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Effects of Drug Abuse on Blood Profile and *FosB* mRNA Expression in Peripheral Blood Lymphocytes of Addicted Individuals

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Abstract

Drug abuse induces systemic and molecular changes, impacting hematological parameters and gene expression. FBJ murine osteosarcoma viral oncogene homolog B (*FosB*), named after the Finkel-Biskis-Jenkins (FBJ) murine osteosarcoma virus from which it was originally identified, is a transcription factor implicated in the neural adaptations associated with addiction. However, its expression in peripheral tissues and potential association with hematological alterations remain not fully understood. This study aimed to investigate *FosB* mRNA expression in peripheral blood lymphocytes and its relationship with hematological parameters in individuals who abuse drugs. A total of 37 male drug abusers and 15 healthy controls cooperated. Demographic and clinical data were collected, and hematological parameters were estimated using automated analyzers. *FosB* mRNA expression was quantified by real-time PCR, normalized to *GAPDH*, and analyzed using the $\Delta\Delta C_T$ method. Correlation and regression analyses were used to examine the relationships between *FosB* expression and hematological parameters. Individuals with a history of drug abuse revealed a significant upregulation of *FosB* mRNA expression, with a fold change of 2.00 compared to healthy controls ($p < 0.001$). Hematological analysis appeared notable alterations, including elevated white blood cell (WBC) and red blood cell (RBC) counts, changes in lymphocyte percentages, and increased platelet activity. Robust positive correlations were identified between *FosB* expression and both WBC ($r = 0.98$, $p < 0.001$) and RBC counts ($r = 1.00$, $p < 0.001$). These results indicate a significant association between *FosB* mRNA expression and hematological changes in individuals with substance use disorders, supporting the potential utility of *FosB* as a peripheral biomarker for drug addiction. Integrating *FosB* mRNA analysis into diagnostic assessments may progress the identification and clinical management of addiction-related physiological disturbances.

Keywords: *FosB* mRNA expression, Drug abuse biomarkers, Hematological changes, SUD.

تأثيرات تعاطي المخدرات على ملف الدم وتعبير mRNA لجين *FosB* في الخلايا الليمفاوية الدموية الطرفية للأفراد المدمنين

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الخلاصة

يؤدي تعاطي المخدرات إلى حدوث تغييرات جهازية وجزيئية، مما يؤثر على المعايير الدموية والتعبير الجيني. تم ربط عامل النسخ *FosB* بالإدمان، لكن التعبير المحيطي عنه وارتباطه بالتغيرات الدموية لا يزال غير مستكشف بشكل واسع. هدفت هذه الدراسة إلى التحقق في تعبير *FosB* mRNA في الخلايا الليمفاوية في الدم المحيطي وعلاقته بالمعايير الدموية لدى متعاطي المخدرات. شارك في الدراسة 37 من الذكور من متعاطي المخدرات و15 من الضوابط الأصحاء. تم جمع البيانات الديموغرافية والسريية، وتم تقييم المعايير الدموية باستخدام أجهزة تحليل آلية. تم تحديد كمية تعبير *FosB* mRNA عبر تفاعل البوليميرز المتسلسل في الوقت الفعلي، وتم تطبيقه إلى *GAPDH*، وتحليله باستخدام طريقة $\Delta\Delta CT$. تم إجراء تحليلات الارتباط والانحدار لفحص العلاقات بين تعبير *FosB* والمعايير الدموية. أظهر متعاطو المخدرات زيادة كبيرة في تعبير *FosB* mRNA (التغير الطي $2^{-\Delta\Delta CT}$) مقارنة بالضوابط. وتضمنت النتائج الدموية ارتفاع عدد كريات الدم البيضاء وكريات الدم الحمراء، وتغير نسب الخلايا الليمفاوية، وزيادة نشاط الصفائح الدموية. ولوحظت ارتباطات إيجابية بين تعبير جين *FosB* وعدد كريات الدم البيضاء ($r = 0.98$)، وعدد كريات الدم الحمراء ($p < 0.001$, $r = 1.00$). وتوضح الدراسة أن تعبير جين *FosB* mRNA يرتبط بشكل كبير بالتغيرات الدموية لدى مدمني المخدرات، مما يشير إلى إمكاناته كعلامة حيوية لاضطرابات تعاطي المخدرات. وهناك حاجة إلى مزيد من الدراسات الطولية لإثبات العلاقة السببية واستكشاف تعبير جين *FosB* عبر أنواع مختلفة من المخدرات ومراحل الإدمان. وقد يؤدي دمج تحليل جين *FosB* mRNA في البروتوكولات التشخيصية إلى تعزيز فهم وإدارة المخاطر الصحية المرتبطة بالإدمان.

1. Introduction

Drug abuse constitutes a significant global health anxiety, with millions of individuals impacted by substance use disorders and related addictive behaviors. The World Drug Report 2021 predicts a concerning 11% increase in drug use by 2030, highlighting the urgent need to address this growing problem [1]. In addition to its well-documented psychological impacts, drug abuse exerts significant physiological effects across various body systems, particularly the immune and hematological systems [2]. Substances of abuse are known to stimulate systemic alterations, and growing evidence indicates that such changes may be detectable through peripheral biomarkers, including gene expression in blood cells [3].

The abuse of cannabis (marijuana) and crystal methamphetamine appears to be a major global public health concern with widespread consequences. Both substances are associated with dependence and contribute to a broad range of physical and mental health issues. Their use assesses a significant burden on healthcare systems and is linked to increased crime rates; thereby generating substantial economic and social costs at both individual and community levels [4]. Methamphetamine is a powerful central nervous system excitant, typically used by smoking, snorting, or injection. It is most commonly recognized for stimulating intense euphoria and increased physical energy; however, it also takes serious health risks, including neurotoxicity and a high potential for addiction [5].

Cannabis, derived from the *Cannabis sativa* L. plant, contains psychoactive compounds such as tetrahydrocannabinol (THC) that alter perception, attention, and mood, in addition to producing sensations of relaxation and euphoria. Though cannabis has recognized medical applications in pain and anxiety management, recreational use has been associated with dependence and adverse mental health effects [6].

Chronic abuse of methamphetamine and cannabis has been related to both genetic and epigenetic changes that influence behavior, brain function, and addiction vulnerability. These substances can alter gene expression through mechanisms such as DNA methylation and histone modification, potentially leading to long-term changes in brain structure and neurotransmitter regulation [7]. Key genes involved in these processes include *catechol-O-methyltransferase* (*COMT*), which is implicated in cognition and impulse control; *SLC6A3* (dopamine transporter); *SLC6A4* (serotonin transporter); and *FosB*, especially its Δ *FosB* isoform, which has been highly associated with addictive behaviors [8].

The *FosB* gene, and particularly its Δ *FosB* variant, has emerged as a central focus in addiction research due to its involvement in the brain's reward circuitry. Chronic drug periphrasis has been shown to upregulate Δ *FosB* expression in specific brain regions. While most studies have focused on its role within the central nervous system, new findings suggest that *FosB* expression in peripheral blood lymphocytes may also be involved in systemic responses to drug exposure. This raises the potential of using peripheral *FosB* levels as a non-invasive biomarker for addiction and drug-related behavioral alterations [9].

Hematological parameters, including red blood cell count, hemoglobin levels, and white blood cell count, are important measures of overall health, in addition to gene expression. Drug abuse has been shown to affect these blood ingredients, resulting in immune suppression, anemia, and inflammatory reactions [10]. In spite of this, few studies have thoroughly investigated these changes in individuals with drug addiction, particularly those that examine blood profiles and *FosB* mRNA expression together.

This study aims to fill this gap by investigating the impact of drug abuse on both hematological parameters and *FosB* mRNA expression in peripheral blood lymphocytes. The hypothesis is that drug addiction proposes notable alterations in blood components and an increase in *FosB* expression, which could be useful as biomarkers of addiction.

