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Methadone therapy modulate the dendritic cells of heroin addicts

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ABSTRACT

Evidence from various studies suggests that narcotics abuse may exert adverse immunomodulatory effects on immune responses. The aim of this research was to understand the effects of detoxification with methadone on the percentage of dendritic cells (DCs) and expression of its markers in heroin addicts. In this study, myeloid DCs ($CD11c^+$) and plasmacytoid DCs ($CD123^+$) were examined in two groups. These groups comprised of 20 healthy volunteers and 20 chronic heroin addicts, before and after detoxification with methadone. The percentages of myeloid DCs and plasmacytoid DCs were lower in addict subjects than in the control. The HLA-DR expression on DCs was significantly lower in addict subjects than in the control, whereas CD11c and CD123 expression in DCs subsets were increased in them. Most of these changes were modified after the methadone therapy. Dendritic cells are essential to the initiation of primary immune responses, therefore the disruption of their function can be one of the reasons for the increased prevalence of infections in heroin addicts. The methadone therapy can improve the imposed changes by heroin.

1. Introduction

Drug addiction is a serious growing problem that affecting members of various social and demographic groups [1]. Narcotics promote the risk of acute and chronic infections, including bacterial pneumonia, urinary tract infection, and tuberculosis [2,3]. In addition, substance abuse can increase the incidence of cancer [4].

One of the reasons for the high susceptibility of drug abusers to infection and increased incidence of cancer is the immunomodulatory impact of these substances [5]. There is a limited number of in-vivo clinical trials evaluating the effects of narcotics on the human immune system, and most of the performed studies in this area are based on morphine and laboratory animals [6,7].

Heroin is one of the most commonly abused substance in Iran [8]. Detoxification is the first step for patients entering drug rehabilitation programs [9]. Rehabilitation, which usually lasts for one to two months, is performed using agonists or antagonists. Methadone is a full agonist used in the treatment of heroin addiction [10,11].

Dendritic cells (DCs) are the most effective stimulating cells of T and B lymphocytes and are necessary for the initiation and regulation of immune responses. They can activate naive T cells through the processing and presentation of antigenic peptides, expression of CD80 and CD86 co-stimulatory molecules, migrate to the lymph tissues, secretion of cytokines, and expression of adhesion molecules. They respond to a wide range of pathogens, including bacteria, viruses, unicellular organs, and tumors [12,13]. Immunomodulatory agents apply their effects on immune responses at early stages through differentiation, suppression, maturity, and activation of dendritic cells [14].

In this study, we aimed to evaluate the effects of detoxification with methadone on the percentage of dendritic cells and expression of its markers in heroin addicts.

2. Materials and methods

2.1. Patients

In this study, two main subsets of dendritic cells, such as myeloid DCs ($CD11c^+$) and plasmacytoid DCs ($CD123^+$) were examined in two groups. These groups comprised of 20 healthy volunteers and 20 chronic heroin addicts, before and after detoxification with methadone. Heroin abusers were matched with the control group. They were kept at the largest addiction treatment center for men in the Arak province (west of Iran) and received a daily oral dose of 40 mg methadone chloride for two months. The control group included 20 people from Arak University of Medical Sciences personnel who had never used narcotics. The general health of the control group was evaluated

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Table 1
Demographic characteristics of volunteers included in the study.

	Control group (n = 20) (Mean ± SD)	Heroin group (n = 20) (Mean ± SD)
Age (years)	28.74 ± 4.60	33.19 ± 5.11
Height (cm)	171.83 ± 7.04	169.97 ± 6.44
Weight (kg)	74.50 ± 6.61	70.32 ± 8.79
BMI (kg/cm^2)	25.67 ± 1.69	24.90 ± 1.41

BMI, body mass index.

through physical examination and performing routine hematologic and biochemical tests.

