

# Impact of Opium Addiction on Levels of Pro- and Anti-inflammatory Cytokines after Surgery

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## Original Article

### Abstract

**Background:** Opium addiction alters immune responses to stresses such as an injury due to changing the secretion of cytokines. The present study assessed the effect of opium addiction on the cytokines [tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), interferon- $\gamma$  (IFN- $\gamma$ ), interleukin-4 (IL-4), and IL-10] before and after laparotomy.

**Methods:** Male rats were randomly divided into control and opium addicted (n = 20). Then, cytokines were measured before surgery, immediately after surgery (within 30-60 minutes) and 24 hours after surgery.

**Findings:** IFN- $\gamma$  was raised in an addicted group in three phases of the study as compared to that of the control group. IL-4 in opium addicted group decreased in two phases after surgery compared to the control group. IL-4 was lower after surgery in comparison to before surgery in the opium addicted group. The difference in IL-10 and TNF $\alpha$  levels was not statistically significant in the all groups measured in three phases of the investigation.

**Conclusion:** The results revealed that opium addiction can increase plasma level of IFN- $\gamma$  in rats and decrease plasma level of IL-4 after surgical stress. It seems that opium addicted rats are a more susceptible to increased inflammation.

**Keywords:** Cytokines; Interleukins; Opium addiction; Laparotomy; Rat

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## Introduction

Abusive drug or opium addiction influences many physiological functions, including the reactions of the immune system.<sup>1</sup> The complexity of the opium's effects results from the wide distribution of opioid receptors. These receptors have been demonstrated in various cell types, including cells of both the nervous and immune systems.<sup>1</sup> Thus, opium can modify the functions of the immune system either straight, by acting on the opioid receptors on lymphocytes and macrophages, or otherwise, it can influence the reactions of the immune system through its effects on the nervous system.<sup>1,2</sup> Three classes of the opioid receptor have been identified and cloned, which all of it are widely expressed in the central nervous system. The distribution of opioid receptors on the immune system cells was the first concerned by the observed ability of opioids to alter the immune function.<sup>2</sup> Opium addiction can change immune responses to stresses in the body due to changes in cytokines or other chemical mediators.<sup>2</sup>

It has been proved that pro- and anti-inflammatory cytokines are essential to the acute phase of the inflammatory and immunologic response. Throughout the fated response, several cells and mediators get involved, either to eliminate infectious agents or remove and repair damaged tissues.<sup>3,4</sup> This mentioned reactions get balanced by anti-inflammatory mechanisms. Moreover, acquiring the correct function by the particular organs and tissues depends on the balance between the mediators of pro- and anti-inflammatory mechanisms. Furthermore, increased levels of pro-inflammatory cytokines after major surgical processes have been related with an increase in the post-operative complications and morbidity.<sup>3,4</sup>

Any surgical intervention impairs homeostasis and initiates various hemodynamic, metabolic, and immunologic reactions. The amount of the disturbances observed in the post-operative period relates with the degree of the tissue injury. Many experimental and clinical studies have shown that surgical trauma is associated with the impaired immune response in the post-operative period.<sup>3,5</sup> This impairment can be connected with altered production of pro-inflammatory cytokines, as well as inhibition in cellular response.<sup>5</sup>

Since the inflammatory responses are dependent on the roles of cytokines, it is proposed that several of the immunomodulatory activities of the nociceptin and opioids are caused, at least in part, by the modulation of cytokine expression. The present study evaluated the interaction of surgery and addiction in the rat on pro- and anti-inflammatory cytokines.

## Methods

Experiments were conducted on male Wistar rats ( $250 \pm 10$  g body weight;  $n = 20$ ). The rats were housed in plastic cages (1 rat per cage,  $50 \times 50 \times 40$  cm). Animals were fed standard pellet diet and ordinary tap dextrose water (D/W 5%) with or without opium supplementation. The care of laboratory animals monitored the guiding principles for Care and Use of Laboratory Animals of the Kerman Neuroscience Research Center, Iran.

The male Wistar rats were randomly divided into two groups of 10: control group and opium-addicted group, control group had access to tap water with dextrose without any additive materials. In the addicted group, opium dependency was induced by adding opium mixture to 1-3 g/l D/W.<sup>6,7</sup> The designed dose of opium for each rat in the first time was 35 mg/kg/day for 5 consecutive days. Naloxone (2 mg/kg) injected intraperitoneally subsequent to naloxone test. Since some of the examined rats did not show withdrawal signs, the opium dosage was increased to 70-100 mg/kg/day (10 mg/kg/day interval) for the following days. For 20 minutes naloxone, injected rats were controlled for withdrawal signs. About 5 days later, naloxone test was repeated and revealed that all the rats in the addicted group had withdrawal signs. Withdrawal signs was determined if four of the following were shown: 1- Jumping; 2- Writhing; 3- Teeth chattering; 4- Ptosis; 5- Headshakes; 6- Paw tremor. A total duration of the handling was 33 days.<sup>8,9</sup>