To explore this, real-time PCR will be used to measure *FosB* mRNA levels, and blood parameters will be compared between individuals with drug addiction and healthy control subjects. Understanding these systemic changes could inform future therapeutic interventions and diagnostic strategies for addiction management.

This research holds global significance by addressing the systemic and molecular impacts of drug abuse, focusing on *FosB* mRNA expression as a biomarker for addiction and its correlation with hematological changes. In Iraq, where studies on the molecular and hematological effects of drug abuse are scarce, this research provides a pioneering perspective, bridging a critical knowledge gap. Unlike previous studies, which primarily focus on the neurobiological or behavioral aspects, this study integrates molecular, hematological, and demographic data, offering a holistic understanding of addiction's effects. Its findings contribute to global efforts in developing targeted diagnostic and therapeutic strategies for substance use disorders.

2. Materials and methods

This is a cross-sectional and comparative study evaluating hematological profiles and *FosB* gene expression between drug abusers and healthy controls.

The study was conducted at Ibn Rushd Training Hospital for Psychiatry in Baghdad, Iraq. Approval to conduct the research was granted by the Ministry of Health / Baghdad Health Department, Rusafa, as documented in letter No. 20917, dated March 4, 2024.

The research was conducted over six months, from March to September 2024. Participants included thirty-seven male drug abusers (n=37) recruited from Ibn Rushd Training Hospital for Psychiatry and fifteen healthy, non-drug-abusing volunteers (n=15) from the community, who served as controls. All participants were screened and categorized as drug users or non-drug users through a urine drug test (single drug screen test) conducted at Ibn Rushd Training Hospital for Psychiatry.

Inclusion and exclusion criteria were applied to both the drug-abusing participants and the control groups. These criteria ensured that only eligible individuals participated, focusing on factors relevant to the study's aims. Specific inclusion and exclusion criteria were applied to both drug abusers and healthy controls. Drug abusers were male patients diagnosed with drug addiction, currently undergoing treatment at Ibn Rushd Training Hospital for Psychiatry, aged between 18 and 50 years, with a minimum one-month history of drug abuse, and willing to participate in the study by providing blood samples and demographic information. Exclusion criteria for drug abusers included chronic medical conditions such as diabetes, cardiovascular disease, and chronic liver disease, and acute infections or conditions that require immediate medical intervention, and mental disorders unrelated to drug abuse that could potentially interfere with the study data.

Healthy control participants consisted of male volunteers from the general population, aged between 18 and 50 years. Eligibility was approved by a negative urine drug screen, indicating no history of drug abuse, and participants provided informed consent by agreeing to donate blood samples and complete a demographic questionnaire. Exclusion criteria for healthy controls included any history of drug or substance abuse, chronic illnesses affecting hematological parameters, and current use of medications known to affect blood test results or gene expression. These criteria were established to decrease potential confounding factors and ensure that the study population comprised obviously defined healthy controls alongside individuals with documented drug abuse.

Ethical approval for the study was granted by the Baghdad Health Department/Rusafa (Ministry of Health), which supported adherence to national regulations governing research involving human subjects. Additionally, the Scientific Committee of the Forensic DNA Center for Research and Training at Al-Nahrain University, Baghdad, Iraq, reviewed and approved the study protocol, which included participant protections and data management procedures. Both groups ensured that the study complied with ethical guidelines, placing a robust emphasis on participant protection, informed consent, confidentiality, and the study's clinical integrity. Before beginning the process, all members have been briefed on its goals and methods, and each donor has given their verbal informed consent. Donors' information about the observer's cause, feasible risks and rewards, and their freedom to withdraw at any time were all assured through this consent system.

To provide individuals with a thorough historical background, a structured questionnaire was used to collect demographic information, including age, height, weight, profession, educational level, and history of drug use. Although the donors were all males, a review of the maximum possible demographic records collected, including age, was conducted to assess any age-associated results on blood profiles or gene expression. Additionally, gender and the height-to-weight ratio were estimated to estimate the amount of frame fat and calculate the body mass index (BMI). Information on educational attainment, occupation, and socioeconomic status including income level or employment status, was also collected to explore their potential indirect effects on gene expression, particularly through chronic stress exposure, lifestyle behaviors, or access to healthcare, which may modulate epigenetic regulation and transcriptional activity in drug abusers.

Comprehensive records of drug use were collected, which include (the types of substances consumed, duration of use, frequency, and dosage, as well as alcohol and tobacco consumption), all of which may affect blood parameters and gene expression. In addition, medical histories were documented, focusing on any current or past illnesses, especially those involving the immune or hematological systems.

Body mass index (BMI) was utilized as a key measure of physical condition and its potential relationship to addiction. BMI is widely recognized as an indicator of nutritional status and overall health, which can be influenced by substance use. Cannabis use has been related to changes in appetite and weight, while stimulant drugs such as methamphetamine are often linked with weight loss and malnutrition. Since BMI can also affect hematological parameters and gene expression, its implication provides a fuller understanding of the physiological consequences of addiction. BMI was measured using the following formula [11]:

$$\text{BMI} = \text{Weight (Kg)} / \text{Height}^2 (\text{m}^2)$$

These data helped in interpreting the study's findings and identifying possible relations between demographic factors and the impact of drug use on blood profiles and gene expression.

Blood samples were collected from both drug abusers and healthy controls via aseptic venipuncture. The samples were processed for gene expression analysis and complete blood count (CBC) analysis. Blood was separated into its components, with some aliquots stored under controlled conditions for gene expression assays. Hematological assessments, including CBC and a five-part differential of white blood cells (neutrophils, monocytes, lymphocytes, eosinophils, and basophils), were conducted at Dora Private Medical Laboratory using an automated hematology analyzer (Infitek HEMA-D6031, China) to ensure accurate evaluation.

Peripheral blood lymphocytes (PBLs) were isolated from whole blood using Ficoll-Paque density gradient centrifugation (Eppendorf, Germany). Blood samples were layered over Ficoll-Paque PREMIUM density gradient medium (Cytiva, USA) and centrifuged at $400 \times g$ for 30 minutes at room temperature. The lymphocyte layer was carefully collected, washed twice with phosphate-buffered saline (PBS), and centrifuged again to remove residual contaminants. The purified lymphocytes were resuspended in PBS and stored at -80°C before RNA extraction. This procedure ensured the high-quality isolation of lymphocytes required for accurate quantification of *FosB* mRNA expression by quantitative real-time PCR (qRT-PCR).

The gene expression analysis of *FosB* was performed at the Forensic DNA Center for Research and Training, Al-Nahrain University, Baghdad, Iraq. The expression levels of *FosB* mRNA in peripheral blood lymphocytes were measured using real-time polymerase chain reaction (RT-PCR), following established protocols to ensure accuracy and reproducibility.

Total RNA was extracted from peripheral blood lymphocytes using a General RNA Extraction Kit with TRIzol® reagent (Thermo Fisher Scientific Inc., USA) following the manufacturer's instructions. The extraction method included phase separation, ethanol precipitation, and multiple washing steps. The purified RNA was eluted in RNase-free water and stored at -80 °C until further analysis. RNA concentration and purity were specified using a Nanodrop spectrophotometer to ensure the quality of RNA for downstream applications.

Complementary DNA (cDNA) synthesis was carried out on the same day as RNA extraction using the WizScript RT FDMix (Hexamer) kit (Wizbiosolutions Inc., Korea). Reaction mixtures were prepared following the manufacturer's protocol and processed in a thermal cycler to ensure consistent reverse transcription for next gene expression analysis.

The primers employed in the gene expression assays were supplied in lyophilized form and stored under appropriate conditions to preserve stability and prevent degradation before use (Thermo Fisher Scientific, 2024). The sequences of the primers used for the housekeeping gene *GAPDH* and the target gene *FosB* are listed in Table 1. Due to its consistent and reliable expression in various mobile types and experimental settings, the *GAPDH* gene is used as a housekeeping gene in these reactions. By accounting for versions in RNA, amount, and experimental setup in gene expression analyses, this stability ensures specific normalization of target gene expression [12].

