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Alcohol dehydrogenase activity and alcohol dehydrogenase 3 (ADH3) gene polymorphism in alcoholics and non-alcoholics Javanese Indonesian subjects

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ABSTRACT

Alcohol is an addictive substance that is often misused worldwide, including in Indonesia. Ninety percent of the alcohol that enters the body will be metabolized in the liver using the alcohol dehydrogenase (ADH) enzyme. It is important to determine the activity of ADH enzyme and ADH3 gene polymorphism on alcoholics and non-alcoholics in Yogyakarta, Indonesia. The aim of the study is to determine ADH activity and identify ADH3 gene polymorphism of alcoholics and non-alcoholics in Yogyakarta, Indonesia. This study was an observational study with a cross-sectional design method. Blood samples were taken from 71 Javanese alcoholics and 71 non-alcoholics of Javanese descent in Yogyakarta, Indonesia. The participants were initially requested to sign an informed consent form. Examination of ADH enzyme activity used the spectrophotometry method and ADH3 gene polymorphism was assessed with PCR-RFLP using *Ssp* I restriction enzyme. The activity of ADH enzyme in all individuals appeared to be a slower type. The average of the ethanol value of alcoholics and non-alcoholics were 0.05554 mM and 0.0758 mM respectively. Gene type of alcoholics were ADH3*2(75.4%), ADH3*1/3*2(21.5%), and ADH3*1(3.1%), and non-alcoholics were ADH3*2(88.6%), ADH3*1/3*2(10.0%), and ADH3*1(1.4%). There were no significant differences between the activity of ADH with polymorphism of ADH3 gene in either alcoholics and non-alcoholics ($p > 0,05$). Conclusion: The activity of ADH enzyme in all participants appeared to be a slower type. Most of the ADH3 gene polymorphism of alcoholics and non-alcoholics were both ADH3*2 (75.4% and 88.6%). There was no differences of ADH enzyme activity with ADH3 gene polymorphism between alcoholics and non-alcoholics of Javanese population in Yogyakarta, Indonesia.

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ABSTRAK

Alkohol merupakan zat adiktif yang sering disalahgunakan di dunia termasuk di Indonesia. Alkohol yang masuk kedalam tubuh 90% dimetabolisme di dalam hati menggunakan enzim alcohol dehidrogenase (ADH). Hal ini perlu dikaji aktivitas enzim ADH dan polimorfisme gen ADH3 pada peminum alkohol dan bukan peminum alkohol di Yogyakarta, Indonesia. Tujuan penelitian ini untuk menentukan aktivitas enzim ADH dan mengidentifikasi polimorfisme gen ADH3 pada peminum alkohol dan bukan peminum alkohol di Yogyakarta, Indonesia. Metode penelitian ini adalah observasional dengan rancang penelitian *crossectional.* Sampel darah vena cubiti diambil dari subyek 71 peminum alkohol dan 71 bukan peminum alkohol suku Jawa di Yogyakarta, Indonesia, setelah menandatangani *informed consent*. Pemeriksaan aktivitas enzim ADH dengan metode Spektrofotometri dan pemeriksaan polimorfisme gen ADH3 dengan metode PCR-RLFP menggunakan enzym restriksi *Ssp* I. Aktivitas enzim ADH semua subyek tipe lambat dengan rata-rata kadar etanol pada peminum alkohol dan bukan peminum alkohol berturut turut adalah 0,05554 mM dan 0,0758 mM. Tipe gen pada peminum alkohol *ADH3*2*(75,4%), *ADH3*1/3*2*(21,5%) dan *ADH3*1*(3,1%), dan pada bukan peminum alkohol *ADH3*2*(88,6%), *ADH3*1/3*2*(10,0%) dan *ADH3*1*(1,4%). Tidak ada perbedaan aktivitas enzim ADH dengan polimorfisme gen ADH3 antara peminum alkohol dan bukan peminum alkohol, nilai $p > 0,05$. Sebagai kesimpulan, aktivitas enzim ADH pada semua subyek mempunyai tipe lambat. Sebagian besar adalah tipe gen *ADH3*2* pada peminum alkohol dan bukan peminum alkohol (75,4% dan 88,6%). Tidak terdapat perbedaan bermakna aktivitas enzim ADH dengan polimorfisme gen ADH3 antara peminum alkohol dan bukan peminum alkohol, suku Jawa, di Yogyakarta Indonesia.

