

Research Article

Elevated Monocyte Chemoattractant Protein-1 and Nuclear Factor Kappa B Biomarkers in Captagon and Methamphetamine Addiction

Mahmood Mehdi Rasheed¹, Hassan Ali Al-Saadi¹, Amer Alhaideri², Yehia Ali Hadi Al-Qhadi³

¹ Department of Clinical Laboratories, College of Applied Medical Sciences, University of Kerbala, Karbala, Iraq

² College of Medicine, University of Kerbala, Karbala, Iraq

³ College of Pharmacy, University of Kerbala, Karbala, Iraq

Article information:

Received: 07-07-2025

Accepted: 10-08-2025

Correspondence: Mahmood Mehdi Rasheed

E-mail: Mahmood.mahdi@s.uokerbala.edu.iq

ORCID: <https://orcid.org/0009-0000-5722-7300>

<https://doi.org/10.70863/karbalajm.v18i2.4921>

Abstract

Background: Addiction is a complex, chronic brain disorder characterized by compulsive drug seeking and use despite harmful consequences. They affect approximately 10% of the population, and this percentage increases over time. This study aimed to investigate the roles of monocyte chemoattractant protein-1 (MCP-1) and nuclear factor kappa B (NF-κB) in patients addicted to captagon and methamphetamine.

Methods: This case-control study was carried out for addicted patients with captagon and methamphetamine at the Rehabilitation Center for Drug and Substance Abusers in the Directorate of Narcotics and Psychotropic Substances in the Karbala governorate from November 2024 to May 2025.

Results: White blood cell (WBC) levels decreased for both unmarried and married patients, while red blood cells (RBC) decreased in unmarried patients. Hematocrit test (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) values increased significantly in both unmarried and married patients with captagon and methamphetamine users compared with healthy individuals. MCP-1 was elevated in married captagon users (470.67 ± 258.41 pg/mL; $p < 0.00001$) and unmarried methamphetamine users (459.40 ± 151.30 pg/mL; $p < 0.00001$). NF-κB was elevated across all user groups ($p < 0.00001$). Receiver operating characteristic (ROC) analysis for MCP-1 (AUC = 99.235%; sensitivity 97.778%, specificity 97.778%) and NF-κB (AUC = 96.716%; sensitivity 94.444%, specificity 95.556%) showed excellent diagnostic accuracy.

Conclusions: MCP-1 levels were higher in married captagon users than in unmarried captagon users, whereas unmarried methamphetamine users exhibited higher MCP-1 than married methamphetamine users. NF-κB levels were markedly higher in both unmarried and married captagon and methamphetamine users.

Key words: MCP-1, NF-κB, captagon, methamphetamine, biomarkers.

Introduction

Addiction is a complex, chronic brain disorder characterized by compulsive drug seeking and use despite harmful consequences. It is recognized as a major public health concern worldwide, affecting approximately 10% of the population and increasing over time [1]. Captagon, a pharmaceutical medication that has evolved into a widely abused illicit substance, represents a complex intersection of medical innovation, geopolitical conflict, and drug trafficking. Once developed as a treatment for conditions including attention deficit hyperactivity disorder (ADHD) and narcolepsy, captagon now cir-

culates primarily as an illegal drug with a significant presence in the Middle East and growing international concerns [2]. Methamphetamine is a synthetic psychoactive compound belonging to the phenethylamine and amphetamine classes, with the chemical formula $C_{10}H_{15}N$. Its International Union of Pure and Applied Chemistry name is (S)-N-methyl-1-phenylpropan-2-amine, characterized by a phenethylamine backbone featuring a methyl group attached to the nitrogen atom and a chiral center conferring stereochemical activity, with the (S)-enantiomer exhibiting greater central nervous system (CNS) potency [3-4].

Human monocyte chemoattractant protein-1 (MCP-1) is located on chromosome 17 (q11.2) and

is composed of 76 amino acids, with a molecular weight of approximately 13 kDa. The primary amino acid sequence of MCP-1 contains a characteristic pattern of cysteine residues that form essential disulfide bonds critical for maintaining its three-dimensional structure and biological activity [5]. The expression of MCP-1 or decreased binge drinking indicates a neuronal TLR4/MCP-1 signaling pathway that governs the onset of voluntary alcohol self-administration [6]. The signal persists during alcohol consumption due to heightened expression of corticotropin-releasing factor and its regulatory feedback on TLR4 expression. This likely contributes to the development of alcohol dependence; however, the relationship between alcohol-induced corticotropin-releasing factor expression and the specific brain sites involved remains ambiguous [7]. MCP-1 plays a critical role in numerous physiological processes, particularly those involved in immune cell trafficking and inflammatory responses. One of its primary functions is to regulate the migration and infiltration of monocytes, memory T lymphocytes, and other immune cells to the sites of injury and infection. This chemotactic activity is fundamental to both the routine immunological surveillance of tissues and targeted responses to inflammation [8-9].

