Original Article

Blood Cytokine Levels in Patients with Alcohol Dependence during Early Withdrawal Treatment

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Abstract

Objectives: Alcohol is known to modulate the immune system, which is associated with the pathophysiology of alcohol dependence (AD). Interleukin-6 (IL-6), soluble IL-6 receptor (sIL-6R), and tumor necrosis factor-alpha (TNF-α) are widely studied immune molecules in neuropsychiatric disorders. In this study, we intended to investigate the differences of blood levels of cytokines between patients with AD during early withdrawal and healthy control (HC). **Methods:** In this study, we included 85 patients diagnosed with AD according to the *DSM-IV* criteria and 49 HC participants. Blood samples were collected from the AD group and HC group on the first and seventh days of withdrawal. Blood levels of IL-6, sIL-6R, and TNF-α were measured using the enzyme-linked immunosorbent assay. **Results:** The baseline blood TNF-α levels in the AD group were significantly higher than those in the HC group (3.0 ± 1.6 pg/mL vs. 1.5 ± 0.9 pg/mL, $p < 0.001$), while blood sIL-6R levels were significantly lower (153.0 ± 37.0 pg/mL vs. 171.4 ± 39.6 pg/mL, $p < 0.01$). The baseline blood IL-6 and TNF-α levels were correlated with alcohol consumption amount and duration of AD. After one week of alcohol withdrawal, the blood TNF-α level of the AD group was still higher than that of the control group, whereas the blood IL-6 and IL-6R were normalized. **Conclusion:** Our findings suggest blood cytokines are dysregulated in patients with AD.

Key words: blood interleukin-6, blood soluble interleukin-6 receptor, blood tumor necrosis factor-alpha, neuro-immune dysregulation
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Introduction

Alcohol consumption poses remarkable risks to public health, resulting in a considerable number of deaths globally [1]. The estimated prevalence of heavy episodic alcohol use among adults is 18.4% worldwide [2]. Recent data from Taiwan's Health Promotion Administration in 2005 have revealed a high prevalence of alcohol consumption among the general population, reaching as high as 36.3% [3]. Furthermore, a previous study has reported a prevalence of alcohol dependence (AD) among patients admitted to general hospitals in Taiwan as 12.6% [4]. AD is characterized by craving, tolerance, a preoccupation with alcohol, and continued drinking despite harmful consequences [5]. AD is also associated with various physical and psychiatric complications, including cancers, cardiovascular diseases, liver diseases, psychosis, suicide, mood disorders, and anxiety disorders, resulting in excessive medical use [1, 6-9]. But the underlying causes of AD are still not fully understood. Some mechanisms have been proposed to explain the development of AD, including compromized prefrontal cortex function, neurodegeneration, disruptions in the neuro-immune balance, and genetic factors [10]. By enhancing our understanding of the pathophysiology of AD, we can acquire valuable insights, to help develop personalized medications and treatment approaches, for the specific requirements of individuals with AD.

Existing evidence consistently suggests that the immune system plays a pivotal rôle in the pathophysiology of AD [11]. Alcohol exerts a potent effect on the immune system,

