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ALDH2 genotype and its association with alcohol intake and transaminase enzyme levels among East Nusa Tenggara residents

Niufti Ayu Dewi Mahila[1]*, Yudha Nurhantari[1], Suhartini[1]

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[1] Department of Forensic and Medico-legal, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

***Correspondence:**
dewymahila@gmail.com

Abstract

Purpose: Alcohol is the most commonly abused substance worldwide, and one of the factors contributing to impaired liver function is the polymorphism of the ALDH2 enzyme gene. The limited research on the relationship between the ALDH2 gene polymorphism and transaminase enzyme levels prompted this study, which focuses on a population in Indonesia with high alcohol consumption: the ethnic group of East Nusa Tenggara (NTT). This study aims to identify the ALDH2 genotype among the NTT ethnic group and to examine its relationship with alcohol consumption and transaminase enzyme levels. **Methods:** A total of 55 ethnic NTT respondents who met the inclusion criteria were included in the study. After the respondents signed the informed consent form, they were interviewed, and their blood samples were collected. The blood sample was then isolated for DNA extraction, examined for DNA levels, subjected to electrophoresis, RFLP, determination of polymorphism using the MboII enzyme, and examination of transaminase levels (SGOT, SGPT, and GGT). Drinking behavior based on routine, frequency, volume, and duration using a questionnaire. Statistical analysis using the chi-square test, then performed a correlation test, and calculated the Odds Ratio. **Results:** Of 55 research subjects, the majority had ALDH2*1 (29 subjects, 52.7%), ALDH2*2 (17 subjects, 30.9%), or ALDH2*1/*2 (9 subjects, 16.4%). Alcohol drinkers were 38 people (69.1%), while non-alcoholic drinkers were 17 people (30.9%). ALDH2*2 subjects were at risk of increasing sgot by 0.54 times (OR=0.54) and sgpt by 1.16 times (OR=1.16), ALDH2*1/*2 subjects at risk of increasing sgpt by 1.08 times (OR=1.08). ALDH2*1 subjects who drink alcohol are at risk of increasing sgpt by 0.52 times (OR=0.52), and ALDH2*2 subjects who drink alcohol are at risk of increasing sgpt by 0.70 times (OR=0.70) and GGT by 1.4 times (OR=1.4). There was no significant proportion between ALDH2 polymorphism and SGOT and SGPT, drinking behavior of alcoholic beverages, and level of alcohol consumption in the alcohol drinking population (p>0.05), but significant with ggt (p=0.045). **Conclusion:** ALDH2 gene polymorphisms are ALDH2*1, ALDH2*2, and ALDH2*1/*2, with ALDH2*1 (52.7%) being the most common in the NTT ethnicity. ALDH2 polymorphism has a significant proportion with ggt (OR=1.4), but not significant with sgot (OR=0.52), and sgpt (OR=0.70).

Keywords: aldehyde dehydrogenase; ALDH2 polymorphism; NTT ethnicity; transaminase enzyme levels

INTRODUCTION

Based on WHO data obtained from the World Bank in 2010, the total population of Indonesian people was 240,000,000, 70% were in the age category above 15 years, and obtained 9.4% men and 1.7% Indonesian women in the age group above 15 years per capita have a habit of consuming alcohol [1]. According to data from the Ministry of Health of the Republic of Indonesia, based on the results of Riskesdas 2018, the age group of 10 years and older in the province of NTT ranked 2nd in alcohol consumption among provinces in Indonesia [2]. Liver enzymes were observed with Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvate Transaminase (SGPT), and Gamma Glutamyl Transferase (GGT). The SGOT/SGPT (De Ritis) ratio is used to support the differential diagnosis of liver diseases. At the same time, GGT is a sensitive marker of hepatobiliary disorders and is useful in evaluating liver function [3]. Further research is needed on the factors that can affect the susceptibility of the liver to alcohol, so it is hoped that it can strengthen education regarding the dangers of alcohol consumption in the community.

