The Role of Connexin in Cutaneous Adverse Drug Reactions (CADRs) in Patients with Increasing Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)

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ABSTRACT

Background: The occurrence of Cutaneous Adverse Drug Reactions (CADRs) is relatively rare but can be fatal when causing organ failure, especially in the liver. The supporting examinations to determine liver injury are aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Connexin-32 (Cx32) and connexin-43 (Cx43) are gap junction proteins that can be found in the liver and allegedly have a role in the mechanism of liver injury. To date, correlations between the level of connexin and aminotransferases enzyme in humans with CADRs cases are still unclear. **Purpose:** To determine the correlations between Cx32/Cx43 and AST/ALT levels in CADRs cases. **Methods:** This was a retrospective study, data collected from inpatient and outpatient's medical records, Department of Dermatology and Venereology of Dr. Sardjito Hospital, from 2011–2015. **Result:** A total of 25 patients with CADRs and 35 healthy controls were included in this study. The levels between Cx32 and AST, Cx32 and ALT, Cx43 and AST, and Cx43 and ALT were not significantly correlated in CADRs cases ($p>0.05$). Both Cx32 and Cx43 were not significantly different between patients with and without CADRs ($p>0.05$). Confounding factors such as gender were not associated with this study ($p>0.05$). **Conclusion:** There was no correlation between levels of Cx32/Cx43 and increasing AST/ALT in CADRs cases. Therefore, further study is necessary to conclude the correlation between connexin and aminotransferase enzyme in CADRs patients.

Keywords: drug eruptions, alanine transaminase, aspartate aminotransferases, connexins, liver dysfunction.

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BACKGROUND

An adverse drug reaction (ADR) is a noxious and unintended reactions to a medication that happens at doses commonly used in the most population.[1] ADRs are a global public health issue that account for a varying percentage of hospital admissions depending on the country. The most common type of ADRs is cutaneous adverse drug reaction (CADR).[3]

According to the Coombs and Gells classification, four types of hypersensitivity underlying the ADRs include type-I hypersensitivity (Ig-E mediated), type-II hypersensitivity (cytotoxic reactions), type-III hypersensitivity (immune-complex mediated), and type-IV hypersensitivity (delayed-type reactions).[4] CADRs with type IV hypersensitivity occurs after it is mediated by T lymphocyte cells, manifesting in various clinical features such as maculopapular eruption (MPE), acute generalized exanthematous pustulosis (AGEP), fixed drug eruption (FDE), drug reaction with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN), drug-induced agranulocytosis (DIA), drug-induced lung injury (DILI).[5]

The liver is the most affected organ in CADRs. Most CADRs patients (61.45%) present with liver dysfunction.[6] Several gap junction proteins, such as connexin-32 (Cx32) and connexin-43 (Cx43), have been reported as essential mediators in liver dysfunction triggered by drugs. Connexin is a gap junction subunit that plays a role in ion exchange, second messengers, and small metabolites in adjacent cells, which is essential for carrying out liver cell homeostasis. Because of this role, connexin has always been involved in the mechanism of liver pathology.[7] To date, liver dysfunction studies in CADRs with connexin levels in the blood are limited, and the correlation is unclear.

Previous in vitro studies on hepatitis cases reported decreased Cx32 levels[8,9] and increased Cx43 levels.[10] A study on cholestasis, liver fibrosis, and acute liver damage reported a similar result.[7] In liver cancer, Cx43 increases the migration, proliferation, and metastasis of cancer cells, while Cx32 occurs in the opposite. Inhibition of Cx43 production and stimulation of Cx32 production abating of cancer cells.[11-13]

The study about connexin involving human subjects, especially in the CADRs, is limited. To the authors' knowledge, this is the first study on the correlations between connexin and aspartate transferase (AST)/alanine transferase (ALT) in CADRs patients. This study was conducted at the Department of Dermatology and Venereology of Dr. Sardjito Hospital in Yogyakarta. The research question was whether Cx32/Cx43 correlates with AST/ALT in CADRs patients.

METHODS

This was a retrospective study at the Department of Dermatology and Venereology of Dr. Sardjito Hospital in Yogyakarta, Indonesia. The data were collected from inpatient and outpatient's medical records medical records of CADRs patients with a type IV hypersensitivity reaction in 2011–2015 using consecutive sampling. Patients who previously had liver problem were excluded.

