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POLYMORPHISM OF ADH2 GENE AND LIVER CELL DAMAGE AMONG ALCOHOL DRINKER IN KUPANG CITY OF INDONESIA

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ABSTRACT

Drinking alcohol is one of the factors for liver dysfunction through the polymorphism of the ADH2 enzyme gene. The small number of studies on the proportion of ADH2 gene polymorphisms with liver function status prompted the authors to examine this in a population that consumes a lot of alcohol in Indonesia, namely the ethnic East Nusa Tenggara (NTT). The aim of this study was to analyze the polymorphisms of the ADH2 gene with impaired liver function associated with drinking alcohol behavior among ethnic NTTs. A total of 60 NTT ethnic respondents were involved in the interview and blood samples were taken for PCR- RFLP analysis, and liver function tests. (SGOT, SGPT, and GGT). Drinking behavior is based on routine, frequency, volume, and duration using a questionnaire. Statistical analysis using chi-square was then performed as a correlation test. From 60 study subjects, genotype ADH2*1 was owned by 1 subject (1.7%), ADH2*2 by 39 subjects (65%), and ADH2*3 by 20 subjects (33.3%). Respondents who drink alcohol are 44 people (73.3%), while 16 people are not alcohol drinkers (26.7%). There was no significant proportion between the ADH2 gene polymorphism and alcoholic drinking behavior (p=0.272) or the level of alcohol consumption in the alcohol-drinking population (p=0.296). ADH2 gene polymorphisms, namely ADH2*1, ADH2*2, and ADH2*3, with ADH2*2 (65%) being the highest number of ethnic groups in NTT. This gene was statistically not a significant proportion of the levels of SGOT, SGPT, GGT, alcohol drinking behavior, and the level of alcohol consumption in the NTT ethnic population.

KEYWORD: Alcohol drinker, ADH2, Polymorphism, Liver Function, ethnic NTT.

1. INTRODUCTION

Alcohol is the most abused substance in the world. According to data in America with the criteria of Applying the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-V), from 2012 to 2013 there were 36% of men and 22.7% of young women experienced disorders due to alcohol use.[2]

According to data from the Ministry of Health of the Republic of Indonesia through the results of Basic Health Research 2018, it was found that the age group of more than 10 years in the province of NTT was ranked 2nd with the habit of consuming excess alcohol compared to other provinces in Indonesia.[14]

Liver enzymes were observed with Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvate Transaminase (SGPT), and Gamma Glutamyl Transferase (GGT). Where the SGOT/SGPT deritis ratio is used to determine the differential diagnosis of liver disease, while the GGT is a sensitive indicator of hepatobiliary disease[16] and is very useful for evaluating liver function.[17]

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. One of the factors thought to influence the individual's response to alcohol consumption is the ADH2 polymorphism. This ADH2 plays an important role in how quickly alcohol metabolizes and how high the production of acetaldehyde is after alcohol consumption. The ADH2 gene is also involved with the occurrence of Alcohol Liver Cirrhosis (ALC).[10] Ethnicity-related polymorphisms, and until now there has been no research on the proportion between ADH2 polymorphisms and liver function status in ethnic groups in Indonesia. Therefore, this study wanted to determine the proportion between ADH2 polymorphisms and liver function status in ethnic NTT who consume a lot of alcohol.

2. MATERIAL AND METHODS

This cross-sectional study took place in Kupang, NTT province in Indonesia, in October 2019. All of the subjects enrolled were above 18 years old and indigenous ethnic NTT, As proved by 3 generations of their families living in NTT. The research was carried out with research ethical principles and has received ethical clearance from the Ethics Commission of the Faculty of Medicine, Public Health, and Nursing, Gadjah Mada University Yogyakarta 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 helath conditions and smoking habits was gained from the subject interviews.