Baltimore first identified NF- κ B in 1986 as a nuclear factor binding to the kappa enhancer of the gene encoding the κ light chain of immunoglobulin in B lymphocytes [10]. The name NF- κ B originated from this initial observation, denoting "nuclear factor binding near the κ light-chain gene in B cells." Subsequent research established that NF- κ B is not exclusive to B cells but rather is present in almost all animal cell types, playing crucial roles across diverse tissues and biological processes [11]. The identification of NF- κ B represents a significant breakthrough in understanding transcriptional regulation and cellular responses to external stimuli, setting the foundation for decades of research [12]. The effects of NF- κ B activation differ substantially across CNS cell types. While neuronal NF- κ B signaling typically serves protective functions by inducing anti-apoptotic genes, such as TNF receptor-associated factors, caspase inhibitors, Bcl-2 family members, and superoxide dismutase, NF- κ B activation in astrocytes and microglia is often associated with more detrimental outcomes [13]. Some studies suggest that NF- κ B activation in oligodendrocyte precursor cells decreases apoptosis and promotes cell maturation, indirectly contributing to myelination in the CNS, whereas

other studies indicate that NF- κ B may be dispensable for certain aspects of oligodendrocyte function [10, 14].

Dysregulated NF- κ B signaling in the central nervous system contributes to various neurodegenerative conditions by promoting neuroinflammation and influencing neuronal survival. As the primary immune response of the central nervous system, microglia exhibit upregulation of NF- κ B upon activation in response to pathological conditions, initiating inflammatory cascades that can exacerbate neurodegeneration [15-16].

This study aimed to investigate the roles of MCP-1 and NF- κ B in captagon- and methamphetamine-addicted patients.

Materials and Methods

Study subject

This case-control study investigated captagon and methamphetamine addiction at the Rehabilitation Center for Drug and Substance Abusers (Directorate of Narcotics and Psychotropic Substances, Karbala Governorate). All the participants provided written informed consent. A total of 135 addicted patients were recruited consecutively upon admission, along with 45 age-matched healthy controls with no history of substance use between November 2024 and May 2025. Inclusion required males aged 15–70 years with a psychologist-confirmed diagnosis of psychostimulant addiction supported by clinical signs (behavioral changes, withdrawal symptoms) and laboratory evidence. Exclusion criteria included age <15 years, incomplete medical/demographic records, and comorbidities (autoimmune diseases and malignancies) that could confound immunological results. Patients were stratified into the following groups: Group 1, who use captagon, subdivided by age (<30 or \geq 30 years); group 2, who use methamphetamine, subdivided similarly; and group 3, who were healthy controls, age-matched to groups 1–2.

Biomarker Assay

Comprehensive diagnostic testing was performed in the laboratory. Hematological (CBC) and immunological biomarkers (MCP-1/monocyte chemoattractant and activating factor (MCAF), NF- κ B) were analyzed in all participants. Biochemical assays were performed utilizing a Dirui fully automated analyzer (e.g., CS-T240 model). Serum samples were centrifuged (3,000 rpm, 10 min) and processed via onboard protocols (15-min runtime). Complete blood count (CBC) was measured from 2 mL of EDTA-anticoagulated venous blood using an automated hematology analyzer (Sysmex XN-

series) with the following reference ranges: (white blood cells (WBC): $4.5\text{--}11.0 \times 10^3/\mu\text{L}$, red blood cells (RBC): $4.5\text{--}5.9 \times 10^6/\mu\text{L}$, hemoglobin (Hb): $13.5\text{--}17.5 \text{ g/dL}$, hematocrit test (HCT): $38.8\text{--}50.0\%$, mean corpuscular volume (MCV): $80\text{--}100 \text{ fL}$, and mean corpuscular hemoglobin (MCH): $27\text{--}33 \text{ pg}$).

Immunological markers were quantified using commercial ELISA kits (Human MCP-1/MCAF and NF-κB; YH Biosearch Laboratory, China; Catalog E0124Hu at a range of $5\text{--}1500 \text{ ng/L}$ and E0690Hu at a range of $0.03\text{--}10 \text{ ng/ml}$, respectively) according to the manufacturer protocols. Samples and standards were incubated in pre-coated wells, developed with HRP-conjugate, and read at 450 nm . All assays included internal quality control and inter-assay calibration to ensure reproducibility.