Initially, blood samples were taken from the retro-orbital sinus from all rats in the intact group in day 10, as well as all rats in the addicted group in day 12 of the study using hairy tube, only after slight sedation by CO<sub>2</sub>. Laparotomy under induction of anesthesia with 100 mg/kg ketamine was performed through midline incision with a length of 3-4 cm and then small bowel handling

was carried out. Fascia and then skin were closed in two layers separately and continuously using nylon 4-0. The second blood sample was, later on, taken from the retro-orbital sinus within 30-60 minutes after surgery, followed by the third blood sample 24 hours later on mild sedation by CO<sub>2</sub>.

All blood samples were centrifuged for 15 minutes in 800 g and the plasma in microtubes was preserved in -80 °C. Cytokines [interleukin-10 (IL-10), IL-4, tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and interferon- $\gamma$  (IFN- $\gamma$ )] were subsequently measured using enzyme-linked immunosorbent assay (ELISA) test.

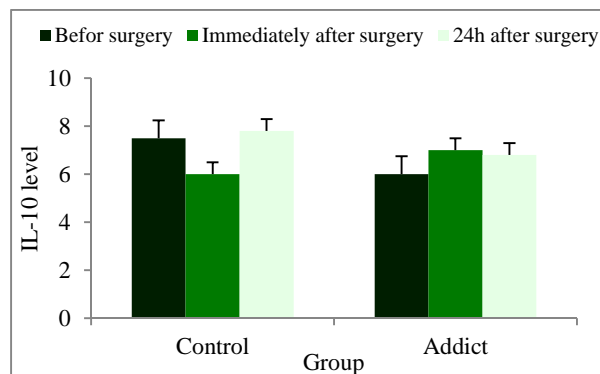
Serum levels of the cytokines in three stages of surgery analyzed with repeated measure ANOVA. Individual comparisons were calculated using Tukey's comparisons test where appropriate. Data are expressed as means  $\pm$  standard error (SE) of mean of 10 animals per group.

## Results

The results showed that the difference in the measured serum level of IL-10 (Figure 1) and TNF $\alpha$  (Figure 2) in the two groups (addicted vs. control), in all phases of the investigation (before and after surgery) was not statistically significant ( $P > 0.050$ ). During the all phases of examination serum level of IFN- $\gamma$  (Figure 3) increased in addicted group compared to control group ( $P < 0.001$ ). Serum level of IL-4 (Figure 4) in the opium addicted group decreased after surgery compared to before surgery ( $P = 0.006$ ) as well as to the control group (within 30-60 minutes after surgery:  $P < 0.010$ ; 24 hours after surgery:  $P < 0.050$ ) (Figure 4). In control group, no difference was found in the measured serum cytokines level after surgery compared to the measured levels before surgery ( $P > 0.050$ ).

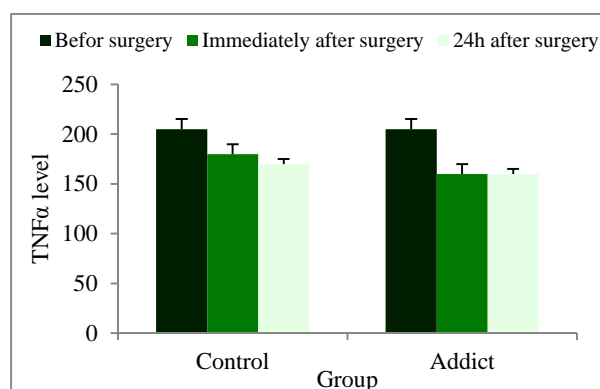
## Discussion

Findings showed that the difference in the measured serum level of TNF $\alpha$  and IL-10 in the opium addicted versus control group, at all phases of the investigation was not statistically significant; however, IFN- $\gamma$  serum level increased, and IL4 decreased in the opium addicted group compared to the control group during all the phases of examination. Accordingly, opium addiction is capable of raising the serum level of IFN- $\gamma$  and reducing IL-4 in comparison to the control group.