A total of 60 research subjects underwent taking 7 ml of blood which was divided into 2 tubes. The first tube is a serum separator tube (SST) to calculate the levels of SGOT, SGPT, and GGT. The second tube contains the anticoagulant ethylene diamine tetra acetic 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 was measured using a nano spectrophotometer. The expected yield of A260:A280 is around 1.8 and 2.0. Genotype identification was carried out using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method[8]. To perform RFLP on ADH2 allele detection, MaeIII Enzyme was used to cut DNA. MaeIII enzyme as much as 1µl was added in a 0.2 ml clean microfuse tube, mixed for a few minutes, then the contents were incubated at 37^{o}C for 15 minutes. Mixing was completed when 2% agarose cells stained with ethidium bromide were digested. MaeIII cleavage sites were forward primer 5'-AATCTTTTCTGAATCTGAACAG-3' and reverse primer 5'-

GAAGGGGGGGTCACCAGGTTG-3'. Where will form indigestible bands in the 92 bp fragment of the ADH2*1 allele, 92, 60 and 30 bp fragments of the ADH2*2 allele, and fragments 60 and 30 bp of the ADH2*3 allele.[11]

Liver function status was analyzed from blood samples inserted into the Plasma Separator Tube (PST), parameters according to the procedure using 4 mL of blood serum for SGOT, SGPT, and GGT analysis.

3. RESULTS AND DISCUSSION

In the ADH2 genotype, subjects were categorized according to general characteristics; the majority of subjects were male (78.3%) and normal weight (58.2%). This study identified the genotypes of 60 research subjects. The majority of NTT ethnic ADH2 genotypes were ADH2*2 (65.0%), followed by ADH2*3 (33.3%) and ADH2*1 (1.7%) (Table 1).

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

ADH2*1				ADH2*2		ADH2*3		n	(%) or median (min-max)	p
Subject		1	1,7%	39	65%	20	33,3%			
Sex/Gender	Men	1	100.0%	29	74.4%	17	85.0%	47	78.3%	0,449
	Women	0	0.0%	10	25.6%	3	15.0%	13	21.7%	
Age			22	23,5 (18-57)		24,5 (19-85)			24 (18-85)	0,536
BMI								20,8 (16,1-31,1)		
BMI Category	Underweight	0	0.0%	5	13.5%	7	41.2%	12	21.8%	0,016*
	Normal weight	1	100.0%	22	59.5%	9	52.9%	32	58.2%	
	Preobese	0	0.0%	0	0.0%	0	0.0%	0	0.0%	
	Obese class I	0	0.0%	8	21.6%	0	0.0%	8	14.5%	
	Obese class II	0	0.0%	2	5.4%	1	5.9%	3	5.5%	
Upper arm circumference			26	26 (20-33)		24 (21-33)			26 (20-33)	0,218
Belly circumference			71	77 (63-102)		73 (62,7-116)			76 (62,7-116)	0,535
Drink Alcohol	Yes	1	100.0%	30	76.9%	13	65.0%	44	73.3%	0,272
	No	0	0.0%	9	23.1%	7	35.0%	16	26.7%	
Drinking frequency	Everyday	0	0.0%	8	27.6%	2	15.4%	10	23.3%	0,057
	2-5x/week	1	100.0%	7	24.1%	1	7.7%	9	20.9%	
	1x/week	0	0.0%	5	17.2%	3	23.1%	8	18.6%	
	1-3x/month	0	0.0%	9	31.0%	5	38.5%	14	32.6%	
	<1x/month	0	0.0%	0	0.0%	2	15.4%	2	4.7%	
Consumption rate	Light-medium	1	100.0%	20	66.7%	11	84.6%			0,296
	High	0	0.0%	6	20.0%	2	15.4%			
	Very high	0	0.0%	4	13.3%	0	0.0%			
Drinking duration (Year)			4	6 (1-30)		6 (1-12)			6 (1-30)	0,746
Regular exercise	Yes	1	100.0%	12	30.8%	8	40.0%	21	35.0%	0,758
	No	0	0.0%	27	69.2%	12	60.0%	39	65.0%	
Smoking	Yes	0	0.0%	15	60.0%	6	66.7%	21	60.0%	0,441
	No	1	100.0%	10	40.0%	3	33.3%	14	40.0%	
Pinang	Yes	0	0.0%	6	24.0%	2	22.2%	8	22.9 %	0,920
	No	1	100.0%	19	76.0%	7	77.8%	27	77.1 %	
SGOT									12 (8-30)	
SGPT									19 (13-39)	
GGT									21 (12-55)	

