

Received: 13.1.2010
Accepted: 28.4.2010

A Review on Hematological Factors in Opioid-Dependent People (Opium and Heroin) after the Withdrawal Period

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Background:	Abstract Long-term use of opioids has acute effects on homeostasis of the body. Discovering the impacts of opioids on hematological parameters of narcotics withdrawal and dependents blood may be helpful in recognizing the homeostasis condition of their body for the useful treatment.
Methods:	In this study a cross-sectional method was applied. The abusers of opium and heroin for more than two consecutive years were considered as opium and heroin dependent groups, respectively. The dependent people, who passed the 1-month withdrawal period, entered the study as opium and heroin withdrawal groups. In this study, hematological factors of heroin and opium dependent and withdrawal groups were investigated.
Findings:	The RBC count remained unchanged in all groups. The WBC count had a significant increase in opium dependent group but in heroin dependent group and withdrawal group there was no significant difference. HGB level had a significant increase only in opium and heroin withdrawal groups. The percentage of HCT had a significant increase in all groups. The MCV increased in heroin and opium dependent groups. MCH level increased significantly in heroin and opium withdrawal groups. MCHC level had a significant increase in all groups. Neutrophil and lymphocyte counts in heroin and opium addicted groups significantly decreased. Platelet, neutrophil and monocyte counts significantly increased in opium dependent group. Monocyte count showed a significant reduction in heroin withdrawal group. Eosinophil count showed no difference in any of the groups.
Conclusion:	The current study indicated that not only the chronic and long-term use of opium and heroin, also withdrawal of addicted people could change hematological parameters related to human serum.
Key words:	Hematological factors, Serum, Addiction, Addiction withdrawal, Heroin, Iran.
Page count:	9
Tables:	2
Figures:	0
References:	45
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Introduction

Addiction is an increasing issue in all over the world. Since heroin and cocaine abuse in western societies are mostly from opioid compounds, most of these countries researches are focused on these two drugs. In Iran, among the opioid compounds, opium has the highest consumption; besides, Iran is the first consumer of the opium in all over the world.¹ The opium which is extracted from the juice of poppy capsules (*papaver somniferum*)² is used as the raw material for the synthesis of some medications such as morphine, noscapine and papaverine (10%, 6% and 1% of opium, respectively).³ The effects of opium are mostly from its morphine, but unexpected intoxications and diseases such as esophageal cancer in abusers of "dross opium" and polyneuropathy due to addition of arsenics in impurities occurred.² Also, since more than 20 alkaloids⁴ and more than 70 ingredients are present in opium,⁵ its impacts can be different in comparison with pure morphine, noscapine and papaverine. Heroin is the very methyl-D-morphine. Heroin may affect the immune system either directly on opioid receptors, lymphocytes and macrophages^{6,7} or indirectly through effecting on nervous system of the immune system.⁸ Many studies have shown that consuming opiates have acute and chronic effects on different body systems. Using narcotics including opium and heroin would lead to changes in body electrolytes,⁹ electrophoretic profile of serum proteins,^{10,11} and coagulation factors.¹² Also, it would inhibit hypothalamus-pituitary-gonadal axis¹³ and consequently reduce the levels of gonadal hormones including testosterone, serum LH, FSH and PTH.¹⁴⁻¹⁶ Moreover, it was indicated that red and white blood cell counts, hemoglobin, hematocrit and platelets level of the opium dependents did not change.¹⁷ In heroin dependent and withdrawal groups, some changes in immune function and blood lymphocytes occurred.¹⁸ Some other studies also indicated that consumption of opioids may cause cofactor role in dependent people for infectious diseases.^{19,20} However, many studies have been done on the effect of acute and chronic opioids compounds on the immune system,^{19,21} but withdrawing of drugs such as morphine and heroin also might have important effects on the immune system in narcotic dependent patients.²² Therefore, it is very

important to discover the main aspect of blood toxicology, especially in drug dependent people and especially after the withdrawal period. Consequently, suitable, efficient and preventive treatments should be used in order to reduce the contingent side effects. So far, in previous studies about narcotic effects especially opium which has the highest rate of narcotic abuse in Iran,¹ no comprehensive study has been done yet on hematological parameters of heroin and morphine dependents in comparison with such parameters of withdrawal people. In this study, we tried to review the effect of narcotic addiction (heroin and opium) on hematological parameters of dependent people in their addiction and withdrawal period in order to create a base for further studies of the researchers about fundamental studies of narcotics, prevention, etiology and particularly its treatments.

