**	**P**
Observed	86	28	4	389	13	
Expected	73,6	34,9	9,4	381,4	20,6	
HW						
N	(68,64%)	(27,96%)	(3,38%)	(96,76%)	(3,23%)	0,137

Criteria: p (sig > 0,05)

In accordance with the results presented in table 3, the p-value is 0.137. Thus, the variant of the SIRT1 gene polymorphism (rs2273773) is considered a polymorphism, as it conforms to the assumptions of the Hardy-Weinberg Equilibrium test.

6.5 The Relationship between Genotype Polymorphism (rs2273773) and Plasma SIRT1 Levels with Frailty

Table 4. Pearson Correlation Test Result of Genotype Polymorphism (rs2273773) and Plasma SIRT1 Levels with Frailty

Hubungan	R	p
Genotipe-Frailty	-0,199	0,030
SIRT1-Frailty	0,177	0,055

Criteria: p (sig) < 0,05

Table 4 displays the correlation between the polymorphism genotype (rs2273773) and frailty, negative (r = -0.199, p = 0.030), indicating a negative relationship between them. However, the correlation is relatively weak, with small changes in one variable affecting the other. There is no association between plasma SIRT1 levels and frailty levels (r = 0.177, p = 0.055), also with a weak correlation

Table 5. Simultaneous F-test Results for Polymorphism (rs2273773) and Plasma SIRT1 Levels with Frailty.

Df	F	p
2	3,433	0.036
115		
117		

Criteria: p (sig) < 0,05

The F-test results indicate a Sig. value of 0.036 < 0.05, signifying a significant relationship between the genotype polymorphism (rs2273773) and plasma SIRT1 levels with frailty. The calculated F-value of 3.433 also exceeds the critical F-table value of 3.08, demonstrating that both independent variables collectively influence frailty.

7. Discussion

In this study, the genotype (rs2273773) was found to be significantly associated with a higher

frailty risk in elderly subjects with a history of poor health, such as diabetes mellitus and stroke. Additionally, age was identified as one of the risk factors related to frailty in this study's elderly subjects. Frailty in this context is characterized by a decline in physiological reserves and an increase in stress levels. With advancing age, elderly individuals experience a reduction in muscle mass and strength, balance and coordination, cardiovascular and respiratory function, all of which can contribute to physical frailty. This is supported by the research findings, which demonstrate a correlation between age and frailty among elderly subjects over the age of 60, particularly in relation to the genotype (rs2273773).

Furthermore, in this study, plasma SIRT1 levels were significantly lower in frail elderly subjects compared to non-frail elderly subjects, even after adjusting for various subject characteristics such as age, gender, diabetes mellitus, hypertension, cognitive impairment, and the number of comorbidities. Specifically, plasma SIRT1 levels also decreased in elderly subjects with a history of or current heart disease, heart attacks, congestive heart failure, kidney disease, and joint pain. However, the levels were even lower in individuals who were frail and had diabetes or a history of stroke. Additionally, plasma SIRT1 levels decreased with age and the number of comorbidities, with a more pronounced decrease observed in frail subjects compared to non-frail elderly individuals. Notably, SIRT1 levels were lower in the frail group compared to the non-frail group.

These findings align with a study by Le Couteur et al, which validated SPR technology results with Western blot analysis, considered the gold standard for protein analysis. The study conducted in the CHAMP population assessed SIRT1 expression in SK Hep1 cells grown in the presence of serum samples obtained from frail and non-frail individuals. Interestingly, no direct correlation was found between frailty and SIRT1 expression in these cells. Post hoc analysis suggested a potential paradoxical relationship between serum-induced low SIRT1 expression and frailty. The authors of the CHAMP study also acknowledged that their results were unexpected, as high tissue SIRT1 expression is generally considered beneficial, influenced by caloric restriction, and expected to be higher in younger subjects [17].

8. Conclusion

Pearson correlation analysis revealed a significant relationship between the genotype polymorphism (rs2273773) in the SIRT1 gene and frailty levels (p-value < 0.05). Conversely, there was no significant association found between plasma SIRT1 levels and frailty levels (p-value > 0.05). Meanwhile, the F-test demonstrated a significant simultaneous association between the genotype polymorphism (rs2273773) and plasma SIRT1 levels with frailty levels (weakness) (p-value < 0.05).

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