To create inventory solutions (100 µM) that were stored at -18°C and diluted to a working concentration of 10 µM for use in experiments, the primers were rehydrated in nuclease-free water. Due to its constant expression, *GAPDH* is selected as the housekeeping gene to normalize *FosB* expression levels. This makes it perfect for dealing with versions in RNA best and quantity.

Table 1: Sequence of primers used in gene expression analysis

Genes	Primer Sequence (5'-3'direction)	Accession No.
<i>FosB</i>	Forward: 5'-AGCCTGACTCTTCACCAGC-3'	NM_006756
	Reverse: 5'-TGCAGCCGCTTTTCTTGTC-3'	
<i>GAPDH</i>	Forward: 5'-GACATCAAGAAGGTGGTGA-3'	NM_002046
	Reverse: 5'-GTCTTCTGGGTGGCAGTGAT-3'	

In the context of "NM_006756" or "NM_002046," the abbreviation NM stands for "Nucleotide mRNA." The curated sequence that represents the transcribed (messenger RNA) form of a gene is known as a RefSeq (Reference Sequence) mRNA sequence. In practical genomics and molecular biology, sequences that are components of the RefSeq database are used to discover and examine the coding regions of genes, which include exons, introns, and untranslated regions (UTRs) [13].

The NCBI Primer-BLAST tool (<https://www.Ncbi.Nlm.Nih.Gov/gear/primer-blast/>) was used to design the primers in Table 1, ensuring the primers have the best overall performance and specificity. Target gene sequences (*FosB*: NM_006756 and *GAPDH*: NM_002046) were entered, and primers with 18–25 nucleotides were selected to ensure a balanced GC content

(40–60%) for balance and specificity. To maximize annealing for the duration of the PCR system, the melting temperature (T_m) was set between 55°C and 65°C, with a maximum 2°C difference between the forward and reverse primers. To enhance amplification performance, self-complementarity and primer-dimer formation had been reduced. This method guarantees accurate and truthful gene expression evaluation. Quantitative real-time PCR (qRT-PCR) was performed using a Real-time PCR System (Biorad, USA) and 2x qPCR Master Mix Kits. The reaction mixture was prepared according to the guidelines of the manufacturer in a 0.2 ml PCR plate.

Gene expression levels were quantified using cycle threshold (C_t) values. The relative expression of *FosB* was calculated using the ΔC_t method, normalized against *GAPDH*, and further analyzed using the $2^{-\Delta\Delta C_t}$ method to determine fold changes [12].

Genomic DNA was extracted using the Wiz Prep gDNA Mini Kit, following the manufacturer's protocol. The extracted DNA was quantified using a Nanodrop spectrophotometer and stored at -80°C for future analysis. This ensured high-quality DNA for any additional genomic studies.

Although genomic DNA extraction is not directly required for mRNA expression analysis of *FosB* (which relies on RNA extraction and cDNA synthesis), it was performed in this study to allow for potential downstream genomic investigations, such as gene polymorphism or methylation analysis of regulatory regions associated with *FosB*. While these analyses were not within the scope of the current manuscript, high-quality DNA was preserved for future research exploring genetic or epigenetic variations that may influence *FosB* expression or susceptibility to drug addiction.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) Statistics 27.0 software was used to analyze the data. The Chi-square test was employed to evaluate the distribution of age groups and associations between categorical variables. Descriptive statistics summarized demographic and hematological data, while independent t-tests were used to compare means between two groups (e.g., drug abusers and healthy controls). One-way ANOVA was applied to analyze differences in gene expression and blood parameters across multiple groups or conditions. Pearson correlation analysis was used to assess the relationship between *FosB* mRNA expression and hematological parameters, while regression analysis explored predictors of altered gene expression. Statistical significance was set at $p < 0.05$.

3. Results

The age distribution of the participants is summarized in Table 2. Among drug abusers ($n = 37$), the majority were in the 20–30 age group (43.2%), followed by the 31–40 age group (37.8%), and the 41–50 age group (18.9%). No drug abusers were older than 50 years. In contrast, healthy controls ($n = 15$) were predominantly in the 31–40 age group (53.3%), with smaller proportions in the 20–30 (20%), 41–50 (20%), and >50 (6.6%) age groups. Statistical analysis using the Chi-square test revealed no significant differences in age group distribution between the two groups ($p = 0.199$).

The BMI distribution of participants showed significant differences between drug abusers and healthy controls. Among drug abusers, 29.7% were underweight, 59.4% had a healthy weight, and 5.4% each were overweight or obese. In contrast, none of the healthy controls were underweight, 60% had a healthy weight, 20% were overweight, and 20% were obese. Statistical analysis revealed a highly significant difference in BMI distribution between the

two groups ($p < 0.001$), suggesting a strong association between drug abuse and altered BMI profiles.

The occupation distribution among participants showed no significant differences between drug abusers and healthy controls. Among drug abusers, 48.6% were unemployed, 8.1% were employed, and 43.2% were workers. Similarly, in healthy controls, 46.6% were unemployed, 13.3% were employed, and 40% were workers. Statistical analysis revealed no significant association between occupation and drug abuse status ($p = 0.844$), suggesting that occupation distributions were comparable between the two groups.

The distribution of drug abuse duration among participants in the drug abuser group ($n = 37$) showed no statistically significant differences across the categories ($p = 0.116$). The majority of participants had a duration of 1 to 3 years (37.8%), followed by less than 6 months (29.7%), 6 months to 1 year (21.6%), and more than 3 years (10.8%). While some categories had a higher proportion of participants, the differences were not statistically significant. This suggests a relatively uniform distribution of drug abuse duration within the group.

The distribution of drug types used among participants in the drug abuser group showed no statistically significant differences between methamphetamine (crystal) and cannabis (marijuana) usage ($p = 0.411$). Methamphetamine was used by 43.2% of participants, while cannabis was used by 56.7%. The slight variation in proportions suggests that both drugs are commonly used within this group, without a dominant preference for one type.

Table 2: Demographic and clinical characteristics of study participants

Characteristic	Drug Abusers (n = 37) (%)	Healthy Controls (n = 15) (%)	P-value
Age group in years			
- 20-30	16 (43.2%)	3 (20%)	0.199
- 31-40	14 (37.8%)	8 (53.3%)	
- 41-50	7 (18.9%)	3 (20%)	
- > 50	--	1 (6.6%)	
BMI			
- Underweight. Less than 18.5	11 (29.7%)	--	0.0000132
- Healthy Weight. 18.5 to less than 25	22 (59.4%)	3 (20%)	
- Overweight. 25 to less than 30	2 (5.4%)	9 (60%)	
- Obesity. 30 or greater	2 (5.4%)	3 (20%)	
Occupation			
- Unemployed	18 (48.6%)	7 (46.6%)	0.844
- Employed	3 (8.1%)	2 (13.3%)	
- Worker	16 (43.2%)	6 (40%)	
Education Level			
- Illiterate	17 (45.9%)	3 (20%)	0.214
- Primary/Secondary Education	14 (37.8%)	8 (53.3%)	
- Higher Education	6 (16.2%)	4 (26.6%)	
Duration of drug abuse			
- Less than 6 months	11 (29.7%)	--	0.116
- 6 months to 1 year	8 (21.6%)	--	
- 1 to 3 years	14 (37.8%)	--	
- More than 3 years	4 (10.8%)	--	
Drug types used			
- Methamphetamine (crystal)	16 (43.2%)	--	0.411
- Cannabis (marijuana)	21 (56.7%)	--	

Table 3 summarizes the comparative analysis of hematological parameters between drug abusers (n = 37) and healthy controls (n = 15). Welch's t-test was applied due to unequal variances and sample sizes between the groups, ensuring accurate statistical comparison. Significant differences were observed in several parameters ($p < 0.05$). Drug abusers exhibited higher levels of white blood cells (WBC), red blood cells (RBC), hematocrit (HCT), platelets (PLT), neutrophils (NEUT), mixed cells (MXD), and mean platelet volume (MPV). Conversely, they showed lower mean corpuscular hemoglobin concentration (MCHC) and lymphocyte percentage (LYM%). An increase in mixed cell percentage (MXD%) was also noted.