Keywords: ADH activtity - ADH3 gene polymorphism – alcoholics - non-alcoholics- Javanese ethnics

INTRODUCTION

The abuse of alcohol leads to the damage of many organs i.e. the liver, pancreas, gastric mucous membrane, and cerebral tissue resulting in the loss of behavior control.[1] To much alcohol consumption may affects the vital body organs especially in the liver, because 80% of the alcohol is metabolized in the liver. However, each individual has varying responses to alcohol exposure, which is caused by the polymorphism of liver enzymes that metabolize alcohol. This enzyme polymorphism affects the metabolism of alcohol in the body and influences an individual's susceptibility for becoming addicted to alcohol. Individuals who have slow-activity of alcohol dehydrogenase (ADH) enzyme to convert alcohol to acetaldehyde eventually will increase their risk of alcoholism. While also increasing risk, the fast-activity of ADH enzyme is likely to accumulate a toxic acetaldyhede in the body.[2,3]

Genetic polymorphism of the ADH3* enzyme is associated with a condition that slowly oxidizes alcohol and is reported to result in an inability to tolerate alcohol, higher levels of high-density lipoprotein (HDL) and a decrease the risk of myocardial infarction.[4] Other studies have reported that genetic polymorphism of the ADH3*2 enzyme allele increases the risk of alcoholism in Mexican-American men and protects against the negative consequences of chronic pancreatitis.[1,5] In addition, further studies have reported that genetic polymorphism of ADH2*2 and ADH3*1 enzymes decreases the risk of alcoholism and that polymorphism of ADH3*1/*1 enzyme augments the risk of colorectal adenoma.[6,7]

Alcohol is widely misused in Indonesia, however no studies have been published about ADH enzyme activtity and type of polymorphism of *ADH3* gene in alcoholics and non-alcoholics in Indonesian subjects. For this research, an alcoholic is operationalized as a person who regularly consumes alcohol and has negative effects over time in their body. Alcohol contributes to more than 60 known diseases including liver cirrohsis, chronic pancreatitis, and myocardial infarction and has many other negative impacts. Hence, there is an urgent need to further study the correlation of ADH3 enzyme activtity and genetic polymorphism of the *ADH3* gene in alcoholics and non-alcoholics of Javanese ethnics in Indonesia.

MATERIALS AND METHODS

Subjects

From November 2014 to July 2015, a cross-sectional analytic study was conducted. Seventy-one adult alcoholics and 71 non-alcoholics of Javanese ethnics participated in the study. Before data were collected, all participants were asked to sign informed consent and the study was approved by the Medical and Health Research Ethic Committee of Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta. Subject characteristics such as gender, age, medical history, alcohol drinking history referring to Alcohol Dependence Score (ADS) instructions were identified and listed in the questionnaire.

Determination ADH enzyme activity and gene polymorphism of ADH3

Six mililiter of blood samples were collected from each participants. ADH enzyme activity were assessed with modified alcohol examination method enzymatically using spectrophotometer.[8] The assessment of ADH3 gene polymorphism in leucocytes DNA used the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. Recognition sequences for amplification were ADH3 321 (5′-GCTTTA AGAGTAAATATTCTGTCCCC-3′) and ADH3 351 (5’-AATCTACCTCTTT CCAGAGC-3′).[9]

The total PCR reaction mixture of 25 µL contained: 0.5 µL recognition sequences, 1.0 µL of DNA, 0.2 µL of dNTP, 2.5 µL of PCR buffer, 0.25 µL of Tag polymerase, 1.25 µL of 50 Mm $MgCl_2$, and 18.80 µL of H_2O. The PCR method used for pre-denaturation of DNA was 2 min at 96 ºC, followed by 20 cycles at 94 ºC for 1 min, 64 ºC for 1 min, 70 ºC for 1.5 min, and then 10 cycles at 92 ºC for 1 min, 64 ºC for 1 min, and 70 ºC for 1.5 min. To perform RFLP for ADH3 allele detection, aliquots of the amplified DNA products were digested with *Ssp*I enzyme at 37 ºC for 16 hour (overnight). Digestion products were subsequently run through electrophoresis. The *Ssp*I recognition sites (cutting sites) were the forward primer 5′...AAT*ATT...3′…and reverse primer 3′...TTA*TAA...5′.[10] The result of the genotype pieces can be seen in FIGURE 1.