Ethical Approval

The study was carried out in accordance with the Declaration of Helsinki, 2013. This study was approved by the Directorate of Narcotics and Psychotropic Substances, Karbala Governorate, under ethical document No. 3798 on 05 Nov 2024. After the Ministry of Health and Environment granted permission to conduct the study, samples were collected, and the study commenced. The study participants gave their permission to collect sociodemographic data and undertake experiments on the selected samples with respect to patient confidentiality.

Statistical Analysis

Data were analyzed using SPSS version 22. Continuous variables are expressed as mean \pm standard deviation (SD). Group comparisons (e.g., control vs. drug users, marital subgroups) were performed using independent samples Student's t-test. For comparisons across more than 2 groups (e.g., control, captagon, and methamphetamine), one-way ANOVA with Bonferroni post-hoc correction was

applied. Statistical significance was set at $p < 0.05$. Receiver operating characteristic (ROC) curves were generated to evaluate biomarker diagnostic accuracy (cutoff values, sensitivity, specificity, and AUC).

Results

Ninety male patients, including captagon and methamphetamine users, demonstrated significantly more unmarried methamphetamine users than captagon users (29 (64.4%) and 20 (44.4%), respectively), while the number of married captagon users was 25 (55.6%) and 16 (35.6%), respectively, and urban methamphetamine users were 31 (68.9%) compared with healthy individuals. Patients aged >30 years who were captagon users were more than methamphetamine users (40.75 ± 9.55 and 38.02 ± 6.84 , respectively) (Table 1). Table 2 presents the hematological parameters (WBC, RBC, Hb, HCT, MCV, and MCH) compared with healthy individuals, captagon users, and methamphetamine users, stratified by marital status. Significant differences ($p < 0.05$) were observed across all parameters; WBC levels decreased for both unmarried and married captagon and methamphetamine users compared with healthy individuals (7.20 ± 0.76 ; 6.44 ± 1.32 ; 6.72 ± 1.24 , and 6.56 ± 1.26 , respectively), with notably low p-values ($p = 0.00001$ for MCV and MCH). Unmarried captagon and amphetamine users exhibited lower RBC (5.40 ± 0.99 and 4.58 ± 1.15 , respectively) than healthy individuals. While methamphetamine users consistently showed reduced RBC regardless of marital status (4.56 ± 1.03) compared with healthy individuals. Hemoglobin (Hb) levels were more elevated in captagon users (unmarried: 15.35 ± 1.56 and married: 14.80 ± 1.58) than in healthy individuals (13.53 ± 1.62).

Table 1: Distribution and characteristics of patients and controls according to the study subjects.

Parameters	Level	Healthy individuals	Captagon	Methamphetamine	Total	p-value
Sex	Male	45	45	45	135	0.00001
		100%	100%	100%	100%	
Marital Status	Unmarried	45	20	29	94	0.00004
		100.0%	44.4%	64.4%	69.6%	
	Married	0	25	16	41	
		0.0%	55.6%	35.6%	30.4%	
Address	urban	27	29	31	87	0.67846
		60.0%	64.4%	68.9%	64.4%	
	rural	18	16	14	48	
		40.0%	35.6%	31.1%	35.6%	
Age Group	Less than 30	36	20	37	93	0.00008
		80.0%	44.4%	82.2%	68.9%	
	Greater than 30	9	25	8	42	
		20.0%	55.6%	17.8%	31.1%	
Age (Mean \pm SD)	Less than 30	23.77 ± 2.88	23.0 ± 2.07	24.94 ± 2.22	24.07 ± 2.56	0.011
	Greater than 30	33.33 ± 1.50	38.84 ± 6.42	40.75 ± 9.55	38.02 ± 6.84	0.049
	Overall	25.68 ± 4.69	31.800 ± 9.36	27.8 ± 7.67	28.41 ± 7.8	0.0165

In the current study, the levels of all immunological markers were significantly increased. MCP-1 levels were significantly elevated in both captagon and methamphetamine users compared to controls ($p < 0.00001$). Married captagon users exhibited higher MCP-1 (470.67 ± 258.41 pg/mL) than unmarried captagon users (403.75 ± 170.66 pg/mL). Conversely, unmarried methamphetamine users showed higher MCP-1 (459.40 ± 151.30 pg/mL) than married users (409.22 ± 127.32 pg/mL). NF-κB levels were markedly higher in both unmarried and married captagon and methamphetamine users (1.72 ± 0.42 , 1.70 ± 0.48 , 1.90 ± 0.44 , and 1.90 ± 0.41 , respectively) than in healthy individuals (Table 3).