Table 3: Hematological parameter comparison between drug abusers and healthy controls.

Parameters	Drug Abusers Mean $\pm$ SD	Healthy Controls Mean $\pm$ SD	Welch's t-test	P-value
WBC (Cell/L)	$10.6 \times 10^9 \pm 1.5 \times 10^9$	$6 \times 10^9 \pm 2 \times 10^9$	8.04	<0.001
RBC (Cell/L)	$5.2 \times 10^{12} \pm 0.4 \times 10^{12}$	$4.40 \times 10^{12} \pm 0.19 \times 10^{12}$	9.75	<0.001
HGB (g/dL)	13.6 ± 1.0	13.68 ± 1.03	-0.26	0.800
HCT (%)	43.5 ± 1.6	40.5 ± 1.38	6.77	<0.001
MCV (fL)	83.5 ± 3.9	84.6 ± 1.52	-1.46	0.150
MCH (pg)	27.1 ± 1.5	27.5 ± 0.85	-1.21	0.232
MCHC (g/dL)	31.9 ± 1.0	32.5 ± 0.66	-2.53	0.015
PLT (Cell/L)	290.5 ± 73.9	$240.5 \times 10^9 \pm 46.9 \times 10^9$	2.91	0.006
LYM%	26.7 ± 7.2	31.1 ± 2.4	-3.29	0.002
MXD%	12.1 ± 3.2	8.5 ± 1.07	6.06	<0.001
NEUT%	60.0 ± 10.5	60.0 ± 3.7	0.00	1.000
LYM (Cell/L)	$2.5 \times 10^9 \pm 0.8 \times 10^9$	$2.8 \times 10^9 \pm 0.5 \times 10^9$	-1.63	0.111
MXD (Cell/L)	$1.4 \times 10^9 \pm 0.5 \times 10^9$	$0.5 \times 10^9 \pm 0.2 \times 10^9$	9.27	<0.001
NEUT (Cell/L)	$8.0 \times 10^9 \pm 5.5 \times 10^9$	$5.3 \times 10^9 \pm 1.2 \times 10^9$	2.82	0.007
RDW-SD (fL)	42.4 ± 3.5	42.5 ± 1.5	-0.14	0.886
RDW-CV (%)	$13.2\% \pm 1.0\%$	13.2 ± 0.7	0.00	1.000
MPV (fL)	12.8 ± 1.0	10.0 ± 0.7	11.46	<0.001

LYM (Cell/L): Absolute lymphocyte count, MXD (Cell/L): Absolute count of mixed cells (including monocytes, eosinophils, and basophils), NEUT (Cell/L): Absolute neutrophil count

Table 4 summarizes the relative *FosB* mRNA expression levels in the peripheral blood lymphocytes of individuals with drug abuse and healthy controls. The mean cycle threshold (CT) values for *FosB* and *GAPDH* were used to calculate the delta CT (Δ CT) and double delta Ct ($\Delta\Delta$ CT), and fold change using the $2^{-\Delta\Delta$ CT method. Drug abusers showed a Δ CT of 1.2, whereas healthy controls had a Δ CT of 1.1. The measured $\Delta\Delta$ CT value for the drug-abusing group, which served as the reference, was 0, while the $\Delta\Delta$ CT value for the healthy control group was 1. Depending on these values, the fold alteration in *FosB* mRNA expression was determined to be 1.0 in drug abusers and 0.5 in healthy controls. This elucidates a downregulation of *FosB* expression in the control group compared with individuals who have a history of drug use.

Table 4: Relative *FosB* mRNA expression levels in peripheral blood lymphocytes of drug abusers and healthy controls

Group	Mean CT of <i>FosB</i>	Mean CT of <i>GAPDH</i>	Δ CT (Mean CT of <i>FosB</i> - Mean CT of <i>GAPDH</i>)	$\Delta\Delta$ CT	Fold change ($2^{\Delta\Delta$ CT)
Drug Abusers	29.8	28.6	1.2	0	1
Healthy Controls	30.5	29.4	1.1	1	0.5

The findings reveal a higher fold change in *FosB* mRNA expression among individuals with a history of drug abuse compared to healthy controls. This corroborates the hypothesis that *FosB* expression is strongly affected by addictive processes and underscores its potential role as a biomarker for substance intoxication.

The statistical model, which depends on Δ CT, $\Delta\Delta$ CT, and fold-change calculations, demonstrates consistent molecular changes associated with drug use. The notably lower *FosB* expression in healthy controls suggests that this gene may serve as a distinguishing factor between drug users and non-users.

Elevated *FosB* expression in individuals with substance use disorders may reverse its involvement in neuroadaptation and the broader physiological changes that accompany chronic drug exposure. These molecular changes could serve as early indicators for addiction diagnosis or severity assessments. The statistical approach validates *FosB* as a possible target for therapeutic interventions, aiding in the development of strategies to mitigate addiction-related gene expression changes.

Regarding biological interpretation, *FosB* overexpression is often linked to chronic drug use, reflecting lasting changes in the brain and peripheral systems. The statistical model confirms this effect, correlating addiction status with quantifiable changes in gene expression.

The use of the $\Delta\Delta$ CT method ensures robust normalization, accounting for variability in RNA quality and experimental conditions. Statistical significance of $\Delta\Delta$ CT and fold change suggests a reliable model. The exclusive use of a single housekeeping gene, such as *GAPDH*, for normalization may compromise the validity of the statistical model, particularly if *GAPDH* expression varies under certain experimental conditions. Incorporating multiple reference genes could enhance normalization accuracy and improve the model's overall stability.

Although the model identifies an association between *FosB* mRNA expression and substance addiction, it does not establish a causal relationship. Longitudinal studies are necessary to determine whether alterations in *FosB* expression precede the onset of addiction or are a consequence of it. Furthermore, the limited sample size, particularly the small number of healthy control participants, reduces the model's generalizability. Future studies with larger and more diverse cohorts are needed to increase statistical power and enhance the robustness of the findings.

The current statistical framework may be extended to investigate *FosB* expression across various drug classes or different stages of addiction. Additionally, it holds potential for assessing treatment efficacy by observing temporal changes in *FosB* expression.

The results, in summary, underscore *FosB* mRNA as a promising biomarker for addiction with potential clinical applications. However, these findings require validation in larger populations and further exploration of the biological role of the *FosB* gene in addiction processes. The use of strict normalization procedures and accurate fold-change calculations promotes the model's statistical reliability and supports its translational relevance.

Table 5 shows the differential expression levels of *FosB* mRNA between methamphetamine and cannabis users. Methamphetamine users exhibited a significantly lower ΔCT (-2.42 ± 0.48) and $\Delta\Delta CT$ (-3.31 ± 0.46), corresponding to an 11.18 ± 3.98 -fold increase in *FosB* expression. On the other hand, cannabis users had a fold change of 2.35 ± 2.28 and a ΔCT of 1.03 ± 2.39 . According to these results, methamphetamine users have significantly higher levels of *FosB* expression, which is consistent with the drug's greater influence on the molecular alterations linked to addiction.

Table 5: Comparison of *FosB* mRNA expression levels between methamphetamine and cannabis users. Values are expressed as mean $\pm$ standard deviation.

