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Alcohol dehydrogenase 1C (*ADH1C*) polymorphism is significantly associated with kidney function status in Nusa Tenggara Timur ethnicity: A cross-sectional study

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ABSTRACT Excessive alcohol consumption is harmful to many human organs, but the association with kidney function is still controversial. The disagreement in findings might be caused by *ADH1C* polymorphism's influence on alcohol metabolism rate. This study aims to determine the correlation between *ADH1C* polymorphism and kidney function status in Nusa Tenggara Timur (NTT) ethnicity, a population with highly prevalent alcohol consumption in Indonesia. We conducted a cross-sectional study of 76 subjects, who are natives of NTT, Indonesia. The genotyping of extracted DNA for *ADH1C* was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using restriction endonuclease SspI. Kidney function status was defined by serum urea level and estimated glomerular filtration rate (eGFR) that had been categorized according to percentiles. The correlation with the ADH1C allele was analyzed using chi-square tests. The genotype of *ADH1C* in NTT ethnicity was *ADH1C*1/*2* (51.3%), *ADH1C*2/*2* (47.4%) and *ADH1C*1/*1* (1.3%). The results showed that the population had the *ADH1C*2* (73.03%) and the *ADH1C*1* (26.97%) allele. There was a significant association between ADH1C polymorphism and eGFR among NTT ethnicity (p=0.005) when eGFR was analyzed at the 25th percentile (74.75 mL/minute/1.73m^2). However, we found no associations when eGFR was analyzed at 50th (p=0.571) and 75th (p=0.335) percentiles. The odds ratio shows that having the *ADH1C*1/*2* genotype escalates the probability of declining eGFR 6.620 times compared to *ADH1C*2/*2* (95% CI: 1.539-28.478), after adjusted for smoking behavior. We found no association between ADH1C polymorphism and serum urea level (p=0.123, 0.421, and 0.335). The majority of NTT ethnicity have the *ADH1C*1/*2* genotype. Populations with *ADH1C*1/*2* have higher odds ratio for eGFR below 74.75 mL/minute/1.73m^2 than those with *ADH1C*2/*2* genotype. There was no association between *ADH1C* polymorphism and serum urea levels.

1. Introduction

Decreased kidney function, although minor, could lead to worsening of patient outcomes in some particular conditions.[1] This alteration could progress to chronic kidney disease (CKD), or even to chronic kidney failure.[2] In Indonesia, data from 2017 showed that hemodialysis therapy for chronic kidney failure had financially burdened the country enormously, equal to more than USD $200,000,000 in a given year.[3]

One of the factors that could affect kidney function is alcohol consumption. Alcohol abuse is the cause of more than 3,000,000 deaths annually.[4] In Indonesia, more than 800,000 people consumed alcohol in 2018, and Nusa Tenggara Timur (NTT) had the highest number of excessive alcohol consumption rate.[5] Home-made liquor that is traditionally produced remains essential parts in many rituals in NTT.[6]

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The effect of alcohol on kidney function is still controversial. A meta-analysis study showed that alcohol had a protective effect on the kidney,[7] while another study showed that excessive alcohol consumption could lead to kidney hyperfiltration, an early marker of kidney problems.[8] We need to define the factors that affect the kidney vulnerability towards alcohol, so the results could be emphasized in the educational programs regarding alcohol preventative measures in society. One of the factors that we presume can vary human body responses to alcohol consumption is the *ADH1C* polymorphism, the gene that encodes the ADH1C enzyme that has a major role in alcohol metabolism,[9] by directly influencing the alcohol metabolism rate. Polymorphism is linked to ethnicity, and there is still no study on the correlation of *ADH1C* polymorphism to kidney function status in the particular NTT ethnicity previously published. Therefore, we conducted this study among the NTT ethnic group, a highly prevalent alcohol consumption population in Indonesia.

2. Method

This cross-sectional study was conducted in Bakunase, Kupang, NTT province in Indonesia, in October 2019. This sub-district was chosen because of the easy access and coordination with the local community health center. All of the subjects enrolled were above 18 years old and belonged to indigenous NTT ethnicity, which was proved by his or her past three generations who all lived in NTT. The subjects were recruited and screened for the inclusion criteria by the local community health center. Approval of this study was given by the Medical and Health Research Ethics Committee of Faculty of Medicine, Public Health and Nursing at Universitas Gadjah Mada (letter number KE/FK/0830/EC/2019). Informed consent was obtained from every subject. We excluded subjects who were undergoing dialysis therapy or suffered from severe kidney-related diseases. Information regarding health conditions and smoking habits was gained from the subjects' interviews.

