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Neuro-Immuno-Endocrine Interactions in Early Life Stress and Heroin Withdrawal Timeline

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Keywords
Early life stress · Inflammation · Heroin abuse · Hypothalamic-pituitary-adrenal axis · Apoptosis

Abstract
Both heroin abuse and early life stress (ELS) affect the immune system and the hypothalamic-pituitary-adrenal (HPA) axis. Additionally, accelerated aging due to mild inflammation has been indicated in these conditions. The present study aims to compare plasma levels of apoptosis markers, inflammatory markers, and stress hormones during early heroin abstinence period. Thirty-one individuals with heroin/opioid use disorder who had heroin-ELS and 26 of their siblings who were not abusing substances (ELS), and 32 individuals with heroin/opioid use disorder without a history of ELS (heroin-no ELS) were included in the study. The levels of interleukin-6, C-reactive protein, erythrocyte sedimentation rate, albumin, alanine transaminase, aspartate transaminase, and white blood cell count were assessed as the inflammatory and biochemistry markers. Also, apoptosis markers including tumor necrosis factor (TNF)-related weak inducer of apoptosis, TNF-related apoptosis-inducing ligand, soluble tumor necrosis factor receptor type I as apoptosis markers were detected by enzyme-linked immunosorbent assay. ELS was simultaneously evaluated using the Childhood Trauma Questionnaire, Minnesota Multiphasic Personality Inventory, and beck depression inventory scales. Besides, heroin craving was assessed by Daily Drinking/Drug Questionnaire score in individuals with heroin use disorder. This is the first study to evaluate the inflammatory, stress, and apoptosis markers during heroin abstinence, supporting the association between ELS and peripheral pro-inflammatory markers' levels and HPA axis.

Introduction

Heroin abuse (drug addiction) is still one of the main problems in today's world. Morphine is a refined extract of the opium plant. Harmful effects of long-term heroin

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inhalation or injection on cell morphology and function have been indicated in many studies [1–3]. It is well known that substance dependence is accompanied by abnormal hypothalamic-pituitary-adrenal (HPA) axis function [4, 5]. Additionally, accelerated aging and apoptosis are some of the side effects of drug addiction which can cause cellular damage [6–8]. Studies have indicated drugs such as cocaine, opiates, and alcohol induce oxidative stress, which contributes to cytotoxicity in different organs [9–11]. In this state, mitochondrial dysfunction and elevated mitochondrial reactive oxygen species accelerate cell death [12, 13]. Oxidative stress and increase of inflammatory cytokines in addicted subjects cause accumulation of toxic agents in the body, which has negative effects on the vascular, pulmonary, and nervous systems [14, 15].

On the other side, risky health behaviors such as unprotected sex and intravenous drug use increase exposure to infections, activate the immune system and inflammatory responses, and accelerate vasculature aging and neuronal toxicity. Also, low socioeconomic status, restricted access to health and follow-up care, lack of sleep, inadequate exercise, and poor nutrition may aggravate age-related changes in individuals with substance use disorder [16]. Along with addiction, these factors may also mediate disturbances in the homeostatic regulation of the neuroendocrine and immune systems, and be associated with the production of pro-inflammatory cytokines including interleukin (IL)-6, tumor necrosis factor alpha (TNF-α), cytokine antagonists, and acute-phase proteins such as C-reactive protein (CRP) [17, 18].

The role of environmental factors and genetics in the etiology of substance use disorders has been indicated. In humans, childhood maltreatment has been shown to be associated with a range of adverse outcomes, including major depression, anxiety disorders, onset of alcohol use disorder, and substance use disorders [19, 20]. Additionally, alterations in the HPA axis is implicated as an outcome of early life stress (ELS) [21–23]. Several hormones, including cortisol, an adrenal steroid, are released by HPA-axis activation. Cortisol is one of the main hormonal end products of the HPA axis that helps to mobilize resources to aid the body filter and increase salient signals, such as stressful stimuli, from the environment [24]. Simultaneously, with cortisol, dehydroepiandrosterone (DHEA) and its sulfated form (DHEA sulfate [DHEAS]), endogenous hormones primarily derived from the HPA axis, are released. These hormones demonstrate a daily rhythm and, as part of the physiological stress response, increase alongside cortisol [25]. Inflammatory immune response and production of pro-inflammatory cytokines such as TNF-α have been indicated in many psychiatric disorders and as a result of adverse experiences during childhood [26, 27].

On the other hand, HPA axis has the pivotal role for homeostasis of the immune system, and its excessive activation has been correlated with several immune-mediated diseases including increased susceptibility to infections and reduced wound healing [28–30]. Conversely, several age-related pathologies have been indicated following excessive glucocorticoid exposure, including hyperglycemia/hyperlipidemia, atherosclerosis, and major depression [31, 32]. Therefore, the interactions between immune mediators and monoamine metabolism, neuroendocrine functions, synaptic plasticity, and neural circuits, play important roles in pathogenesis in the individuals with a history of ELS and also in individuals with substance use disorder. TNF-α is a pro-inflammatory cytokine with an important role in the innate host response to infection and injury. Alterations in the TNF-α system have been indicated in a number of psychiatric disorders such as schizophrenia and depression as well as in individuals with cocaine use disorder [33–37].