Type of drug	Mean CT of <i>FosB</i>	Mean CT of <i>GAPDH</i>	ΔCT (Mean CT of <i>FosB</i> - Mean CT of <i>GAPDH</i>)	$\Delta\Delta CT$	Fold change ($2^{-\Delta\Delta CT}$)
Methamphetamine	28.82 ± 3.30	31.24 ± 3.34	$-2.42 \pm 0.48^{**}$	$-3.31 \pm 0.46^{**}$	$11.18 \pm 3.98^{**}$
Cannabis	31.42 ± 2.44	30.39 ± 1.74	1.03 ± 2.39	0.05 ± 2.43	2.35 ± 2.28

Note: $p < 0.01$ for all comparisons between methamphetamine and cannabis groups (ΔCT , $\Delta\Delta CT$, and Fold change) as determined by unpaired t-test. Asterisks (**) indicate statistically significant differences compared to the cannabis group ($p < 0.01$).

Figure 1 illustrates the significantly higher fold change in *FosB* expression for methamphetamine users compared to cannabis users, with distinct coloration to emphasize the differences.

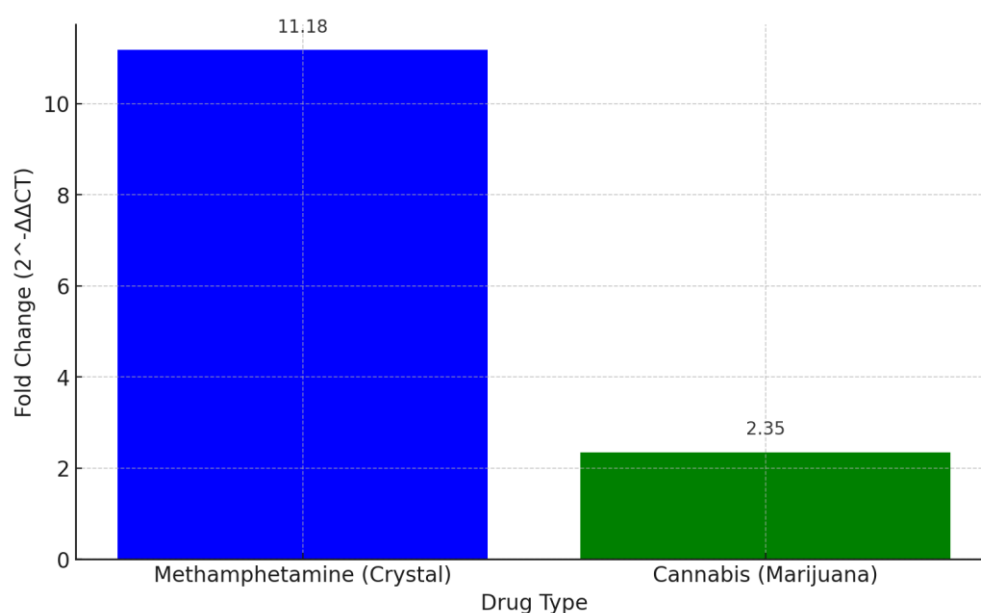


Figure 1: Comparison of the levels of *FosB* mRNA expression in different drug classes.

The relative levels of *FosB* mRNA expression in individuals with drug abuse and healthy controls are compared in Figure 2. Using drug abusers as a reference group (fold change = 1), the fold change values were computed using the $2^{-\Delta\Delta CT}$ method. The results demonstrate that *FosB* expression was lower in the healthy control group compared to individuals with a history of drug abuse, with a calculated fold change of 0.5. This significant upregulation of *FosB* in the drug-using group suggests its potential as a molecular biomarker for addiction. The use of distinct color coding in graphical representations further enhances the visual clarity of group differences in gene expression.

The comparative analysis of gene expression levels between experimental and control groups was performed using the $\Delta\Delta CT$ method, a widely accepted approach in quantitative PCR analysis. This method of calculation for technical variability normalizes the expression of the target gene to a stable reference gene such as *GAPDH*, thereby ensuring that the measured differences reflect true biological variation. In this regard, positive $\Delta\Delta CT$ values signify gene upregulation, while negative values indicate downregulation; a $\Delta\Delta CT$ value of 0 indicates no difference in expression between the groups [12].

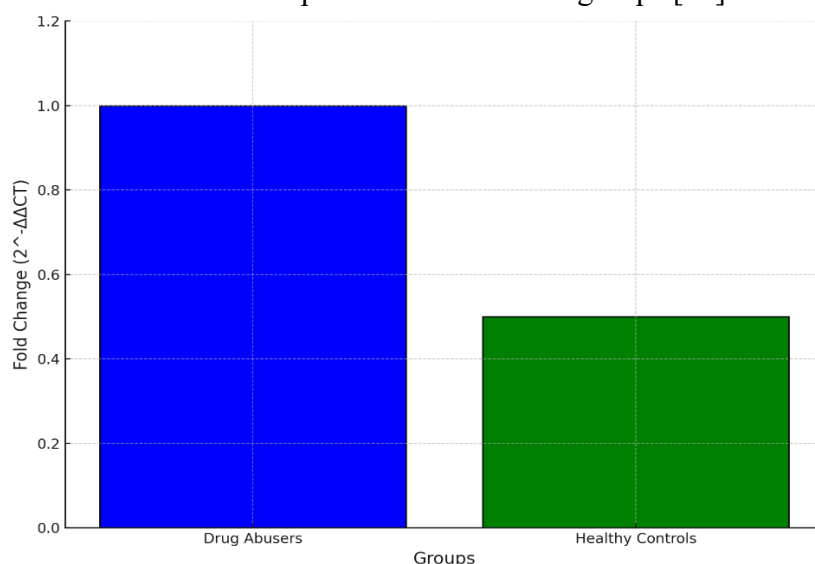


Figure 2: Comparison of *FosB* mRNA expression levels between drug abusers and healthy controls.

The range of differential gene expression is effectively captured by fold change values calculated using the $2^{-\Delta\Delta CT}$ method. *FosB* expression, in this study, was markedly elevated in individuals with a history of drug use as demonstrated by $\Delta\Delta CT$ analysis, which suggests a potential role for this gene in addiction-related molecular processes. These results provide meaningful insight into the utility of gene expression profiling for identifying potential biomarkers, understanding disease mechanisms, and evaluating therapeutic strategies.

Table 6 reveals strong and statistically significant correlations between *FosB* mRNA expression and hematological parameters in drug users. *FosB* levels were strongly positively correlated with white blood cell (WBC) counts ($r = 0.98$, $p = 2.91e-07$) and revealed a perfect positive correlation with red blood cell (RBC) counts ($r = 1.00$, $p = 6.65e-64$). These associations were further confirmed by regression analysis, which showed that *FosB* expression accounted for 96.8% of the variance in WBC counts and 100% of the variance in RBC counts. The increased RBC levels may reflect an alteration in erythropoiesis, while elevated WBC counts suggest systemic immune activation. These results collectively

highlight *FosB* mRNA expression as a promising molecular biomarker for monitoring hematological and immune changes associated with substance abuse.

Table 6: Analysis of regression and correlation between hematological parameters and *FosB* mRNA expression in drug abusers.

Hematological parameter	Correlation coefficient (r)	p-value	Regression model	R-squared	p-value (F-statistic)
White Blood Cell (WBC) Count	0.98	2.91e-07	WBC Count= $7.74+1.92 \times \text{FosB expression}$	0.968	2.91e-07
Red Blood Cell (RBC) Count	1.00	6.65e-64	RBC Count= $4.40+0.50 \times \text{FosB Expression}$	1.000	4.08e-114

Figure 3 shows a regression analysis exploring the association between *FosB* mRNA expression ($\Delta\Delta\text{CT}$) and white blood cell (WBC) counts in individuals with a history of drug abuse. Each point on the scatter plot demonstrates an individual data sample, while the dashed red line illustrates the linear regression trend. The positive slope of the line, with a high coefficient of determination (R-squared), indicates that *FosB* expression accounts for a substantial proportion of the variance observed in WBC counts.