In the proportion of characteristics with alleles, there was no significant relationship between sex, age, upper arm circumference, belly circumference, drinking alcohol, drinking frequency, duration of drinking, regular exercise, smoking, areca nut, and SGOT with alleles ($p>0.05$). Most patients from ADH2*3 came from underweight (41.2%) compared to ADH2*2 (13.5%) and ADH2*1 (0.0%) with a significant proportion ($p=0.016$) (Table 1).

Table 2: Proportion of ADH2 with SGOT, SGPT, and GGT.

SGOT								
		Normal		High		p	OR	CI 95%
		n	%	n	%			
Type of ADH2	ADH2*1	1	100	0	0.0	0,952	-	-
	ADH2*2	38	97.4	1	2.6	0,567	2,00	0,12-33,76
	ADH2*3	19	95.0	1	5.0			
SGPT								
		Normal		High		p	OR	CI 95%
		n	%	n	%			
Type of ADH2	ADH2*1	1	100.0	0	0.0	0,857	-	-
	ADH2*2	36	92.3	3	7.7	0,325	2,12	0,39-11,60
	ADH2*3	17	85.0	3	15.0			
GGT								
		Normal		High		p	OR	CI 95%
		n	%	n	%			
Type of ADH2	ADH2*1	1	100.0	0	0.0	0,857	-	-
	ADH2*2	38	97.4	1	2.6	0,108	6,71	0,65-69,22
	ADH2*3	17	85.0	3	15.0			

There is no significant proportion between ADH2 genotype and liver function status. Subjects with ADH2*3 had higher SGOT than ADH2*1 and ADH2*2 (p>0.05), but OR=2.00 means that ADH2*3 subjects were at risk of an increase in SGOT of 2.00 times. Subjects with ADH2*3 had higher SGPT than ADH2*1 and ADH2*2 (p>0.05), but OR=2.12 means that ADH2*3 subjects were at risk of an increase in SGPT of 2.12 times. ADH2*3 subjects had higher GGT than ADH2*1 and ADH2*2 (p>0.05), but OR=6.71 means that subjects with ADH2*3 had a 6.71-fold increased risk of GGT (Table 2).

There was no significant proportion between the ADH2 genotype and drinking behavior (p>0.05) (Table 3) and the level of alcohol consumption (p>0.05) (Table 4).

Table 3: Proportion of ADH2 genotypes with drinking behavior.

Type of ADH2	n	Drinking behavior		p
		Yes n (%)	No n (%)	
ADH2*1	1	1 (100)	0 (0)	0,272
ADH2*2	39	30 (76,9)	9 (23,1)	
ADH2*3	20	13 (65,0)	7 (35,0)	

Table 4: The proportion of ADH2 genotype with the level of alcohol consumption.

Alcohol consumption rate	ADH2*1 n (%)	ADH2*2 n (%)	ADH2*3 n (%)	p
Light-Medium	1 (100)	20 (66,7)	11 (84,6)	0,296
High	0 (0,0)	6 (20,0)	2 (15,4)	
Very High	0 (0,0)	4 (13,3)	0 (0,0)	

There is no significant proportion between the ADH2 polymorphism relationship in alcoholic drinkers, non-alcoholic drinkers, and liver function status. ADH2*2 subjects who drank alcohol had higher SGOT than non-drinkers (p>0.05), and ADH2*3 subjects who drank alcohol experienced higher SGOT than non-drinkers (p>0.05). ADH2*2 subjects who drink alcohol have less high SGPT than non-drinkers (p>0.05), but OR=0.15 means that ADH2*2 subjects who drink alcohol are at risk for an increase in SGPT of 0.15 times. ADH2*3 subjects who drink alcohol have a higher SGPT than non-drinkers (p>0.05), but OR=0.27 means that ADH2*3 is at risk of an increase in SGPT of 0.27 times.