Cx32 and Cx43 levels were obtained by Enzyme-linked Immunosorbent Assay (ELISA) test, while AST and ALT levels were obtained by spectrophotometric test. The patients' peripheral blood was taken at their first admission, and the blood samples were then stored in the laboratory. The data were collected from medical records. Eligible subjects were selected in accordance with the following inclusion criteria; having AST and ALT values data when CADRs with a type IV hypersensitivity reaction present (MPE, AGEP, FDE, DRESS, SJS, SJS/TEN, TEN), having Cx32 and Cx43 values data, and age ≥ 18 years old.

Controls were obtained from healthy subjects, and their Cx32 and Cx43 levels were compared to CADRs patients. The inclusion criteria for controls were have no history of drug eruption with type-IV hypersensitivity reaction, have similar characteristics to eruption case based on history taking and patients with a history of liver disease were excluded from this study.

The minimum sample size was 28, and we used the consecutive sampling technique. A total of 25 patients with CADRs and 35 healthy controls were included in this study.

The correlation between connexin (Cx32 and Cx43) levels and aminotransferases enzyme (AST and ALT) levels in CADRs patients was analyzed using the Spearman bivariate analysis test. Meanwhile, the comparison between Cx32 and Cx43 levels in CADRs patients and healthy controls was analyzed using the Mann-Whitney test. The Spearman test and Mann-Whitney test were selected due to uneven data distribution.

The ethics committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada has approved this research per a research approval number KE/FK/1150/EC.

RESULT

In 2011–2015, 25 patients with type IV hypersensitivity drug eruption including MPE, AGEP, SJS, SJS/TEN, DRESS, DIA, DILI were included in this study. The baseline characteristics of the research participants are presented in Table 1. The research participants were at least 18 years. Patients with drug eruption who included in this study had more than 18 years of age. The average of the CADRs patient group was 40 ± 13 years old. Of 25 patients in the CADRs patient group, 60% of patients were female, and 40% were male. Based on the classification of diagnoses, patients with drug eruption consisted of 40% MPE, 4% AGEP, 16% SJS, 4% SJS/TEN, 28% DRESS, and 8% were not specific. Based on the suspected drugs, we found 12 groups of drugs with a proportion of 29.27% beta-lactam antibiotics, 14.63% anticonvulsants, 12.20% NSAIDs, 12.20% acetaminophen, 12.20% antituberculous drugs, 7.32% allopurinol, 4.88% antipsychosis, 2.44% other antibiotics, 2.44% calcium channel blockers, and 2.44% proton pump inhibitors.

The mean of the results of Cx32, Cx43, AST, and ALT examinations in CADRs patients is shown in Table 2. To determine the comparison between Cx32 and Cx43 levels in CADRs patients and non-CADRs patients, a control group consisting of 35 participants was included in this study. The comparison between characteristics in CADRs patient group and control group is shown in Table 3. Based on the characteristics table, gender was not a confounding variable in the study because the gender differences between the CADRs group and the control group were not significantly different after the appropriate descriptive test, the Chi-square test.

The Cx32, Cx43, ALT, and AST data were processed using the SPSS software version 22. The appropriate tests to analyze the correlation of numerical data was the Pearson test, as a parametric test, and alternatively, the Spearman test, as a non-parametric test. The Kolmogorov-Smirnov normality test showed a p-value of ≤ 0.05 both in the Cx32 and Cx43 levels. The data representing Cx32, ALT, and AST levels did not pass the parametric test requirements because they did not have a normal distribution. However, the patient's Cx43 level data passed the parametric test requirements because they had a normal distribution. Therefore, the appropriate test to determine the correlation between Cx32 levels and ALT, Cx32

levels and AST, cx43 levels and ALT, and Cx43 and AST in CADRs patients was the Spearman test.