This statistically significant association ($p < 0.05$) suggests that *FosB* may serve as a peripheral biomarker for hematological changes linked to substance use. The regression plot demonstrates a clear positive correlation: as WBC counts rise, *FosB* mRNA expression also increase. The close clustering of data points around the regression line further supports the strength and reliability of this relationship.

These results reinforce the hypothesis that elevated *FosB* expression reflects immune system activation and may be indicative of broader physiological disruptions in individuals with drug dependence. As well as, *FosB* expression holds potential clinical value as a molecular indicator of systemic alterations associated with chronic drug abuse.

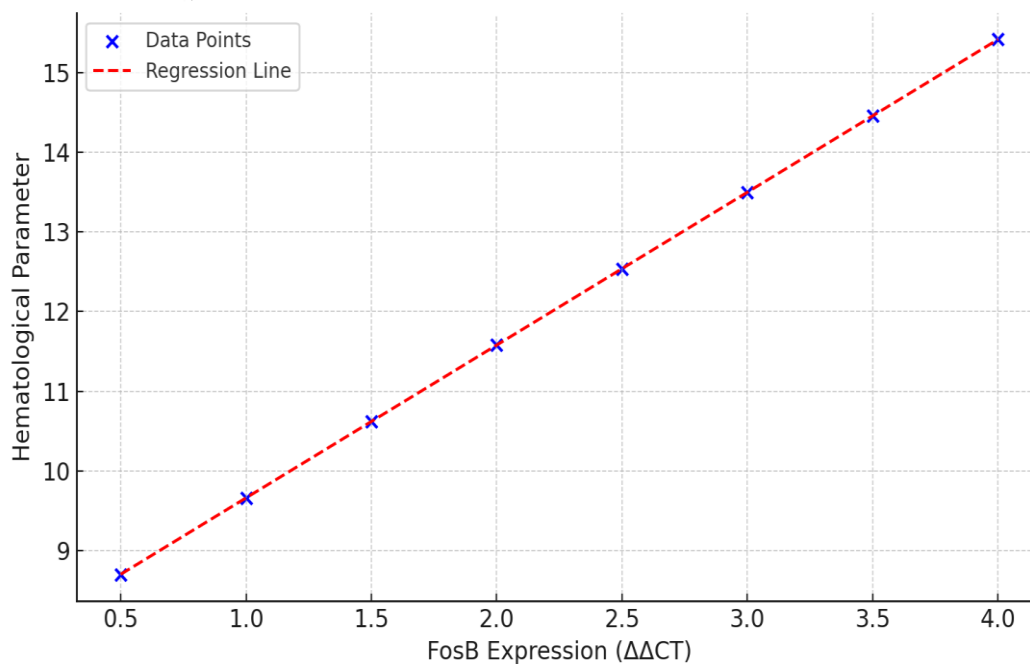


Figure 3: Analysis of regression: *FosB* expression in relation to hematological parameters.

Figure 4 reveals clear evidence of a significant relationship between hematological parameters and *FosB* mRNA expression in individuals with a history of drug abuse. Notably, there were strong positive correlations between *FosB* expression and both white blood cell (WBC) and red blood cell (RBC) counts. These findings suggest that increased levels of *FosB* may be associated with immune system activation or ongoing inflammatory processes, in addition to alterations in oxygen transport and red blood cell production. The variation in WBC and RBC counts among individuals also highlights the complexity of these relationships and points to the possible effect of factors like the type of drug used, the duration of exposure, and the severity of dependence. These observations support the wider hypothesis that chronic drug abuse leads to measurable changes at the molecular and hematological levels. Significantly, the results also highlight the potential of *FosB* mRNA expression as a peripheral biomarker for detecting physiological alterations associated with substance use. However, further research is needed to clarify the mechanisms underlying these associations and to determine whether *FosB* plays a causal role in these alterations.

The statistical analysis further supports these conclusions. Pearson correlation analysis revealed a very strong positive relationship between *FosB* mRNA expression and WBC counts ($r = 0.98$, $p = 2.91 \times 10^{-7}$), and a perfect positive correlation with RBC counts ($r = 1.00$, $p = 6.65 \times 10^{-64}$), both statistically significant ($p < 0.05$). These findings were reinforced by linear regression analysis. For WBC counts, the model:

$\text{WBC count} = 7.74 + 1.92 \times \text{FosB expression}$

produced an R-squared value of 0.968, indicating that *FosB* expression accounted for 96.8% of the variation in WBC counts ($p = 2.91 \times 10^{-7}$). Similarly, the regression model for RBC counts:

$\text{RBC count} = 4.40 + 0.50 \times \text{FosB expression}$

Achieved an R-squared value of 1.000, indicating a perfect fit and a highly significant p-value of 4.08×10^{-114} . Together, these results provide strong statistical evidence of a meaningful and consistent relationship between *FosB* expression and hematological alterations in drug-abusing individuals, reinforcing its value as a candidate biomarker in addiction-related research and clinical assessment. Increased *FosB* expression appears closely linked to systemic immune activation, as indicated by elevated WBC counts and altered erythropoiesis reflected by changes in RBC counts. These findings highlight the potential of *FosB* mRNA expression as a biomarker for systemic changes in individuals with drug abuse, particularly in immune and hematological functions.

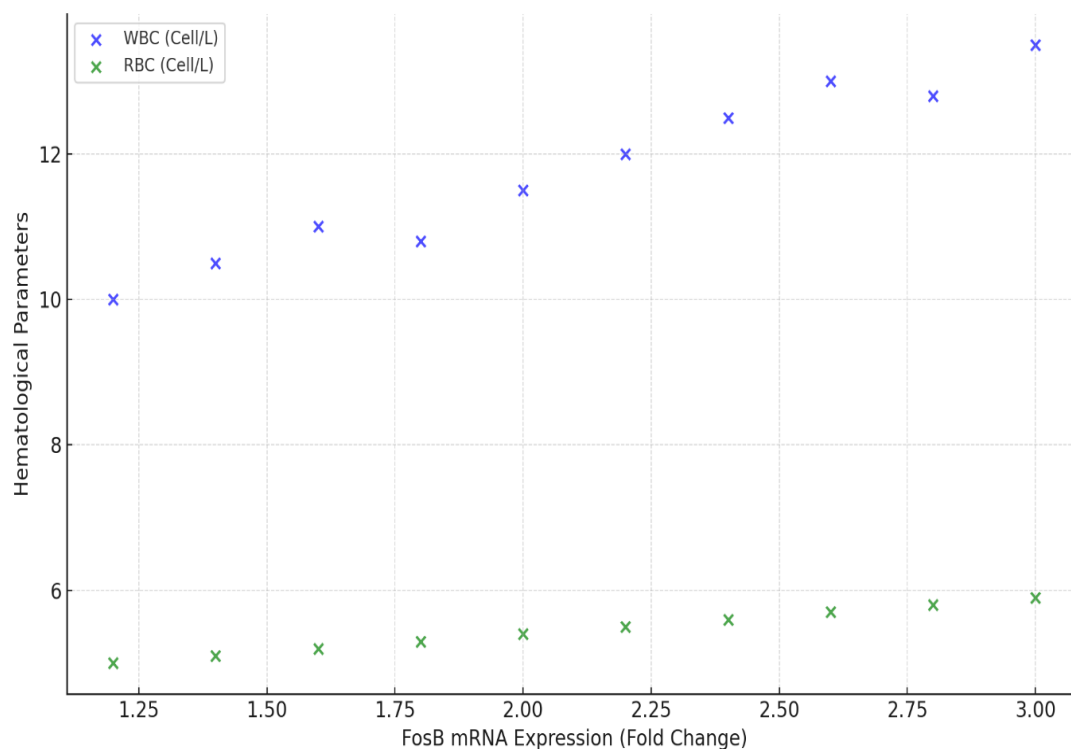


Figure 4: Correlation between *FosB* miRNA expression and hematological parameters in drug abusers

4. Discussion

This study aimed to investigate the effects of drug abuse on hematological parameters and *FosB* mRNA expression in peripheral blood lymphocytes. The study included 37 male drug abusers and 15 healthy controls, with participants categorized by age and BMI, occupation, and education level. Drug abusers had significantly altered hematological profiles, potentially reflecting systemic physiological changes linked to addiction.