ADH2*2 subjects who drink alcohol have higher GGT than non-drinkers (p>0.05), ADH2*3 subjects who drink alcohol have higher GGT than non-drinkers (p>0.05), but OR=1,08 means that ADH2*3 subjects who drink alcohol are at risk of increasing GGT by 1.08 times (Table 5).

Table 5: Proportion of ADH2 polymorphisms in alcoholics, non-drinkers, and liver function status.

SGOT										
				High		Normal		p	OR	CI 95%
				n	%	n	%			
Type of ADH2	ADH2*1	Alcohol	Yes	0	0.0	1	100.0	-	-	-
		Drinker	No	0	0.0	0	0.0			
	ADH2*2	Alcohol	Yes	1	3.3	29	96.7	0,769	-	-
		Drinker	No	0	0.0	9	100.0			
	ADH2*3	Alcohol	Yes	1	7.7	12	92.3	0,650	-	-
		Drinker	No	0	0.0	7	100.0			
SGPT										
				High		Normal		p	OR	CI 95%
				n	%	n	%			
Type of ADH2	ADH2*1	Alcohol	Yes	0	0.0	1	100.0	-	-	-
		Drinker	No	0	0.0	0	0.0			
	ADH2*2	Alcohol	Yes	1	3.3	29	96.7	0,127	0,15	0,02 – 1,47
		Drinker	No	2	22.2	7	77.8			
	ADH2*3	Alcohol	Yes	1	7.7	12	92.3	0,270	0,27	0,03 – 2,47
		Drinker	No	2	28.6	5	71.4			
GGT										
				High		Normal		p	OR	CI 95%
				n	%	n	%			
Type of ADH2	ADH2*1	Alcohol	Yes	0	0.0	1	100.0	-	-	-
		Drinker	No	0	0.0	0	0.0			
	ADH2*2	Alcohol	Yes	1	3.3	29	96.7	0,769	-	-
		Drinker	No	0	0.0	9	100.0			
	ADH2*3	Alcohol	Yes	2	15.4	11	84.6	0,730	1,08	0,12 – 9,89
		Drinker	No	1	14.3	6	85.7			

In multivariate analysis of ADH2 genotypes with smoking habits, ADH2*2 had 1 smoker with high SGOT and 2 non-smokers with high SGPT. The ADH2*3 subjects had 1 non-smoker with high SGT and 1 smoker with high GGT (Table 6).

Table 6: Proportion of smoking with SGOT, SGPT, and GGT in each allele.

SGOT										
			Normal		High		p	OR	CI 95%
			n	%	n	%			
ADH2*1	Smoking	Yes	0	0.0	0	0.0	-	-	-
		No	1	100.0	0	0.0			
ADH2*2	Smoking	Yes	14	93.3	1	6.7	1,000	-	-
		No	10	100.0	0	0.0			
ADH2*3	Smoking	Yes	6	100.0	0	0.0	0,333	-	-
		No	2	66.7	1	33.3			
SGPT									
			Normal		High		p	OR	CI 95%
			n	%	n	%			
ADH2*1	Smoking	Yes	0	0.0	0	0.0	-	-	-
		No	1	100.0	0	0.0			
ADH2*2	Smoking	Yes	15	100.0	0	0.0	0,150	-	-
		No	8	80.0	2	20.0			

ADH2*3	Smoking	Yes	6	100.0	0	0.0	0,333	-	-
		No	2	66.7	1	33.3			
GGT									
			Normal		**High**		**p**	**OR**	**CI 95%**
			n	**%**	**n**	**%**			
ADH2*1	Smoking	Yes	0	0.0	0	0.0	-	-	-
		No	1	100.0	0	0.0			
ADH2*2	Smoking	Yes	15	100.0	0	0.0	1,000	-	-
		No	10	100.0	0	0.0			
ADH2*3	Smoking	Yes	5	83.3	1	16.7	1,000	-	-
		No	3	100.0	0	0.0			