Table 4 and Figure 1 summarize the diagnostic performance of three biomarkers (MCP and NF-κB)

using receiver operating characteristic (ROC) analysis. All biomarkers demonstrated excellent diagnostic accuracy, with area under the curve (AUC) values exceeding 96%: TNF-α (97.556%), MCP (99.235%), and NF-κB (96.716%). Sensitivity and specificity were consistently high across biomarkers, ranging from 94.444% to 97.778% and 95.556% to 97.778%, respectively. Asymptotic significance values ($p < 0.002$) confirmed statistical reliability, while narrow 95% confidence intervals (TNF-α: 0.952–0.999) indicated precise estimates. The optimal cutoff points for MCP and NF-κB distinguishing cases were 157.418 and 0.870, respectively, and exhibited the highest AUC and balanced sensitivity-specificity (97.778% and 94.444%), suggesting superior discriminatory power.

Table 2: Comparison of hematological parameters between control, captagon, and methamphetamine groups stratified by marital status.

Parameter	Group	Control		Captagon		Methamphetamine		p-value
		Mean	SD	Mean	SD	Mean	SD	
WBC	Unmarried	9.37	1.41	7.20	0.76	6.44	1.32	0.0084
	Married	9.37	1.41	6.72	1.24	6.56	1.26	0.0199
RBC	Unmarried	7.33	1.28	5.40	0.99	4.58	1.15	0.0003
	Married	7.33	1.28	7.32	1.77	4.56	1.03	0.0003
Hb	Unmarried	13.53	1.62	15.35	1.56	14.13	1.43	0.0003
	Married	13.53	1.62	14.80	1.58	14.81	1.27	0.0019
HCT	Unmarried	40.93	4.45	44.75	2.07	43.93	2.37	0.0002
	Married	40.93	4.44	43.44	2.27	45.00	1.86	0.0003
MCV	Unmarried	84.09	6.81	91.45	1.60	91.82	2.28	0.00001
	Married	84.09	6.81	91.72	1.99	92.25	1.77	0.00001
MCH	Unmarried	26.67	2.31	32.00	2.79	33.13	5.51	0.00001
	Married	26.67	2.31	33.44	2.08	32.06	1.28	0.00001

WC: white blood cells, RBC: white red blood cells, Hb: hemoglobin, HCT: hematocrite test, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin

Table 3: Comparison of serum inflammatory marker levels (MCP-1 and NF-κB) among control, captagon, and methamphetamine users stratified by marital status

Parameter	Group	Control		Captagon		methamphetamine		p-value
		Mean	SD	Mean	SD	Mean	SD	
MCP-1	Unmarried	19.15	3.55	403.75	170.66	459.40	151.30	<0.00001
	Married	19.15	3.55	470.67	258.41	409.22	127.32	<0.00001
NF-κB	Unmarried	0.28	0.04	1.72	0.42	1.90	0.44	<0.00001
	Married	0.28	0.04	1.70	0.48	1.90	0.41	<0.00001

MCP-1: monocyte chemoattractant protein-1, NF-κB: nuclear factor kappa B

Table 4: Performance metrics of TNF-α, MCP, and NF-κB biomarkers, including diagnostic accuracy, sensitivity, and specificity

Metrics		MCP	NF-κB
Std. Error		0.005	0.015
Asymptotic Sig.		0.001	0.001
Asymptotic 95% Confidence Interval	Lower Bound	0.982	0.937
	Upper Bound	1.000	0.997
Cutoff Point		157.418	0.870
Area Under Curve (AUC)		99.235%	96.716%
Sensitivity		97.778%	94.444%
Specificity		97.778%	95.556%

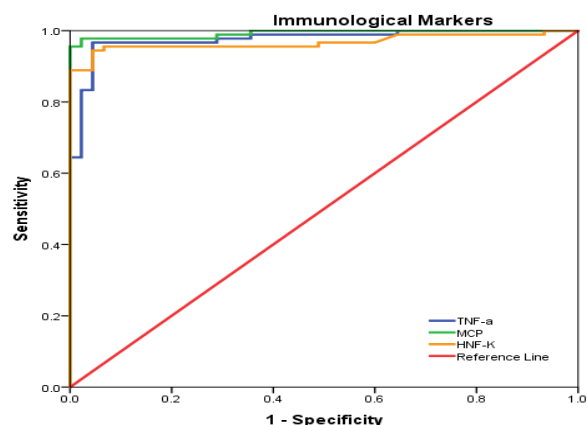


Figure 1: Receiver Operating Characteristic curves for TNF- α , MCP, and NF- κ B biomarkers

