BioSocial Health J 2024; 1(2): 90-97
doi:10.34172/bshj.24
https://biosocialhealthjournal.com

BioSocial
Health Journal

Original Article

The effects of alcohol on recovery from musculoskeletal trauma and injuries

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Abstract

Introduction: This study aimed to investigate the association between alcohol misuse (abuse and dependence) and clinical outcomes including infection, length of stay (LOS), and in-hospital mortality (IHM) among patients with musculoskeletal injuries.

Methods: A retrospective analysis was conducted using California Hospital Discharge Data for 2018. The study included patients aged 18 years or older with musculoskeletal injuries categorized by injury sites (head/neck, trunk, and extremities) and alcohol misuse (abuse or dependence). Multivariate logistic regression analysis was used to assess the independent association of alcohol misuse with the outcome variables, controlling for age, gender, ethnicity, and insurance status.

Results: Among 3.7 million discharges, 207 623 (40.2%) had alcohol abuse, and 58.8% had alcohol dependence. The findings revealed that among musculoskeletal injury discharges, those with alcohol abuse, compared to alcohol dependence, had higher odds of infection (OR = 1.25; CI = 1.07-1.45). However, they had lower odds of LOS of ≥ 4 days (OR = 0.78; CI = 0.77-0.79), and lower odds of IHM (OR = 0.91; CI = 0.86-0.96). Extremity injuries were associated with higher odds of infection, longer hospital stays, and lower IHM compared to head/neck and trunk injuries. Males compared to females and patients aged 67 or older compared to their 18-34 age group encounters were more likely to experience infection, LOS of ≥ 4 days, and IHM. Hispanic and Asian patients experienced less infection and fewer days in the hospital but presented with higher odds of IHM.

Conclusion: Our results reveal the burden of alcohol misuse in treatment outcomes among patients undergoing hospitalization for treatment of musculoskeletal injuries as they related to infection, length of hospital stay, and IHM. These findings also highlight the potential economic implications of alcohol-related musculoskeletal injuries. Our findings emphasize the necessity for an approach that goes beyond treating immediate physical injury, but considering a patient's history of alcohol abuse and providing appropriate support and interventions to improve treatment outcomes for individuals affected by musculoskeletal injuries and alcohol misuse.

Keywords: Alcohol drinking, Fractures, Bone, Musculoskeletal system, Wounds and injuries, Hospitalization, Length of stay, Infections

Received: April 4, 2024, **Accepted**: May 23, 2024, **ePublished**: July 4, 2024

Introduction

Alcohol use disorder (AUD) is a pattern of alcohol misuse characterized by an individual's inability to control alcohol consumption due to both physical and emotional dependence.[1] AUD has significant public health consequences.[2] According to the 2018 World Health Organization's (WHO's) Global Status Report on Alcohol and Health alcohol misuse was responsible for approximately three million deaths in 2016, accounting for 5.3% of all fatalities.[3] This surpasses the combined impact of hypertension and diabetes. Projections indicate that alcohol consumption is expected to rise in the next decade.[4] Epidemiologic studies have constantly reported a link between AUD and lower income levels, as well as a higher prevalence in urban areas compared to rural regions.[5] Additionally, individuals with lower socioeconomic status are more likely to experience adverse outcomes related to alcohol injuries.[5] Between 2006 and 2014, visits to the emergency department due to alcohol-related incidents increased by 47%.[6] In 2010, AUD resulted in an economic burden of $249.0 billion in the United States.[7] The majority of alcohol-related cases involve injury to the musculoskeletal system, particularly fractures and dislocations.[8] These injuries could arise from

various causes, including traffic accidents, falls, gunshot wounds, and other forms of personal injuries (i.e., physical altercations).[9] Among preventable behaviors, AUD impairs balance, judgment, and ultimately could lead to accident-related fractures.[10,11] Managing fractures in in individuals with alcohol misuse poses a challenging task given that up to 40% of orthopedic trauma patients have a positive blood alcohol content (+BAC), and alcohol misusers (i.e., alcohol abuse and/or alcohol dependency) have a four times higher fracture rate compared to non-users.[12-15]

Numerous histological studies have demonstrated the contribution of chronic alcohol consumption contributing to the development of osteoporosis. Both animals and human studies following chronic alcohol exposure consistently reveal low bone mass and decreased bone formation rate.[16] Alcohol's inhibitory role in the skeletal system is understood in the context of bone remodeling, a process involving the coordinated activities of osteoblasts (bone formation cells) and osteoclasts (bone resorption cells).[17] Alcohol hampers bone remodeling by suppressing osteoblasts, which are responsible for building new bone. Consequently, reduced bone formation leads to decreased in bone mineral density and increased fracture incidence. Contrary to the alternative explanation, studies have not supported the notion that alcohol stimulates osteoclasts to increase bone resorption.[17] This cellular understanding of alcohol's impact on bone remodeling has provided the foundation for further investigations into alcohol's effect on fracture healing.