Data analysis

Data were presented as mean ± standard deviation (SD) or as median (minimum-maximum) depending on the types of data. The mean difference between groups was tested using unpaired t-test. The differences between groups were considered statistically significant if a p value < 0.05.

RESULTS

The characteristics of subjects were presented in TABLE 1. The mean age of alcoholics and non-alcoholics was not

significantly different (p= 0.062). Most of the alcoholics were male (87.3%, p=0.01), the median period of alcohol consumption was 16 years, half of them had daily frequent drinking behavior (50.7%), most of the types of alcohol consumed were categorized as non-combination (73.24%).

TABLE 1. Characteristics of subjects alcoholics and non-alcoholics of Javanese people in Yogyakarta, Indonesia

Variable	Alcoholics	Non- alcoholics	p
Age (mean ± SD)	44.27 ± 13.57	48.73 ± 14.29	> 0.05
Gender (n/%)			
• Man	62 (87.32)	28 (41.80)	< 0.05
• Female	9 (12.68)	39 (58.20)	
Duration of use (years)			
• Minimal	1		
• Median	16		
• Maximum	49		
Frequency of drinking (n/%)			
• Day	36 (50.70)		
• Week	24 (33.80)		
• Month	6 (8.45)		
• Sometimes	5 (7.04)		
Type alcohol (n/%)			
• Combination	19 (26.76)		
• Non-combination	52 (73.24)		

Types of gene polymorphism of ADH3 pieces using enzymes Ssp1 result can be seen in FIGURE 1. The relation between age and alcohol consumption duration can be seen in FIGURE 2. There is a significant relationship between age and duration of alcohol consumption ($p < 0.001$).