Discussion

This study highlights a significant male predominance in substance use. 100% of captagon and methamphetamine users were male, consistent with findings from Baghdad, where 95% of participants were male [17]. Similarly, Curran *et al.* (2022) noted that methamphetamine users are disproportionately male and often of lower socioeconomic status [18]. Unmarried patients constituted the majority of the substance users. For instance, 64.4% of methamphetamine users were unmarried, consistent with broader conclusions that Iraqi meth users are predominantly single [17], and the risk factors for amphetamine use include peer use, a history of violence, unfavorable friends, and low levels of education. Males, singles, and urban residents were prevalent among substance users (68.9% for methamphetamine and 64.4% for captagon). It was reported that 80% of participants lived in urban areas [17]. According to Derefinco *et al.* (2018), rural residents had lower rates of alcohol and marijuana usage than their urban counterparts when they were freshmen, but by their junior year, they had caught up with urban students [19]. The methamphetamine users were younger (82.2% less than 30 years). Westbrook *et al.* (2020) emphasized that early adulthood is a critical period for methamphetamine exposure [20]. Adolescent vulnerability is further supported by Guerin *et al.* (2023) [21], who linked methamphetamine use in youth (10–25 years) to impulsivity and poor educational outcomes.

The mean age of methamphetamine users (24.94 ± 2.22 for those under 30) reflects a younger demographic. Al-Imam *et al.* (2023) identified withdrawal symptoms and psychotic traits in this group, underscoring the age-specific risks. Low educational attainment and socioeconomic status are strongly associated with substance use [22]. Over 61% of methamphetamine users had only primary

education, unemployment, and low education as key risk factors [23]. Guerin *et al.* (2023) highlighted cognitive impairments [21], whereas Hanan *et al.* (2025) [24] linked adolescent use to psychosocial issues. The rise in methamphetamine use poses economic strain, as substance users are less productive and more likely to be unemployed [25]. This contrasts with prior studies reporting elevated WBC counts in methamphetamine users and amphetamine/cocaine addicts [26–27]. RBC counts and Hb levels were significantly lower in methamphetamine groups, aligning with findings of decreased Hb, HCT, and albumin in methamphetamine users [26, 28]. Methamphetamine groups demonstrated elevated HCT and markedly higher MCV and MCH. Marital status stratified differences emerged: unmarried captagon users had lower WBC and RBC counts than their married counterparts, while married methamphetamine users showed marginally higher HCT and MCV. Such stratification underscores the role of socio-demographic factors in hematological outcomes, as highlighted in studies of amphetamine-cannabis poly-drug users [29–30].

MCP-1 elevation in cerebrospinal fluid (CSF) has been linked to dementia progression in humans, implicating chemokine signaling in neurodegenerative processes [31]. In alcoholic liver disease, MCP-1 is upregulated in hepatocytes, although its functional role remains unclear. Further research is needed to elucidate its cell-specific modulation during injury, proposing a model in which alcohol upregulates MCP-1 expression and activates CCR2 signaling, leading to activation of pro-inflammatory transcription factors such as AP-1 and NF- κ B through GSK3 β or JNK pathways, ultimately causing neuroinflammation and neuronal death [32]. The role of MCP1/CCR2 in alcohol-induced brain damage remains unclear. Recent evidence indicates that alcohol exposure increases the activity of MCP-1/CCR2 in both mature and developing central nervous systems (CNS) [32]. Clinically, MCP-1 serves as a diagnostic and prognostic marker in diabetic kidney disease, with levels strongly associated with nephropathy severity, progression, and outcome [33–34]. A cross-species translational study examining immune dysregulation in methamphetamine users found that MCP-1 levels were similarly increased in the plasma of methamphetamine-exposed mice and humans. This study demonstrated that mice with methamphetamine-induced changes in peripheral immune molecule expression also showed significant brain region-specific changes in pro-inflammatory cytokines, chemokines, and ICAM-1 [31]. We hypothesized

that MCP-1 upregulation promotes monocyte infiltration into the CNS and perpetuates neurotoxicity. This aligns with murine studies, where METH increased hippocampal MCP-1 expression, concomitant with microglial activation and spatial memory deficits [35].

Recent studies have highlighted the prognostic and diagnostic utility of inflammatory biomarkers for various renal and hepatic pathologies. Patients presenting with acute-on-chronic liver failure (ACLF) exhibit significantly reduced circulating levels of tumor necrosis factor- α (TNF- α) and macrophage inflammatory protein-1 α (MIP-1 α) [36]. A recent study investigated the addiction treatment on inflammatory markers and found a significant decrease in MCP-1 levels in patients receiving medium doses of both medications. This research reinforced the understanding that MCP-1 plays a crucial role in drug addiction by participating in dopamine neuron activation in the reward system and affecting hypothalamic-pituitary-adrenal (HPA) axis deregulation through corticotrophin-releasing factor secretion [37]. Another study supported previous findings that buprenorphine may reduce MCP-1 by inhibiting monocytes by blocking the integrin pathway and by controlling addiction and neuroinflammatory process deterioration, while methadone showed more severe effects on intracellular functions [38].