Table 1. Baseline characteristics of subjects

Variable	n (%)
Age (years), mean±SD	40 ± 13
Sex	
Male	10 (40)
Female	15 (60)
Diagnosis	
MPE	10 (40)
AGEP	1 (4)
SJS	4 (16)
SJS/TEN	1 (4)
DRESS	7 (28)
Non-specific	2 (8)
Suspected drugs	
Beta-lactam	12 (29.27)
Anticonvulsant	6 (14.63)
NSAID	5 (12.20)
Acetaminophen	5 (12.20)
Antituberculous drug	5 (12.20)
Xanthine oxidase inhibitor	3 (7.32)
Antipsychotic	2 (4.88)
Other antibiotics	1 (2.44)
Calcium Channel Blocker	1 (2.44)
Proton Pump Inhibitor	1 (2.44)

SD = standard deviation; MPE = maculopapular eruption
AGEP = acute generalized exanthematous pustulosis
SJS = Stevens-Johnson syndrome
TEN = toxic epidermal necrolysis
DRESS = drug reaction with eosinophilia and systemic symptoms
NSAID = non-steroidal anti-inflammatory drug.

Table 2. The result of Cx32, Cx43, AST, and ALT examinations in CADRs patients

Variable	Mean			
	Cx32 (ng/mL)	Cx43 (ng/mL)	AST (IU/L)	ALT (IU/L)
Sex				
Male (n = 10)	3.35	41.58	180	484
Female (n = 15)	2.76	44.88	98	85
Diagnosis				
MPE (n = 10)	6.05	46.84	64	87
AGEP (n = 1)	0.23	46.74	89	122
SJS (n = 4)	1.65	43.49	38	35
SJS/TEN (n = 1)	5.29	51.14	182	209
DRESS (n = 7)	1.61	42.05	282	634
Non-specific (n = 2)	0.88	49.53	114	170

Cx32 = connexin-32
Cx43 = connexin-43
AST = aspartate aminotransferase
ALT = alanine aminotransferase
CADRs = cutaneous adverse drug reactions
MPE = maculopapular eruption
AGEP = acute generalized exanthematous pustulosis

SJS = Stevens-Johnson syndrome
TEN = toxic epidermal necrolysis
DRESS = drug reaction with eosinophilia and systemic symptoms.

The correlation test result on Cx32 and AST levels was not statistically significant (p = 0.512). The correlation test result on Cx32 and ALT was not statistically significant (p = 0.481). The test result on Cx43 and AST levels in patients with drug eruption did not show a significant correlation (p = 0.939). The test result on Cx43 and ALT did not show a statistically significant correlation (p = 0.994).

The data consisted of Cx32 level of the CADRs patient group, Cx32 level of the control group, Cx43 level of the CADRs patient group, and Cx43 level of the control group. The appropriate test for comparing unpaired numerical data was unpaired T-tests, as a parametric test, and alternatively, the Mann-Whitney test, as non-parametric tests. The Kolmogorov-Smirnov normality test result on Cx32 levels in the CADRs group and control group showed a p-value of 0.05 and a p-value of > 0.5 on Cx43 levels. The normality test result showed that the Cx32 level of the CADRs group and control group did not pass the parametric test requirements because the data was not normally distributed. The data were analyzed using the Mann-Whitney test. The Cx43 level of the CADRs group and control group was qualified for the parametric test requirements because it was normally distributed. Therefore, the data were analyzed using the T-test, not in pairs.

Table 3. Comparison between drug eruption characteristics in sufferers and healthy controls

Variable	CADRs patient group (n = 25) n (%)	Control group (n = 35) n (%)	p-value
Sex			
Male	10 (40)	13 (37.1)	0.822
Female	15 (60)	22 (62.9)	
Diagnosis			
MPE	10 (40)	0	
AGEP	1 (4)	0	
SJS	4 (16)	0	
SJS-TEN	1 (4)	0	
DRESS	7 (28)	0	
Non-specific	2 (8)	0	
Control	0	35 (100)	

CADRs = cutaneous adverse drug reactions
MPE = maculopapular eruption
AGEP = acute generalized exanthematous pustulosis
SJS = Stevens-Johnson syndrome
TEN = toxic epidermal necrolysis
DRESS = drug reaction with eosinophilia and systemic symptoms.