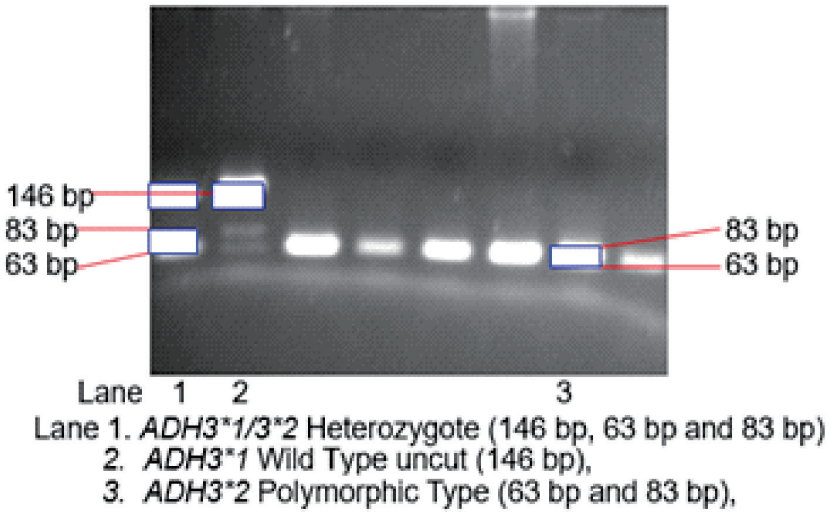

FIGURE 1. Schematic determination of allele gene ADH3

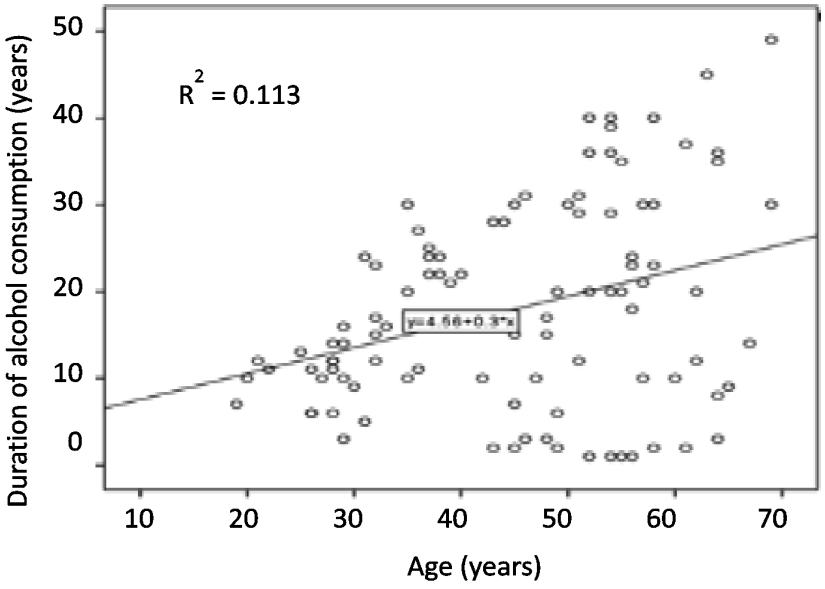

FIGURE 2. Relations between age and alcohol consumption duration

ADH enzyme activity of alcoholics and non-alcoholics can be seen in TABLE 2. ADH activity of non-alcoholics was higher than alcoholics, however this difference couldn't be analyzed statistically due to slow activity in both alcoholics and non-alcoholics (Km ≤ 0.6 mM). The averages of ADH activity of alcoholic and non-alcoholics were 0.05554 mM and 0.0758 mM, respectively.

TABLE 2. ADH activity of alcoholics and non-alcoholics of subjects (ethanol concentration in mM)

ADH activity	Alcoholics	Non-Alcoholics
Number of subjects (n)	71	71
Minimum activity	0.00	0.00
Maximum activity	0.27	0.34
Median	0.0334	0.0427
Mean	0.05554	0.0758
Standard Deviation	0.06253	0.07881

The distribution of ADH activity of alcoholics and non-alcoholics can be seen in FIGURE 3 and 4, respectively.