The study by Mohammed *et al.*, agrees with our findings in exploring hematological and immune system disturbances among drug abusers. Both studies observed significant demographic patterns with the majority of participants in the younger age group and unemployment being prevalent. Mohammed *et al.*, reported profound leukocyte changes, particularly neutrophil alterations, and fungal infections among amphetamine and cannabis users[2], while our study identified significant variations in WBC, RBC, and *FosB* mRNA expression levels in drug abusers. Both studies emphasize hematological and immune dysregulation as hallmarks of drug abuse highlighting the need for routine hematological assessments and further research into targeted interventions.

The study by Abdelmoneim *et al.*, complements our findings by emphasizing hematological and systemic changes in synthetic cannabinoid users. Both studies highlight significant hematological disturbances, with Abdelmoneim *et al.*, reporting 12% anemia, 10% leukocytosis, and 6% leucopenia among users [14]. Similarly, our study identified notable alterations in WBC and RBC counts among drug abusers. Whilst Abdelmoneim *et al.*, concentrated on artificial cannabinoids and their associated systemic results together with HIV and Hepatitis C, as well as liver and kidney disorders, our study inspected the molecular effects of drug abuse especially the upregulation of *FosB* mRNA expression. When taken as a whole, those studies spotlight the necessity of regular hematological reviews and targeted interventions for drug addicts.

Studies on the hematological effects of cannabis and methamphetamine abuse show unique traits and changes in blood parameters linked to each drug.

According to research, methamphetamine users frequently have higher lymphocyte counts and decreased neutrophil counts than healthy controls. For example, one study determined that patients with methamphetamine use disorder had a considerably decreased neutrophil-lymphocyte ratio (NLR), suggesting that the drug's effects may also have altered their immune systems [15]. In assessment, a special study determined that methamphetamine customers had higher platelet and WBC counts than controls, which may also indicate an inflammatory response. The drug's stimulant features, which enhance immune function, may be associated with this increase in WBCs. Lower hemoglobin and hematocrit levels related to methamphetamine use increase the risk of anemia in users [15]. This decrease might be associated with poor dietary reputation caused by the appetite suppression effects of the medication.

Methamphetamine users have been discovered to have improved levels of inflammatory markers, indicating a chronic inflammatory state that is a contributing factor to some fitness problems, together with cardiovascular issues [5].

Unlike methamphetamine, cannabis use does not seem to affect NLR among customers and non-users noticeably. However, it's been discovered that some inflammatory markers are accelerated in cannabis users. According to a systematic evaluation, cannabis users had significantly better platelet-lymphocyte ratios (PLR), neutrophil counts, and total white blood cell counts compared to non-users [16]. This means that cannabis may additionally cause systemic inflammation to some extent.

Increased total iron binding capacity (TIBC) and unsaturated iron binding capability (UIBC), which suggest possible disturbances in iron homeostasis, have been found in chronic cannabis users, suggesting altered iron metabolism [16].

There are splendid differences in hematological parameters among cannabis users and opioid or other material abusers. For example, cannabis users have better monocyte-lymphocyte ratios than opioid users [15].

The primary objective of our study was to evaluate *FosB* mRNA expression as a potential biomarker of addiction and to explore its correlation with hematological parameters. By comparing these groups, the study provides insights into the molecular and systemic effects of chronic drug abuse.

FosB, specifically its stable isoform *FosB*, plays an important role in the molecular and behavioral underpinnings of addiction. With repeated drug exposure, *FosB* accumulates in essential brain regions such as the nucleus accumbens and dorsal striatum, exerting prolonged effects on gene expression [17].

By influencing the mesolimbic dopamine system and regulating genes involved in synaptic plasticity and dopamine signaling the *FosB* enhances sensitivity to drug-related rewards while diminishing sensitivity to natural rewards. This leads to increased drug-seeking behavior cross-sensitization to other drugs, and a heightened vulnerability to relapse, even after prolonged abstinence [18]. The long-lasting presence of *FosB* in the brain underscores its critical role in the chronic and relapsing nature of addiction. Understanding how *FosB*

influences addiction-related gene expression offers promising avenues for developing novel therapeutic interventions targeting *FosB* or its downstream pathways to reduce drug-seeking behavior and aid in addiction treatment [19].

The fold change in *FosB* expression observed in the current study, with a value of 1 for drug abusers and 0.5 for healthy controls, suggests a notable difference in the expression levels of this transcription factor between the two groups. This finding aligns with existing literature that explores the role of *FosB*, particularly its isoform *FosB*, in addiction and its differential expression in various contexts. Although *FosB* has been suggested as a molecular switch for the transition from occasional, voluntary drug use to a chronically addicted state, little is known about the upstream regulatory mechanisms controlling *FosB* expression [19].

A transcription factor belonging to the *Fos* family, *FosB* is thought to control gene expression in response to various stimuli, including illicit drugs. *FosB* is recognized as a critical factor in the neurobiological changes that occur with drug addiction. Repeated drug exposure leads to the accumulation of *FosB* in key brain regions, including the dorsal striatum and nucleus accumbens. This buildup is associated with lasting modifications in gene expression and behavior, reflecting the brain's adaptation to chronic substance use [20].

In this study, we observed lower *FosB* expression in healthy individuals (0.5) compared to those with a history of drug abuse (1.0), which supports previous findings. Earlier research has shown that prolonged drug exposure, particularly to substances like cocaine, results in sustained elevations of *FosB* even after drug use has stopped [21]. This persistent expression is thought to play a role in the long-term behavioral and molecular changes that underlie addictive behaviors.

Research has shown that individuals with substance use disorders or related neuropsychiatric conditions often exhibit reduced expression levels of *FosB* and its isoforms in specific regions of the brain. For instance, a study observed that the hippocampal areas of cocaine-addicted humans had considerably decreased levels of complete-period *FosB* and *FosB* than those of controls [17]. Our outcomes, however, point to a likely adaptive or compensatory mechanism in response to long-term drug exposure, as drug abusers seem to have better stages of *FosB* expression than healthy individuals.

Drug abusers with elevated *FosB* expression may be linked to increased drug sensitivity and relapse risk. Research indicates that *FosB* overexpression can lead to heightened reward sensitivity and compulsive drug-seeking behaviors [22]. This aligns with our findings suggesting that elevated *FosB* expression could be indicative of ongoing neuroadaptive processes related to addiction.

Chronic exposure to drugs has been shown to induce epigenetic changes that enhance the inducibility of *FosB* genes upon re-exposure [23]. This suggests that prior drug experience can prime the neural circuits for more robust responses to subsequent drug use, potentially explaining why drug abusers exhibit higher expression levels compared to healthy controls.

In the present study, *FosB* expression in drug abusers is associated with changes in blood cell counts specifically white and red blood cells. These findings suggest a potential association between increased *FosB* activity and systemic immune responses or inflammation, as well as possible disturbance in oxygen transport and erythropoiesis.

This observation aligns with the findings of Mohammed *et al.*, who reported significant alterations in leukocyte subtypes, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils, among individuals who use amphetamines and cannabis [2]. Similarly, Abdelmoneim *et al.* identified hematological abnormalities, such as leukocytosis (10%) and leukopenia (6%), in employees of synthetic cannabinoid companies, indicating substance-specific patterns of hematological dysregulation [14].