Production of TNF-α in inflammatory immune response recruits and activates immune cells and induces the production of other pro- and anti-inflammatory cytokines, such as IL-1, IL-6, IL-8, and IL-10 [38]. This cytokine is implicated as a regulating factor in a broad spectrum of biological conditions, including cell proliferation, differentiation, apoptosis, and coagulation [39]. TNF-α acts by 2 different receptors with different functional endpoints, which can be cleaved from the surface of different types of cells and are detectable in serum in soluble forms [39]. TNF receptor type I (TNFR I) is engaged in the recruitment of associated death domain protein-mediated apoptosis and activation of nuclear factor-kappa B (NF-kB) signaling pathway. While TNFR I is only associated with NF-kB activation, it plays the main role in regulating TNF-mediated inflammatory responses and sheds soluble TNFR I (sTNFR I) from the cell surface in different conditions such as apoptosis and inflammation [40, 41].

TNF-related weak inducer of apoptosis (TWEAK), another TNF superfamily ligand, mediates immune responses against tissue injury. Serum TWEAK levels in previous studies in psychiatric disorders, including schizophrenia, bipolar disorder, and ELS, have been assessed as possible pathophysiological factors in inflammatory and immune response changes [42–44]. Additionally, TNF-related apoptosis-inducing ligand (TRAIL)

is another TNF superfamily ligand which causes apoptosis by binding to specific death receptors, TRAIL receptors 1 and 2 [45–47].

Despite the recent evidence of chronic pro-inflammatory state in both stress and substance use as well as indications of changes in the peripheral levels of TNF superfamily members in ELS and crack cocaine withdrawal, to our knowledge, neuro-immuno-endocrine processes have never been investigated in individuals with heroin use disorder. Accordingly, the history of anxiety, depression, and addiction status in all the subjects was assessed using different tests. Also, the levels of usual markers of inflammation such as CRP, erythrocyte sedimentation rate (ESR), white blood cell (WBC) counts, IL-6, cortisol, DHEAS, and cell death markers such as sTNFR I, TRAIL, and TWEAK in individuals with heroin use disorder with a history of ELS (heroin-ELS) were compared with the corresponding values in their siblings who did not abuse substances (ELS), and individuals with heroin use disorder without a history of ELS (heroin-no ELS).

Materials and Methods

Participants

The study participants (89 men and women), selected by trained staff using simple selection methods in Isfahan, Iran, included 31 heroin-dependents with ELS (heroin-ELS group from Shahid Khabushani camp), 26 of their siblings who were not addicted (ELS group), and 32 heroin-dependents without a history of ELS (heroin-no ELS; heroin group from an abstinence addiction therapy center). During a leading period (a minimum of 6–18 days), subjects were asked to abstain from using any narcotics including illicit drugs and medications. Drug use, medical, and psychiatric assessments were conducted, and drug urine tests were carried out to ensure subjects remained drug-free during the leading and study periods. To be eligible to participate, individuals had to have 20–60 years of age and meet Diagnostic and Statistical Manual of Mental disorders, also known as DSM-IV criteria for individuals with heroin use disorder. Exclusion criteria included other current substance abuse or dependence, the presence of a current major Axis I disorder, use of any psychoactive medication or any medication known to alter HPA axis function, and presence of current infectious diseases or history of autoimmune, endocrine or coronary heart disease, rheumatoid arthritis, and neurological disorders.

Study Design

The procedure was fully explained to each subject before the start of the project, and a written informed consent was obtained. The Structured Clinical Interview, which was previously designed in the Iranian National Center for Addiction Studies, was used to assess psychiatric exclusions, history of drug abuse, social status, and medical information. Subjects were also instructed to abstain from heroin and other drug use during the sample collection.

Clinical Assessment

ELS was assessed through validated Iranian version of Childhood Trauma Questionnaire (CTQ) [48] which assesses the history of sexual, physical, and emotional abuse, as well as physical and emotional neglect during childhood. Beck Depression Inventory (BDI) score [49], Minnesota Multiphasic Personality Inventory test [50], and Hamilton Rating Scale for Depression [51] were evaluated in all subjects. Also, heroin craving was assessed by Daily Drinking/Drug Questionnaire (DDQ) [52] in individuals with heroin use disorder.

Laboratory Analyses

Blood Withdrawal

An indwelling cannula was inserted at least 3 h before sampling. Whole blood was collected between 11:00 and 11:30 a.m., after 3 h of fasting, in order to minimize differences due to biological variations. Ten milliliters of blood was drawn from each participant for assessment of complete blood count, ESR, serum levels of albumin (Alb), and liver enzymes (aspartate transaminase [AST] and alanine transaminase [ALT]). In order to separate serum from blood, blood was immediately centrifuged at 1,800 *g* and 4 °C for 10 min. Serum was collected and stored at –80 °C until assayed.

Detection of Routine Indicators

Complete blood count was carried out using automated blood cell analyzer. The output included leukocyte count, neutrophils and lymphocytes percentages, and hemoglobin. Liver function tests, including ALT and AST, were carried out by colorimetry using an automated analyzer. CRP was tested by turbidimetric inhibition immunoassay, and ESR was detected by the Westergren method. IL-6 was measured by electrochemiluminescent immunoassay using an automated analyzer. Cortisol and DHEAS blood sample were collected at 9 a.m., and baselines for DDQ, Clinical Opiate Withdrawal Scale [53], VAS [54], and Hamilton Check list were established. Basal cortisol and DHEAS levels were assessed using a competitive immune analysis method on the COBAS E 411 device. Reference intervals for morning cortisol were 4.82–19.5 μg/dL and for DHEAS 148–407 μg/dL. Albumin level was measured using a nephlometer.