Methods

In this study, a cross-sectional method was applied. The subjects included 180 males between 25 to 45 year-old, addicted to opium and heroin referred to the medical center for a 3-month withdrawal period. Those who were the abusers of opium (smoking, inhaling and opium pipe method) and heroin (inhaling method) were considered as opium dependent and heroin dependent groups, respectively. The subjects of these groups were considered in withdrawal group if they stopped the drug abuse (opium and heroin) for one month. Some of the non-addicted volunteers, who were in compliance with this population in terms of age, were selected as the control group. According to DSM-IV criteria, only the opium and heroin dependent people were allowed to enter the study. The individuals who abused several drugs simultaneously and other abusers as substance abusers, recreational abusers and trial abusers were excluded. The test subjects who already suffered from syphilis, hepatitis, AIDS or any infectious disease with detectable clinical signs such as skin diseases also were excluded. In order to ensure about continuous abuse of opioids compound, urine sample for diagnostic specimens were obtained from individuals who were considered as opioid-dependents. At the beginning, one screening test (rapid immunochromatographic test) was conducted on the samples. Thereafter, in order to approve opium and heroin abuse, solid-liquid

column chromatography test and then, thin layer chromatography was conducted on positive cases of screening (Baharafshan, Tehran, Iran). In order to ensure that control and withdrawal groups did not use any opioids drugs, experiment investigation of opioids compounds was conducted on the urine of mentioned individuals (only RSA was done using rapid immunochromatographic technique). First, the aim of this study was explained to the test subjects. Then, regarding to anonymity, consent form was obtained consciously before blood and urine sampling. Meanwhile, this research was approved by Ethics Committee of Kerman Neuroscience Research Center at Kerman University of Medical Sciences by moral code number EC/KNRC/88-31. Demographic data about the age, type of drug, last date of consumption and duration of addiction were collected. Given a special code to each individual, hematological parameters were obtained by trusted researchers. In order to determine hematological parameters, the following stages were carried out: 2 ml fresh venous blood was collected in test tubes containing specific EDTA anticoagulant, and then the following tests were carried out on the samples utilizing Coulter Counter Sysmex: complete blood cell count (CBC) for red and white blood cell, hemoglobin level (HGB), hematocrit percentage (HCT) and calculating cell indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), differential leukocyte count (lymphocytes, monocytes, basophiles, eosinophils) and blood platelets.

Statistical analysis

The data were analyzed by SPSS_{11.5} software and presented in mean (standard deviation). Parametric test was also used for comparison between the groups. Moreover, the significant level of 0.05 was considered.

Results

The comparison of hematological factors in opium dependent and opium withdrawal groups

One-way ANOVA indicated that in the period of opium dependence and its related withdrawal, red blood cell count remained unchanged both toward each other and in comparison with that in the control group. The white blood cell count actually had a significant increase in comparison

with that in control group ($P < 0.05$), but in the subsequent withdrawal group it showed a non-significant decrease. The platelet, neutrophil and monocyte counts were significantly increased in opium dependents ($P < 0.01$, $P < 0.001$, and $P < 0.05$ respectively). In the opium withdrawal group, the number of platelets, neutrophils and monocytes were decreased in comparison with those in addiction period and the reduction in neutrophil count was significant ($P < 0.001$). The lymphocyte count had a significant reduction in opium dependent group ($P < 0.001$) and had reached to the level of that in control group. In opium dependence and subsequent withdrawal period, the number of eosinophils (EOS) showed no difference toward each other and in comparison with that in control group. The level of hematocrit in opium dependence and subsequent withdrawal group was significantly increased ($P < 0.001$) in comparison with that in control group; however in subsequent withdrawal group there was no significant difference. The hemoglobin and MCH level in opium dependent group had no difference in comparison with those in control group but, in subsequent withdrawal group, the HGB and MCH level had a significant increase both in comparison with those in dependency period ($P < 0.001$) and control group ($P < 0.001$). The mean corpuscular volume (MCV) in opium dependent group also had a significant increase in comparison with that in control group ($P < 0.05$). Although in withdrawal period, the MCV increased compared to that in control group, but the increase was not significant. The MCHC significantly decreased in opium dependent group in comparison with that in control group ($P < 0.001$) but in subsequent withdrawal opium group, it showed a significant increase in comparison to that both in dependent and control groups ($P < 0.001$).