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contributing to the developing multi-organ diseases such as cancers, cardiovascular diseases, liver cirrhosis, infectious diseases, and neuropsychiatric disorders [1]. Numerous explanations have been postulated to clarify how alcohol impacts immune function [12]. One potential pathway involves alcohol-induced changes in cytokine expression, such as interleukin (IL)-1-α, IL-1-β, IL-6, IL-8, IL-12, tumor necrosis factor-alpha (TNF-α), and monocyte chemoattractant protein-1 [13]. The pro-inflammatory cytokine IL-6 plays a crucial rôle in various biological processes, including the regulation of monocyte differentiation into macrophages, augmentation of B-cell immunoglobulin production, and suppression of dendritic cell maturation [14]. TNF-α regulates immune responses through interacting with receptors to control inflammation and cell death. Excessive or inappropriate TNF-α production can contribute to diseases, for example, rheumatoid arthritis, inflammatory bowel disease, psoriatic arthritis, psoriasis, and noninfectious uveitis [15]. Hence, both IL-6 and TNF-α play a rôle in developing chronic inflammatory diseases and may also be involved in developing AD [16]. In line with these facts, evidence from animal studies showed that chronic alcohol administration increases the cytokine level, and the increase is observed not only in the central nervous system but also in the peripheral blood [17, 18]. Similar observations were also found that persons with chronic alcohol consumption have elevated blood levels of IL-6 and TNF-α [19-21]. Moreover, Laso et al. found that individuals following chronic alcohol consumption but without liver disease also have increased production of inflammatory cytokines levels, such as IL-6 and TNF-α, in peripheral blood [19]. These findings suggest that AD disrupts cytokine homeostasis. In addition, the interaction between soluble IL-6 receptor (sIL-6R) and IL-6 enhances the bioactivity of IL-6, triggering intracellular signaling [14]. Elevated blood levels of sIL-6R in individuals are linked to an increased risk of inflammation when blood IL-6 levels are high. This increased blood sIL-6R level has been observed in neurological disorders like Alzheimer's disease, suggesting a potential connection between receptor activity and neurodegeneration. It is hypothesized that sIL-6R might also be involved in alcohol-induced neurodegenerative processes [22]. But limited research has explored the rôle of sIL-6R in the pathophysiology of alcohol's impact on the immune system.

Alcohol withdrawal syndrome, a hallmark for the development of AD, typically manifests within hours after a reduction or cessation of alcohol consumption. Dysregulation in pro-inflammatory cytokines has been observed in patients with AD during alcohol withdrawal but gradually recovered thereafter [23]. But a scarcity of research exists to examine the changes of systemic neuroinflammatory cytokines in patients with AD during early alcohol withdrawal. In particular, the existing data for blood IL-6R are lacking. Therefore, we intended to examine the differences in various neuroinflammatory cytokines, including blood IL-6, sIL-6R, and TNF-α, between the patients with AD group and healthy controls (HC). In addition, we also intended to understand the alterations of these cytokines following alcohol withdrawal. We hypothesized that chronic alcohol consumption would disrupt the homeostasis of cytokines, and the dysregulation of pro-inflammatory cytokines would be gradually recovered over time.

Methods

Study participants

The study protocol obtained approval from the institutional review board of the Taipei City Psychiatric Center (IRB No = 1000406 and date of approval = 1217, 2008), requiring to obtain informed consents from the study participants.

The study participants who were admitted to an alcohol detoxification treatment ward at Taipei City Psychiatric Center, Taipei City Hospital, were enrolled to participate in the study if they met the following inclusion criteria. Patients with severe known medical conditions or those developing severe liver cirrhosis or acute infections were excluded and directed to appropriate medical management facilities. The inclusion criteria were: (a) age between 20 and 60 years, (b) fulfilling the *DSM-IV* diagnosis criteria of AD that were verified by two board-certified psychiatrists (MCH and HMC).

Exclusion criteria included patients who had (a) a history of schizophrenia, bipolar disorder, major depressive disorder, or current treatment with antipsychotics or mood stabilizers (such as lithium, valproic acid, or carbamazepine); (b) concurrent substance use disorders, excluding nicotine dependence; (c) remarkable systemic or physical illnesses, including infectious, autoimmune, cardiovascular diseases, or diabetes mellitus; and (d) a history of neurological disorders, such as primary seizure disorder, cerebrovascular disease, Parkinson's disease, or dementia. Following an initial clinical interview to assess their psychiatric status, patients received a physical examination and urine toxicology test to screen for illicit drug use, and excluded other substance use disorders. Eligible patients were provided with a comprehensive explanation of the study and enrolled after giving written informed consent.

Patients with alcohol intoxication

With interviews with eligible patients, we collected sociodemographic data and alcohol consumption history, including age at first drink, duration of AD, and average daily alcohol consumption amount. The duration of AD was defined as the number of years during which patients met the criteria for an AD diagnosis. The average daily alcohol consumption amount was calculated based on the past 90 days of consumption. Alcohol consumption was immediately and completely discontinued on admission. All patients received a standardized alcohol withdrawal treatment regimen, consisting of 2–6 mg of oral lorazepam administered four times on the admission day, with doses adjusted for alcohol withdrawal syndrome management. In addition, patients were given 50–100 mg of oral trazodone at night to treat insomnia commonly experienced during alcohol withdrawal. Multivitamins and oral thiamine supplementation (150 mg/day) were provided to prevent severe neurological complications like Wernicke–Korsakoff syndrome. Patients who continued to

use nicotine were given nicotine patches for smoking cessation, with a dosage tailored to their daily tobacco use.