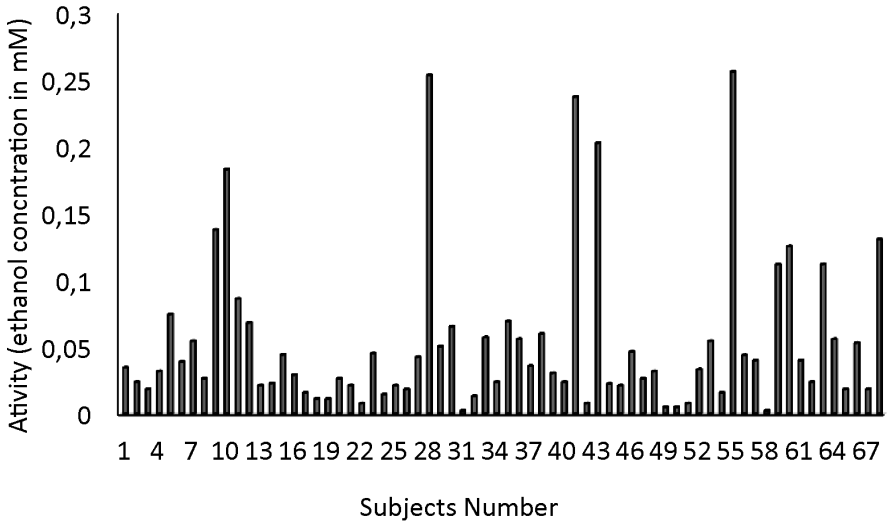

FIGURE 3. ADH activity of alcoholics subjects

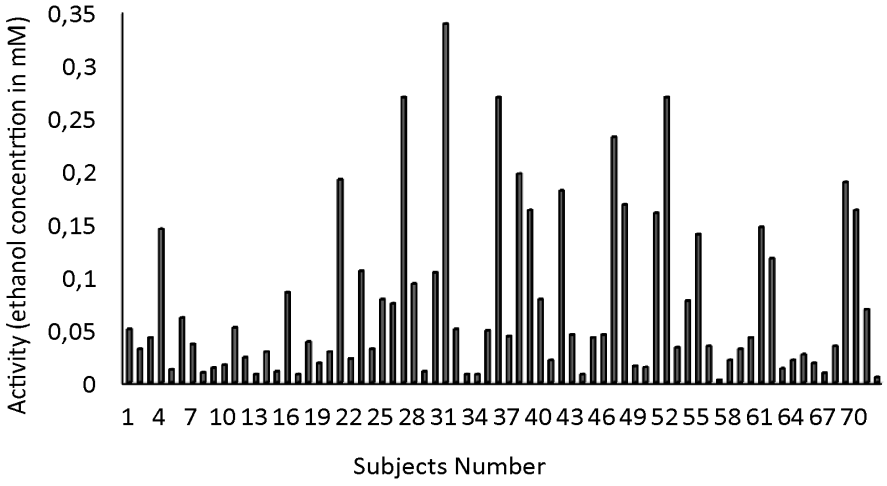

FIGURE 3. ADH activity of non-alcoholics subjects

The distribution of ADH activity based on the group low (≤ 0.1 mM), moderate (0.11-0.20), and high (≥0.21 mM) among alcoholics and non-alcoholics is shown in FIGURE 5.

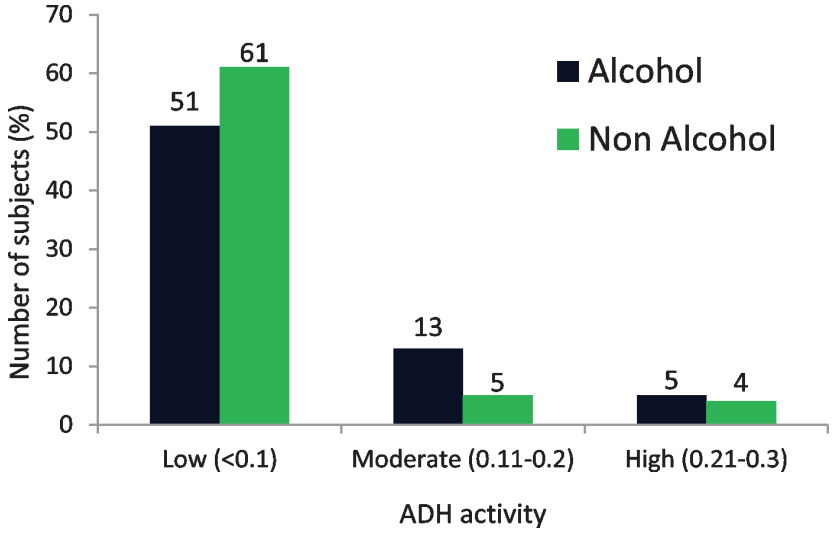

FIGURE 5. Total distribution of ADH activity on alcoholics and non-alcoholics

ADH3 polymorphism gene type with ADH activity among alcoholics and non-alcoholics of Javanese ethnics in Yogyakarta is shown in TABLE 3.

TABLE 3. ADH3 polymorphism type with ADH activity in alcoholics and non-alcoholics of Javanese ethnics in Yogyakarta, Indonesia

Variable	ADH3			
	Wild type *ADH3*1* n(%)	Polymorphic Type *ADH3*2* n(%)	Heterozygote *ADH3*1/3*2* n(%)	Total n(%)
Slow-rate activity (<0.6mM)	3(2.2)	111(82.2)	21(15.6)	135(100.0)
Total	3(2.2)	111(82.2)	21(15.6)	135(100.0)

The most common polymorphic gene type was ADH3*2 (82.2 %). ADH activity was divided based on above or below mean value with ADH3 polymorphism gene type among either alcoholics and non-alcoholics and can be seen in TABLE 4. The result of both alcoholics and non-alcoholics showed that most have ADH3*2 polymorphic type, which was 49 (75.4%) and 62 (88.6%), respectively. However, there was no significant difference statistically between ADH activity with ADH3 polymorphism type, either in alcoholics (p=0.359) and non-alcoholics (p=0.172), or between alcoholics and non-alcoholics (p =0.377).