Aldehyde dehydrogenase 2 (ALDH2) is an enzyme that plays an important role in alcohol metabolism, converting acetaldehyde to acetate and reducing NAD+ to NADH [4]. Aldehyde dehydrogenase (ALDH) is a group of genes encoding proteins and catalyzing the conversion of substrates from aldehydes to carboxylates via NAD+. Acetaldehyde has a toxic effect, injuring mitochondria by altering cell membranes, causing DNA injury, reducing oxygen use, and decreasing Reactive Oxygen Species (ROS). Hepatocyte death due to alcohol consumption causes liver fibrosis and results in fibrotic regeneration [5]. Ethnicity-related polymorphisms may influence enzyme activity. However, to date, no studies have investigated the relationship between ALDH2 polymorphisms and transaminase enzyme levels across ethnic groups in Indonesia. Therefore, this study aims to determine the association between ALDH2 polymorphisms and transaminase enzyme levels in individuals of NTT ethnicity who consume high amounts of alcohol.

METHODS

This cross-sectional study was conducted in Kupang, NTT Province, Indonesia, in October 2019. All subjects enrolled were over 18 years old and indigenous to NTT, as evidenced by their families having lived in NTT for 3 generations. The research was conducted in accordance with ethical principles and received ethical clearance from the Ethics Commission of the Faculty of Medicine, Public Health, and Nursing, Gadjah Mada University, Yogyakarta, under the number KE/FK/0830/EC/2019. Data collection of research subjects begins with the informed consent process. Exclusion was carried out in subjects with severe disease related to liver function. Information regarding health conditions and smoking habits was gained from the subject interviews.

A total of 55 research subjects underwent blood collection, with 7 mL of blood drawn and divided into two separate tubes. The first tube is a serum separator tube (SST) to calculate transaminase enzyme levels. The second tube contains the anticoagulant ethylenediaminetetraacetic acid (EDTA), and the blood sample in this tube is used for DNA analysis. DNA was isolated using the Wizard® Genomic DNA Purification Kit (Promega, Inc.). The integrity and purity of the isolated DNA were measured using a nanospectrophotometer. The expected A260:A280 ratio is around 1.8-2.0. Genotype identification was carried out using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method6. To perform RFLP on the detection of the ALDH2 allele, the amplified DNA was digested using the MboII enzyme. Digestion products are then continued into electrophoresis, the cutting locations are forward primer 5'...GAAGA(N)8*...3'… and the reverse primer 3'...CTTCT(N)7*...5'. The ALDH2 gene is 135 bp (base pairs) long. If truncated with the MboII enzyme, it yields 125-bp and 10-bp fragments. The wild-type allele (ALDH2*1) was truncated, while the polymorphic allele (ALDH2*2) was not. So the results were grouped into 3 genotypes: ALDH2*1 Wild type, ALDH2*2 (polymorphic homozygous), and ALDH2*1/*2 (polymorphic heterozygous). Transaminase enzymes were analyzed from blood samples inserted into the Plasma Separator Tube (PST), with parameters determined according to the procedure, using 4 ml of blood serum for SGOT, SGPT, and GGT analysis.

RESULTS

The majority of the subjects were male (76.4%) and had a normal weight (49.1%). Most NTT ethnic ALDH2 genotypes were ALDH2*1 Wild type (52.7%) (Table 1). There were no significant differences in the distribution of sex, age, upper arm circumference,

abdominal circumference, alcohol consumption, drinking frequency, drinking duration, regular exercise, smoking, areca nut consumption, and SGOT levels across different alleles (p> 0.05). The frequency of daily alcohol consumption was higher in the ALDH2*2 and ALDH2*1/*2 genes (60%), while the consumption of 1-5x/week (45%) in the ALDH2*1 gene showed a significant proportion (p<0.05) (Table 1).

Table 1. Proportion of characteristics by allele (Spearman correlation for ordinal and numeric data, Mann-Whitney for nominal data)