Healthy control participants

The HC group consisted of individuals recruited from the hospital's physical check-up unit. Participants received screening using the Mini-International Neuropsychiatric Interview [24], done by a trained research assistant, to ensure the absence of any known physical or psychiatric illnesses. Control group participants did not meet the diagnostic criteria for alcohol abuse or dependence in the past and had abstained from alcohol consumption for the preceding three months. In addition, all healthy control participants were able to read Mandarin Chinese and provided informed consent before their involvement in the study.

Blood laboratory assays

Venous blood samples were collected at 8: 00 – 9: 00 a.m. after overnight fasting. For the patients with AD, the blood drawn on admission (baseline) as well as that drawn after one week of withdrawal treatment was delivered to the Taipei Institute of Pathology (Taipei, Taiwan) for the analyses of laboratory panels. Laboratory panels included chronic alcohol consumption, including blood levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and mean corpuscular volume (MCV). With the E_{max} Immunoassay System Kit, we measured levels of blood IL-6, sIL-6R, and TNF-α according to the manufacturer's instructions (R and D Systems, Taipei, Taiwan). We measured levels of blood IL-6, sIL-6R, and TNF-α of all study participants on the same day.

Statistical analysis

Description statistics, including the mean and standard deviation, were presented. The Student's *t*-test (for continuous data) and proportion test (for binary data) were used to test the difference for demographic and clinical characteristics of patients. The differences of blood levels of IL-6, sIL-6R, and TNF-α levels were tested using the Student's *t*-test (for AD groups between different time points) and analysis of covariance (ANCOVA) (for the AD group and the HCs at baseline and one week after alcohol withdrawal treatment). We adjusted four covariates (age, sex, body mass index [BMI], and tobacco use) in the ANCOVA model. Furthermore, we used those tests to compare the blood levels between two AD groups at different time points. Spearman's correlation tests were used to analyze the association between drinking variables (drinking consumption, duration of drinking years, and duration of years of AD) and immunocytokine markers. Correlations among the immunocytokine markers were also examined. Variables that did not follow a normal distribution were logarithmically transformed before analysis.

The analyses were done using R version 4.2.2 and the Statistical Package for the Social Science software version 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The differences between groups were considered significant if *p*-values were smaller than 0.05.

Results

Table 1 shows the demographic and clinical characteristics of the participants in the AD and HC groups. The AD group consisted of 85 participants, while the HC group had 49 participants. The two groups were significantly different in sex distribution, with a significantly higher proportion of males in the AD group compared to the HC group (91.8% vs. 59.2%, $p < 0.001$). The mean age of participants showed no statistically significant difference between the two groups ($p < 0.001$). But the BMI was also significantly lower in the AD group (kg/m^2) compared to the HC group (22.6 ± 3.5 kg/m^2 vs. 24.1 ± 4.0 kg/m^2, $p < 0.05$). Regarding to tobacco use, the AD group was reported a significantly higher number of cigarettes smoked per day compared to the HC group (20.4 ± 13.2 vs. 1.3 ± 4.2, $p < 0.001$). In alcohol drinking variables, the AD group had an average drinking onset age of 17.1 ± 3.4 years, a total duration of drinking of 26.7 ± 7.9 years, and an age of meeting the diagnosis of AD at 28.1 ± 8.0 years. The duration of AD was 11.0 ± 7.5 years. The average drinking consumption amount in the AD group was 25.9 ± 13.3 drinks per day. In addition, the biochemical data showed that the AD group had elevated levels of GGT (291.3 ± 502.6 U/L) and AST (76.8 ± 119.5 U/L) compared to normal reference ranges. The levels of ALT (59.0 ± 104.4 U/L), blood urine nitrogen (10.7 ± 3.5 mg/dL), creatinine (0.8 ± 0.2 mg/dL), and MCV (91.8 ± 9.1 fL) were within the normal range in both groups.