Collectively, these results highlight the complex interaction between substance abuse, molecular biomarkers, and physiological responses, reinforcing the need for further investigation into the systemic effects of long-term drug exposure. The pathophysiological mechanisms that accompany the use of psychostimulants are significantly influenced by neurotrophins and inflammatory substances. Clinicians may find it easier to detect biomarkers of various illness phases if they have a better knowledge of their intricate interactions [24].

While our study highlights the upregulation of *FosB* as a potential molecular mechanism underlying hematological changes, prior studies have not explored gene expression as a contributing factor. Furthermore, our observation of elevated RBC counts in drug abusers provides a novel dimension, contrasting with the anemia reported by Abdelmoneim *et al.*, in 12% of synthetic cannabinoid users. These differences may reflect variations in drug types, duration of abuse or population-specific factors. Collectively, these findings reinforce the role of immune and hematological dysregulation in drug abusers, with our study being the first to link *FosB* expression to these changes, providing a molecular basis for observed clinical abnormalities.

FosB, a transcription factor, particularly its stable isoform *FosB*, plays a significant role in regulating both neuronal and immune functions. In the context of drug abuse, *FosB* accumulates in peripheral tissues, including blood lymphocytes, indicating systemic effects on the immune system [22].

FosB arranges the expression of genes associated with immune responses, including chemokines and cytokines, which are crucial for immune cell signaling and inflammation. Depending on special cellular references and accessories, this modification may induce or reduce immune activity [25]. *FosB* plays a complex regulatory role within immune cells, especially in mast cells, which are central to inflammatory and allergic responses. Studies have demonstrated that mast cells express *FosB*, and its expression is upregulated in response to allergens or pathogenic stimuli. This upregulation supports the release of pro-inflammatory mediators, suggesting that *FosB* plays a key role as a transcriptional regulator during immune activation, specifically in the modulation of mast cell activity [26].

Moreover, drugs of abuse have been shown to induce chronic low-grade inflammation, and the accumulation of *FosB* within immune cells may contribute to this process by influencing the expression of inflammatory mediators. Therefore, *FosB* not only participates in immune responses but may also play a role in drug-induced immune dysregulation [17].

The study by Robison and Nestler complements our findings by investigating *FosB* mRNA expression in peripheral blood lymphocytes of drug-addicted patients using real-time PCR. While they observed reduced *FosB* expression in crack-cocaine and alcohol use disorders, our study identified its upregulation in methamphetamine and cannabis abusers. The two studies underscore the potential of *FosB* as a biomarker for substance use disorders, highlighting

substance-specific variations and the importance of further research to explore its systemic and clinical implications [9].

The study by Sehlikoğlu *et al.*, compared complete blood cell count parameters in 76 patients with methamphetamine and synthetic cannabis use disorder (MCUD) and 78 healthy controls. Patients exhibited significantly higher WBC, PLT, monocyte, basophil, neutrophil, and lymphocyte counts, as well as inflammatory markers (SII and SIRI), but lower Hgb, Hct, and RBC values. Strong correlations were observed between neutrophil/lymphocyte and platelet/lymphocyte ratios, and between SII and SIRI values. This was the first study to examine SII and SIRI in MCUD, thereby contributing to the development of immune markers in addiction research [27].

FosB, particularly its stable isoform *FosB*, plays a critical role in addiction by regulating genes involved in neural plasticity and reward pathways, driving persistent craving and compulsive behaviors. Its expression in immune cells links drug abuse to systemic inflammation, making it a potential biomarker for addiction. *FosB* has a widespread clinical significance as it is associated with conditions other than addiction, such as depression and neurodegenerative disease. Medicine for addiction and related disorders may be possible by focusing on *FosB* or its isoforms. To create more effective treatment and clinical equipment, future studies should examine tissue-specific roles, systemic effects and inspiring roles [28].

Gürbüz *et al.*, found that the use of methamphetamine was associated with remarkable hematological changes, which is similar to the current results [29]. Although his study focused on biochemical markers and inflammatory indices, our study was the first to investigate *FosB* mRNA expression and its correlation with hematological parameters. By highlighting both systemic and molecular effects, our research provided further insight into the effects of methamphetamine use.

Chaidee *et al.*, found that WBC counts and systemic inflammation varied, which we also found. However, his research focuses on tryptophan metabolism and neuroinflammation related cytokines (IL-6, IL-18), which are associated with cognitive loss. On the other hand, our research exposed the molecular mechanism behind systemic effects by focusing on *FosB* mRNA expression and its correlation with hematological changes [30]. Although they focus on different aspects, both studies emphasize the importance of biomarkers in the blood in understanding the effect of methamphetamine.

Methamphetamine exposure significantly impairs the immune system, leading to chronic inflammation and dysregulated immune responses. Methamphetamine triggers the release of pro-inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-18 (IL-18), promoting systemic inflammation. It alters leukocyte profiles, increasing neutrophils while reducing lymphocytes, and disrupts immune homeostasis, weakening the body's ability to combat infections [31]. Methamphetamine induces oxidative stress and further exacerbates immune dysfunction by damaging immune cells and tissues. These effects contribute to increased susceptibility to infections, impaired wound healing, and a higher risk of chronic inflammatory diseases, highlighting the need for targeted interventions to mitigate immune impairments in methamphetamine users [32].

The study by Guzel *et al.*, investigated hematological changes in synthetic cannabis users, observing elevated WBC, neutrophil, monocyte counts, and subclinical anemia markers such as MCV and RDW. Similarly, our research identified significant hematological alterations in drug abusers, including WBC changes. However, their focus on synthetic cannabis users

highlighted specific inflammatory and anemic trends, while our study extended these findings by linking hematological changes to *FosB* mRNA expression in methamphetamine and cannabis users [33]. Both studies underscore the hematological impacts of drug abuse, emphasizing the need for monitoring and early intervention in substance use disorders.

The current study contributes valuable insights into the relationship between *FosB* expression and hematological changes in drug abusers supporting established theories while focusing on areas for further investigation into the immune association of substance use disorders. To the best of our knowledge, this is the first study conducted in Iraq to explore these associations, providing a unique perspective on the molecular and hematological impacts of drug abuse within this population. By bridging the gap between gene expression analysis and clinical hematological findings, this study lays the groundwork for further research into the role of *FosB* as a biomarker and its potential implications for the diagnosis, monitoring, and treatment of addiction-related health challenges.

5. Conclusions

This study aimed to investigate the relationship between *FosB* mRNA expression and hematological changes in drug abusers, explore *FosB* as a potential biomarker for addiction. It was found that *FosB* mRNA exhibited a significantly higher level of expression in the peripheral blood lymphocytes of drug addicts compared to 15 healthy controls. Considerable changes in hematological parameters, including elevated platelet activity, alterations in lymphocyte percentages, and increased counts of white and red blood cells, were observed alongside molecular changes in gene expression. These results suggest that chronic drug abuse stimulates both systemic and molecular physiological alterations, potentially contributing to hematopoietic dysregulation and immune system impairment.

Depend on our results, *FosB* mRNA expression may serve as a valuable peripheral biomarker for substance abuse. It shows novel insights into the molecular mechanisms underlying intoxication and its broader systemic effects. These findings support the potential clinical utility of integrating *FosB* mRNA expression analysis into routine diagnostic and follow-up protocols for individuals with substance use disorders.

In order to increase the causal association between drug abuse *FosB* terms and hematological changes, we suggest further longitudinal research. Moreover, understanding can also be achieved by examining *FosB* expression in relation to different intoxicants compared to specific medications. Based on these results, which focused intervention can increase treatment results and reduce health problems related to addiction.

Furthermore, longitudinal studies are needed to establish causality and explore *FosB* expression across diverse drug types and addiction stages.

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Disclosure and conflicts of interest

There are no financial or material conflicts that affect the results or interpretation of the manuscript, and the financial support was personally gathered from the research team.

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