The comparison of hematological factors in heroin dependent and heroin withdrawal groups

One-way ANOVA indicated that in heroin dependence and heroin withdrawal periods, red blood cell count did not change toward each other and in comparison with that in the control group. The white blood cell count in heroin dependent group and heroin withdrawal group had a non-significant difference toward that in the control group. The platelet and neutrophil counts in heroin dependents increased; this

increase in neutrophils was significant toward that in control group ($P < 0.01$). In subsequent heroin withdrawal, the number of platelets and neutrophils decreased in comparison with those in dependency period and this reduction was significant in neutrophils ($P < 0.001$). The monocyte count decreased in heroin dependent and withdrawal groups and this reduction was significant in withdrawal period in comparison with that in the control group ($P < 0.01$). The number of lymphocytes had a significant reduction in heroin dependent group ($P < 0.01$). The lymphocyte count in subsequent withdrawal period had a significant increase in comparison with that in dependency period ($P < 0.001$). However, no significant difference was seen in neutrophil count between the control and withdrawal groups. In heroin dependence and its subsequent withdrawal period, the number of eosinophils had no difference toward each other and in comparison with that in the control group. The hematocrit level in heroin dependent and subsequent withdrawal groups had a significant increase in comparison with that in the control group ($P < 0.001$), however no significant difference was seen in hematocrit level between heroin

dependent and subsequent withdrawal groups. The hemoglobin level did not change in heroin dependent group in comparison with that in the control group but, in the subsequent withdrawal, the hemoglobin level had a significant increase in comparison with that both in dependency period ($P < 0.05$) and control group ($P < 0.001$). The MCH level in heroin dependent group non-significantly increased in comparison with that in the control group, but in subsequent withdrawal group it had a significant increase in comparison with that in the control group ($P < 0.001$). In heroin dependence period and its subsequent withdrawal period, no significant difference was seen in MCH level. MCH increased in heroin dependency period and its subsequent withdrawal period and this increase in heroin dependent group had a significant difference in comparison with that in control group ($P < 0.001$). The MCHC volume significantly decreased in heroin dependent group in comparison with that in the control group ($P < 0.001$). However, MCHC level in subsequent withdrawal group significantly increased compared to that both in dependent and control groups ($P < 0.001$).

Table 1. Hematologic factors in control, opium dependent and opium withdrawal groups

Hematologic factors	Groups		
	Control	Opium Dependent	Opium Withdrawal
WBC	7.33 (0.14)	8.43 (0.56)*	7.88 (0.34)
RBC	5.25 (0.5)	5.1 (0.11)	5.19 (0.1)
HGB	14.46 (0.15)	14.19 (0.3)###	16.34 (0.23)***
HCT	41.91 (0.39)	46.0 (0.94)***	45.41 (0.64)***
MCV	84.7 (0.65)	89.53 (1.99)*	88.03 (1.08)
MCH	27.62 (0.24)	27.81 (0.72)###	31.69 (0.47)***
MCHC	32.93 (0.92)	30.69 (0.35)***###	35.90 (0.22)***
PLT	242.97 (5.27)	289.1 (14.05)**	253.15 (9.18)
NEUT	57.78 (0.67)	66.034 (2.3)#####	56.32 (1.53)
LYMPH	38.42 (0.66)	30.47 (2.17)***###	40.47 (1.56)
MONO	2.79 (0.15)	4.92 (0.21)*	2.17 (0.25)
EOS	2.68 (0.13)	2.65 (0.65)	2.31 (0.24)

Standard error of the mean was used in data.

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ vs. Control Group, ###: $P < 0.001$ Opium Withdrawal vs. Opium dependent

Table 2. Hematologic factors in control, heroin dependent and heroin withdrawal groups