Table 2 presents the results of difference tests for blood levels of IL-6, sIL-6R, and TNF-α in the HC and AD groups. At baseline (W0), the AD group showed higher levels of IL-6 compared to HC (4.0 ± 3.1 pg/mL vs. 1.6 ± 2.3 pg/mL, nonsignificant). After one week of alcohol withdrawal (W1), the IL-6 levels decreased but remained higher than in the control group without statistical significance (2.7 ± 1.8 pg/mL). The decrease in IL-6 levels from W0 to W1 in the AD group showed no significance. For sIL-6R levels at W0, the AD group had significantly lower levels compared to HC (153.0 ± 37.0 pg/mL vs. 171.4 ± 39.6 pg/mL, $p < 0.01$). At W1, sIL-6R levels were slightly increased but remained lower than in the control group (161.4 ± 37.4 pg/mL, nonsignificant). Regarding to blood TNF-α levels at W0, the AD group had significantly higher levels compared to the control group (3.0 ± 1.6 pg/mL vs. 1.5 ± 0.9 pg/mL, $p < 0.001$). After one week of withdrawal, TNF-α levels remained elevated in the AD group (2.7 ± 1.8 pg/mL, $p < 0.001$). The differences in TNF-α levels from W0 to W1 in the AD group were statistically significant ($p < 0.01$).

Table 3 describes the correlation between alcohol drinking consumption, duration of drinking years, duration of years of AD, and cytokine markers. For blood IL-6 level, a positive correlation was found with drinking consumption (correlation = 0.41, $p < 0.01$) and duration of years of AD (correlation = 0.49, $p < 0.01$). But there was no significant correlation between IL-6 and the duration of drinking years. Regarding

Table 1. Demographic and clinical characteristics of patients with alcohol dependence and healthy control participants (N = 134)

	HC (n = 49)	AD (n = 85)
Sex, n (%)		
Male	29 (59.2)	78 (91.8)***
Female	20 (40.8)	5 (5.9)
Age (year old), mean ± SD	41.6 ± 12.2	44.1 ± 8.1
BMI (kg/m^2), mean ± SD	24.1 ± 4.0	22.6 ± 3.5*
Tobacco use (cigarette/day), mean ± SD	1.3 ± 4.2	20.4 ± 13.2***
Current smokers, n (%)	5 (10.2)	63 (74.1)***
Alcohol drinking variables, mean ± SD		
Onset age of alcohol drinking (year old)		17.1 ± 3.4
Total years of alcohol drinking (year)		26.7 ± 7.9
Age of meeting diagnosis of AD (year)		28.1 ± 8.0
Duration of AD (year)		11.0 ± 7.5
Average daily alcohol consumption amount in the past 90 days (drinks[b]/day)		25.9 ± 13.3
Biochemical data, mean ± SD		
BUN (5.0–24.0 mg/dL)		10.7 ± 3.5
Creatinine (0.7–1.2 mg/dL)		0.8 ± 0.2
AST (10–39 U/L)		76.8 ± 119.5
ALT (7–42 U/L)		59.0 ± 104.4
GGT (0–26 U/L)		291.3 ± 502.6
MCV (80.0–100.0 fL)		91.8 ± 9.1

*p < 0.05; **p < 0.01; ***p < 0.001 significantly different between groups, tested using the Student's t-test or proportion test when appropriate
[a]Analyses were based on 134 subjects, including 85 patients with AD and 49 HC; [b]One drink is defined by 10 g of pure alcohol.
AD, alcohol dependence; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urine nitrogen; GGT, γ-glutamyl transferase; HC, healthy control; MCV, mean corpuscular volume; SD, standard deviation

Table 2. Analysis of Difference Tests for blood level of interleukin-6, soluble interleukin-6 receptor, and tumor necrosis factor-α in healthy control and alcohol-dependent groups (N^a = 134)

Serum concentration	Control, mean ± SD[a] (n = 49)	AD, mean ± SD[a] (n = 85)	
		Baseline (W0)	Follow-up (W1)
IL-6 (pg/mL)	1.6 ± 2.3	4.0 ± 3.1	2.7 ± 1.8
sIL-6R (pg/mL)	171.4 ± 39.6	153.0 ± 37.0**[,b]	161.4 ± 37.4
TNF-α (pg/mL)	1.5 ± 0.9	3.0 ± 1.6***[,b]	2.7 ± 1.8**[,b]