Enzyme-Linked Immunosorbent Assay Analysis

Serum concentrations of sTNFR I (Abcam, UK), TWEAK (Hoelzel, Germany), and TRAIL (Ebioscience, USA) were determined in duplicate using commercially available enzyme-linked immunosorbent assay kits according to the procedures supplied by the manufacturer for the respective receptors. Detection limits were defined at <1 pg/mL for sTNFR I, 5 pg/mL for TWEAK, and 10 pg/mL for TRAIL. Concentrations were expressed in pg/mL.

Statistical Analyses

Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software Inc., USA) statistical package. Data normality and homogeneity were checked by Shapiro-Wilk and Levene tests, respectively. As the data were normally distributed, a comparison of the results between heroin-ELS group, ELS group, and heroin group was performed using ANOVA and ANCOVA models using the Tukey-Kramer adjustment for multiple comparisons.

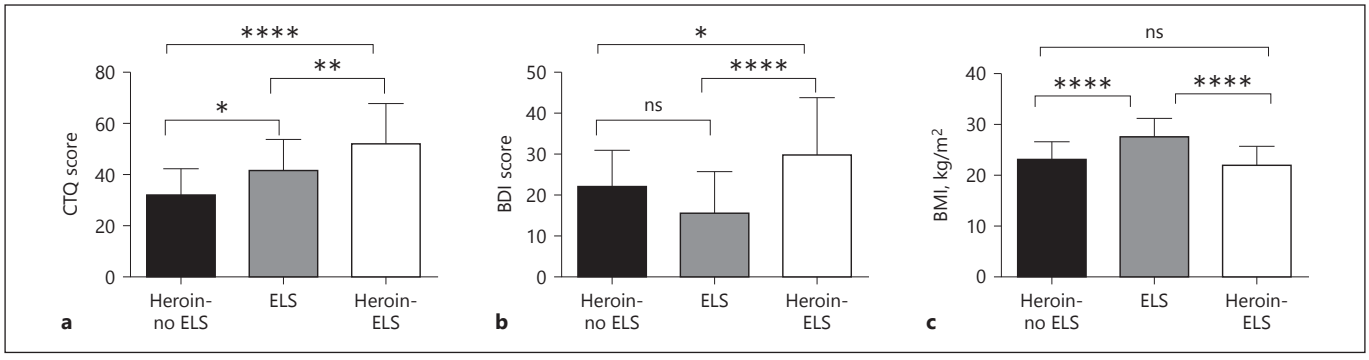

Fig. 1. Clinical assessment of subjects. ELS was assessed by CTQ score (**a**) and depression status was determined by BDI score (**b**) and also BMI (**c**) was assessed in all groups. *p* values represent a test of crude differences between groups using ANOVA using the Tukey-Kramer adjustment for multiple comparisons. Data are presented as mean and SD. Statistically significant differences are indicated: * $p < 0.05$, ** $p < 0.05$, and **** $p < 0.0001$. Heroin-no ELS: $n = 32$, ELS: $n = 26$, heroin-ELS: $n = 31$. CTQ, Childhood Trauma Questionnaire; ELS, early life stress; BDI, Beck Depression Inventory; BMI, body mass index; ns, not significant.

The interrelationship between the 2 parameters was tested by Pearson correlation analysis. The level of statistical significance was $p \leq 0.05$, and the data is reported as mean ± SD.

Results

Sociodemographic and Clinical Data

There were significant differences between groups in marital status ($p < 0.0001$), while we did not find significant differences in the ages of participants (Table 1). ELS was assessed through CTQ score, and we found significant differences between groups ($F_{2, 86} = 18.29$, $p < 0.0001$) in CTQ scores. Post hoc analyses showed CTQ score to be significantly higher in ELS ($p < 0.0001$) and heroin-ELS compared to heroin-no ELS group (Fig. 1a). Also, assessment of BDI score in groups revealed significant differences between groups ($F_{2, 86} = 11.3$, $p < 0.0001$) and post hoc analyses indicated higher BDI score in heroin-ELS group compared to ELS ($p < 0.0001$) and heroin-no ELS ($p < 0.05$) groups (Fig. 1b). Body mass index (BMI) analysis in 3 groups showed significant differences ($F_{2, 86} = 18.59$, $p < 0.0001$) and post hoc analysis showed BMI to be significantly higher in ELS group compared to heroin-ELS ($p < 0.0001$) and heroin-no ELS groups ($p < 0.0001$; Fig. 1c). There were no significant differences between groups in Minnesota Multiphasic Personality Inventory, DDQ, and Hamilton scores (Table 1).

Serum Cortisol and DHEAS Levels as the Endocrine Markers

The cortisol levels differed significantly among groups ($F_{2, 86} = 36.65$, $p < 0.001$) and post hoc analyses revealed increased cortisol levels in both heroin-no ELS ($p < 0.001$) and heroin-ELS groups ($p < 0.05$) when compared to ELS group. Also, cortisol levels were significantly higher in heroin-no ELS group compared to heroin-ELS group ($p < 0.0001$; Fig. 2a).

DHEAS was assessed because it is an essential marker of endocrine function and also for its role in antagonizing many glucocorticoid-related changes. The DHEAS levels differed significantly in the 3 groups ($F_{2, 86} = 8.81$, $p < 0.003$). Post hoc analyses indicated increased DHEAS levels in both heroin-no ELS ($p < 0.001$) and heroin-ELS groups ($p < 0.05$) when compared to the ELS group. We did not find significant differences in DHEAS level between heroin-no ELS group and heroin-ELS group (Fig. 2b). Also, the cortisol/DHEAS ratio did not differ among the groups ($F_{2, 86} = 2.77$, $p = 0.068$; Fig. 2c).