4. DISCUSSION

The majority of the samples were men with normal body weight. According to the general Japanese population, BMI, weekly alcohol consumption, and Fasting Plasma Glucose (FPG) levels were significantly higher in 492 men compared to 183 women. The majority of the genotypes of the Austro-Melanesian NTT ethnic group were polymorphic heterozygous ADH2*2 (Table 1). ADH2*2 allele is more dominant in the Southeast Asian population than in the white and Indian populations. [3,13,7]

In ADH2*3 subjects the risk of an increase in SGPT was 2.12 times (Table 2), according to a study of East Indian populations, Afro-Trinidadian and Indo-Trinidadian ethnicities, ADH2*3 was significantly less addicted to alcohol ($p=0.018$), low alcohol ($p<0.05$), and alcohol drinkers significantly increased the SGPT value ($p<0.05$).[4]

In ADH2*3 subjects the risk of an increase in GGT was 6.71 times (Table 2), this is different from the study in the Japanese population the percentage of alcohol drinkers, alcohol consumption, SGOT, SGPT, and Gamma-GTP were higher in ADH2*1/1 than the ADH2*1/2 or ADH2*2/2 groups ($p<0.05$).[18]

There is no significant proportion between the ADH2 genotype and alcohol-drinking behavior (Table 3), similar to the study of Zhang, *et al.* (2013) that the ADH2 genotype was not associated with drinking status or frequency, or other risk factors such as smoking, physical activity, education, occupation, age, BMI, total cholesterol, LDL, triglycerides, and fasting blood sugar.[20] However, there are studies to the contrary, namely in the Jewish population ADH2*2 is predicted to have less alcohol and is protective of heavy drinkers.[9]

ADH2*3 subjects who drink alcohol are at risk of an increase in SGPT of 0.27 times and the risk of an increase in GGT is 1.08 times (Table 5), this is the opposite of a study in 1588 Japanese male alcohol drinkers aged >40 years, the last drinking alcohol for 14 days found that higher urine ketone levels were associated with higher serum total bilirubin, SGOT, SGPT, and GGT levels, where ADH2*1 subjects who consumed whiskey or shocu, hypoglycemia, low BMI, and smoking were significant in determining ketosis.[18]

There was no significant proportion in the multivariate analysis between smoking behavior and liver function status for each ADH2 allele (Table 6). This is consistent with a study in a population in Japan that there was no statistically significant proportion in the distribution of ADH2 genotypes according to sex, age, drinking status, or smoking, although these drinkers were more common among the ADH2 Arg/Arg (ADH2*1) genotypes than among the ADH2 genotypes.[12]

GGT has the highest sensitivity of 42-86% and the lowest specificity of 40-84% when compared to SGOT and SGPT, so in this study, the OR value of the increase in GGT was higher than SGOT and SGPT.[1,5] Oxidative metabolism of ethanol and methanol always involves two enzymes and different functions, namely ADH and ALDH.[15] Alcohol metabolism goes through a complex process of absorption, distribution, and elimination wherein the liver metabolizes alcohol into acetaldehyde by ADH, cytochrome P450-2E1 (CYP450E1) and catalase, then acetaldehyde is converted to acetate and water by ALDH2.[6] Alcohol is a process that is played by many enzymes, and in this research it will be linked with other enzyme researchs, so that comprehensive research results are obtained.

5. CONCLUSSION

This study showed that ethnic NTT had the most genotypes with the order ADH2*2 (65.0%), ADH2*3 (33.3%), and ADH2*1 (1.7%). From 1 ADH2*1 subject, all of them were alcoholics, from 39 ADH2*2 subjects there were 30 alcohol drinkers (76.9%), and from 20 ADH2*3 subjects there were 13 alcohol drinkers (65%). ADH2*3 subjects who drink alcohol are at risk of an increase in SGOT by 2 times, an increase in SGPT by 2.12 times, and an increase in GGT by 6.71 times in subjects with other genotypes. This study demonstrates a genetic susceptibility to decreased liver function in alcohol drinkers of NTT ethnicity.

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DECLARATION OF INTEREST

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