*p < 0.05; **p < 0.01; ***p < 0.001 significantly different between AD group and HC group; †p < 0.05; ‡p < 0.01; §p < 0.001 significantly different for AD groups in W0 and W1; [a]Analyses were based on 134 subjects, including 85 patients with AD and 49 healthy controls; [b]p-values were calculated based on using the Student's t-test and ANCOVA adjusted for covariates (age, sex, BMI, and tobacco use).
SD, standard deviation; W0, screening at baseline; W1, follow-up at week one; ANCOVA, analysis of covariance; AD, alcohol dependence; SD, standard deviation; IL-6, interleukin-6; sIL-6R, soluble IL-6 receptor; TNF-α, tumor necrosis factor α

Table 3. The correlation test[a] between cytokines and alcohol drinking variables (N = 134)

	Correlation coefficient		
	Total years of alcohol drinking (years)	Duration of AD (years)	Average daily alcohol consumption amount in the past 90 days (drinks[b]/day)
IL-6 (pg/mL)	−0.02	0.49**	0.41**
sIL-6R (pg/mL)	−0.09	−0.19*	−0.18
TNF-α (pg/mL)	−0.03	0.48***	0.37***

*p < 0.05; **p < 0.01; ***p < 0.001 significantly different, tested using Spearman's correlation test when appropriate; [a]Analyses were based on 134 subjects, including 85 patients with AD and 49 healthy controls; [b]One drink is defined by 10 g of pure alcohol.
AD, alcohol dependence; IL-6, interleukin-6; sIL-6R, soluble IL-6 receptor; TNF-α, tumor necrosis factor α; AD, alcohol dependence

to the sIL-6R level, no significant correlations were observed with drinking consumption or duration of drinking years. But a significant negative correlation was found with the duration of years of AD (correlation = −0.19, p < 0.05). For blood TNF-α level, a positive significant correlation was observed with drinking consumption (correlation = 0.37, p < 0.001) and duration of years of AD (correlation = 0.48, p < 0.001). But no significant correlation was found between the blood levels of TNF-α and the duration of drinking years.

Discussion

Our study included 134 participants, with 85 participants diagnosed with AD and 49 HC. Analysis of blood levels of IL-6, sIL-6R, and TNF-α showed that the AD group had higher baseline blood levels of IL-6 and TNF-α, but lower blood levels of sIL-6R compared to HC. After one week of alcohol withdrawal, blood IL-6 levels were decreased in the AD group but remained higher than HC, while blood TNF-α levels remained elevated. As shown in Table 3, correlation analysis revealed a significant positive association between IL-6 and TNF-α blood levels and alcohol consumption amount and the duration of years of AD ($p < 0.01$). But no significant correlation was found between IL-6 blood levels and the duration of drinking years. The sIL-6R blood levels did not show significant correlations with alcohol consumption amount or the duration of drinking years. These findings suggest that a relation existed between alcohol consumption, duration of AD, and blood levels of IL-6 and TNF-α.

We observed a significant alteration in inflammatory cytokines among individuals with AD (Table 2). Specifically, patients with AD showed significantly higher baseline blood levels of serum TNF-α ($p < 0.001$). These findings agree with existing evidence indicating elevated blood levels of various cytokines in AD, for example, blood levels of IL-2, IL-4, IL-5, IL-6, IL-10, and TNF-α. Specifically, our results are consistent with one previous study that reported increased blood levels of IL-6 and TNF-α in patients with AD without liver diseases, suggesting that the elevation in blood levels of IL-6 and TNF-α might be due to chronic alcohol consumption *per se* instead of other underlying physiological problems [25]. Our investigation into IL-6 blood level did not reveal any significant differences both at baseline and after withdrawal when compared to the HC group (Table 2). But, existing evidence indicates that a range of factors may impact the blood levels of IL-6, such as sex [9, 26], BMI [27], metabolic factors [28], smoking history [29], and underlying subclinical physical illnesses could confound results, thus may confound our results and preclude us from definitive conclusions.