One prevailing theory regarding the impact of alcohol's role on fracture healing suggests that it impairs the stage of callus formation.[18] Fracture healing occurs through stages, starting with an inflammatory response and progressing to bone regeneration at the injury site. An intermediate step in this process involves the mineralization of cartilage. The deposition of the mineral calcium hydroxyapatite, which reflects light and is visible on plain film X-rays, allows for the observation of early signs of fracture healing, which is known as.[18] Brown et al conducted an animal study to assess the effect of alcohol on fracture healing. They administered ethanol intragastrically to rats and examined the impact of new bone formation in intact and injured bone in separate experiments. In both scenarios, they observed a reduction in quality (mineral content) and quantity of newly formed bone.[19] Kristensson et al pioneered investigation into callus formation in human subjects. They demonstrated through serial plain film X-rays that callus formation was defective in alcoholic individuals.[18]

This underscores the need for further investigation into musculoskeletal injuries among patients with a history of alcohol misuse. Given that ethical constraints limits conducting controlled trials to assess the impact of alcohol in humans, several important retrospective studies have examined the relationship between alcohol misuse and factors such as wound rupture, duration of hospital stay, and re-operation rate. In one such study the investigator reviewed the trauma registry to examine the prevalence of alcohol and drug abuse among adult patients with fractures and dislocations.[11] Out of the 1126 patients who underwent blood alcohol concentration (BAC) testing in this study, 33% (335) had a BAC of 0.10% or higher. The highest prevalence of alcohol use was observed among men aged 21 to 33. The authors reported that the alcohol-positive group had higher average injury severity scores and longer hospital stays.[11] In a different retrospective study the authors focused on ankle fractures and alcoholism.[20] They compared 90 alcohol abusers with 90 controls to investigate postoperative morbidity in patients with malleolar fractures. Within the first 14 days after surgery, the alcohol group experienced significantly more complications compared to the control group, including infection (most frequent), wound rupture, longer hospital stays, and higher re-operation rates.[20] These studies collectively demonstrate an inhibitory relationship between alcohol misuse and the recovery process following fractures and dislocations.

While a substantial body of existing research has explored the molecular and physiological effects of alcohol on bone, this study aims to contribute to our understanding of alcohol misuse and fracture healing by focusing on clinical outcomes such as hospital stay duration, infection rates, and in-hospital mortality (IHM). Although Tonnesen et al examined these parameters in their retrospective analysis, their focus was limited to ankle fractures and alcoholism.[20] In contrast, the present study will encompass all musculoskeletal injuries affecting the head, neck, trunk, and extremities. Additionally, this study will differentiate between different drinking patterns, including alcohol abuse and alcohol dependence (also referred to as alcohol misuse). The aims of the present study are to investigate: (1) the association between alcohol abuse and infection, LOS of ≥ 4 days, and IHM among hospital discharges in 2018. (2) The association between alcohol dependence and infection, LOS of ≥ 4 days, and IHM among hospital discharges in 2018, and (3) the independent predictive role of alcohol misuse on infection, LOS of ≥ 4 days, and IHM when controlling for injury sites and other demographic variables. Our hypothesis is that patients diagnosed with alcohol dependence will experience significantly longer hospital stays, higher rates of infection, and increased IHM compared to patients diagnosed with alcohol abuse. The knowledge gained from this study will enhance our understanding of the burden of alcohol misuse experienced by hospitalized patients undergoing treatment for musculoskeletal injuries.

Materials and Methods

Study design, database, and sample

This retrospective analysis is based on California Hospital Discharge Data obtained from the California Department of Health Care Access and Information. The database, maintained by the California Health Facilities Commission, consists of comprehensive records of inpatient hospital discharges across California. The data is collected by the hospitals primarily for billing and payment purposes. For this study, we used discharge data in 2018. We included discharges individuals of all racial and ethnic backgrounds who were 18 years of age or older and met the ICD-10 codes for musculoskeletal injuries in the following categories: head and neck (ICD-10 codes S00-S19), trunk (ICD-10 codes S20-S49), and extremity musculoskeletal (ICD-10 codes S50-S99) injuries. We also used ICD-10 codes for alcohol abuse (F101) and alcohol dependence (F102). Discharge data for patients under 18 years of age were excluded from this study.