Biochemistry Blood Markers

Liver enzymes (AST/ALT) and albumin levels did not show significant differences between groups (Table 2).

Immune System and Soluble Apoptosis Markers

WBC counts differed significantly among groups ($F_{2, 86} = 7.16$, $p < 0.01$) and post hoc analyses revealed higher WBC counts in heroin-no ELS ($p < 0.01$) and heroin-ELS groups compared to ELS group ($p < 0.01$; Fig. 3a). However, no statistically significant differences among the heroin-no ELS, ELS, and heroin-ELS groups emerged in terms of ESR, IL-6, and CRP levels (Table 2).

Although no significant statistical difference was found for serum TWEAK among groups ($F_{2, 85} = 1.51$, $p > 0.05$; Fig. 3b), higher levels of sTNFR I were found in heroin-no ELS group compared to heroin-ELS group ($p < 0.01$) and

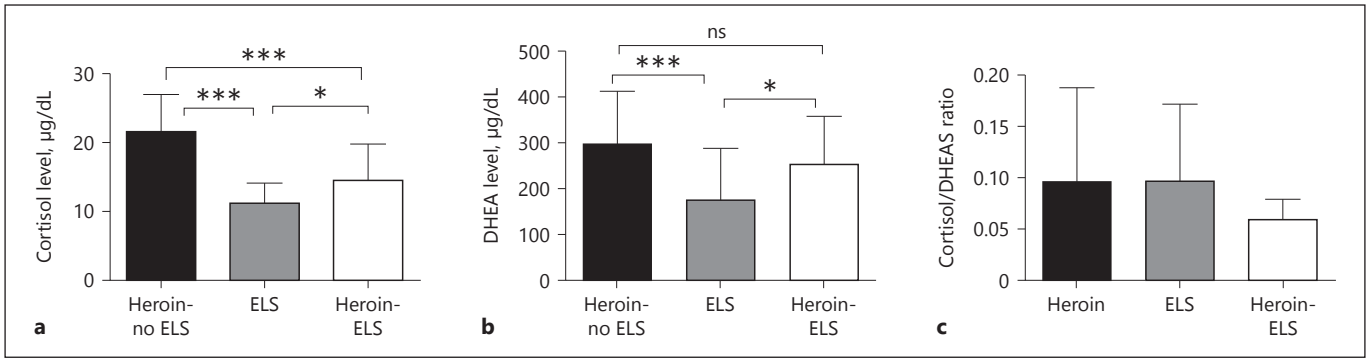

Fig. 2. Serum evaluation of adrenal secreted hormones. Cortisol and DHEAS levels were assessed in duplicate using a competitive immune analysis method. Cortisol levels (**a**) and DHEAS levels (**b**) were determined from a single morning serum collection (9 a.m.). **c** Cortisol/DHEAS ratio was determined by calculating the relation between the morning (9 a.m.) serum levels of both hormones. *p* values represent a test of crude differences between groups using ANCOVA using the Tukey-Kramer adjustment for multiple comparisons. Data are presented as mean and SD. Statistically significant differences are indicated: * $p < 0.05$ and *** $p < 0.001$. Heroin-no ELS: $n = 32$, ELS: $n = 26$, heroin-ELS: $n = 31$. ELS, early life stress; DHEAS, dehydroepiandrosterone sulfate.

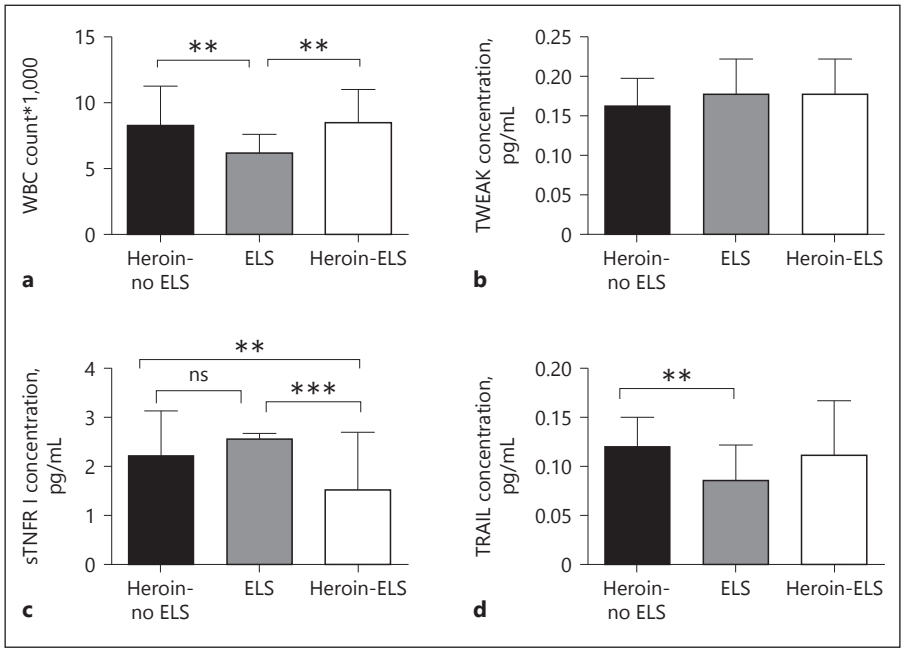

Fig. 3. Immune system and apoptosis markers. **a** WBC counts of heroin, ELS and heroin-ELS groups. **b** TWEAK, (**c**) sTFNR I and, (**d**) TRAIL serum levels of heroin, ELS, and heroin-ELS groups' comparisons. Data are presented as mean and SD. *p* values represent a test of crude differences between groups using ANOVA using the Tukey-Kramer adjustment for multiple comparisons: ** $p < 0.01$ and *** $p < 0.001$. Heroin-no ELS: $n = 32$, ELS: $n = 26$, heroin-ELS: $n = 31$. WBC, white blood cell; ELS, early life stress; TWEAK, TNF-related weak inducer of apoptosis; TRAIL, TNF-related apoptosis-inducing ligand; sTNFR I, soluble tumor necrosis factor receptor type I; ns, not significant.

in ELS group compared to heroin-ELS group ($p < 0.0001$; Fig. 3c). Also, heroin group had a significantly higher concentration of TRAIL compared to ELS group ($p < 0.01$; Fig. 3d).