All the subjects were male, aged between 20 and 40 years and none had recent infections and active inflammatory disease and were free of drugs affecting the immune system. The inclusion criteria in the control group was n*egative urine test* for opiates. Urine samples of the control group were negative for opiates by ACON 10 Panel Drug Screening Test Card (ACON Laboratories, San Diego, CA). The urine samples were collected one month before, and a second was collected at the commencement of the study. The inclusion criteria for the addict group included consumption of heroin for at least 1 year, lack of addiction to other drugs, such as opium, marijuana, cocaine and methamphetamine for more than three months in the past, lack of alcohol dependency in the past for more than six months, and the existence of > 5.5 μg/ml of morphine in urine. The exclusion criteria for the addict group included allergic reaction to methadone and unwillingness to continue the study. After approval by the Ethics Committee of the University (92-156-23) and obtaining written informed consent, 20 ml of venous blood was drawn from each volunteer and was transferred to test tubes.

2.2. Flow cytometry

Five ml peripheral blood was prepared on 1000 units/ml heparin. The pellet containing monocytes and lymphocytes was separated by ficoll-hypaque and cells were counted by hemocytometer. The cell viability was determined by trypan blue exclusion test, and 25×10^6 cells/ml concentration was prepared by adding FACS buffer. Twenty μl of this concentration, which is equal to 5×10^5 cells, was added to FACS tube. Fifteen μl Lin-1 (Lineage cocktail 1 contains antibodies against CD3, CD14, CD16, CD19, CD20, CD56) in combination with 5 μl anti-CD34 (FITC-labelled), and 5 μl antibody against HLA-DR (PerCP-labelled), was added to each tube.

Five μl of CD123 (PE-labelled) was added to a tube for identifying the percentage of Lin$^-$/HLA-DR$^+$/CD123$^+$ plasmacytoid DCs. In addition, 5 μl CD11c antibody (PE-labelled) was added to another tube for determining the percentage of Lin$^-$/HLA-DR$^+$/CD11c$^+$ myeloid DCs. These tubes were then incubated for 30 min at 4 °C [15]. All antibodies