Study Measures

The outcome variables were as follows: Length of stay (LOS) measured as the number of days in the hospital from admission to discharge date and coded as LOS of four days or more (≥4) versus less than 4 days (<4); infection, indicated by the presence or absence of soft tissue or bone infection using ICD-10 codes T814); and IHM. The predictor variables included two categories: (1) discharges identified by the ICD-10 code as for alcohol abuse; and (2) discharges identified by the ICD-10 code for alcohol dependence. Additionally, we used age (18-34, 35-64, and 65+), sex (male or female), race/ethnicity (White, Black, Hispanic, and Asian/other), and insurance status as the confounding variables.

Analysis plan

Descriptive statistics (percentages, means, and standard deviations) were used to depict the study sample's characteristics. We conducted bivariate analyses, using the chi-square test, to examine the association between independent variables (i.e., alcohol abuse or dependence) and dichotomized dependent variables (i.e., infection as yes or no, LOS of≥4 days or 4<days, and IHM as yes or no). We performed multivariate analysis using adjusted logistic regression analysis to determine the independent association of each predictor variable with the outcome variables, controlling for age, gender, ethnicity, and insurance. We used a significance level of $P \leq 0.05$ for all tests in the study, and a 95% confidence interval was reported. We used SAS 9.3 for statistical analysis.

Results

Sample characteristics

In 2018, there were 3.7 million discharged records, of which 207 623 were with alcohol abuse or alcohol dependence. The ethnic distribution of the sample is presented in Table 1, with 113 158 discharges (54.0%) corresponding to White patients, 21 854 discharges (10.4%) to Black patients, 58,683 discharges (28.0%) to Hispanic patients, and 15 900 discharges (7.6%) to Asian/others. The male patient discharges accounted for 71.4% of the discharges, and the 35-64 age group sample, accounted for 63.2%. (Table 1). Regarding alcohol misuse, 40.2% of the discharges were with patients diagnosed with alcohol abuse, and 58.8% were with patients diagnosed with alcohol dependence.

Bivariate association tests

According to Table 2. the Chi-square test results indicate the association between the injury sites and infection, LOS of≥4 days, and IHM among discharges where patients were diagnosed with alcohol abuse. Head/neck and trunk injuries showed associations with infection and LOS of≥4 days ($P \leq 0.05$) but not IHM. Extremity injuries showed associations with all three outcome variables: infection, LOS of≥4 days, and IHM ($P \leq 0.05$).

Table 3 displays the association between injury sites and infection, LOS of≥4, and IHM among discharges where patients were diagnosed with alcohol dependence, based on the chi-square test results. Head/neck injuries were associated with LOS of≥4 days and IHM ($P = < 0.05$). Trunk injuries showed an association with infection and IHM ($P \leq 0.05$). Similarly, extremity injuries in patients with an alcohol dependent were associated with all three outcome variables; infection, LOS of≥4 days, and IHM ($P \leq 0.05$).

Multivariate association test

Table 4 presents the adjusted logistic regression analysis results testing the independent association of alcohol misuse (abuse & dependence) with infection, LOS of≥4 days, and IHM. We controlled for injury sites and other demographic variables. The findings revealed that among musculoskeletal injury discharges, those with alcohol abuse, compared to alcohol dependence, had higher odds of infection (OR=1.25; CI=1.07-1.45). However, they had lower odds of LOS of≥4 days (OR=0.78; CI=0.77-0.79), and lower odds of IHM (OR=0.91; CI=0.86-0.96).

