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Experimental Research Article

Interactions between hemoglobin, age, Sirtuin1, and rs7069102 on fatigue in older people with chronic illness. A moderated mediation analysis

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ABSTRACT

Introduction: Fatigue is a common issue among the elderly with chronic illnesses. This pilot study examined the effects of hemoglobin, age, Sirtuin1 (SIRT1), and rs7069102 on fatigue in older persons with chronic illness in Indonesia.

Methods: A cross-sectional study of 129 elderly individuals with chronic illnesses was conducted using a moderated mediation analysis. A questionnaire was used to evaluate fatigue, SIRT1 levels were measured using ELISA, and the genotypes of rs7069102 were identified using PCR-CTPP. Hayes' approach was used in both the mediation and moderated mediation studies.

Results: Hemoglobin levels were negatively correlated with fatigue ($r = -0.182$, $p = 0.038$), but age was positively correlated with fatigue ($r = 0.183$, $p = 0.039$). The mediation analysis revealed that age had no significant effect on the relationship between hemoglobin level and fatigue. However, moderated mediation analysis indicated a significant indirect effect of the rs7069102 GG genotype on the relationship between hemoglobin, age, and fatigue at all SIRT1 levels (β(mean-1SD) = -0.100, $p = 0.010$; β(mean) = -0.112, $p = 0.007$; β(mean+1SD) = -0.122, $p=0.006$).

Conclusion: This study shows that the rs7069102 GG genotype at all SIRT1 plasma levels acts as a mediator, reducing the effect of age-related hemoglobin on fatigue. These findings indicate the intricate interaction between genetic factors, physiological parameters, and fatigue perception in these individuals, suggesting further investigation.

Keywords:
Fatigue
Hemoglobin
SIRT1
SNP rs7069102
Elderly

Introduction

The global issue of chronic fatigue in the elderly is important. Chronic illness often causes severe fatigue (Goërtz et al., 2021). 85% of the elderly population in developed regions have experienced at least one disease, with more than 60% having multiple chronic conditions

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(Quiñones et al., 2016). Approximately 40–74% of older patients with chronic illnesses experience fatigue (Menting et al., 2018). Chronic illnesses and complex health issues among older adults significantly affect their participation in family, community, and social growth (Jiang and Liu, 2023).

Hemoglobin helps transport oxygen and synthesize energy (Dunn et al., 2016). Low levels can cause chronic diseases. The most common hematological abnormalities in older adults are anemia, reduced physical performance, and increased mortality (Fukushima et al., 2019; Liu et al., 2021; Marzban et al., 2021). Previous studies have revealed that chronic inflammatory processes are part of a pathophysiological pathway that correlates anemia to decreased physical function (Gi et al., 2020).

Aging and chronic illnesses can also lead to fatigue. Age increases the risk of chronic illnesses such as heart disease, respiratory disease, and dementia. Previous studies demonstrated that fatigue increases with age. Heart failure, multiple sclerosis, rheumatoid arthritis, chronic kidney disease, and COPD result in significant energy loss during fatigue (Jaime-Lara et al., 2020).

Sirtuin1 (SIRT1) is often called a "longevity gene" because it makes people live longer and slows the aging process. SIRT1 is a highly conserved nicotinamide adenine dinucleotide $(NAD)^+$-dependent histone deacetylase (HDAC) (Yao et al., 2021) that affects many biological processes, such as inflammation (He et al., 2023), stress responses (Scisciola et al., 2020), insulin regulation (Li et al., 2023), autophagy (Balarastaghi et al., 2022), cellular senescence, and lifespan (Zhu et al., 2022). These processes affect aging and chronic illnesses, such as cancer (Alves-Fernandes and Jasiulionis, 2019), neurodegenerative diseases (Zhang et al., 2020), cardiovascular diseases (Khayatan et al., 2022), chronic kidney disease (Zbroch et al., 2020), and metabolic diseases (Lu et al., 2023). The likelihood of fatigue is significantly affected by chronic conditions, particularly in older adults.