Variable	ALDH2*1		ALDH2*2		ALDH2*1/*2		n	%	Median (min-max)	p
	n	%	n	%	n	%				
Subject	29	52.7	17	30.9	9	16.4				
Sex/Gender										
Men	23	79.3	12	70.6	7	77.8	42	76.4		0.793
Women	6	20.7	5	29.4	2	22.2	13	23.6		
Age		22 (18-74)		24 (19–57)		22 (19–85)			22 (18–85)	0.575
BMI (Body Mass Index)										
Underweight	6	20.7	3	17.6	3	33.3	12	21.8		0.489
Normal weight	14	48.3	8	47.1	5	55.6	27	49.1		
Pre obese	7	24.1	4	23.5	1	11.1	12	21.8		
Obese class I	1	3.4	0	0.0	0	0.0	1	1.8		
Obese class II	0	0.0	1	5.9	0	0.0	1	1.8		
Obese class III	1	3.4	1	5.9	0	0.0	2	3.6		
Drinking alcohol										
Yes	23	79.3	10	58.8	5	55.6	38	69.1		0.220
No	6	20.7	7	41.2	4	44.4	17	30.9		
Consumption rate										
Light-Medium	19	82.6	9	90.0	3	60.0	31	81.6		0.506
High	3	13.0	1	10.0	0	0.0	4	10.5		
Very High	1	4.3	0	0.0	2	40.0	3	7.9		
Drinking frequency										
Everyday	3	13.6	6	60.0	3	60.0	12	32.4		0.007*
1-5x/week	10	45.5	1	10.0	2	40.0	13	35.1		
1-3x/month	7	31.8	3	30.0	0	0.0	10	27.0		
<1x/month	2	9.1	0	0.0	0	0.0	2	5.4		
Drinking duration		5 (1–10)		6 (2–30)		6 (4–9)	—	—	5 (1–30)	0.317
Regular exercise										
Yes	10	34.5	6	35.3	1	11.1	17	30.9		0.372
No	19	65.5	11	64.7	8	88.9	38	69.1		
SGPT	19	(13–39)	21	(15–30)	21	(18–27)	—	—		0.422
SGOT	13	(9–30)	12	(8–32)	15	(11–21)	—	—		0.376
GGT	21	(14–35)	21	(14–44)	19	(17–32)	—	—		0.888

Table 2. Proportion of ALDH2 with SGOT, SGPT, and GGT

	Variable	SGOT				p	OR	95% CI
		Normal		High				
		n	%	n	%			
Gene	ALDH2*1	26	89.7	3	10.30			
	ALDH2*2	16	94.1	1	5.90	1.000	0.54	0.05-5.66
	ALDH2*1/*2	9	100.0	0	0.00	1.000	-	-
	Variable	**SGPT**						
		Normal		High		p	OR	95% CI
		n	%	n	%			
Gene	ALDH2*1	26	89.7	3	10.30			
	ALDH2*2	15	88.2	2	11.80	1.000	1.16	0.17-7.72
	ALDH2*1/*2	8	88.9	1	11.10	1.000	1.08	0.09-11.92
	Variable	**GGT**						
		Normal		High		p	OR	95% CI
		n	%	n	%			
Gene	ALDH2*1	29	100.0	0	0.00			
	ALDH2*2	14	82.4	3	17.60	0.045	-	-
	ALDH2*1/*2	9	100.0	0	0.00	-	-	-

In this study, transaminase enzyme levels were assessed based on normal and elevated SGOT, SGPT, and GGT values. The results showed that subjects with the ALDH2*2 genotype had a higher proportion of elevated SGOT levels (5.9%) compared to those with the ALDH2*1/*2 genotype (0%) (p > 0.05). Additionally, subjects with the ALDH2*1 genotype showed a higher proportion of elevated SGOT (10.3%) than those with ALDH2*2 (5.9%) (p > 0.05). However, the odds ratio (OR = 0.54) indicates that individuals with the ALDH2*2

genotype had a 0.54 times lower risk of elevated SGOT levels.

Subjects with the ALDH2*2 genotype also exhibited a higher proportion of elevated SGPT (11.8%) compared to those with ALDH2*1 (10.3%) ($p > 0.05$). The calculated OR of 1.16 suggests that ALDH2*2 subjects had a 1.16-fold greater risk of elevated SGPT levels. Similarly, subjects with the ALDH2*1/*2 genotype showed a slightly higher SGPT level (11.1%) than ALDH2*1 (10.3%) ($p > 0.05$), with an OR of 1.08, indicating a 1.08-fold increased risk of elevated SGPT in ALDH2*1/*2 carriers. Furthermore, GGT levels were significantly higher in ALDH2*2 subjects (17.6%) compared to ALDH2*1 and ALDH2*1/*2 subjects (0%) ($p < 0.05$) (Table 2).