TABLE 4. *ADH3 polymorphism gene type with* ADH activity (low and high) of alcoholics and non-alcoholics subjects

Variable/Polymorphism type	ADH activity			p
	Low (≤mean) n (%)	High (>mean) n (%)	Total n (%)	
Alcohlics				
Wild Type (*ADH3*1*)	2(100.0)	0(0.0)	2(3.1)	
Polymorphic (*ADH3*2*)	31(63.3)	18(36.7)	49(75.4)	>0.05
Heterozygote(*ADH3*1/3*2*)	8 (57.1)	6(42.9)	14(21.5)	
Total	41(63.1)	24(36.9)	65(100.0)	
Non-alcoholics				
Wild Type (*ADH3*1*)	1(100.0)	0(0.0)	1(1.4)	
Polymorphic (*ADH3*2*)	49(79)	13(21.0)	62(88.6)	>0.05
Heterozygote (*ADH3*1/3*2*)	7(12.3)	0(0.0)	7(10.0)	
Total	57(81.4)	13(18.6)	70(100.0)	
Alcoholics-non-alcoholics				
Wild Type (*ADH3*1*)	3(100.0)	0(0.0)	3(2.2)	
Polymorphic (*ADH3*2*)	80(72.1)	31(27.9)	111(82.2)	>0.05
Heterozygote (*ADH3*1/3*2*)	15(71.4)	6(28.6)	21(15.6)	
Total	98(72.6)	37(27.4)	135(100.0)	

DISCUSSION

Alcohol consumption was more common among male participants, which may be associated with the cultural practice that men more often take part in activities outside the home compared to women. After the data was analyzed through logistic regression analysis, it was concluded to be significantly different between alcoholics and non-alcoholics in men and men are more likely than women by 8.176 times to consume alcohol (CI 95% 4.079-16.386). Men also have a ratio of alcohol-related burden of disease at 7.4%, much higher compared to women at 1.4%.[11] From 71 subjects (100%) included in the study, it was found that 9 subjects (12.8%) were women. This number indicates that the current use of alcohol in the Javanese community is changing. In fact, during the sampling from one couple, both are found to consume alcohol regularly while even carrying a toddler who is still breast-feeding. Such situation is very alarming because of not only affecting the individual, but also their own blood and flesh, or to put it in a bigger picture, the future generations of the country. Therefore, it is understandable that the Indonesian government is taking the issue seriously. The concern is also reflected in several Regional Regulations about liquor, eventhough there are still some issues of the supervision on its circulation within the market as shown in the 2013 regulation.[12]

As shown in TABLE 1, half ot the sample population have daily alcohol consumption (50.7%), and the average consumption period was 16 years. The period of condition ranging from at least one year to a maximum of 49 years. There was a significant relationship between age and period of consumption.

The older the person, the longer the duration in which they consume alcohol (FIGURE 2). Statistically, there was a tendency to consume alcohol 7.398 times higher as a person get older. Most alcoholic types was non combination (73.24%). These facts create a contrast to studies in Western countries for instance, that already have a culture of drinking alcohol, hence a relatively high per-capita consumption and arguably more dependable data.[13]

In this study, ADH enzyme activity either in alcoholics and non-alcoholics had slow activity (TABLE 2). This causes the ethanol that enters the cell to be converted slowly to acetaldehyde, so acetaldehyde accumulation doesn't happen. As a result, it will decrease the risk of getting diseases but may increase the risk of alcoholism. Our finding was similar to a study conducted by Parlesak *et al.* that found activity level did not merely depend on the level of the gene expression but also on the allele variation among the population.[14] Of the Javanese people being studied results showed ADH activity was slow. It is supported by the fact that most of them have ADH3*2 gene, which is characterized by slow conversion of ethanol to acetaldehyde (TABLE 3).[11]

ADH activity distribution is shown in FIGURE 5. ADH activity both in alcoholics and non-alcoholics was similar, and all was the slower type. When we categorized the result into above and below the mean, there was no significant difference statistically (TABLE 4). ADH activity distribution which was divided into low, moderate and high level can be seen in FIGURE 5, and it was found that mostly the levels were low, both in alcoholics and non-alcoholics. This finding was consistent with distribution of ADH3 allele: ADH3*2 (82.2%), ADH3*1/3*2 (15.6%) and ADH3*1 (2.2%). In this study, we correlated the phenotype of ADH activity with ADH3 allele of adults Javanese alcoholics. Since Indonesia has a limited history of alcohol consumption as a culture, only few researches have been conducted to study alcoholism-related diseases based on genetic variation. This situation is contrasted with Europe or America where alcohol consumption is a long-standing culture.