NF- κ B activation is a hallmark of METH-induced toxicity, as evidenced by the increased nuclear translocation and transcriptional activity in exposed models [39]. Human studies corroborate these findings, showing marked NF- κ B overexpression in chronic METH users [40]. Beyond neurotoxicity, NF- κ B regulates inflammatory and apoptotic pathways in diverse pathologies, including acute liver failure, via its canonical pathway [40]. A groundbreaking study demonstrated the primary neurotransmitter affected by all addictive substances directly activates NF- κ B in primary human macrophages, resulting in the induction of downstream targets, including the NLRP3 inflammasome and the inflammatory cytokine IL-1 β [41]. The researchers found that NF- κ B activation is required for dopamine-mediated increases in IL-1 β production, as an NF- κ B inhibitor completely abrogates the effects of dopamine on cytokine production [41].

A comprehensive review examined the functions of NF- κ B in addiction processes and demonstrated that NF- κ B can induce the expression of diverse gene targets beyond inflammatory mediators, including opioid receptors and neuropeptides involved in addictive processes [42]. The authors

presented evidence that NF- κ B mediates complex behaviors, including learning and memory, stress responses, anhedonia, and drug reward processes that may extend beyond the classic neuroimmune response traditionally associated with this transcription factor [42]. Researchers found that the NF- κ B antagonist sodium diethyldithiocarbamate trihydrate significantly reversed cocaine-induced expression changes in the amphetamine addiction pathway, with genes demonstrating differential expression in response to cocaine treatment being enriched for the axon guidance pathway [43]. Another study revealed that the NF- κ B homodimer motif could be mapped to 86 of the genes reversed by NF- κ B inhibition, suggesting that NF- κ B directly modifies the expression of axon guidance pathway members, leading to cocaine sensitization and uncovering the molecular mechanisms by which NF- κ B drives changes in the addicted brain [44]. A recent study examined the synergistic cardiotoxic effects of captagon (a fenethylamine) and azithromycin. The researchers demonstrated that both substances, alone and in combination, caused cardiotoxicity by activating several inflammatory pathways and observed a significant increase in NF- κ B expression in both the captagon-only and captagon with azithromycin groups, with dose-dependent effects [45]. NF- κ B signaling has emerged as a pivotal driver of renal fibrosis. Accumulating evidence suggests that NF- κ B is a central modulator of fibrotic pathways, and its activation is implicated in extracellular matrix remodeling and chronic kidney disease progression [46].

Conclusions

Captagon and methamphetamine addiction induce hematological dyscrasias and robust upregulation of MCP-1/NF- κ B, with marital status influencing MCP-1 expression. These biomarkers demonstrate high diagnostic accuracy and are mechanistically linked to neuroinflammation, hepatic/renal damage, and addiction pathophysiology, positioning them as critical predictors for organ disease in substance use disorders.

Acknowledgement

The authors greatly appreciate the support of the medical staff at the Addiction Treatment Center for their cooperation and support, which greatly contributed to the successful completion of this research, and also extend their thanks to the patients and healthy individuals for their kind response and

for providing the blood samples and essential information that played a significant role in accomplishing this study.

Funding: No funds were received to fulfill this work.

Competing interests: The author declare that they have no competing interests

Author Contribution: Conceptualization: M.M.P., H.A.A., and A.A.; Methodology: M.M.P., H.A.A., and A.A.; Formal analysis: M.M.P., H.A.A., A.A., and Y.A.H.; Investigation: M.M.P., H.A.A., and A.A.; Supervision: H.A.A. and A.A.; Resources: M.M.P., H.A.A., and A.A.; Writing: M.M.P., H.A.A., and A.A.