The single-nucleotide polymorphism (SNP) rs7069102 C > G is related to the SIRT1 gene, which encodes SIRT1. The SNP rs7069102 has been identified in intron 4 of the SIRT1 gene and is related to promoter polymorphisms, which could affect the activity of the SIRT1 gene by modifying promoter activity and thus SIRT1 expression (Shimoyama et al., 2011). Patients with the rs7069102 polymorphism may benefit from eating well. The presence of this SNP can help manage oxidative stress and inflammation in individuals with coronary heart disease, which could lead to a slower aging process and associated diseases (Hidalgo-Moyano et al., 2022).

Age, anemia, and fatigue have all been studied in relation to older individuals; however, there is limited information on the particular processes by which these variables interact with SIRT1 plasma levels and the rs7069102 genotype in older people with chronic illnesses. To acquire prospective relationships and recognize hypothetical interactions, we used a moderated mediation model. We hypothesized that (H1) Hemoglobin is negatively associated with fatigue, (H2) Age is positively correlated with fatigue, (H3) the relationship between fatigue and hemoglobin is mediated by age, and (H4) SIRT1 levels in plasma and the rs7069102 genotype act as mediators, reducing the effect of age-related hemoglobin on fatigue.

The objective of this study was to investigate the effect of SIRT1 levels in plasma and the rs7069102 genotype on the relationship between age, hemoglobin levels, and fatigue in older Indonesians with chronic illnesses.

Material and Methods

Data collection

This cross-sectional observational study (cross-sectional) followed the STROBE and AGReMA guidelines with moderated mediation analysis to examine the effects of the relationship. Mediation analysis of observational research and trials with randomization could provide knowledge on how interventions and exposure could affect health outcomes by exploring the underlying mechanisms (Lee et al., 2021). Data were collected from patients receiving outpatient care at a university hospital between January 2022 and May 2022. This study included 129 participants who strictly adhered to the eligibility criteria. Participants included both men and women, aged ≥ 60 years. We had a complete electronic medical record, including laboratory and medical test results, as well as information about chronic diseases from the previous year. There were no physical or mental obstacles to answering the survey questions. Exclusion criteria include acute illness or infection at the time of the study, having undergone a significant surgical procedure within the previous three months, or participation in another clinical trial that could interfere

with the study results. The study participants were selected using purpose sampling, and the following formula was used to determine the sample size (Charan and Biswas, 2013):

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

According to previous studies (p), the expected proportion of the population was 18.7 % (Setiati et al., 2021), $Z_{1-\alpha/2}^{2}$ (type I error 5 %) = 1.96, and absolute error (d)= 0.05.

Social demographic, medical, and laboratory information

Trained observers conducted in-depth interviews using a local-language questionnaire. Personal, demographic, medical, and laboratory data from a database of electronic health records were reviewed after each participant's clinical diagnosis one year earlier.

Fatigue assessment

A questionnaire presented in the subjects' native language was used to find information about their previous year's fatigue history. 'How often did you feel tired in the last four weeks?' was the question provided. 1 indicated "always," 2 indicated "most of the time," 3 indicated "some of the time," 4 indicated "not at all," and 5 indicated "never." Answers to 1 or 2 were assigned a value of 1, and all other answers were assigned a value of 0. This question was adapted from the Indonesian version of the FRAIL Scale and determined to be valid (Dwipa et al., 2021).

SIRT1 assay

SIRT1 levels in plasma were assessed using a monoclonal antibody and a commercial human SIRT1 ELISA kit from Elabscience®, Houston, USA (E-EL-H1546).. Human SIRT1 antibodies were applied to the microtiter plates. Secondary antibodies and avidin-conjugated HPP were used as secondary antibodies. The procedure involves pipetting 100-µL of the plasma and control samples into the appropriate wells. To quantify horseradish peroxidase, an ELISA reader (Thermo Fisher Scientific®, Finland) was used at 450 nm. A standard curve was created using pure SIRT1 at concentrations of 20, 10, 5, 2.500, 1.250, 0.630, and 0.31 ng/ml.