Our results showed that the majority of alcoholics (75.4%) and non-alcoholics (88.6%) were found to have the *ADH3*2* (polymorphic type) allele (TABLE 4). Genetic polymorphism of the ADH enzyme and aldehyde dehydrogenase in humans are also linked to alcohol consumption and the incident of alcohol abuse. Research conducted by Quertemont concluded that the accumulation of blood acetaldehyde causes unpleasant effects that prevent further drinking of alcohol, which is also related to alcohol drinking habits and the incidence of alcohol abuse.[15]

In Indonesia, many medical cases document patients' death caused by high alcohol intake and the mixing of alcoholic beverages with other harmful substances, such as glue and methanol. Adding to the concern, alcohol consumption is not only consumed by adults, even now it is consumed by adolescents; a previous study reported that 4.6% of adolescents aged between 10 and 18 years have consumed alcohol.[16] Yogyakarta is one of the popular cities visited by a large number of tourists. This tourism traffic increases the possibility of alcoholic beverages being distributed among and consumed by the residents of Yogyakarta, who are mainly of the Javanese community. The most commonly consumed alcoholic beverage in urban areas among the Indonesian adult population is beer (33.6%), while the second-most consumed alcoholic drink is wine (27.1%), then liquor (14.4%) with the remaining involving traditional alcoholic beverages (24.9%).[11]

Alcoholism as a disease is a maladaptive pattern of alcohol use that causes clinical problems as a result of developing a tolerance for alcohol, withdrawal symptoms and an inability to cease consumption. Alcohol tolerance is a decreased biological or behavioral response resulting from repeated alcohol use that causes alcoholics to need increasing amounts of alcohol to achieve the same effects. Withdrawal symptoms are a group of physical and psychological symptoms that arise upon ceasing the continuous use of alcohol.[17] There are also individual factors such as genetic types that effect how the body reacts to alcohol. Polymorphism of the *ADH3*2* allele gene for example has been reported to protect against the negative consequences of chronic pancreatitis. Therefore, the prevalent allele type of *ADH3*2* that was found among alcoholics and non-alcoholics in Yogyakarta may play a role in preventing the negative impact of chronic pancreatitis and in reducing the risk of myocardial infarction.[1]

Differences between class I and II *ADH* are shown with nucleotides in exon 8, isoleucin on *ADH3*1* and valine at *ADH3*2*. There are two loci *ADH3* variants: *ADH3*1* and *ADH3*2* that encode a subunit γ1 and γ2 sequentially. *ADH3*1* metabolizes alcohol with speed a maximum of 88 μM / min, while *ADH3*2* with speed 35 μM / min. This difference makes *ADH3*2* relative slower to *ADH3*1* and is a slow metabolizing enzyme.[18] Alcohol metabolism produces acetaldehyde as a toxic substance in the body. Among the ADH3 enzymes, *ADH3*1* type is faster at metabolizing ethanol into acetaldehyde. There were only 3 male alcoholics (2.2% of all participants) that possessed the *ADH3*1* type (TABLE 4). Further studies about the genetic subtypes of *ALDH*, as an enzyme that metabolizes acetaldehyde to acetic acid[19] are needed to confirm our findings. Research conducted by Wanandi demonstrated that the 70 subjects (70%) have the *ALDH2* wild-type allele, while 29 (29%) subjects were with atypical heterozygous *ALDH2* alleles and only 1 (1%) were homozygous atypical. This result may be related to the ethnic diversity of the population found in Indonesia.[20]

The predominant gene type found in the literature was *ADH3*1*, whereas our study only found this genotype in 2.2% of men and none in women (of all participants). Through in-depth interviews, it was found that among those who regularly consume alcohol, most usually engage in drinking once or twice weekly on the weekend; while some respondents only consumed alcohol during senior high school and then quit and others abstained from drinking since getting married. Our study found that ADH activity enzyme in all participants had a slower type and the predominant gene type among alcoholics and non-alcoholics was *ADH3*2*. No significant differences were observed in ADH activity enzyme and *ADH3 gene polymorphism* either in alcoholics and non-alcoholics (TABLE 4).

CONCLUSION

ADH activity in all participants had a slower type. Most of the polymorphism type of alcoholics and non-alcoholics were both ADH3*2 (75.4% and 88.6%). There were no significant differences of ADH enzyme activity with ADH3 gene polymorphism in either alcoholics or non-alcoholics of the sampled Javanese population in Yogyakarta, Indonesia.

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