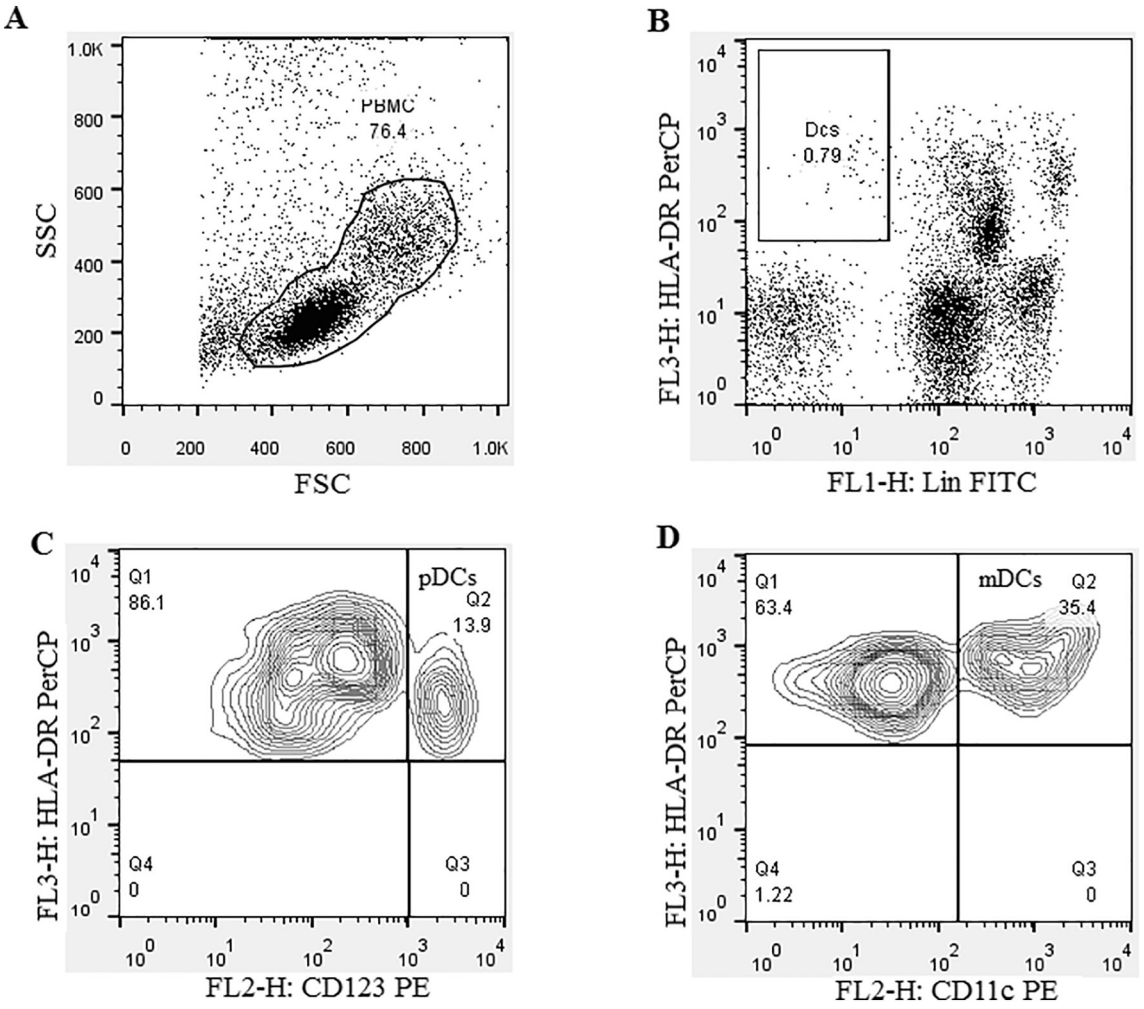

Fig. 1. Example of flow cytometry analysis and gating strategy for identification and quantification of blood dendritic cell subsets. The PBMC (peripheral blood mononuclear cell) population is identified by a combination of forward/side scatter characteristics (A), and DC were identified within the lineage (CD3, CD14, CD16, CD19, CD20, CD56, and CD34)-negative (Lin$^-$) HLA-DR$^+$ (B). One representative experiment is shown to demonstrate the gating strategy used to identify DC subsets (C and D). mDCs: myeloid DCs (CD11c$^+$), pDCs: plasmacytoid DCs (CD123$^+$).