DNA isolation

White blood cells' genomic DNA was extracted using a Wizard® DNA isolation kit (A1120, Madison, WI, USA). After blood samples from each subject were collected in tubes containing EDTA. According to product usage guidelines, pure DNA samples were kept at temperatures ranging from 2 °C to 8 °C before PCR applications for SIRT1 genotypes.

Identification of the SNP genotype rs7069102 in SIRT1

The rs7069102 polymorphism was selected based on its considerable prevalence as a polymorphism in SIRT1 and its association with several diseases.

The genotype of rs7069102 in intron 4 was investigated using minimally modified PCR-CTPP assays (Shimoyama et al., 2011; Yin et al., 2012). A primer targeted to the genotype of the rs7069102 polymorphism site was used to amplify SIRT1 using PCR. In summary, a reaction solution consisting of Taq solution with $(NH_4)_2SO_4$ was used to prepare 25 ml PCR mixtures. These mixes included 100-200 ng of DNA, 10.0 pmol of each primer, 1.0 mM deoxynucleotide triphosphate (dNTP), 25 mM MgCl2, and 2.5 U Taq DNA polymerase. PCR was conducted with the specified primers (Hamajima et al., 2000):

Forward primer 1: 5'-GTA GCA GGA ACT ACA GGC CTG-3'

Reverse primer 1: 5'-CTA TCT GCA GAA ATA ATG GCT TTT CTC-3'

Forward primer 2: 5'-GAG AAG AAA GAA AGG CAT AAT CTC TGC-3'

Reverse primer 2: 5'-GAT CGA GAC CAT CCT GGC TAA G-3'

The rs7069102 C > G polymorphism was genotyped after an initial denaturation at 95 °C for 10 min, followed by 30 cycles at 95 °C for 1 min and 63 °C. The PCR results were analyzed on a 2 % agarose gel using ethidium bromide staining. Three distinct band patterns of the rs7069102 genotype are shown in Figure 1. These patterns corresponded to the GG genotype at 391 and 167 bp, the CC genotype at 391 and 277 bp, and the CG genotype at 391, 277, and 167 bp, respectively (Shimoyama et al., 2011).

Statistical evaluation

Two alleles were tested using the Hardy-Weinberg equilibrium calculator (https://www.had2know.org).

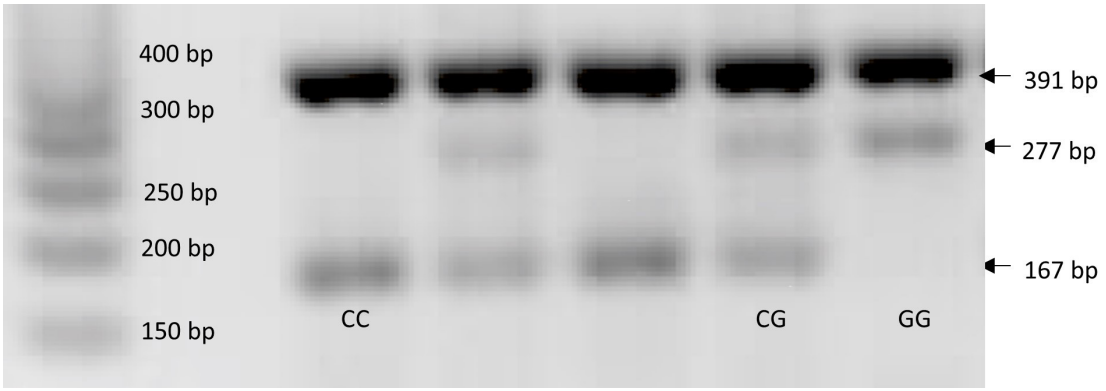

FIGURE 1. Representative PCR gel electrophoresis of SIRT1 SNP rs7069102 C>G.
The figure shows three distinct banding patterns corresponding to the CC, CG, and GG genotypes of the SIRT1 SNP rs7069102. The CC genotype was represented by bands at 391 and 277 bp, the CG genotype by bands at 391, 277, and 167 bp, and the GG genotype by bands at 391 and 167 bp. A 50 bp DNA marker was included as a reference.