Hematologic factors	Groups		
	Control	Heroin Dependent	Heroin Withdrawal
WBC	7.33 (0.14)	8.91 (1.05)	7.38 (0.27)
RBC	5.25 (0.5)	4.99 (0.19)	5.24 (0.13)
HGB	14.46 (0.15)	14.75 (0.37) [#]	16.54 (0.26) ^{***}
HCT	41.91 (0.39)	47.32 (1.14) ^{***}	45.52 (0.62) ^{***}
MCV	84.7 (0.65)	95.3 (2.21) ^{***}	88.31 (1.41)
MCH	27.62 (0.24)	29.67 (0.72)	31.75 (0.66) ^{***}
MCHC	32.93 (0.92)	31.13 (0.34) ^{***###}	36.13 (0.29) ^{***}
PLT	242.97 (5.27)	291.14 (23.5)	246.3 (10.04)
NEUT	57.78 (0.67)	68.3 (3.54) ^{***###}	54.19 (1.43)
LYMPH	38.42 (0.66)	28.5 (3.28) ^{***###}	43.04 (1.47)
MONO	2.79 (0.15)	1.86 (0.46)	1.65 (0.17) ^{**}
EOS	2.68 (0.13)	2.43 (0.95)	2.15 (0.18)

Standard error of the mean was used in data.

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ vs. Control Group, #: $P < 0.05$, Heroin Withdrawal vs. Heroin dependent; ###: $P < 0.001$

Discussion

The results showed that in opium and heroin-dependence and withdrawal period of opioids, red blood cell counts had no change. White blood cell count however increased in opium-dependent group but decreased in withdrawal group. WBC count had a non-significant increase in heroin dependent group, while in subsequent withdrawal and control group did not have any significant difference. In a study on morphine dependent dogs and their withdrawal period (studies in chronic morphine poisoning in dogs), RBC count did not change in dependency period, but decreased in withdrawal period. The above-mentioned study also showed that WBC count had no change in dependency period but decreased in withdrawal period.²³ The current study determined that lymphocyte count significantly decreased in opium and heroin dependent groups. In previous studies, opposite results were reported; i.e., both increase and decrease in peripheral lymphocyte counts of heroin dependent people were demonstrated. In another study it was indicated that in heroin addicted people, the total number and percentage of lymphocytes and T cells construction decreased in peripheral blood circulation.²⁴ On the contrary, another report indicated that in heroin addicted people who had no malnutrition, T cell count increased in their blood.²⁵ It also showed that the average number of leucocytes increased in heroin dependent people; however, none of them had

any infectious disease.²⁶ Louria (1976) reported leukocytosis and fever without visible symptoms of infectious disease in heroin addicted people.²⁷ Isbell (1968) and Sapira (1947) observed that heroin dependent people had an increase in leucocytes and erythrocytes sedimentation rate in comparison with that in the control group. These observations led to the conclusion that improper feeding, lack of self-care, inadequate diet and low level of hemoglobin would cause anemia and infection in heroin dependent group.^{28,29} In another study, the effect of 6-month heroin consumption was shown on some of the blood parameters such as reduced the lymphocyte percentage.³⁰ Morphine is the agonist of μ opioids receptors and the main and active metabolite of heroin. It was indicated that morphine and heroin in dose dependent way could reduce the proliferation of lymphocytes. Moreover, these inhibitory effects disappeared by naloxone.^{18,31} The possible mechanism of immune suppression by morphine might regulate the immune system either directly via mu opiate receptors located on the immune cells, or indirectly through a central pathway with the activity of the mu receptors in the central nervous system (CNS).³² Opioid compounds may affect CNS through HPA descending axis activity and sympathetic nervous system.³³ HPA axis activity may release the repressive glucocorticoids on immune system, while increase the peripheral release of sympathetic activity of epinephrine,

norepinephrine and dopamine from the adrenal center. Norepinephrine and glucocorticoids both act as negative regulators of immune system by affecting the leucocytes.^{34,35} The ability of immune system in rhesus monkeys may take two years as a result of morphine dependency.³⁶ It was indicated that withdrawing after long-term and chronic use of opioids may cause immunosuppression. A study on the rats determined that morphine withdrawal after 8 hours caused inhibition of T cells activity, inhibition of B cells proliferation and production of interleukin 2.³⁷ Another study also showed that 24 hours after stopping exposure to chronic morphine and cocaine, corticosterone plasma level increased and immune system was suppressed. Around 3 days after withdrawal, despite that corticosterone level reached to its basic level, the suppression of immune system still continued. Therefore, although there would be still some stressing effects of cocaine and morphine withdrawal along with HPA axis activity, there were still other accurate mechanisms in creating these long effects on the immune system, which are not yet identified.³⁸ Moreover, in the current study, one month after initiating withdrawal of opium and heroin, the number of lymphocytes had a significant increase compared to that in the addiction period and besides it reached to the control group level. Immune system function in heroin addicted people who initiated withdrawal period, generally reduces in 15- to 21- days and 6- to 24-months. But it seemed that in withdrawing period, immunological parameters came back to normal after passing 2 years.¹⁸ In a study which was about the effects of cocaine withdrawal on the immune system, it was shown that the proliferation of blood circulation lymphocytes decreased from 2 hours to 6 days after cocaine withdrawal.³⁹