Clinical Correlates of Endocrine and Immune Variables

First, we assessed clinical correlates of endocrine changes reported here. No correlations were found between BDI or Hamilton scores with cortisol levels and morning DHEAS levels in any groups. However, in the assessment of relationship between clinical and immune variables, BDI scores related positively to sTFNR I in heroin-noELS group ($r = 0.369$, $p = 0.037$; Fig. 4a) and BMI related positively to sTFNR I in the ELS group ($r = 0.4066$, $p = 0.0393$; Fig. 4b).

Also, in ELS group, TRAIL levels was found positively related to DHEAS levels ($r = 0.535$, $p = 0.0048$; Fig. 5a) and negatively related to cortisol/DHEA ratio ($r = -0.534$, $p = 0.0049$; Fig. 5b). Interestingly, in heroin-ELS group, TRAIL levels had positive correlation with cortisol levels ($r = 0.641$, $p = 0.0001$; Fig. 5c) and DHEAS levels ($r = 0.453$, $p = 0.009$; Fig. 5d). Also, the negative correlation

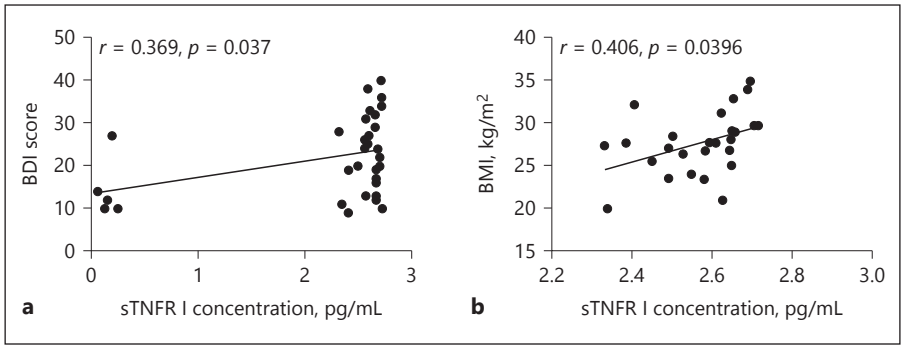

Fig. 4. Clinical correlates of endocrine and immune variables. **a** Positive correlation between BDI score and serum levels of sTFNR I in heroin group. **b** Positive correlation between BMI and serum levels of sTFNR I in ELS group. Heroin-no ELS: n = 32, ELS: n = 26. BDI, Beck Depression Inventory; BMI, body mass index; sTNFR I, soluble tumor necrosis factor receptor type I.