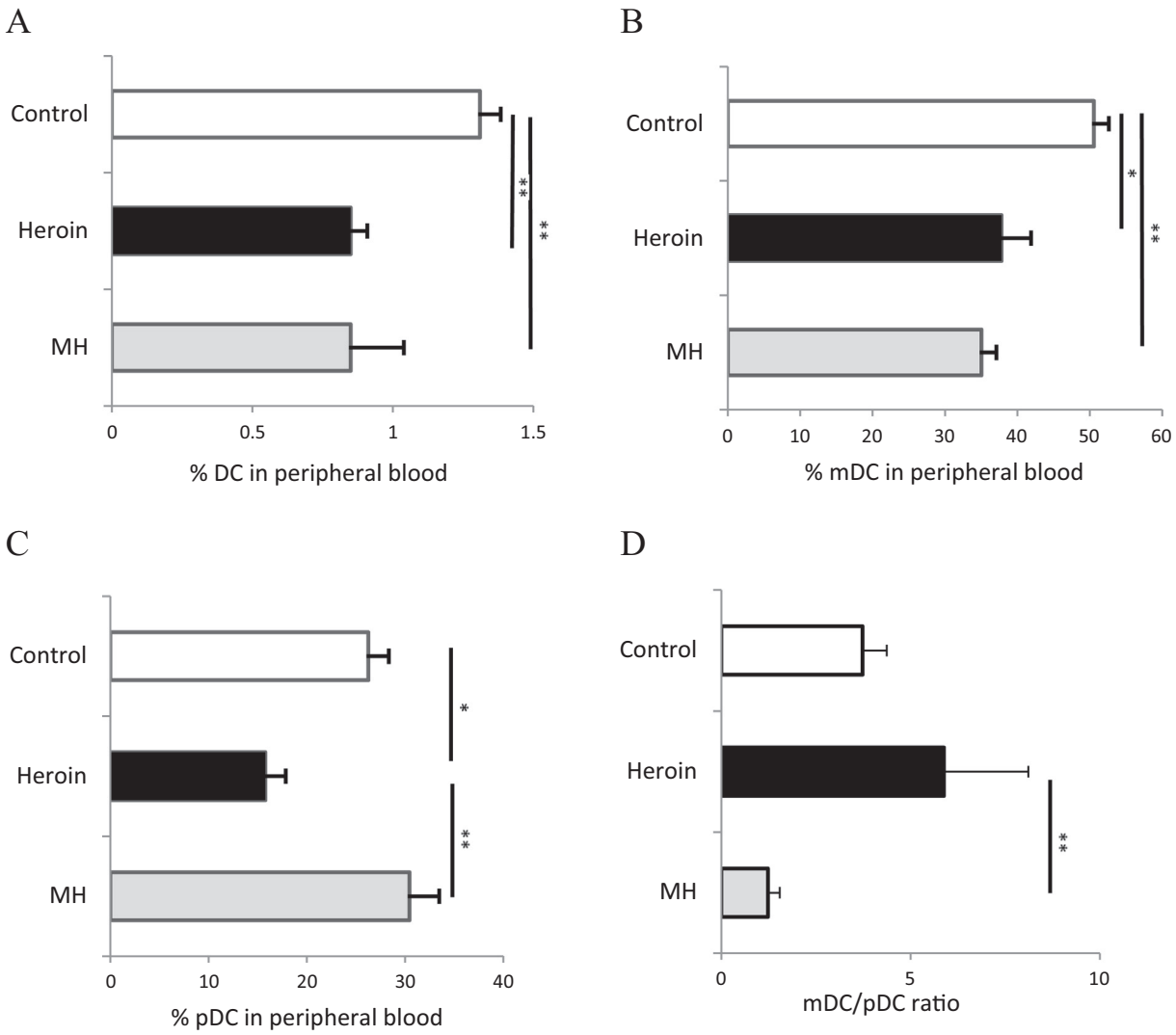

Fig. 2. (A) The percentage of dendritic cells (DC), (B) myeloid DCs ($CD11c^+$), (C) plasmacytoid DCs ($CD123^+$) and (D) myeloid DCs/plasmacytoid DCs ratio in peripheral blood of control subjects and heroin addicts before and after methadone intake. Error bars represent standard deviations. Comparisons between groups were performed by one-way ANOVA with Tukey post hoc comparisons. *p*-Values < 0.05 were considered statistically significant. MH; methadone hydrochloride-treated addicts. $*p < 0.05$, $**p < 0.01$.

were purchased from Becton Dickinson. Samples were analyzed using the Becton Dickinson FACSAria™ cytometer (BD Biosciences, San Jose, CA) equipped with a 15-mW aircooled 488-nm argon laser. Routinely, 100,000 events per sample were collected. Data were analyzed with FlowJo software (Tree Star). Flow cytometric data were expressed as the relative proportion (%) and mean fluorescence intensity (MFI) of cells expressing each antigen.

2.3. Statistical analysis

Statistical analysis of the data was performed with the SPSS software (version 17.0.1, SPSS Inc., Chicago, IL). The one-way analysis of variance (ANOVA) with Tukey post hoc comparisons was used for the determination of statistical significance between experimental groups. All data were expressed as mean ± SEM. *p*-Values < 0.05 were considered statistically significant. $*p < 0.05$ and $**p < 0.01$.

3. Results

There was not a statistically significant difference between age, height, weight, and body mass index (BMI) of the volunteers (Table 1). The general health of the control group was confirmed by physical examination and performing routine hematologic and biochemical tests (data not shown).

In this study, the two major DC subsets were identified in PBMC as $Lin^-HLA\text{-}DR^+CD11c^+$ mDC or $Lin^-HLA\text{-}DR^+$ $CD123^+$ pDC (Fig. 1).

The frequency of dendritic cells was markedly decreased in the peripheral blood of heroin addicts compared to control subjects (0.85 ± 0.059% vs. 1.31 ± 0.074%, $p < 0.001$) (Fig. 2A). Detoxification with methadone hydrochloride had no effects on the decreased percentage of dendritic cells of peripheral blood of heroin addicts (0.85 ± 0.189% vs. 0.85 ± 0.059%, $p = 0.996$) (Fig. 2A).