In the current study, the neutrophil count significantly increased in opium and heroin dependent groups. But, in the subsequent opium and heroin withdrawal groups, neutrophil decreased compared to that in dependency period. The monocytes significantly increased in opium dependent people. But, in opium withdrawal group, monocytes decreased non-significantly in comparison to that in the control group. The monocyte count decreased in heroin dependent and withdrawal period and this reduction was significant in comparison to that in

the control group. In heroin and opium dependent and its subsequent withdrawal period, the number of EOS did not change toward each other and in comparison with that in the control group. Unlike the current study, in a study which was carried out on the monkeys in morphine dependent period, the total neutrophil, monocyte and lymphocyte counts did not change compared to the original level of these factors before being addicted; but, in withdrawal period, the total lymphocyte count decreased and neutrophils significantly increased in comparison with those in the addiction period.⁴⁰ The difference between the results of mentioned studies and those of the current study might be due to difference in the type of material and method of injection, addiction time and the duration of withdrawal period. In those studies, monkeys were studied in-vitro and under controlled condition and their addiction period took 3 months. In this study, the animals were given oral morphine in meals. Then, hematological factors were assessed two weeks after morphine and syndrome withdrawal. In another study on the morphine-dependent dogs, the number of WBC did not change in morphine dependent dogs, but in withdrawal period, the multi-core neutrophil count increased. Naturally, WBC and RBC counts in dogs are higher than those in humans.²³ There are also several reports which suggested after prescription of heroin and morphine, some parameters of immune system would strengthen. So that in mice it was shown that the production of some of the cytokines increased a few minutes after using morphine.⁴¹ The other studies showed that the monocyte count was higher than normal level in heroin dependent patients.^{30, 42} The current study also indicated that HGB increased only in opium and heroin withdrawal groups. The HTC percentage significantly increased in all dependent and withdrawal groups. MCV level increased in opium and heroin dependent groups, but in opium and heroin withdrawal groups, this increase was not significant in comparison with those in the control group. MCH value did not change in opium and heroin dependent groups, but it significantly increased in heroin and opium withdrawal group. The MCHC significantly decreased in opium and heroin dependent groups in comparison with that in the control group. But in opium and heroin withdrawal groups, it increased significantly both in comparison with those in the relative dependent and control groups. Platelets count increased in opium and

heroin dependent groups in comparison with that in the control group and this increase was significant in opium dependent group. In opioid withdrawal group, no significant difference was observed between withdrawal and control groups. In another study in parallel with the current study it was shown that blood hemoglobin increased in heroin addicted people, but in people who used methadone, the amount of hemoglobin decreased to its normal level.⁴³ On the other hand, six-months heroin consumption did not cause major changes in erythrocyte parameters such as RBC count and hemoglobin and hematocrit levels.³⁰ It was also indicated that low blood platelets and hemoglobin would decrease in heroin dependent people.²⁹ Another study which was done on morphine-dependent dogs showed that blood hemoglobin content did not change, but in withdrawal period, it decreased in parallel with RBC.²³ Sapira also showed that hemoglobin concentration decreased in morphine dependent people.⁴⁴ Also, in a similar observation reported by Pastone et al, it

was indicated that hemoglobin and hematocrit levels decreased in heroin injection abusers.⁴⁵ Finally, it is worth mentioning that different parameters such as the level of education, culture, diet type, medicine prescription method, method and duration of withdrawal and its conditions which were very complicated and sometimes uncontrollable in human studies might be involved in such changes in opioid dependence period and its subsequent withdrawal. Nevertheless, the aim of the current study was to facilitate new ideas which lead to rapid progression and development in order to have effective treatments of diseases associated with drugs.

Conflict of interest: The Authors have no conflict of interest.

Acknowledgment

This article was the result of the research project No. 88-31/A/K at Neuroscience Research Center of Kerman. The authors of this article would appreciate the above-mentioned center.

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