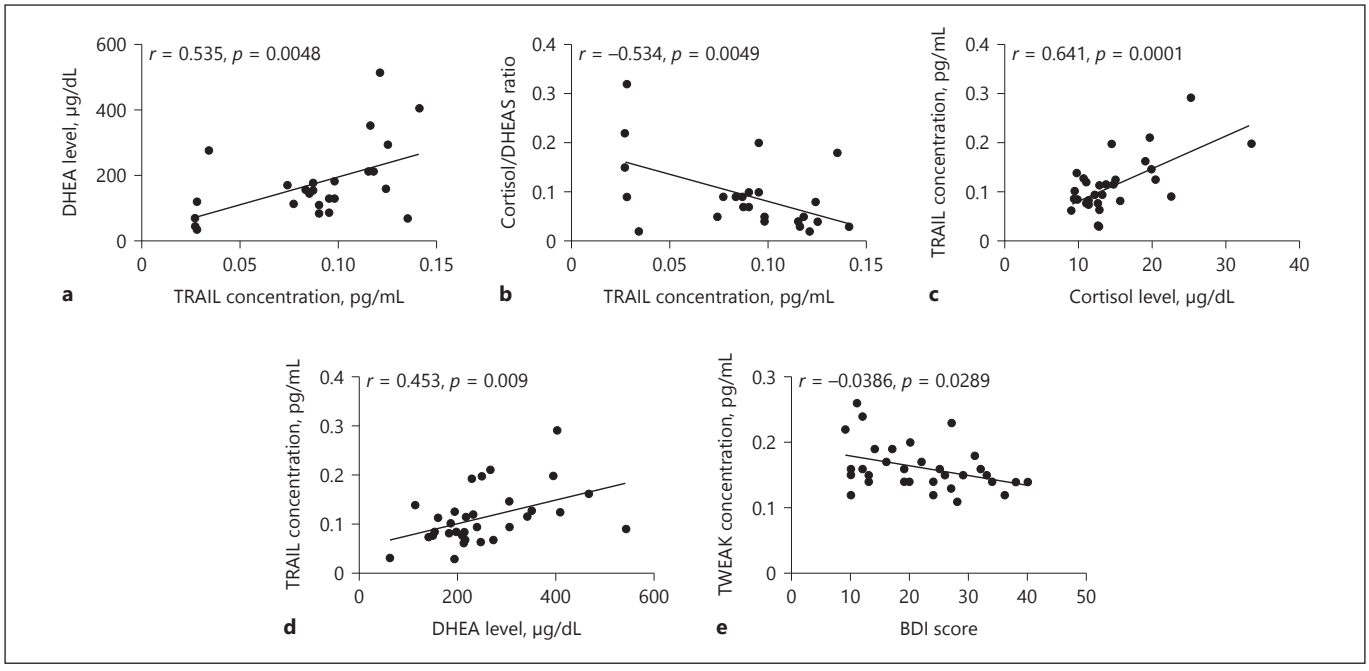

Fig. 5. Correlation of endocrine and immune variables. **a** Positive correlation between TRAIL serum levels and DHEAS in ELS group. **b** Negative correlation between TRAIL serum levels and cortisol/DHEAS ratio in ELS group. **c** Positive correlation between TRAIL serum levels and cortisol in heroin-ELS group. **d** Positive correlation between TRAIL serum levels and DHEAS in heroin-ELS group. **e** Positive correlation between TWEAK serum levels and BDI in heroin group. Heroin-no ELS: n = 32, ELS: n = 26, heroin-ELS: n = 31. DHEA, dehydroepiandrosterone; TRAIL, TNF-related apoptosis-inducing ligand; DHEAS, dehydroepiandrosterone sulfate; BDI, Beck Depression Inventory; TWEAK, TNF-related weak inducer of apoptosis.

between BDI and TWEAK concentrations was found in heroin-no ELS group (r = –0.0386, p = 0.0289). In addition, in ELS group, TRAIL serum level was found positively related to CRP (r = 0.396, p = 0.044; Fig. 6a) and ALT (r = 0.433, p = 0.027; Fig. 6b). A positive correlation was also found between sTNFR I and ESR levels in the heroin group (r = 0.396, p = 0.024; Fig. 6c). The remaining immune variables were not found to correlate with adrenal hormones or clinical indices in the 3 groups.

Discussion

In the present study, we investigated some inflammatory and biologic markers including CRP, ESR, WBC, IL-6, ALT, AST, sTNFR I, TWEAK, TRAIL, and stress hormones (cortisol and DHEAS) in heroin-dependent patients without a history of ELS (Heroin-no ELS group), heroin-dependent patients with a history of ELS (Heroin-ELS group), and their siblings who were not addicted (ELS group).

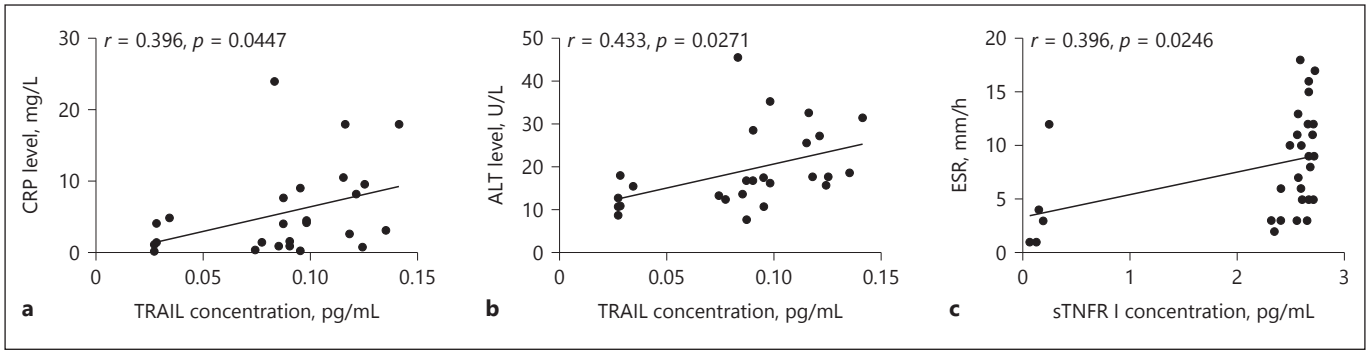

Fig. 6. Correlation of immune variables. **a** Positive correlation between TRAIL serum levels and CRP in ELS group. **b** Positive correlation between TRAIL serum levels and ALT in ELS group. **c** Positive correlation of sTNFR I with ESR levels in heroin group. Heroin-no ELS: $n = 32$, ELS: $n = 26$. CRP, C-reactive protein; TRAIL, TNF-related apoptosis-inducing ligand; ALT, alanine transaminase; ESR, erythrocyte sedimentation rate; sTNFR I, soluble tumor necrosis factor receptor type I.

Table 1. Demographic and clinical characteristics of the study participants

Variables	Heroin-ELS group ($n = 31$)	Heroin-no ELS group ($n = 32$)	ELS group ($n = 26$)	p value
Age, years	31.00±8.149	36.16±9.109	31.96±7.49	0.39
Marital status, % (n)				
Permanent marriage	32.26 (10)	37.5 (12)	96.1 (25)	
Single/never married	19.35 (6)	21.88 (7)	–	0.0001
Separated/but not divorced	25.80 (8)	21.88 (7)	–	
Divorced	22.56 (7)	18.74 (6)	3.9 (1)	
BMI, kg/m^2	22.10±3.63	23.10±3.58	27.66±3.63	0.0001
BDI score	29.77±14.13	21.91±9.20	15.31±10.52	0.0001
DDQ score	–8.0.03±17.7	–1.9±24	–	0.261
MMPI score	26.13±5.39	23.28±6.15	28/85±14/22	0.076
Hamilton	17.90±11.64	15.52±10.62	–	0.348
CTQ score	53.7±16.6	32.09±10.1	51.96±15.8	0.0001

Values are showed as mean ± SD.

p value obtained from ANOVA (comparison of means).