The percentages of myeloid DCs ($CD11c^+$) and plasmacytoid DCs ($CD123^+$) were lower in addict subjects than in the control (37.81 ± 4.08% vs. 50.56 ± 2.07% & 15.78 ± 2.07% vs. 26.26 ± 2.08%, respectively, $p < 0.05$) (Fig. 2B and C). Detoxification had no effects on the percentage of myeloid DCs of peripheral blood of heroin addicts (35.04 ± 2.06% vs. 37.81 ± 4.08%, $p = 0.869$) (Fig. 1B) but cell percentage was corrected for plasmacytoid DCs when compared to control subjects (30.45 ± 3.03% vs. 26.26 ± 2.08%, $p = 0.746$) (Fig. 2C).

The myeloid DCs/plasmacytoid DCs ratio was higher in heroin

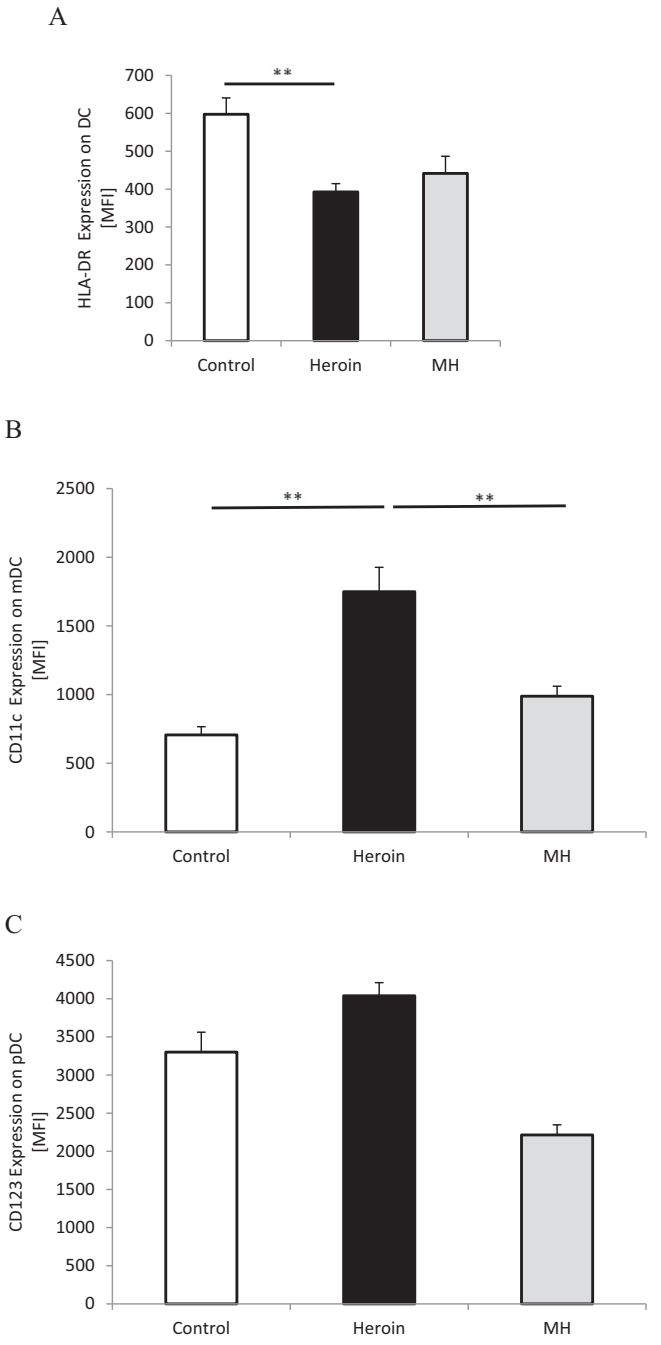

Fig. 3. (A) The expression of HLA-DR as mean fluorescence intensity (MFI) in DC, (B) expression of CD11c in myeloid DCs and (C) expression of CD123 in plasmacytoid DCs of peripheral blood of control subjects and heroin addicts before and after methadone intake. Error bars represent standard deviations. Comparisons between groups were performed by one-way ANOVA test with Tukey post hoc comparisons. p-Values < 0.05 were considered statistically significant. MH; methadone hydrochloride-treated addicts. $^{**}p < 0.01$.

addicts than in that of control, but the difference was not significant (5.88 ± 2.23 vs. 3.73 ± 0.64, $p > 0.05$) (Fig. 2D). Detoxification with methadone significantly reduced the ratio of these cells compared to control subjects (1.23 ± 0.31 vs. 5.88 ± 2.23, $p < 0.01$) (Fig. 2D).