Additional findings

Furthermore, among musculoskeletal injury discharges, those with head/neck injuries, compared to other injury locations (truck and extremities), had lower odds of infection (OR=0.49; CI=0.35-0.70), lower odds of LOS of≥4 days (OR=0.90; CI=0.87-0.93), and increased odds of IHM (OR=1.23; CI=1.11-1.37). Discharges with trunk injuries, when compared to other injury locations (head/neck and extremities), showed increased odds of infection (OR=1.66; CI=1.24-2.22), increased odds of LOS of≥4 days (OR=1.36; CI=1.30-1.41), and increased odds of IHM (OR=1.21; CI=1.06-1.38). Lastly, discharges with

Table 1. Demographics: Sample size (n = 209 623)

Sample demographics	
Age	
18-34	35907 (17.1%)
35-64	132392 (63.2%)
65 +	41324 (19.7%)
Gender	
Male	149709 (71.4%)
Female	59874 (28.6%)
Insurance status	
Medicare	55024 (26.3%)
Medicaid	89930 (42.9%)
Private	48629 (23.2%)
Other insurance	16040 (7.6%)
Ethnicity	
White	113158 (54.0%)
Hispanic	58683 (28.0%)
Black	21854 (10.4%)
Asian/Others	15900 (7.6%)
Alcohol Use	
Alcohol abuse	84296 (40.2%)
Alcohol dependent	123304 (58.8%)
Unspecified	2023 (1.0%)
Length of hospital stay (LOS)	
< 4 days	102034 (48.7%)
≥ 4 days	107589 (51.3%)
Injury	
Head and neck	16100 (7.7%)
Truck	10876 (5.2%)
Extremities	13851 (6.6%)
Procedure	
Head and neck	891 (5.5%)
Truck	66 (0.6%)
Extremities	2515 (18.2%)
In-hospital mortality	
No	204799 (97.7%)
Yes	4824 (2.3%)
Infection	
Yes	699 (0.3%)
No	208924 (99.7%)

extremity injuries, compared to other injury locations (head/neck and trunk), exhibited increased odds of infection (OR = 2.04; CI = 1.60-2.59), increased odds of LOS of ≥ 4 days (OR = 1.28; CI = 1.23-1.33), and lower odds of IHM (OR = 0.43; CI = 0.36-0.51). Other variables in the regression model, such as age, gender, and ethnicity, also demonstrated associations with increased odds of infection, LOS of ≥ 4 days, and IHM. Males compared to females and patients aged 67 or older compared to their 18-34 age group encounters were more likely to experience infection, LOS of ≥ 4 days, and IHM. Hispanic and Asian patients experienced less infection and fewer days in the hospital but presented with higher odds of IHM.

Discussion

In this study, we investigated the prevalence of alcohol abuse and alcohol dependence among patients with musculoskeletal injuries and explored their impact on clinical outcomes. Our data revealed that over one-third of the musculoskeletal injury discharges were diagnosed with alcohol abuse, while nearly 60% were diagnosed with alcohol dependence.

We hypothesized that patients diagnosed with alcohol dependence would experience significantly higher rates of infection, longer hospital stays, and increased IHM compared to patients diagnosed with alcohol abuse. Our results contradicted our hypothesis, as patients with alcohol abuse had higher odds of experiencing an infection during their hospital admission compared to patients with alcohol dependence. Based on the findings of previous studies, the underlying mechanism of alcohol's effect on immune response and infection susceptibility requires further investigation.[21,22]

Our findings that patients qualifying with the diagnosis of alcohol abuse had lower odds of experiencing a LOS of ≥ 4 days and IHM could be reflecting that a less severe drinking pattern carries less burden regarding LOS and could reduce IHM risk, compared to alcohol dependence. Several investigators have suggested that achieving a reduction in the consumption of alcohol among alcohol-dependent individuals who may not consider entering treatment could reduce the overall burden associated with alcohol dependence,[23,24] reinforcing the validity of harm reduction through reduced alcohol consumption as a treatment goal.[25]

Moreover, our findings revealed that patients with head and neck injuries were more likely to experience IHM compared to their counterparts with other injury locations such as truck and extremities. It is possible that this association is due to the severity of such injuries, including skull fractures that may lead to fatal brain bleeds, which require further investigations. On the other hand, patients with extremity injuries were less likely to experience IHM suggesting these injuries are less likely to be life-threatening. Furthermore, our findings highlight that being aged 67 or older, male, of Hispanic or Asian ethnicity, and having Medicare or Medicaid could increase the vulnerability to adverse health outcomes among patients hospitalized for musculoskeletal injury treatment. These outcomes indicate potential risks and disparities in healthcare access and quality of care among certain demographic groups subject to injuries.[26-28]

Table 2. Bivariate analysis of alcohol abuse with infection, LOS ≥ 4 days, and IHM