Statistical analysis revealed no significant association between ALDH2 polymorphisms and drinking behavior (Table 3) or alcohol consumption level (Table 4) ($p > 0.05$). There was also no significant association between ALDH2 polymorphisms and transaminase enzyme levels among alcoholics and non-drinkers. However, among ALDH2*2 subjects, those who consumed alcohol had a higher proportion of elevated GGT (20%) than non-drinkers (14.3%) ($p > 0.05$). The OR = 1.40 indicates that ALDH2*2 alcohol consumers were 1.4 times more likely to have elevated GGT levels (Table 5).

Table 3. Proportion of the ALDH2 genotype with drinking behavior

Type of ALDH2	n	Drinking alcohol (n, %)		p
		Yes	No	
ALDH2*1	29	23 (79.3)	6 (20.7)	0.220
ALDH2*2	17	10 (58.8)	7 (41.2)	
ALDH2*1/*2	9	5 (55.6)	4 (44.4)	

Table 4. Proportion of ALDH2 genotype with level of alcohol consumption

Alcohol consumption rate	n (%)			p
	ALDH2*1	ALDH2*2	ALDH2*1/*2	
Light-medium	19 (82.6)	9 (90.0)	3 (60.0)	0.506
High	3 (13.0)	1 (10.0)	0 (0.0)	
Very high	1 (4.3)	0 (0.0)	2 (40.0)	

Table 5. Relationship of ALDH2 polymorphisms in alcoholics, non-drinkers, and liver function status

		Variable		SGOT n (%)		p	OR	95% CI
				High	Normal			
Gene	ALDH2*1	Drinking alcohol	Yes	3 (13.0)	20 (87.0)	0.485	-	-
			No	0 (0.0)	6 (100.0)			
	ALDH2*2	Drinking alcohol	Yes	0 (0.0)	10 (100.0)	0.412	-	-
			No	1 (14.3)	6 (85.7)			
	ALDH2*1/*2	Drinking alcohol	Yes	0 (0.0)	5 (100.0)	0.444	-	-
			No	0 (0.0)	4 (100.0)			
		Variable		SGPT n (%)		p	OR	95% CI
				High	Normal			
Gene	ALDH2*1	Drinking alcohol	Yes	2 (8.7)	21 (91.3)	0.515	0.52	0.06 – 4.83
			No	1 (16.7)	5 (83.3)			
	ALDH2*2	Drinking alcohol	Yes	1 (10.0)	9 (90.0)	0.669	0.70	0.05 – 9.41
			No	1 (14.3)	6 (85.7)			
	ALDH2*1/*2	Drinking alcohol	Yes	0 (0.0)	5 (100.0)	0.444	-	-
			No	1 (25.0)	3 (75.0)			
		Variable		GGT n (%)		p	OR	95% CI
				High	Normal			
Gene	ALDH2*1	Drinking alcohol	Yes	0 (0.0)	23 (100.0)	-	-	-
			No	0 (0.0)	6 (100.0)			
	ALDH2*2	Drinking alcohol	Yes	2 (20.0)	8 (80.0)	0.640	1.40	0.16 – 12.60
			No	1 (14.3)	6 (85.7)			
	ALDH2*1/*2	Drinking alcohol	Yes	0 (0.0)	5 (100.0)	-	-	-
			No	0 (0.0)	4 (100.0)			

DISCUSSION

The most common genotype in this study was ALDH2*1 (wild type; Table 1). This differs from findings in Indonesia, where the allele frequencies of ALDH2*2 and ALDH2*1/*2 were reported to be higher than those in the Caucasoid population [13]. In Yogyakarta, for example, the majority of subjects were found to carry the ALDH2*2 allele [7]. The frequency of daily alcohol consumption was higher among individuals with the ALDH2*2 and ALDH2*1/*2 genotypes (60%). In contrast, alcohol consumption of 1–5 times per week

was more common among those with the ALDH2*1 genotype (45%) ($p < 0.05$) (Table 2). This finding is consistent with previous data from Yogyakarta, where the most prevalent genotype among both alcoholic drinkers (82.3%) and non-drinkers (63.1%) was ALDH2*2 [7]. However, this contrasts with data from Japan, where individuals with the ALDH2*1 genotype, both male and female, were reported to consume more alcohol than those with the ALDH2*1/*2 genotype [8].