In this study, mean fluorescence intensity (MFI) of CD123, CD11c, and HLA-DR at the level of dendritic cells in peripheral blood of volunteers was evaluated. The MFI of HLA-DR on DCs was significantly lower in addict subjects than in the control (392.60 ± 21.91% vs. 597.63 ± 43.01, $p < 0.01$) (Fig. 3A and Fig. 4). Detoxification with

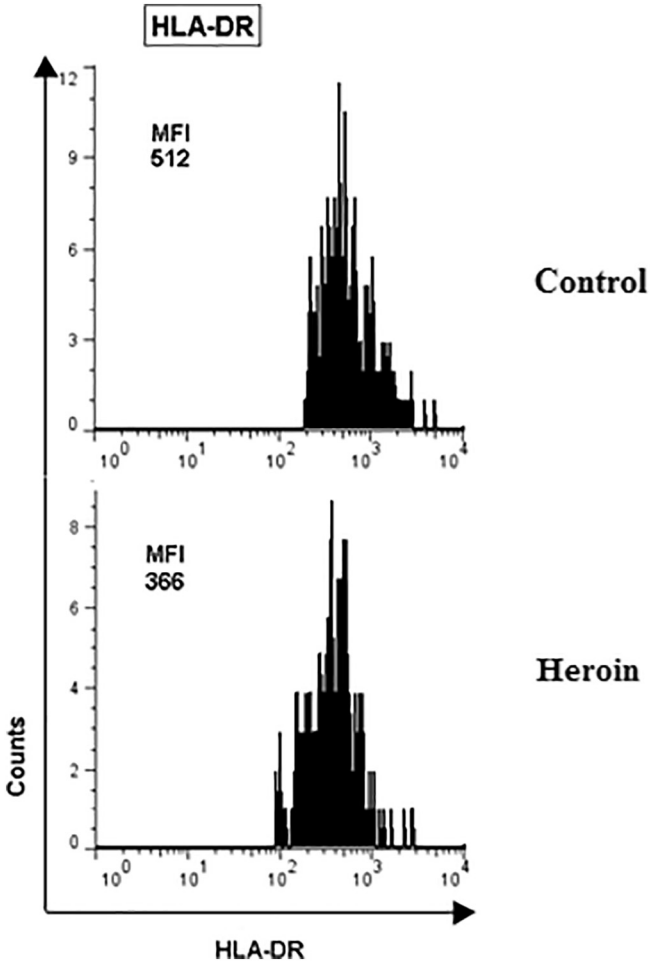

Fig. 4. Comparison of HLA-DR expression in DC of peripheral blood of a control subject and a heroin addict as histogram plot.

methadone corrects the decrease the MFI of HLA-DR in addict subjects (441.50 ± 45.25 vs. 597.63 ± 43.01, $p > 0.05$) (Fig. 3A).

However, unlike HLA-DR, the MFI of CD11c and CD123 on DCs was increased in addict subjects than in the control (1749.16 ± 177.43 vs. 706.09 ± 59.81, $p < 0.001$ & 4038 ± 171.99 vs. 3299.72 ± 261.77, $p > 0.05$, respectively) (Fig. 2B and C). Detoxification with methadone hydrochloride corrects the increase the MFI of CD11c and CD123 in heroin addicts (988.00 ± 72.81 & 2214.50 ± 132.42, respectively) (Fig. 3B and C).

4. Discussion

Two main subsets of dendritic cells are $CD123^+$ and $CD11c^+$ dendritic cells [16]. The dendritic cells produced in bone marrow, migrate to various peripheral tissues through blood flow [13]. Therefore, peripheral blood dendritic cells may be an indicator of systemic immune status. For instance, in the study of Lissoni et al. reduced levels of blood dendritic cells were significantly linked to the immunosuppressive condition of patients with metastatic malignant tumors [17].

Different pathogens may activate distinct subsets of dendritic cells, and the activation of these subsets depends on immune response stages [18]. The myeloid DCs are thought to mediate cellular immune response by inducing TH1 (T helper-1) cells while plasmacytoid DCs mediate antibody production by inducing the TH2 response. The myeloid DCs/plasmacytoid DCs ratio might be a good indicator of immune status [19].