The Jamovi statistical software ver.2.3 was used for all analyses, with a significance level of $p<0.05$. Descriptive statistics were used to analyze the characteristics of each participant. Non-normalized variables were reported as medians and ranges, whereas normalized data were presented as means and standard deviations. Qualitative characteristics are described in terms of frequency and number.

A correlation plot (Figure 2) was used to examine the correlations between several important variables, and (Epskamp et al., 2012), a simple mediation analysis proposed by Hayes (Hayes, 2018) was conducted to examine the role of age in mediating the relationship between hemoglobin levels and fatigue (Figure 3A). However, a moderate mediation analysis was performed to investigate whether the plasma levels of SIRT1 and the rs7069102 genotype moderated the role of age in mediating the relationship between hemoglobin and fatigue (Figure 3B). Both mediation and moderate mediation analyses were proposed by Hayes (Hayes and Rockwood, 2017) and analyzed using the jAMM GLM mediation model (Hayes and Rockwood, 2020).

All interactions are shown before the hypotheses were tested. This model employs covariates to predict the expression of "standardized" hemoglobin and SIRT1. Dummy variable for rs7069102 genotype (CC=1, CG=2, GG=3). The delta method employs the central limit theorem approximation to assess the significance of the indirect, direct, and total effects. The effects were considered statistically significant if the confidence interval (CI) was 0 (Deng et al., 2018).

Several steps were taken to address possible sources of bias in this study. Validated questionnaires, established processes, and objective measurements were used to reduce information bias and ensure data consistency. Strict adherence to the eligibility criteria during participant selection reduced selection bias. Statistical analysis was adjusted for possible confounders to account for their impact on the observed relationships. However, the cross-sectional design restricts causal inference, and unmeasured confounding variables may potentially influence the findings.

Ethics approval

All participants gave their written consent after receiving information about the study's objective, the procedure by which it would be done, any possible risks and benefits, procedures to protect their personal information, and their right to terminate the study at any time without any consequences. The ethics committee investigated the written permission forms and methods and gave their approval. The study followed the Declaration of Helsinki's principles on ethics.

Results

Tests of Hardy-Weinberg equilibrium

This study found a Hardy-Weinberg equilibrium for the rs7069102 genotype ($p = 0.69$). This shows a strong Hardy-Weinberg equilibrium, and that the SNP-tested allele frequencies were stable and unaffected by the main evolutionary factors in the studied population (Abramovs et al., 2020).

TABLE 1: Sociodemographic, medical, and laboratory characteristics of the study participants (n = 129).

Sociodemographic Data		**Laboratory Data**	
Female	67 (51.9)[B]	Sirtuin1 Plasma (ng/ml)	57.97 (2.65-199.2)[A]
Age	65 (60-85)[B]	Hemoglobin (g/dL)	12.4 (6.8-17.1)[A]
Marital Status		HbA1c	7.4 (5-16.9)[A]
Married	86 (66.7)[B]	Total Cholesterol (mg/dL)	194.92 (43.91)[A]
No Spouse	43 (33.3)[B]	Triglyceride (mg/dL)	132 (53-669)[A]
Medical		High-density Lipoprotein (mg/dL)	50.7 (39.32)[C]
Fatigue [*]	0: 86 (66.7) ; 1: 43 (33.3)[B]	Low-density Lipoprotein (mg/dL)	133 (54-282)[A]
Systolic BP (mmHg)	75.13 (10.88)[C]	Blood Urea Nitrogen (mg/dL)	25.9 (19.87)[C]
Diastolic BP (mmHg)	138.71 (21.87)[C]	Creatinine (mg/dL)	1.54 (2.4)[C]
Comorbidity			
Diabetes Mellitus	80 (62.0)[B]	**SNP rs7069102**	
Hypertension	57 (44.2)[B]	Genotype:	
Arthritis	48 (37.2)[B]	CC	52 (40.3)[B]
Stroke	48 (37.2)[B]	CG	62 (48.1)[B]
Heart Attack	28 (21.7)[B]	GG	15 (11.6)[B]
Angina	19 (14.7)[B]	Allele:	
Kidney Diseases	17 (13.2)[B]	C	114 (59.69)[B]
Chronic Lung Disease	11 (8.5)[B]	G	77 (40.31)[B]
Asthma	11 (8.5)[B]		
Cancer	7 (5.4)[B]		