Table 4. Results of comparative bivariate analysis of Cx32 and Cx43 levels in CADRs patients group and healthy controls group

Variable	Mean ± SD		p-value
	CADRs patients (n = 25)	Healthy controls (n = 35)	
Cx32 (ng/ml)	3.42 ± 4.94	2.68 ± 4.08	0.476
Cx43 (ng/ml)	45.34 ± 7.94	41.06 ± 7.49	0.795

CADRs = cutaneous adverse drug reactions
Cx32 = connexin-32
Cx43 = connexin-43.

The comparative bivariate analysis of Cx32 and Cx43 levels in the CADRs patients group and healthy controls group are shown in Table 4. The comparison result of Cx32 levels in CADRs patients and healthy

control and the comparison between Cx43 levels in CADRs patients and healthy control showed no statistically significant mean differences (respectively $p = 0.476$ and $p = 0.795$).

DISCUSSION

Based on the results of this study, the levels of Cx32 with AST and the levels of Cx32 with ALT in patients with drug eruption did not show a statistically significant correlation. Several in vitro studies of drug-induced liver toxicity produced lower levels of the enzyme aminotransferase in rodents with Cx32 mutations than in wild-types.[14,15] The histopathological appearance of the liver showed that inflammation and liver damage were more minimal in rodents with Cx32 mutations compared to wild-type.[14] A propylthiouracil-induced cytotoxicity study using BRL-3A rat liver cells showed reduced necrosis in cells with Cx32 knockdown using small interfering RNA.[16] Those results showed that the role of Cx32 was a vital signal channel of cell death to restore liver homeostasis.[7] However, this result differed from other studies conducted in Japan; there was no significant difference between rodents with Cx32 mutations and wild-type in both histopathological appearance and aminotransferase enzymes. The study also found a decrease in glutathione expression proportional to the dose of acetaminophen as a toxicity agent in both groups.[17]

Based on the results of this study, the levels of Cx43 with AST and the levels of Cx43 with ALT in patients with drug eruption did not have a statistically significant correlation. In an in vitro study, it was found that hepatic Cx43 expression has increased in rats with an overdose of acetaminophen and allegedly had a role in the mechanism of cell death.[16,20] Other studies explained that cell death, oxidative stress, and inflammation were more significant in mice with Cx43 mutations than wild-type in acetaminophen overdoses.[18] Cx43 is the most common isoform in the heart, being present in ventricular and atrial cardiomyocytes, endothelial cells, smooth muscle cells, and fibroblasts.[19,20] Hemichannel from Cx43 was usually closed in a healthy myocardium but can open if electrical and chemical stimuli are present due to inflammatory or ischemic conditions. Hemichannel of Cx43 could open long enough, causing loss of ion gradient, excess Ca2 + ions that enter, swelling, and cell damage. Therefore, based on other studies, pharmacological interventions that inhibit Cx43 expression can be a new strategy in preventing cell damage.[21]

Based on the data of this research, the comparison result of Cx32 level between the CADRs patient group and the control group did not show statistically significant mean differences. Other results revealed that the expression of Cx32 on liver biopsy was lower than hepatitis patients compared to healthy controls. Unlike healthy controls, the Cx32 expression in hepatitis patients was primarily found in the cytoplasm, not cell membranes. This result was due to the degraded Cx32 by lysosomes. The decreased expression of Cx32 in hepatitis could develop into cirrhosis and hepatocellular carcinoma.[8,9] In the heart organ, hypoxic cardiomyocytes decreased the expression of Cx43.[22,23] In the first 15 minutes of hypoxia, there was no visible decrease in Cx43 expression. However, after a few hours, there was a significant decrease in Cx43 expression and an internalization process.[24,25]

This insignificant research was expected to occur due to the examination of Cx32/Cx43 levels that did not coincide when a drug eruption occurs but when the patient's condition had improved. Therefore, the amount of Cx32/Cx43 levels had significantly reduced. Besides, an insufficient number of samples was estimated to cause uneven data distribution. The different characteristics of the subjects between the drug eruption group and the control group also played a role, especially in the mean age of the subjects.

In conclusion, there was no correlation between levels of Cx32/Cx43 and increasing AST/ALT in CADRs cases. For further investigation, it is necessary to conduct a similar study where sample collection and drug eruption happen within a determined timeframe and involve an adequate number of samples.

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