		Infection (%)	*P* value	LOS (≥4 days) greater or equal(%)	*P* value	In-hospital mortality (%)	*P* value
Overall		312 (0.37%)		40212 (47.70%)		1774 (2.10%)	
Age	18-34	37 (0.21%)	0.0004	7936 (45.06%)	<0.0001	95 (0.54%)	<0.0001
	35-64	211 (0.41%)		24277 (47.71%)		1104 (2.17%)	
	65+	64 (0.41%)		7999 (50.67%)		575 (3.64%)	
Gender	Male	239 (0.39%)	0.1889	29099 (47.94%)	0.0657	1316 (2.17%)	0.1003
	Female	73 (0.31%)		11106 (47.10%)		458 (1.94%)	
Ethnicity	White	180 (0.45%)	0.0023	19206 (48.43%)	<0.0001	878 (2.21%)	<0.0001
	Hispanic	80 (0.31%)		11839 (45.59%)		538 (2.07%)	
	Black	33 (0.29%)		5760 (50.16%)		157 (1.37%)	
	Asian/Other	19 (0.27%)		3398 (47.41%)		200 (2.79%)	
Insurance Status	Medicare	89 (0.39%)	0.0762	11331 (53.41%)	<0.0001	626 (2.95%)	<0.0001
	Medicaid	154 (0.39%)		19137 (47.87%)		728 (1.82%)	
	Private	56 (0.33%)		7225 (43.01%)		302 (1.80%)	
	Other	13 (0.21%)		2519 (39.93%)		118 (1.87%)	
Injury Site	Head and neck	16 (0.20%)	0.0082	3537 (44.00%)	<0.0001	189 (2.36%)	0.0964
	Trunk	35 (0.61%)	0.0018	3025 (52.98%)	<0.0001	118 (2.07%)	0.8361
	Extremities	48 (0.67%)	<0.0001	3741 (52.29%)	<0.0001	71 (0.99%)	<0.0001

Limitations

We acknowledge certain limitations in this study, including potential confounders not fully controlled for, and the lack of detailed information on specific patient lifestyle factors and comorbidities. Future studies should consider the inclusion of the severity of injuries and other potential confounding factors to provide a more comprehensive understanding of the independent association of alcohol misuse in assessing patient outcomes. Also further studies are required to elucidate the dosage and duration of alcohol consumption necessary to impair bone formation and fracture healing.

Implications and future directions

The observed associations between alcohol misuse and clinical outcomes underscore the importance of tailored

Table 3. Bivariate analysis of alcohol-dependent with infection, LOS ≥ 4 days, and IHM

		Infection (%)	*P* value	LOS (≥4 days) (%)	*P* value	In-hospital mortality (%)	*P* value
Overall		386 (0.31%)		67036 (53.91%)		3040 (2.44%)	
Age	18-34	21 (0.12%)	<0.0001	9085 (50.77%)	<0.0001	104 (0.58%)	<0.0001
	35-64	262 (0.32%)		43356 (53.38%)		1868 (2.30%)	
	65+	103 (0.41%)		14595 (57.85%)		1068 (4.23%)	
Gender	Male	295 (0.33%)	0.0693	47604 (53.82%)	0.4427	2256 (2.55%)	0.0006
	Female	91 (0.25%)		19421 (54.14%)		783 (2.18%)	
Ethnicity	White	255 (0.35%)	0.0065	40598 (55.56%)	<0.0001	1712 (2.34%)	<0.0001
	Hispanic	84 (0.26%)		16327 (50.27%)		875 (2.69%)	
	Black	18 (0.18%)		5427 (53.56%)		180 (1.78%)	
	Asian/Other	29 (0.34%)		4679 (54.13%)		273 (3.16%)	
Insurance Status	Medicare	126 (0.38%)	0.0114	19648 (58.66%)	<0.0001	1154 (3.45%)	<0.0001
	Medicaid	160 (0.32%)		25049 (50.47%)		1167 (2.35%)	
	Private	77 (0.24%)		16700 (53.02%)		586 (1.86%)	
	Other	23 (0.24%)		5639 (57.96%)		133 (1.37%)	
Injury Site	Head and neck	21 (0.27%)	0.5009	4513 (57.89%)	<0.0001	222 (2.85%)	0.0174
	Trunk	23 (0.46%)	0.0463	3191 (64.49%)	<0.0001	148 (2.99%)	0.0111
	Extremities	38 (0.59%)	<0.0001	4063 (62.93%)	<0.0001	78 (1.21%)	<0.0001

Table 4. Independent association of alcohol misuse with Infection, LOS, and IHM using adjusted logistic regression analysis (n = 207 623)