[A]=median (min–max); [B]=n (%); [C]= mean (±SD). [*]0=some or little of the time or none; 1 = all or most of the time.

Subject characteristics

Table 1 shows the characteristics of 129 patients with chronic illnesses, whose ages varied from 60 to 85 years, with a median age of 65 years. The median plasma Sirtuin1 level was 57.97 ng/ml, with values ranging from 2.65 - 199.2 ng/ml. The median hemoglobin level was 12.4 g/dL, which varied from 6.8-17.1 g/dL. The distribution of the rs7069102 genotypes was as follows: CC (40.3%), CG (48.1%), and GG (11.6%). The frequencies of the C and G alleles were 59.69% and 40.31%, respectively. 33.3% of individuals reported experiencing fatigue all or most of the time.

Interaction of the Potential Major Variables

Figure 2 shows that lower hemoglobin levels had a significant and strong negative association with fatigue (r = -0.182, p = 0.038). Furthermore, age and fatigue were significantly correlated (r=0.183, P=0.039). Based on our findings, we accepted both hypotheses that hemoglobin is negatively correlated with fatigue (H1) and that age is positively associated with fatigue (H2).

Mediation Analysis

The association between hemoglobin and fatigue, as shown in Figure 3A and Table 2, did not consider age as a mediator, as the indirect effect was not statistically significant (p=0.114). Nevertheless, there was a total effect between hemoglobin levels and fatigue (r= -0.203, p = 0.019), indicating that higher hemoglobin levels were related to reduced fatigue. A significant correlation was observed between age and hemoglobin levels. There was a tendency to associate the adverse effect of hemoglobin level with fatigue and the positive effect of age on fatigue; however, none of these associations achieved statistical significance. Therefore, additional investigations and data are necessary. The complex nature of the interactions, along with the potential absence of additional factors in the analysis, may have affected