The mechanisms underlying the lower blood levels of sIL-6R observed in patients with AD have not been fully clear. But, review articles suggest that the bioactivity of the blood level of IL-6 is enhanced through forming complexes with sIL-6R, leading to trans-signaling within cells [14]. In the blood, sIL-6R and sgp130 act as a buffer for IL-6, protecting against overstimulation of IL-6 [30]. Similar findings have been reported in studies involving patients with type 2 diabetes mellitus, where chronic high blood levels of IL-6 overload the buffer system, leading to a reduction in both sIL-6R and sgp130 serum levels and disrupting the buffering mechanism [31]. These observations provide evidence that sIL-6R plays a rôle in neutralizing circulating blood IL-6 levels, and persistent high blood levels of IL-6 may overwhelm the buffer system, contributing to reduced sIL-6R blood levels. We speculate that downregulating sIL-6R blood levels might be a protective mechanism in the inflammatory process, but further studies are needed to confirm this hypothesis, particularly in the context of alcohol consumption.

During the alcohol withdrawal phase, we observed reduced IL-6 blood levels with nearly significance after one week of alcohol abstinence. The finding is compatible with previous evidence that indicates blood levels of IL-6 are decreased during alcohol abstinence [16]. When compared to controls, blood TNF-α levels at W1 remained elevated in patients with AD, suggesting that one week of abstinence is not sufficient for TNF-α levels to be normalized. In line with this point, previous research has also shown that elevated cytokine levels do not reverse until after four weeks of abstinence from alcohol [32]. On the other hand, for sIL-6R blood levels, patients with AD showed the downregulation of sIL-6R at baseline but restored to comparable blood levels with the HC group at W1. This suggests that normalization in sIL-6R blood levels could be observed as early as one week into treatment. Collectively, our data showed that one week of alcohol abstinence might result in a partial recovery of the neuroinflammatory cytokine dysregulation associated with chronic and heavy alcohol consumption. This observation is in line with previous research, also indicating short-term abstinence can partially restore the cytokines imbalance [23]. We cannot clarify whether the partial recovery results from alcohol discontinuation itself or the withdrawal treatment which includes the use of benzodiazepine and multi-vitamins. Cornwell et al. revealed that lorazepam can stimulate the production of IL-6 production [33]. Therefore, it is possible that discontinuation and withdrawal treatment may jointly contribute to the improvement of cytokine dysregulation. Future studies should study the changes or reversibility of the dysregulation following long-term abstinence.

Regarding to the alcohol drinking-related variables, although we did not observe a significant correlation between cytokine levels and total years of alcohol drinking, we found a significantly more positive correlation between the duration of AD (years) and IL-6 ($p < 0.01$) or TNF-α ($p < 0.001$) blood levels (Table 3). The results indicated that a longer duration of addiction on alcohol produced higher blood levels of these two cytokines. On the contrary, we found an opposite direction of the correlation, i.e., the duration of AD is negatively correlated to sIL-6R blood levels. This result suggests a potential protective or modulating rôle for sIL-6R against overstimulation of cytokines. Similarly, we found that a positive correlation existed between average daily alcohol consumption and blood levels of IL-6 or TNF-α. The result is consistent with previous reviews, indicating that higher consumption is linked to increased cytokine blood levels [16, 34].

Regarding to TNF-α, the blood levels are higher in hospitalized patients with AD compared to HCs, regardless of alcohol consumption levels [35]. From our findings (Table 3), we further found that a significant relationship existed between alcohol consumption and TNF-α elevation ($p < 0.001$), and that significantly more dose–response effect existed, i.e., the more one drinks, the higher the TNF-α blood

levels become ($p < 0.001$). In summary, these outcomes underscore the potential impact of both AD duration and alcohol consumption levels on cytokine dysregulation, implying that excessive and chronic alcohol intake could suppress immune function.

Study limitations

The readers are cautioned not to overgeneralize the study results because this study has five study limitations:

- A relatively small sample size may introduce selection bias.
- A significant proportion of patients with AD were smokers, which could impact IL-6 and TNF-α blood levels.
- The study was limited to assessing blood levels of IL-6, sIL-6R, and TNF-α over a one-week alcohol withdrawal period. Effects of prolonged sobriety on these cytokines remain unknown and warrant investigation.
- Consideration is needed for diverse biological activities, cell sources, and potential inducers of synthesis for each cytokine in cytokine studies.