References

- Heilig M, MacKillop J, Martinez D, Rehm J, Leggio L, Vanderschuren LJMJ. Addiction as a brain disease revised: why it still matters, and the need for consilience. *Neuropsychopharmacology*. 2021;46(10):1715–23.
- Eugene V, Magnusson P, EL-Tallawy SN, Wagner M, Rania S, Giustino V. The emergence of the old drug Captagon as a new illicit drug: a narrative review. *Cureus*. 2024;16(2).
- Logan BK. Methamphetamine-effects on human performance and behavior. *Forensic Sci Rev*. 2002;14(1):133–51.
- Courtney KE, Ray LA. Methamphetamine: an update on epidemiology, pharmacology, clinical phenomenology, and treatment literature. *Drug Alcohol Depend*. 2014 Oct;143:11–21.
- Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interf Cytokine Res*. 2009; 29(6):313–26.
- Mulholland BS, Forwood MR, Morrison NA. Monocyte chemoattractant protein-1 (MCP-1/CCL2) drives activation of bone remodelling and skeletal metastasis. *Curr Osteoporos Rep*. 2019;17:538–47.
- Bianconi V, Sahebkar A, Atkin SL, Pirro M. The regulation and importance of monocyte chemoattractant protein-1. *Curr Opin Hematol*. 2018;25(1):44–51.
- Singh S, Anshita D, Ravichandiran V. MCP-1: Function, regulation, and involvement in disease. *Int Immunopharmacol*. 2021; 101(Pt B):107598.
- Yoshimura T, Li C, Wang Y, Matsukawa A. The chemokine monocyte chemoattractant protein-1/CCL2 is a promoter of breast cancer metastasis. *Cell Mol Immunol*. 2023;20(7):714–38.
- Yu H, Lin L, Zhang Z, Zhang H, Hu H. Targeting NF- κ B pathway for the therapy of diseases: mechanism and clinical study. *Signal Transduct Target Ther*. 2020;5(1):209.
- Albensi BC. What is nuclear factor kappa B (NF- κ B) doing in and to the mitochondrion? *Front cell Dev Biol*. 2019;7:154.
- Guo Q, Jin Y, Chen X, Ye X, Shen X, Lin M, et al. NF- κ B in biology and targeted therapy: new insights and translational implications. *Signal Transduct Target Ther*. 2024;9(1):53.
- Ebrahimi N, Abdulwahid AHRR, Mansouri A, Karimi N, Bostani RJ, Beiranvand S, et al. Targeting the NF- κ B pathway as a potential regulator of immune checkpoints in cancer immunotherapy. *Cell Mol Life Sci*. 2024;81(1):106.
- Trares K, Ackermann J, Koch I. The canonical and non-canonical NF- κ B pathways and their crosstalk: A comparative study based on Petri nets. *Biosystems*. 2022;211:104564.
- Singh S, Singh TG. Role of nuclear factor kappa B (NF- κ B) signalling in neurodegenerative diseases: an mechanistic approach. *Curr Neuropsychopharmacol*. 2020;18(10):918–35.
- Babkina II, Sergeeva SP, Gorbacheva LR. The role of NF- κ B in neuroinflammation. *Neurochem J*. 2021;15(2):114–28.
- Chachan NE, Al-Hemiary NJ. Socio-demographic, clinical characteristics and risk factors of crystal meth use in Baghdad/Iraq. *Muthanna Med J*. 2023;10(1):53–66.
- Curran L, Nah G, Marcus GM, Tseng Z, Crawford MH, Parikh NI. Clinical correlates and outcomes of methamphetamine-associated cardiovascular diseases in hospitalized patients in California. *J Am Heart Assoc*. 2022;11(16):e023663.
- Derefinko KJ, Bursac Z, Mejia MG, Milich R, Lynam DR. Rural and urban substance use differences: effects of the transition to college. *Am J Drug Alcohol Abuse*. 2018;44(2):224–34.
- Westbrook SR, Carrica LK, Banks A, Gulley JM. AMPed-up adolescents: The role of age in the abuse of amphetamines and its consequences on cognition and prefrontal cortex development. *Pharmacol Biochem Behav*. 2020;198:173016.
- Guerin AA, Bridson T, Plapp HM, Bedi G. A systematic review and meta-analysis of health, functional, and cognitive outcomes in young people who use methamphetamine. *Neurosci Biobehav Rev*. 2023;153:105380.
- Al-Imam A, Motyka MA, Hoffmann B, Al-Ka'aby H, Younus M, Al-Hemiary N, et al. Risk factors of suicidal ideation in Iraqi crystal methamphetamine users. *Brain Sci*. 2023;13(9):1279.
- Muslim SH, Al-Jumaili AA, Al-Ani HA. Identifying the factors influencing the completion of institutional detoxification period among patients with substance use disorder in Iraq. *Al-Rafidain J Med Sci*. 2024;7(2):202–8.
- Elrassas HH, Hassan YAEM, Abdelqader MAE, Abdelghani AAL. Methamphetamine use and psychiatric comorbidities among Egyptian adolescents: a cross-sectional study. *Middle East Curr Psychiatry*. 2025;32(1):31.
- Al-Imam A, Michalak M. Iraq's meth crisis: prioritizing research to combat suicidal ideation. *Al-Kindy Coll Med J*. 2024;20(1):78–9.
- Salo R, Nordahl TE, Natsuaki Y, Leamon MH, Galloway GP, Waters C, et al. Attentional control and brain metabolite levels in methamphetamine abusers. *Biol Psychiatry*. 2007;61(11):1272–80.
- Richards JR, Farias VF, Clingan CS. Association of leukocytosis with amphetamine and cocaine use. *Sci World J*. 2014;2014(1):207651.
- Tavasolian F, Abdollahi E, Samadi M, Vakili M. Analysis of hematological and biochemical parameters in methamphetamine addicts compared with healthy individuals. *Med Lab J*. 2015;9(4).
- Mohammed AH, Aljarallah AS, Huq M, Mackawy AMH, Alharbi BF, Almutairi KS, et al. Evaluation of the immune system status and hematological dyscrasias, among amphetamine and cannabis abusers at Eradah Hospital in Qassim, Saudi Arabia. *Sci Rep*. 2024;14(1):106.