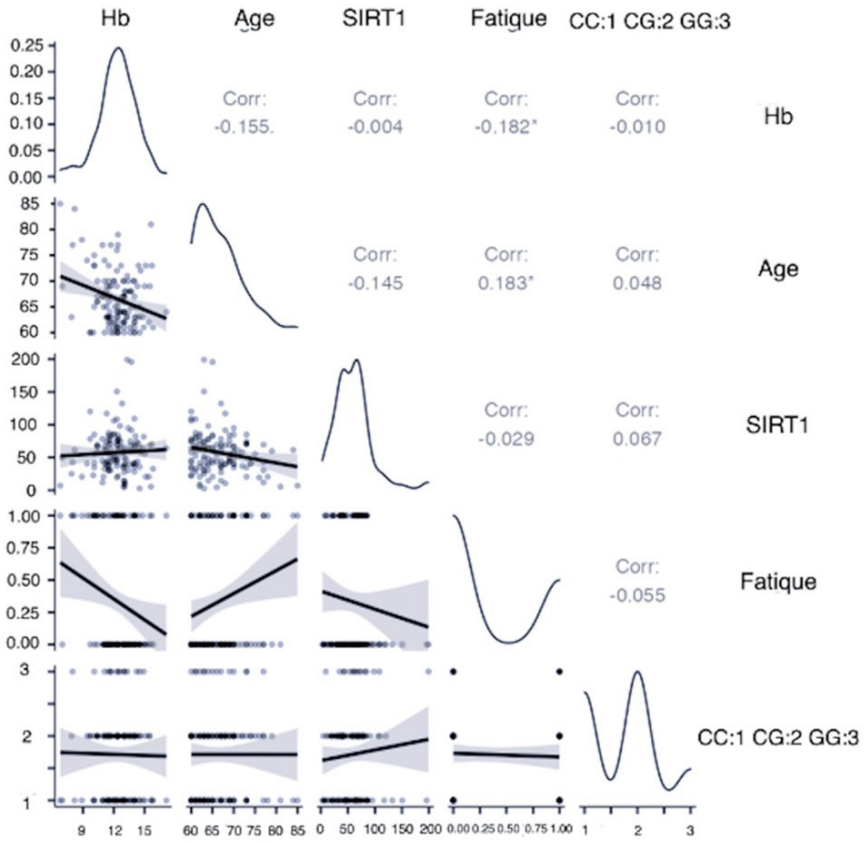

FIGURE 2. Correlation plot between the main possible variables.
The figure presents a correlation plot visualizing the relationship between hemoglobin (Hb), age, sirtuin1 (SIRT1), fatigue, and the rs7069102 genotype (CC: 1, CG: 2, GG: 3). Significant correlations (P<0.05) are indicated by asterisks. The color and size of the circles indicate the strength and direction of the correlation, respectively.

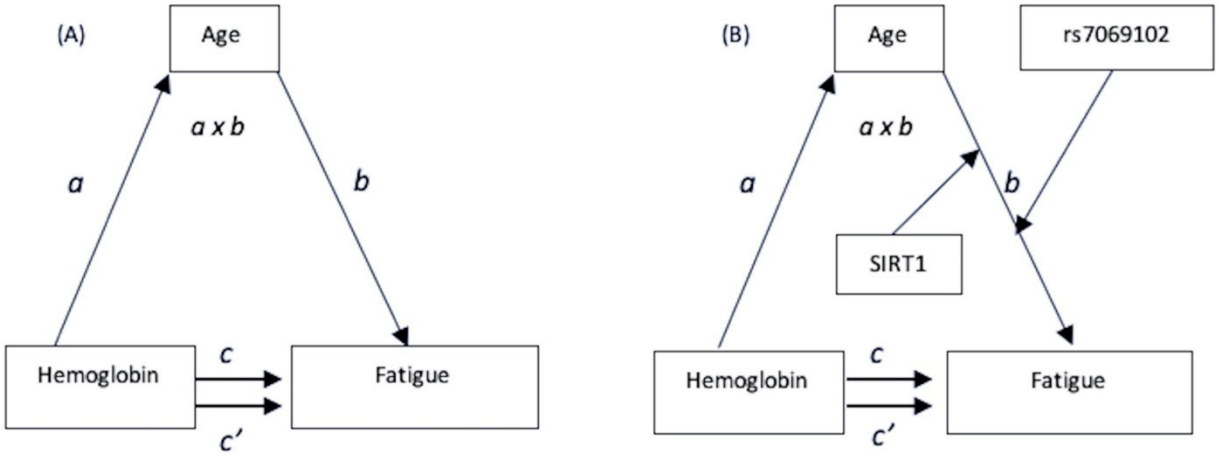

FIGURE 3. Graphs of the relationship between variables in the mediation and moderated mediation models.
(A) Mediation model: Age as a mediator between hemoglobin and fatigue. Pathways: a (hemoglobin and age), b (age and fatigue, hemoglobin controlled), c (direct effect: hemoglobin on fatigue, age controlled), and c' (total effect: hemoglobin on fatigue).
(B) Moderated mediation model: SIRT1 genotype and SNP rs7069102 as moderators, and age influencing fatigue. Indirect effects: a × b, showing the influence of moderators on the relationship among hemoglobin, age, and fatigue.

TABLE 2: Relationship between hemoglobin and fatigue mediated by age.