Seven milliliters of venous blood were drawn from 76 subjects, and divided into 2 vacutainers. The first vacutainer was a serum-separator tube. The blood was utilized to assess the serum creatinine and urea levels, using an automatic chemistry analyzer (Cobas® 6000, Roche, Rotkreuz, Switzerland). The second vacutainer was coated with ethylenediaminetetraacetic acid (EDTA). The blood in the second vacutainer was prepared for DNA analysis. DNA was isolated using a Wizard® Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA). The integrity and purity of isolated DNA were checked by a nano spectrophotometer with the expected result of A260:A280 with around 1.8. Genotyping was done using the PCR-RFLP method as previously described with some modifications.[10] The recognition sequences were: ADH3321 (5'-GCTTTAAGAGTAAATATTCTGTCC-3') and ADH3351 (5'-AATCTACCTCTTTCCAGAGC-3') as forward and reverse primers, respectively. PCR was performed using a 30 µl reaction mixture, comprised of 3µl isolated DNA, 15 µl of the DreamTaq Green PCR master mix (Thermo Fisher, Massachusetts, USA) , 8 µl of water, and 2 µl of each primer. The PCR conditions involved initial denaturation at 95^0C for 5 min, followed by 34 cycles of denaturation, annealing, and elongation at 94^0C for 1 min, 55^0C for 1 min, and 72^0C for 1 min. The last step was the final elongation at 72^0C for 10 min. PCR product was then digested with FastDigest™ SspI kit (Thermo Fisher, Massachusetts, USA) that has recognition site at 5'…AAT*ATT…3' and 3'…TTA*TAA…5' for forward and reverse primer, respectively. The incubation was done at 37^0C for 20 min. The product then underwent an electrophoresis process and visualized under UV light. The *ADH1C**1/*1 shows an undigested band at 146 bp, the *ADH1C*1/*2* shows three bands at 146, 83, and 63 bp, and *ADH1C**2/*2 shows two bands at 83 and 63 bp.

Genotypic frequencies were calculated and crude odds ratios (OR) were determined using chi-square tests with 95% confidence intervals (CI) if the requirements were met. The alternatives were the Fisher exact or Kruskal-Wallis tests. Kidney function status was analyzed according to 25^{th}, 50^{th}, and 75^{th} percentiles of eGFR and serum urea levels.

Estimated GFR was calculated based on serum creatinine levels using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. To determine the effect modifiers or confounding

variables involved bivariate analysis (chi-square) to suggest potential variables, continued with multivariate analysis using logistic regression to adjust the OR.

3. Result

Among 76 study subjects, the most frequent genotype was *ADH1C**1/*2 (51.3%), followed by *ADH1C**2/*2 (47.4%) and *ADH1C**1/*1 (1.3%), respectively. Hence, the majority of the population had the *ADH1C**2 allele (73.03%) (Table 1). There was no statistically significant difference between observed and expected results of genotype distribution were according to the Hardy-Weinberg equilibrium equation (p=0.653).

The demographical and clinical characteristics that consisted of sex, age, body mass index (BMI), smoking habit, and hypertension were similar between *ADH1C*1/*2* and *ADH1C*2/*2* groups. A comparison could not be made between *ADH1C**1/*1 with other *ADH1C* genotypes because there was only 1 subject in the group (Table 2).

There was a significant association between *ADH1C* genotype and eGFR when analyzed at the 25th percentile (p=0.005), which was at 74.75 ml/min/1.73m^2 (Table 3). When *ADH1C**2/*2 was used as a reference genotype, only *ADH1C*1/*2* was correlated significantly, with a crude OR of 4.480 (95% CI: 1.312-15.299) (Table 4). After adjusted for smoking habit, having *ADH1C*1/*2* showed an OR of 6.620 compared to *ADH1C**2/*2 (95% CI: 1.539-28.478). Smoking habit modified the association with an OR of 0.191 (95% CI: 0.051-0.708).

There were no significant results concerning the correlation between *ADH1C* and eGFR when assessed at the 50th (p=0.571) and 75th percentiles (p=0.335), which were at 92.90 ml/min/1.73m^2, and 116.82 ml/min/1.73m^2, respectively (Table 3). There were no associations between *ADH1C* and serum urea levels, in all percentiles (Table 5).