Summary

These findings suggest that AD is linked to immunocytokine dysregulation, particularly elevated IL-6 and TNF-α levels. The study provides evidence in the potential connection between alcohol consumption and cytokine shifts, particularly blood levels of IL-6, sIL-6R, and TNF-α. The observed correlations between alcohol consumption and cytokine markers, along with the duration of drinking years and AD, reinforce the cytokines' rôle in AD. But further research is needed to better understand their involvement in AD and withdrawal, including the duration of recovery from alcohol withdrawal.

Data Availability Statement

The datasets produced and/or examined in the course of this present study can be obtained by contacting the corresponding author through a reasonable request.

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Conflicts of Interest

All authors have no conflicts of interest to report.

References

1. Im PK, Wright N, Yang L, et al.: Alcohol consumption and risks of more than 200 diseases in Chinese men. *Nat Med* 2023; 29: 1476-86.
2. Peacock A, Leung J, Larney S, et al.: Global statistics on alcohol, tobacco and illicit drug use: 2017 status report. *Addiction* 2018; 113: 1905-26.
3. Health Promotion Administration, Ministry of Health and Welfare, Taiwan: *Alcohol Consumption Rate among Adults Aged 18 and Above*. Taiwan: Health Promotion Administration, Ministry of Health and Welfare, Taiwan, 2023.
4. Chen CH, Chen WJ, Cheng AT: Prevalence and identification of alcohol use disorders among nonpsychiatric inpatients in one general hospital. *Gen Hosp Psychiatry* 2004; 26: 219-25.
5. National Institute for Health and Clinical Excellence (NICE). *Alcohol-Use Disorders: Diagnosis, Assessment and Management of Harmful Drinking (High-Risk Drinking) and Alcohol Dependence, Clinical Guideline*. UK: National Institute for Health and Clinical Excellence, 2011.
6. Skryabin VY, Martinotti G, Franck J, et al.: Acute alcoholic hallucinosis: A review. *Psychopathology* 2023; 56: 383-90.
7. Chen WY, Chen YY, Liu HH, et al.: The possible mediating effect of alcohol dependence on the relationship between adverse childhood experiences and attempted suicide. *Alcohol* 2018; 73: 9-15.
8. Hsu HE, Chen PY, Chang HM, et al.: Incidence of and risk factors for alcohol dependence in bipolar disorder: a population-based cohort and nested case-control study. *Aust N Z J Psychiatry* 2023; 57: 725-35.
9. Chang HM, Pan CH, Chen PH, et al.: Sex differences in incidence and psychiatric comorbidity for alcohol dependence in patients with panic disorder. *Drug Alcohol Depend* 2020; 207: 107814.
10. Vetreno RP, Crews FT: Current hypotheses on the mechanisms of alcoholism. *Handb Clin Neurol* 2014; 125: 477-97.
11. Cui C, Shurtleff D, Harris RA: Neuroimmune mechanisms of alcohol and drug addiction. *Int Rev Neurobiol* 2014; 118: 1-12.
12. Kovacs EJ, Messingham KA: Influence of alcohol and gender on immune response. *Alcohol Res Health* 2002;26:257-63.
13. Achur RN, Freeman WM, Vrana KE: Circulating cytokines as biomarkers of alcohol abuse and alcoholism. *J Neuroimmune Pharmacol* 2010; 5: 83-91.
14. Kang S, Narazaki M, Metwally H, et al.: Historical overview of the interleukin-6 family cytokine. *J Exp Med* 2020; 217: e20190347.
15. Jang DI, Lee AH, Shin HY, et al.: The role of tumor necrosis factor lpaha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics. *Int J Mol Sci* 2021; 22: 2719.
16. Heberlein A, Käser M, Lichtinghagen R, et al.: TNF-α and IL-6 serum levels: Neurobiological markers of alcohol consumption in alcohol-dependent patients? *Alcohol* 2014; 48: 671-6.
17. Jiang C, Zhang Y, Tang X, et al.: IL-6 and IL-1β upregulation and tau protein phosphorylation in response to chronic alcohol exposure in the mouse hippocampus. *Neuroreport* 2021;32:851-7.
18. Tiwari V, Kuhad A, Chopra K: Suppression of neuro-inflammatory signaling cascade by tocotrienol can prevent chronic alcohol-induced cognitive dysfunction in rats. *Behav Brain Res* 2009; 203: 296-303.
19. Laso FJ, Vaquero JM, Almeida J, et al.: Production of inflammatory cytokines by peripheral blood monocytes in chronic alcoholism: relationship with ethanol intake and liver disease. *Cytometry B Clin Cytom* 2007; 72: 408-15.
20. Deviere J, Content J, Denys C, et al.: High interleukin-6 serum levels and increased production by leucocytes in alcoholic liver cirrhosis. correlation with IgA serum levels and lymphokines production. *Clin Exp Immunol* 1989; 77: 221-5.
21. Khoruts A, Stahnke L, McClain et al.: Circulating tumor necrosis factor, interleukin-1 and interleukin-6 concentrations in chronic alcoholic patients. *Hepatology* 1991; 13: 267-76.
22. Quillen D, Hughes TM, Craft S, et al.: Levels of soluble interleukin 6 receptor and Asp358Ala are associated with cognitive performance and Alzheimer disease biomarkers. *Neurol Neuroimmunol Neuroinflamm* 2023; 10: e200095.
23. Huang MC, Chung RH, Lin PH, et al.: Increase in plasma CCL11 (eotaxin-1) in patients with alcohol dependence and changes during detoxification. *Brain Behav Immun* 2022; 99: 83-90.
24. Sheehan DV, Lecrubier Y, Sheehan KH, et al.: The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 1998; 59 (Suppl 20): 22-33.
25. Laso FJ, Vaquero JM, Almeida J, et al.: Chronic alcohol consumption is