Effect	95% C.I.*		β	p
	Lower	Upper		
a x b	-0.0449	0.00481	-0.0423	0.114
a	-2.3202	-0.50977	-0.2604	0.002
b	-0.000886	0.0292	0.1626	0.065
c	-0.1581	0.00534	-0.1614	0.067
c'	-0.1767	-0.01617	-0.2038	0.019

n = 129. ***a*** × ***b***: Indirect effect of hemoglobin on fatigue as mediated by age. The paths are shown as follows: ***a***: hemoglobin and age, ***b***: age and fatigue (hemoglobin controlled), ***c***: direct effect (hemoglobin on fatigue, age controlled), and ***c':*** total effect (hemoglobin on fatigue). *Confidence intervals computed using the standard delta method.

TABLE 3: Conditional mediation and moderation levels for the SIRT1 plasma levels and rs7069102 genotypes modulate age-related hemoglobin and fatigue.

Conditional Mediation									
Moderator levels		SIRT1 (Mean-1·SD)			SIRT1 (Mean)			SIRT1 (Mean+1·SD)	
		Genotypes			Genotypes			Genotypes	
		CC	CG	GG	CC	CG	GG	CC	CG	GG
		Indirect effect							
95% C.I.*	Lower	-0.028	-0.006	-0.052	-0.036	-0.010	-0.060	-0.043	-0.016	-0.068
	Upper	0.001	0.018	-0.007	-0.002	0.013	-0.009	-0.004	0.008	-0.011
	β	-0.051	0.022	-0.100	-0.068	0.004	-0.112	-0.084	-0.015	-0.122
	p	0.062	0.33	0.01	0.028	0.853	0.007	0.016	0.519	0.006
		Direct effect							
95% C.I.*	Lower	-0.078	-0.080	-0.079	-0.079	-0.080	-0.079	-0.080	-0.080	-0.079
	Upper	0.014	0.015	0.014	0.014	0.015	0.014	0.015	0.015	0.014
	β	-0.120	-0.119	-0.109	-0.117	-0.121	-0.105	-0.114	-0.122	-0.100
	p	0.169	0.184	0.171	0.175	0.184	0.172	0.182	0.184	0.175
		Total effect							
95% C.I.*	Lower	-0.098	-0.098	-0.098	-0.098	-0.098	-0.098	-0.098	-0.098	-0.098
	Upper	-0.008	-0.008	-0.008	-0.008	-0.008	-0.008	-0.008	-0.008	-0.008
	β	-0.198	-0.198	-0.198	-0.198	-0.198	-0.198	-0.198	-0.198	-0.198
	p	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021

n = 129. Indirect effect of hemoglobin on fatigue according to age**.** Direct effect (hemoglobin on fatigue, age-controlled) and total effect (hemoglobin on fatigue). *Confidence intervals computed using the standard delta method.

the outcome. (Zhao et al., 2010). The mediation model analysis rejected the hypothesis (H3) that age mediates the relationship between fatigue and hemoglobin.

Moderated mediation analysis.

Figure 3B and Table 3 show that age as a mediator appears to influence the relationship between hemoglobin and fatigue differently between genotypes and SIRT1 plasma levels. In particular, the GG genotype had significant indirect effects on all SIRT1 plasma levels ($\beta_{(mean-1SD)} = -0.100$, $p = 0.010$), ($\beta_{(mean)} = -0.112$, $p = 0.007$), and $\beta_{(mean +1SD)} = -0.122$, $p = 0.006$). However, the CG

and CC genotypes did not exhibit this pattern. Meanwhile, the direct effect of hemoglobin on fatigue was not significant for all genotypes at each SIRT1 level. This suggests that the GG genotype strengthens the age-mediated relationship between hemoglobin and fatigue by increasing SIRT1 levels. Moderate mediation analysis supports the hypothesis (H4) that the level of plasma SIRT1 and the genotype rs7069102 reduce hemoglobin-induced fatigue with age.

Discussion

The findings of this study provide information on the intricate genetic–biological interactions that generate fatigue in elderly adults with chronic diseases.