4. Discussion

The most frequent *ADH1C* genotype in this study was the heterozygote polymorphic type, that has similarity with Papua ethnicity that is a Melanesian

Table 1. Genotype distribution and allele frequency of *ADH1C* in NTT ethnicity

Genotype	n (%)	Allele	n (%)
*ADH1C*1/*1*	1(1.3)	*ADH1C*1*	41 (26.97)
*ADH1C*1/*2*	39 (51.3)	*ADH1C*2*	111 (73.03)
*ADH1C*2/*2*	36 (47.4)		
Total	76 (100)		152 (100)

Table 2. Demographical and clinical characteristics of NTT ethnicity according to *ADH1C* genotype

Characteristics	ADH1C		
	1/*1 **n (%)**	***1/*2*** **n (%)**	***2/*2*** **n (%)**
Sex			
Male, n=57	1 (100)	28 (71.8)	28 (77.8)
Female, n=19	0 (0)	11 (28.2)	8 (22.2)
Age (years old)			
<22, n=31	0 (0)	14 (35.9)	17 (47.2)
≥22, n=45	1 (100)	25 (64.1)	19 (52.8)
BMI (kg/m^2)			
<25, n=56	1 (100)	29 (74.4)	26 (72.2)
≥25, n=20	0 (0)	10 (25.6)	10 (27.8)
Cigarette Smoking			
Yes, n=45	1 (100)	24 (61.5)	20 (55.6)
No, n=31	0 (0)	15 (38.5)	16 (44.4)
Hypertension			
Yes, n=19	0 (0)	9 (23.1)	10 (27.8)
No, n=57	1 (100)	30 (76.9)	26 (72.2)

BMI: body mass index.

Table 3. The association between *ADH1C* genotype and eGFR

ADH1C	**n**	**eGFR-25**		*p*
		<74.75 n (%)	**≥74.75 n (%)**	
**1/*1*	1	1 (100)	0 (0)	0.005
**1/*2*	39	14 (35.9)	25 (64.1)	
**2/*2*	36	4 (11.1)	32 (88.9)	
ADH1C	**n**	**eGFR-50**		***p***
		<92.90 n (%)	**≥92.90 n (%)**	
**1/*1*	1	1 (100)	0 (0)	0.571
**1/*2*	39	20 (51.3)	19 (48.7)	
**2/*2*	36	17 (47.2)	19 (52.8)	
ADH1C	**n**	**eGFR-75**		***p***
		<116.82 n (%)	**≥116.82 n (%)**	
**1/*1*	1	1 (100)	0 (0)	0.335
**1/*2*	39	27 (69.2)	12 (30.8)	
**2/*2*	36	29 (80.6)	7 (19.4)	

eGFR values are in ml/min/1.73m²

Table 4. The crude odds ratio of *ADH1C* genotype in correlation with eGFR-25

ADH1C	**n**	**eGFR-25**		***p***	**Crude OR**
		<74.75 n (%)	**≥74.75 n (%)**		
**1/*1*	1	1 (100)	0 (0)	0,135[1]	-
**1/*2*	39	14 (35.9)	25 (64.1)	0,012[2]	4.480
**2/*2*	36	4 (11.1)	32 (88.9)	Ref.	Ref.

[1]By Fisher's Exact test. [2]By chi-square test.

racial profile.[11] The genotype that was also widely found in NTT ethnicity was *ADH1C**2/*2. This homozygote polymorphic type is considered the majority genotype in Javanese ethnicity, an Austronesian racial profile. Java ethnicity also has *ADH1C**2 alleles dominating, as found in this study.[10] These comparable findings might be due to modern human migration in ancient times. Nusa Tenggara Timur ethnicity in their ancestors were derived from transethnic interactions of Austronesian peoples that came from western and Melanesian peoples from eastern regions of Indonesia who gave rise to the Austro-Melanesian racial profile of the indigenous NTT ethnicity.[12] These findings were different from studies that were conducted in Europe and China that showed *ADH1C**1 is the most frequent allele carried by the populations.[13,14]

The genotype distribution in NTT ethnicity was in the Hardy-Weinberg equilibrium. Therefore, we conclude that our study subjects met the Hardy-Weinberg equilibrium assumptions, to wit: random mating, and no genetic mutations nor natural selections happened.[15]

The demographical and clinical characteristics that consisted of sex, age, BMI, smoking habit, and hypertension had balanced distribution between *ADH1C**1/*2 and *ADH1C**2/*2 groups. Hence, these two groups were comparable. We could not include *ADH1C**1/*1 in the comparison due to the lack of subjects in the mentioned group.