In the current study, we evaluated the total percentage of dendritic

cells and their two major subsets in peripheral blood of heroin addicts. The expression level of HLA-DR, CD11c, and CD123 markers on their surface were calculated as MFI. Our findings indicated that the total percentage of dendritic cells and the percentage of $CD123^+$ and $CD11c^+$ dendritic cells in heroin addicts were significantly lower than healthy controls, which can imply a defect in their immune systems. This phenomenon might be secondary to reduced dendritic cell production in the bone marrow, impaired response of monocytes to stimulators of monocyte differentiation to dendritic cells, inhibition of the stimulating factors of monocyte differentiation to dendritic cells, or induction of apoptosis in dendritic cells [20–22]. Even though further studies are required to prove these possibilities, our findings propose the involvement of dendritic cells in the immunopathogenesis of developed disorders in heroin addicts.

Monocytes can differentiate into dendritic cells or macrophages in the human body. The proximity of monocytes to stimulators such as GM-CSF and IL-4 differentiates them to dendritic cells. Roy et al. also reported interrupted response of monocytes of morphine-treated rats to M-CSF stimulator in the culture medium, which was reversible with naloxone. They demonstrated that chronic use of morphine in rats could lead to an inhibition of M-CSF in the bone marrow [23]. Some of the studies argue that morphine induces apoptosis in macrophages by increasing TGF-β concentration as well as expression of p38 MARK13, Fas, and Fas-L [24,25]. In the study of Weed et al., drinking water containing morphine for a few months by monkeys decreased the absolute number and percentage of NK cells [26]. Morphine was used in the mentioned studies, while in our study heroin was applied. Detoxification with methadone had no effect on the total percentage of dendritic cells and percentage of $CD11c^+$ dendritic cells of peripheral blood in heroin addicts. Methadone treatment caused an increase in the percentage of $CD123^+$ dendritic cells.

Antigen presentation to CD4 + T helper cells is mediated by HLA-DR molecules (MHC class II molecules). This process mediated by the recycling of surface HLA-DR molecules. The quantitative expression of this molecule can affect the initiation of specific immune responses. Therefore, the decrease of this molecule or lack of its expression might lead to the disarmament of antigen-presenting cells, which results in decreased antigen presentation and cytokine secretion [27]. The HLA-DR expression is regulated by cytokines in an antagonistic manner so that IFN-γ increases the expression of HLA-DR, which in turn, results in the increase of cellular immune responses, while IL-10 inhibits its expression [28].

Our data indicated that HLA-DR is downregulated in dendritic cells of heroin addicts. The HLA-DR molecule is one of the most important molecules of surface dendritic cells that stimulate CD4 + T helper cells, and the reduction of its expression on the surface of cells can be an indicator of immune paralysis. This reduction can impair the function of dendritic cells, and consequently, increasing the risk of infection in addicts. IL-10 can lower the expression of HLA-DR in the surface of dendritic cells through re-endocytosis and cellular sequestration of HLA-DR [29]. Our previous study showed that serum levels of IL-10 increase in opium addicts [30]. Because heroin can induce inflammation [31], a possible explanation is that an immunoregulatory response is occurring with the upregulation of IL-10 in an attempt to control the inflammation. Reduced expression of HLA-DR in opium addicts was improved after consumption of methadone hydrochloride, which may be due to the normalization of elevated serum IL-10.

This study suggested that heroin can significantly increase expression of CD11c molecules on the surface of $CD11c^+$ dendritic cells of peripheral blood of heroin addicts. Detoxification with methadone could normalize the increased expression of CD11c. Heroin had no significant effect on the expression of the CD123 molecule on the surface of $CD123^+$ dendritic cells.

5. Conclusion

Our data show changes in the percentage of dendritic cells and expression of their surface markers. This phenomenon can have temporary immunosuppressive effects on the ability of dendritic cells in the create a quick response and removal of pathogens. The dendritic cells of heroin addicts might lose their ability to develop specific immune responses to attacking pathogens, which prove increased susceptibility to infection in addicts. The methadone chloride can improve the imposed changes by heroin.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Acknowledgments

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