The Hardy–Weinberg equilibrium value of 0.69 for SNP rs7069102 matched the expected consistency of allele distribution in a population without evolutionary influences (Hao and Storey, 2019; Li and Graubard, 2009). Various investigations have discovered that allele frequencies in comparable cohorts are aligned, supporting the theory that genetic variables such as SNP rs7069102 are balanced in specific populations (Hidalgo-Moyano et al., 2022; Letonja et al., 2021).

Our results showed a clear inverse relationship between hemoglobin levels and fatigue. This inverse relationship corresponds with another study that suggests that anemia is an important contributor to fatigue in elderly populations (Guralnik et al., 2022). This relationship may be attributed to the reduced oxygen-carrying capacity that occurs in individuals with anemia, which could influence muscle metabolism and lead to increased fatigue (Katsumi et al., 2021). In contrast, age was shown to have a significant positive correlation with fatigue, indicating that older people experience more fatigue. This is consistent with the literature, which suggests that fatigue increases with age (Torossian and Jacelon, 2021).

The mediation analysis in this study found that age did not significantly moderate the relationship between hemoglobin level and fatigue. Age may have contributed to the lack of a significant mediation. This is possible because the complex multifactorial nature of fatigue in elderly people with chronic illnesses may have obscured the specific role of age in the hemoglobin-fatigue relationship, which may have a greater impact than age alone.

Although age played no role in the relationship between hemoglobin and fatigue, the moderated mediation analysis showed some interesting things about how SIRT1 levels and the rs7069102 genotype moderated the relationship between age and fatigue. The results showed that the GG genotype of rs7069102 significantly strengthened the age-mediated relationship between hemoglobin and fatigue, particularly at higher SIRT1 levels.

This finding suggests that individuals with the GG genotype and higher SIRT1 levels may be more susceptible to the fatiguing effects of low hemoglobin levels as they age. Owing to its role in cellular regulatory processes such as aging and inflammation, SIRT1 has been associated with age-related illnesses (Xu et al., 2020; Yang et al., 2022). This interaction emphasizes the intricate nature of genetic influences on phenotypic outcomes and the importance of considering genetic origins when evaluating biological interactions (Garcia-Casal et al., 2023). Rather, fatigue is probably the result of an intricate combination of interrelated variables such as physiological, psychological, and environmental factors (Azzolino et al., 2020; Bendak and Rashid, 2020; Habay et al., 2021). Our study is consistent with the idea that fatigue in the elderly is a multifaceted construct that is affected by an intricate combination of factors (Belza et al., 2018).

Limitation

This study has various limitations that should be emphasized when considering the results. However, these results may not apply to other populations because of the limited sample size. The cross-sectional approach limits causal inferences because unmeasured confounding variables may explain the observed relationships. Fatigue is complicated, and therefore, a single questionnaire item may not adequately describe it. This study revealed the genetic and biological causes of fatigue; however, further research is required to precisely investigate these pathways. The sample's high rates of comorbid conditions and potential medication use may have affected fatigue levels, and the lack of a healthy comparator group made it difficult to determine whether the relationships observed were specific to chronic disease populations or age-related.

Conclusion

In a preliminary investigation involving elderly In-

donesians with chronic diseases, we found that hemoglobin levels were negatively correlated with fatigue assessments, but age was positively associated with fatigue. Mediation analysis showed that age had no significant effect on the relationship between hemoglobin levels and fatigue. However, moderated mediation analysis revealed that the rs7069102 GG genotype increased the indirect effect of hemoglobin on fatigue with age by enhancing SIRT1 levels. These results provide insights into the complex genetic-biological interactions that underpin fatigue in these individuals, emphasizing the need for further research to better understand these interactions.

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Conflict of interest

Dedi Ardinata received funding from Universitas Sumatera Utara. However, the funding organization had no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript. The remaining authors declare that they have no competing financial or non-financial interests in relation to the work described in this manuscript.

Ethics approval

This study was reviewed and approved by the Ethics Committee for Health of Sumatera Utara University. The reference number for the approval is 1097/KEPK/USU/2022. All procedures performed in this study 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.

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