Heroin-ELS, Heroin Dependents with history of Childhood Maltreatment; Heroin, Heroin Dependents without history of Childhood Maltreatment; ELS, Subjects with history of Childhood Maltreatment; BMI, body mass index; BDI, Beck Depression Inventory; DDQ, Daily Drinking/Drug Questionnaire; MMPI, Minnesota Multiphasic Personality Inventory; CTQ, Childhood Trauma Questionnaire.

The relationship between posttraumatic stress disorder and substance use disorders and also higher rate of posttraumatic stress disorder in substance use disorder subjects in the process of detoxification highlights the role of stress in substance use disorders [55]. Our findings demonstrated increased cortisol and DHEAS levels in both heroin and heroin-ELS groups when compared to the ELS group. These increases were significantly higher in heroin-no ELS group compared to heroin-ELS group. Previous findings have indicated ELS has long-term effects on neurophysiological pathways and may have profound consequences up to adulthood [56]. Elevated levels of cortisol and DHEA have been reported in ELS exposure (e.g., parental depression, marital conflict, family upheaval) in multiple studies [57–61].

In addition, Walter et al. [62] have shown that heroin has an acute effect on the HPA axis response compared to placebo when administered to healthy controls. They also found that cortisol levels were higher in patients compared to healthy controls, and its levels decreased in heroin-dependent patients after heroin administration [62].

Table 2. Serum levels of biomarkers in ELS-heroin, heroin, and ELS groups

Biomarkers	ELS-heroin group (n = 31)	Heroin-no ELS group (n = 32)	ELS group (n = 26)	p value
Cortisol*	14.51±5.37	21.66±5.39	11.18±2.96	0.0001
DHEAS*	251.87±107	296.87±116	172.46±114	0.0001
Albumin	4.24±0.79	3.96±0.73	4.08±0.85	0.389
TWEAK	0.176±0.044	0.161±0.035	0.178±0.44	0.239
TRAIL	0.111±0.055	0.120±0.029	0.085±0.036	0.007
sTNFR I	1.51±1.17	2.21±0.90	2.56±0.11	0.0001
WBC	8.52±2.52	8.32±3.00	6.26±1.38	0.001
ALT	19.19±18.15	20.78±10.91	19.26±9.23	0.874
AST	18.64±18.83	22.38±15.07	18.73±9.25	0.548
ESR	7.81±8.06	8.00±4.83	11.23±5.79	0.088
CRP	3.13±4.17	4.92±6.51	5.51±6.24	0.257

Values are showed as mean ± SD.

p values represent a test of crude differences between groups using ANOVA using the Tukey-Kramer adjustment for multiple comparisons.

* Cortisol and DHEAS resulted from ANCOVA and adjusted were made for sex.

Heroin-ELS, Heroin Dependents with history of Childhood Maltreatment; Heroin, Heroin Dependents without history of Childhood Maltreatment; ELS, Subjects with history of Childhood Maltreatment; DHEAS, dehydroepiandrosterone sulfate; TWEAK, TNF-related weak inducer of apoptosis; TRAIL, TNF-related apoptosis-inducing ligand; sTNFR I, soluble tumor necrosis factor receptor type I; WBC, white blood cell; ALT, alanine transaminase; AST, aspartate transaminase; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ANCOVA, analysis of covariance.

Therefore, in our study, an increase in cortisol and DHEAS levels in heroin-no ELS group, compared to heroin-ELS group, maybe due to abstinence from heroin before the start of the research in this group. Also, in the absence of the suppressor effect of heroin on HPA axis, cortisol and DHEAS levels in heroin-ELS group were significantly higher in comparison to the ELS group. Accordingly, our findings are consistent with previous studies that have shown the association of opioid withdrawal with increased stress hormone secretion [63–65].

Also, in the present study, WBC count was significantly higher in heroin-no ELS and heroin-ELS groups compared to ELS group. However, no statistically significant difference among heroin, ELS, and heroin-ELS groups emerged in terms of ESR, IL-6, and CRP levels.

Previous studies have shown that the production of some inflammatory cytokines rise a few minutes after morphine administration [66, 67].

Additionally, the increase in IL-6 level has been indicated after morphine treatment in patients receiving morphine for pain management [68]. In another study, Chan et al. [96] showed that the production of IL-1β, IL-6, and IL-8 were significantly higher in a group of methadone-maintained patients compared to a healthy control group. On the other hand, various studies in heroin-dependent patients have indicated that opioids consistently cause immunosuppression [69–71]. IL-6 production can be induced by psychological stress in animals, and its elevation can produce other cytokines such as TNF-α and IL-1β [72, 73]. However, in our study, the lack of significant differences between groups in ESR, IL-6, and CRP levels was probably due to the heroin abstinence period and the elimination of its inflammatory effects.

Cortisol displays anti-inflammatory and immunosuppressive effects through inhibition of pro-inflammatory cytokines and induction of thymocyte apoptosis. Accordingly, cortisol increase in heroin and heroin-ELS groups in our study may have reduced the heroin-induced inflammatory response [74–76].