Kidney function status in this study was presented in the declining eGFR and elevating serum urea levels

Table 5. The association between *ADH1C* genotype and serum urea levels

ADH1C	n	Serum Urea Level-25		p
		≥20.1 n (%)	<20.1 n (%)	
*1/*1	1	1 (100)	0 (0)	0.123[1]
*1/*2	39	11 (28.2)	28 (71.8)	
*2/*2	36	6 (16.7)	30 (83.3)	

ADH1C	n	Serum Urea Level-50		*p*
		≥29.1 n (%)	<29.1 n (%)	
*1/*1	1	1 (100)	0 (0)	0.421[1]
*1/*2	39	20 (51.3)	19 (48.7)	
*2/*2	36	16 (44.4)	20 (55.6)	

ADH1C	n	Serum Urea Level-75		*p*
		≥36.1 n (%)	<36.1 n (%)	
*1/*1	1	1 (100)	0 (0)	0.335[1]
*1/*2	39	27 (69.2)	12 (30.8)	
*2/*2	36	29 (80.6)	7 (19.4)	

Serum urea levels are in mg/dl. [1]By Kruskal Wallis test.

that were analyzed at 25th, 50th, and 75th percentiles. The only significant association was found between *ADH1C* genotype and eGFR while analyzed at the 25th percentile (eGFR below 74.75 ml/min/1.73m^2). Estimated GFR less than 74 ml/min/1.73m^2 has increased mortality rate in populations at risk if albumin-to-creatinine urine ratio was more than 30 mg/g.[16]

Crude OR of 4.480 was generated from comparing *ADH1C**1/*2 to *ADH1C**2/*2 for declining eGFR at the 25th percentile. To ensure there were no confounding or effect modifier variables, bivariate and multivariate analyses were done on all of the external variables. The OR was adjusted for smoking habit and yielded 6.620 times the probability of having declining eGFR below 74.75 ml/min/1.73m^2 for *ADH1C**1/*2 compared to *ADH1C**2/*2 carriers.

This finding is probably due to the heterodimer ADH1C enzyme that is encoded by *ADH1C**1/*2. This enzyme has γ_1 subunit with a faster alcohol to acetaldehyde metabolism rate compared to the γ_2 subunit, which is encoded by the *ADH1C**2 allele.[17] The faster metabolism rate increases the acetaldehyde accumulation risk, although slower acetaldehyde to acetate metabolism rate is needed for this accumulation to occur. Acetaldehyde, which is toxic, could induce some pathogenic symptoms,[18] and is suspected to harm the kidney. Previous studies found that having *ADH1C**1 allele increased the risk for liver cirrhosis, alcoholic pancreatitis, and breast cancer in the alcoholic population.[19,20]

Smoking habit was an effect modifier variable in this study. The inverse OR between smoking habit and declining eGFR could be related to the previous study by Wang et al. This study adduced that smokers had larger kidney volume.[21] However, to conclude that smoking has beneficial value towards kidney function should not be recklessly made. A 10-year interval cohort study in living kidney donor populations found that smoking lowered postoperative eGFR, thus it increased chronic kidney disease risk.[22]

There was no association between *ADH1C* polymorphism and serum urea level, as found in an animal study that compared alcohol consumption and serum urea levels.[23] On the other hand, the previous study in humans showed that drinkers tended to have higher serum urea levels,[24] though our study suggests that this association is not correlated to *ADH1C* polymorphism.

The statistically insignificant result might occur because serum urea level is an unreliable indicator in kidney function status since it is easily changed by internal and external factors, i.e. protein intake, muscle catabolism, and limited blood absorption from gastrointestinal tract bleeding.[25] Therefore, we recommend a more accurate kidney function indicator, such as serum cystatin c levels or moreover, radioisotope markers.

However, our study had some limitations in the sampling methods. We suggest using a larger sample size in the next research regarding *ADH1C* in the NTT ethnic group so that subjects in the *ADH1C**1/*1 group possibly meet the minimal sample size to be analyzed. This study also could be improved with the random sampling method so that it could accurately represent the actual population.

Conclusions

This study found that the majority of ethnic NTT have the *ADH1C**1/*2 genotype. Having *ADH1C**1/*2

tended to have 6.620 times lower eGFR when the cut-off was at 74.75 ml/min/1.73m^2 compared to *ADH1C**2/*2, after adjusted for the smoking habit. This study showed the genetic vulnerability of alcohol-related declining kidney function in most of the ethnic NTT population. Hopefully, these results will provide an evidence-based stimulus to encourage alcohol cessation or moderation in the NTT population.

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Conflict of interests

The authors declare that they have no conflict of interests.

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