Respondents with underweight status were more common among those with the ALDH2*1/*2 genotype (33.3%) compared to those with ALDH2*1 (20.7%) and ALDH2*2 (17.6%) ($p > 0.05$) (Table 1). This finding contrasts with reports from East Asian populations, where individuals with the ALDH2*2/*2 genotypes tend to have a higher risk of obesity and fatty liver [8]. Subjects with the ALDH2*2 genotype in this study had a 1.16 times higher risk of elevated SGPT levels (Table 2). This differs from findings in the Japanese population, where SGPT levels in ALDH2*2 individuals were reportedly lower than normal after alcohol consumption. However, excessive alcohol intake in ALDH2*2 individuals has been associated with a higher cancer risk compared to ALDH2*1/*2 individuals [9]. Furthermore, in individuals with the ALDH2*2 genotype, the presence of the G rs671 allele has been associated with a 10.60 U/L increase in GGT levels for every 10 g/day increment in ethanol consumption [10].

Statistical analysis showed no significant difference in the proportion of drinkers and non-drinkers across ALDH2 genotype groups ($p > 0.05$) (Table 3). This finding contrasts with studies in the Japanese population, where both men and women with the ALDH2*1 genotype were found to consume significantly more alcohol than those with the ALDH2*1/*2 genotype [8], and the ALDH2*2 genotype was reported to inhibit alcohol consumption strongly [11]. However, the results are consistent with a study in the Yogyakarta population, in which individuals with the ALDH2*1 genotype comprised 17.7% of drinkers and 35.9% of non-drinkers, and blood chemistry analysis showed no statistically significant differences ($p > 0.05$) [7].

Among ALDH2*2 subjects, those who consumed alcohol had a higher proportion of elevated GGT levels (20%) compared to non-drinkers (14.3%) ($p > 0.05$), with an odds ratio (OR) of 1.40, indicating a 1.4 times greater risk of elevated GGT among alcohol-consuming ALDH2*2 carriers (Table 5). This finding aligns with studies conducted in Southeast Chinese populations, which reported that the oriental ALDH2*504Lys (*2) variant is associated with a higher risk of alcoholic liver disease, late-onset Alzheimer's disease, colorectal cancer, and esophageal cancer [12].

The OR for increased GGT levels was higher than for SGOT and SGPT, likely because GGT has the highest sensitivity (42–86%) and the lowest specificity (40–84%) among the three biomarkers [14,15]. The oxidative metabolism of ethanol and methanol involves two main enzymes with distinct roles: alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) [16]. Alcohol metabolism includes the processes of absorption, distribution, and elimination, in which the liver metabolizes alcohol into acetaldehyde via the actions of ADH, cytochrome P450 2E1 (CYP2E1), and catalase. Subsequently, acetaldehyde is further converted into acetate and water by ALDH2 [5] Given that alcohol metabolism involves multiple enzymatic pathways, the findings of this study may be relevant for comparison and integration with future research exploring other related enzymatic mechanisms.

CONCLUSION

This study found that the most common genotype among individuals of NTT ethnicity was ALDH2*1 (52.7%), followed by ALDH2*2 (30.9%) and ALDH2*1/*2 (16.4%). Subjects with the ALDH2*2 genotype had a 0.54 times lower risk of elevated SGOT and a 1.16 times higher risk of elevated SGPT. ALDH2*1/*2 carriers had a 1.08-fold increased risk of elevated SGPT. Among ALDH2*1 individuals, alcohol drinkers had a 0.52 times higher risk of elevated liver enzymes compared to non-drinkers. Meanwhile, ALDH2*2 alcohol drinkers had a 0.70 times increased risk of elevated SGPT and a 1.4 times increased risk of elevated GGT compared to ALDH2*2 non-drinkers. These findings suggest a genetic susceptibility associated with altered transaminase enzyme levels among alcohol drinkers of NTT ethnicity. This study is part of an umbrella project titled 'Relationship between ADH2, CYP450E1, and ALDH2 Gene Polymorphisms with Liver Enzyme Levels in Alcoholic Drinkers of Papuan and East Nusa Tenggara Ethnicities,' which was presented at the Second Conference on Community Empowerment for Health in Yogyakarta on November 28, 2020.

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