- 2024;14(1):10600.
30. Dominic P, Ahmad J, Awwab H, Bhuiyan MS, Kevil CG, Goeders NE, et al. Stimulant drugs of abuse and cardiac arrhythmias. *Circ Arrhythmia Electrophysiol.* 2022;15(1):e010273.
 31. Loftis JM, Choi D, Hoffman W, Huckans MS. Methamphetamine causes persistent immune dysregulation: a cross-species, translational report. *Neurotox Res.* 2011 Jul;20(1):59–68.
 32. Zhang K, Luo J. Role of MCP-1 and CCR2 in alcohol neurotoxicity. *Pharmacol Res.* 2019;139:360–6.
 33. Liu Y, Xu K, Xiang Y, Ma B, Li H, Li Y, et al. Role of MCP-1 as an inflammatory biomarker in nephropathy. *Front Immunol.* 2024;14:1303076.
 34. Swaminathan SM, Rao IR, Bhojaraja M V, Attur RP, Nagri SK, Rangaswamy D, et al. Role of novel biomarker monocyte chemo-attractant protein-1 in early diagnosis & predicting progression of diabetic kidney disease: A comprehensive review. *J Natl Med Assoc.* 2024;116(1):33–44.
 35. Davidson M, Mayer M, Habib A, Rashidi N, Filippone RT, Fraser S, et al. Methamphetamine induces systemic inflammation and anxiety: the role of the gut-immune-brain axis. *Int J Mol Sci.* 2022 Sep;23(19).
 36. Zhou J, Yang Z, Yang X, Wang Z. Changes in TNF- α , IL-33, and MIP-1 α before and after artificial liver support treatment and their prognostic value. *Am J Transl Res.* 2024;16(3):988.
 37. Leventelis C, Veskoukis AS, Rojas Gil AP, Papadopoulos P, Garderi M, Angeli A, et al. Methadone and buprenorphine as medication for addiction treatment diversely affect inflammation and craving depending on their doses. *Pharm (Basel, Switzerland).* 2025;13(2).
 38. Fang Y, Wang B, Pang B, Zhou Z, Xing Y, Pang P, et al. Exploring the relations of NLR, hsCRP and MCP-1 with type 2 diabetic kidney disease: a cross-sectional study. *Sci Rep.* 2024;14(1):3211.
 39. Mostafizar M, Cortes-Pérez C, Snow W, Djordjevic J, Adlimoghaddam A, Albensi BC. Challenges with methods for detecting and studying the transcription factor nuclear factor kappa B (NF- κ B) in the central nervous system. *Cells.* 2021;10(6):1335.
 40. Li H, Niu X, Zhang D, Qu MH, Yang K. The role of the canonical nf-kb signaling pathway in the development of acute liver failure. *Biotechnol Genet Eng Rev.* 2023;39(2):775–95.
 41. Nolan RA, Reeb KL, Rong Y, Matt SM, Johnson HS, Runner K, et al. Dopamine activates NF- κ B and primes the NLRP3 inflammasome in primary human macrophages. *Brain, Behav Immun - Heal.* 2020 Feb;2.
 42. Nennig SE, Schank JR. The role of NFkB in drug addiction: beyond inflammation. *Alcohol Alcohol.* 2017;52(2):172–9.
 43. Wang Y, Teng H, Sapozhnikov DM, Du Q, Zhao M. Transcriptome sequencing reveals candidate NF- κ B target genes involved in repeated cocaine administration. *Int J Neuropsychopharmacol.* 2018;21(7):697–704.
 44. Gürbüz N, Güler MC, Tör IBH. Methamphetamine use disorder and inflammation: a case-control study. *Psychiatry Investig.* 2024;21(5):513–20.
 45. Shehata SA, Abd El-Fadeal NM, Fattah IOA, Hagrass AM, Mostafa EMA, Abdel-Daim MM, et al. Synergistic cardiotoxic effects of Captagon and azithromycin in rat via oxidative stress, apoptosis and upregulation of the PI3K/AKT/NF-kB pathway. *Toxicol Lett.* 2025;408:77–94.
 46. Ren N, Wang WF, Zou L, Zhao YL, Miao H, Zhao YY. The nuclear factor kappa B signaling pathway is a master regulator of renal fibrosis. *Front Pharmacol.* 2024;14:1335094.