associated with changes in the distribution, immunophenotype, and the inflammatory cytokine secretion profile of circulating dendritic cells. *Alcohol Clin Exp Res* 2007; 31: 846-54.
26. Sperry JL, Friese RS, Frankel HL, et al.: Male gender is associated with excessive IL-6 expression following severe injury. *J Trauma* 2008; 64: 572-8.
27. El-Mikkawy DM, EL-Sadek MA, EL-Badawy MA, et al.: Circulating level of interleukin-6 in relation to body mass indices and lipid profile in Egyptian adults with overweight and obesity. *Egypt Rheumatol Rehabil* 2020; 47: 1-7.
28. Bastard JP, Jardel C, Bruckert E, et al.: Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab* 2000; 85: 3338-42.
29. Al-Tameemi S, Hameed N, Gomes K, et al.: Cigarette smoking increases plasma levels of IL-6 and TNF-α. *Baghdad J Biochem Appl Biol Sci* 2022; 3: 60-8.
30. Rose-John S, Jenkins BJ, Garbers C, et al.: Targeting IL-6 trans-signalling: Past, present and future prospects. *Nat Rev Immunol* 2023; 23: 666-81.
31. Aparicio-Siegmund S, Garbers Y, Flynn CM, et al.: The IL-6-neutralizing sIL-6R-sgp130 buffer system is disturbed in patients with type 2 diabetes. *Am J Physiol Endocrinol Metab* 2019; 317: E411-20.
32. Yen CH, Ho PS, Yeh YW, et al.: Differential cytokine levels between early withdrawal and remission states in patients with alcohol dependence. *Psychoneuroendocrinology* 2017; 76: 183-91.
33. Cornwell AC, Tisdale AA, Venkat S, et al.: Lorazepam stimulates IL6 production and is associated with poor survival outcomes in pancreatic cancer. *Clin Cancer Res* 2023; 29: 3793-812.
34. Adams C, Conigrave JH, Lewohl J, et al.: Alcohol use disorder and circulating cytokines: a systematic review and meta-analysis. *Brain Behav Immun* 2020; 89: 501-12.
35. Gonzalez-Quintela A, Campos J, Loidi L, et al.: Serum TNF-alpha levels in relation to alcohol consumption and common TNF gene polymorphisms. *Alcohol* 2008; 42: 513-8.