		Infection OR (95% CI)	LOS (≥4 days) OR (95% CI)	In-hospital mortality OR (95% CI)
Alcohol misuse	Alcohol abuse	1.25 (1.07-1.45)	0.78 (0.77-0.79)	0.91 (0.86-0.96)
	Alcohol dependent	Ref	Ref	Ref
Age	18-34	Ref	Ref	Ref
	35-64	2.18 (1.65-2.87)	1.08 (1.05-1.10)	4.07 (3.52-4.70)
	65+	2.30 (1.62-3.27)	1.01 (0.97-1.04)	7.91 (6.72-9.32)
Gender	Male	1.33 (1.11-1.59)	1.03 (1.01-1.05)	1.07 (1.00-1.15)
	Female	Ref	Ref	Ref
Ethnicity	White	Ref	Ref	Ref
	Hispanic	0.72 (0.59-0.86)	0.87 (0.85-0.89)	1.19 (1.11-1.27)
	Black	0.58 (0.43-0.78)	1.01 (0.98-1.04)	0.71 (0.63-0.80)
	Asian/Other	0.81 (0.60-1.09)	0.98 (0.94-1.01)	1.44 (1.30-1.59)
Insurance Status	Medicare	1.19 (0.92-1.54)	1.36 (1.32-1.41)	1.04 (0.94-1.15)
	Medicaid	1.31 (1.06-1.61)	1.03 (1.01-1.05)	1.18 (1.08-1.28)
	Private	Ref	Ref	Ref
	Other	0.84 (0.58-1.22)	1.09 (1.05-1.13)	0.88 (0.76-1.01)
Injury site	Head and neck	0.49 (0.35-0.70)	0.90 (0.87-0.93)	1.23 (1.11-1.37)
	Trunk	1.66 (1.24-2.22)	1.36 (1.30-1.41)	1.21 (1.06-1.38)
	Extremities	2.04 (1.60-2.59)	1.28 (1.23-1.33)	0.43 (0.36-0.51)

interventions for patients undergoing hospitalization for musculoskeletal injury treatment. Additional treatments, such as electrical bone stimulation, may hold promise for improving healing in difficult-to-treat fractures in this population. [29] Also, our results shed light on the important role of physicians, including orthopedic surgeons, in treating not only the musculoskeletal complaints of patients but also addressing their overall health, including any history of alcohol abuse. A patient's hospital admission for a musculoskeletal injury presents an opportune moment for healthcare professionals to identify and address alcohol-related issues that may otherwise go unnoticed. By incorporating routine alcohol screening and referral for treatment, physicians can offer valuable support and interventions to patients struggling with alcohol misuse.[30,31] It is especially important for healthcare providers to extend stronger efforts to reach and support patients with limited access to healthcare services and resources, therefore contribute to more holistic and effective patient care.

Conclusion

Our results reveal the burden of alcohol misuse in treatment outcomes among patients undergoing hospitalization for treatment of musculoskeletal injuries as they related to infection, length of hospital stay, and IHM. These findings also highlight the potential economic implications of alcohol-related musculoskeletal injuries. Our findings emphasize the necessity for an approach that goes beyond treating immediate physical injury, but considering a patient's history of alcohol abuse and providing appropriate support and interventions to improve treatment outcomes for individuals affected by musculoskeletal injuries and alcohol misuse.

Acknowledgments

We also would like to acknowledge the support of Hamed Yazdanshena, MD, Assistant Professor, Department of Surgery, CDU/UCLA, for his initial mentorship.

Authors' Contribution

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Competing Interests

The authors have no conflicts of interest to declare.

Consent for publication

All respondents permitted publication, provided anonymity was ensured.

Data Availability Statement

The dataset analyzed during the current study is publicly available and downloadable from (https://www.cdc.gov/nchs/ahcd/datasets_documentation_related.htm) and also available from the corresponding author on reasonable request.

Ethical Approval

This study did not require institutional review board approval or patient consent because no identifying patient data was collected. We used publicly available national. However, all methods were carried out by relevant guidelines and regulations (e.g., the Helsinki Declaration). Informed consent was obtained from all participants in the study. The study was performed in line with the principles of the Helsinki Declaration.

Funding

Research for this article was supported in part by NIH Accelerated Excellence in Translational Sciences (AXIS) grant number 2U54MD007598-07; and the University of California at Los Angeles (UCLA) Clinical and Translational Science Institute (CTSI), grant number UL1TR001881.

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