TNF receptors are characterized by the ability to bind TNF and become soluble only after they are cleaved and released in plasma. TNFR I has a death domain and plays an essential role in apoptosis (programmed cell death) and neurotoxicity [77]. The serum level of sTNFR I is an indirect indicator of TNF levels, and studies have shown that in response to an increase in TNF, soluble receptors are shedding from cell surfaces to neutralize TNFα effects such as apoptosis and inflammation [78, 79].

We found that sTNFR I was significantly lower in the heroin-ELS group compared to heroin-no ELS and ELS groups. These results are consistent with the cortisol test results in our study.

Consistent with our findings, Levandowski et al. [44] have found lower sTNFR I in crack-ELS patients compared to crack-addicted subjects. Therefore, it seems sTNFR I levels decrease in the presence of cortisol probably due to cortisol anti-inflammatory effect. However, we did not find a significant correlation between cortisol and sTNFR I in all groups. In the present study, there was a positive correlation between BMI and sTNFR I in ELS group.

ELS influence physical health and are associated with increased inflammation [80]. Raposa et al. [81] have found a positive correlation between BMI and sTNFR II and CRP in subjects with ELS. Accordingly, the positive relationship between BMI and sTNFR I in ELS group may be related to the inflammatory response in ELS group. Additionally, lower levels of cortisol and its anti-inflammatory effects in ELS group compared to the other groups may have affected the results.

Regarding the inflammation parameters, a positive association was found between sTNFR I and BDI in heroin-no ELS group. Moderate to severe depressive symptoms are usually common in heroin-dependent patients [82–84]. Additionally, in a study, Tunler et al. [85] found that antidepressant response on mirtazapine was associated with a highly significant increase of sTNFR I.

Therefore, it seems sTNFR I increases in response to cortisol and contributes to a decrease in depression and inflammation in addicted patients. Concomitant to the decrease in sTNFR I serum levels, TRAIL levels had increased in the heroin-no ELS group compared to the ELS group. Also, there was a positive relationship between TRAIL serum levels and DHEAS in ELS group; and TRAIL had a positive correlation with cortisol and DHEAS serum levels in the heroin-ELS group.

TRAIL has an important role in the activation of the apoptotic pathway and also in inflammatory pathways associated with NF-kB [45]. Previous studies have shown that heroin and morphine induce apoptosis in neurons and microglia [86–88]. Additionally, accelerated biological aging at both cellular and brain system levels have been indicated in heroin abuse subjects [8]. In an animal model study, Cunha-Oliveira et al. [89] found that heroin induces apoptosis in rat cortical neurons. The rate of apoptosis is higher in most of aging cell populations and organs, including the brain, immune system, eyes, endocrine system, intestines, and reproductive system [90]. On the other hand, aging phenotypes are associated with mild inflammation (inflammaging) in many conditions such as changes in body composition, energy production and utilization, metabolic homeostasis, immune senescence, and neuronal health [91, 92]. Therefore, according to the involvement of neuro-immune-endocrine interactions in heroin addiction and also in ELS, an increase of TRAIL in heroin-no ELS group and its positive correlation with cortisol and DHEAS in ELS and heroin-ELS groups may be related to chronic inflammation in heroin-addicted patients and also in ELS subjects. Additionally, the anti-apoptotic effects of DHEA and DHEAS on different cells have been indicated in many studies [93]. Recently, Ding et al. [94] showed that pre-treatment of Leydig cells with DHEA inhibited early apoptosis by reduction of pro-apoptotic protein Bax, caspases-9, and caspases-3 mRNA levels. Interestingly, apoptosis through TRAIL is exerted by 2 signaling pathways and activation of caspases-8, caspases-9, and caspases-3 [95]. Therefore, DHEAS production in ELS and heroin-ELS groups is likely to reduce TRAIL apoptotic effects. Also, our data demonstrated that TRAIL levels were positively associated with CRP in ELS group. As mentioned before, since TRAIL can activate pro-inflammatory pathways through NF-kB signaling, it was expected that this relationship would be observed.

In spite of no statistically significant alterations in TWEAK levels, our findings demonstrate that TWEAK levels were negatively associated with BDI in heroin-no ELS subjects; this result has been found to be consistent with the study by Levandowski et al. [44].

This study has several limitations. First, the sample size may have been small, and thus, larger scale studies are recommended to confirm these results. Second, the histories of ELS were collected retrospectively and are subject to recall bias. Finally, it is important to test the other apoptosis, immune system, and endocrine system biomarkers in heroin-addicted patients, different stressor conditions, and also in people who abuse other substances.

Conclusion

Based on a novel integration of peripheral inflammation, molecular and endocrine system measures, and depression severity, the present study presents evidence that both heroin abuse and ELS affect the neuro-immuno-endocrine system. Specifically, heroin and ELS induces chronic inflammation, and stress hormones levels in-

crease in response to stress and inflammation. Also, inflammaging in heroin and ELS condition accelerate biological aging. These findings constitute a significant contribution to our understanding of how heroin abuse and ELS influences the neuro-immuno-endocrine system and lays an important foundation for studies that seek to characterize further the mechanisms that mediate substance abuse, ELS, inflammation, and biological aging. Understanding such mechanisms raises the possibility of reversing the detrimental effects of drug addiction and ELS.

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Statement of Ethics

The study protocol was approved by the Ethics Committee of Kashan University of Medical Sciences.

Disclosure Statement

All other authors declare that they have no conflicts of interest.

Author Contributions

N.E., M.M., and H.R.B. designed the study and wrote the protocol. H.R.M. conducted the statistical analysis, literature searches, and provided summaries of previous research studies. M.M. and N.E. performed all laboratory tests. N.E. wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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