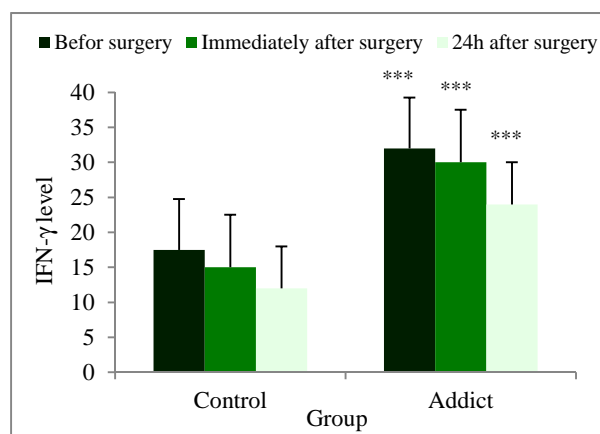
**Figure 1.** Serum levels of the interleukin-10 (IL-10) in opium addicted vs. control group

There was not statistically different in IL-10 level between two groups measured in all phases of examination (before surgery, within 30-60 minutes after surgery and 24 hours after surgery)



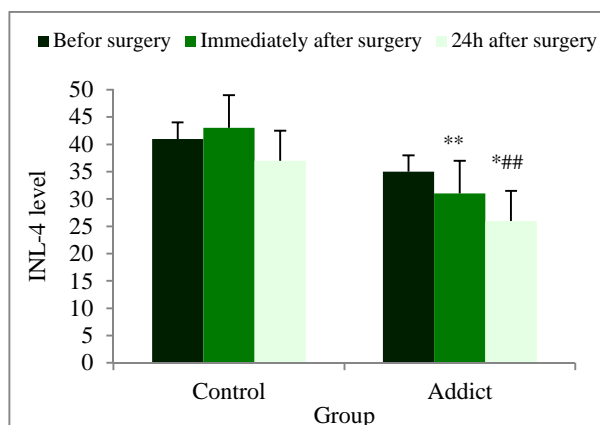
**Figure 2.** Serum levels of the tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) in opium addicted versus control group

There was not statistically different in TNF $\alpha$  level measured in before surgery, immediately after surgery and 24 hours after surgery between two groups



**Figure 3.** Serum levels of the interferon- $\gamma$  (IFN- $\gamma$ ) in opium addicted cases and healthy controls

IFN- $\gamma$  was raised in opium addicted group in all phases of study as compared to control group. \*\*\* $P < 0.001$ , indicate statistically significant differences between cases compared with control group



**Figure 4.** Serum levels of the interleukin-4 (IL-4) in opium addicted cases and healthy controls

IL-4 decreased in opium addicted group in phases of immediately and 24 hours after surgery. Serum level of the IL-4 also was declined 24 hours after surgery in addicted than before surgery.

\* $P < 0.050$ , \*\* $P < 0.010$ , as compared to control group, ### $P < 0.010$ , as compared to before surgery in opium addicted group

Surgical stress decreased serum level of IL-4 in the addicted group but not in the control group. IFN- $\gamma$  is a pro-inflammatory cytokine and IL-4 is an anti-inflammatory cytokine, thus an increased level of IFN- $\gamma$  and a decreased level of IL-4 (after surgical stress) can make the addicted rats susceptible to inflammatory reactions. This effect (in some conditions such as infection and sepsis) can increase the rate of acute lung injury, adult respiratory distress, and multi-organ failure. Surgical stress in the addicted group through decreasing the serum level of IL-4 can make rats susceptible to side effects of the increased inflammatory reactions after surgery. Opium can also change IL-1, IL-2, IL-4, IL-5, IL-10, TNF $\alpha$ , and IFN- $\gamma$  as shown in other studies.<sup>10,11</sup> IL-4 appears to increase macrophage susceptibility to the anti-inflammatory properties of glucocorticoids.<sup>12</sup> In addition, it induces class switching of differentiating B lymphocytes to produce predominantly immunoglobulins (IGs) which are important in allergic responses.<sup>12</sup>

The immunomodulatory properties of the nociceptin and opioids are not completely identified, yet. It is possible that the altered appearance of some cytokines can be due to the opioid-induced manufacture of regulatory mediators which, in turn, leads to altered levels of cytokines such as IL-2 and IFN- $\gamma$ .<sup>11</sup>

Opioids are well-known due to their ability to induce analgesia, however, it has shown that

opioids control the function of cells involved in the immune response. Regulation of cytokine and cytokine receptors is a critical element for immunomodulatory effects of the opioids.<sup>11</sup> Sacerdote et al.<sup>13</sup> have been reported that opioid peptides affect different immune functions. They showed that these effects could be mediated by the modulation of Th1/Th2 cytokine production.

While, activation of the  $\mu$  opioid receptor favors a pro-inflammatory response, activation of the kappa opioid receptor induces anti-inflammatory responses through the down-regulation of chemokine, cytokine, and chemokine receptors.<sup>11</sup> Increase or decrease in the level of cytokines can change inflammatory body response to stress or infection that may be caused by hemodynamic instability, shock or immunodeficiency.<sup>14</sup> An increase in tissue cytokine following shock can associate with raising in endogenous opioids, and this elevation is consistent with their possible modulatory role in immune response.<sup>15</sup> Other studies have shown adverse effects of opium on the immune system with an increased rate of infections and sepsis.

Malviya et al.<sup>2</sup> suggested that opium addicts suffer a much higher grade of post-operative illness as compared to non-addicts. According to their study, opium addicts had a significantly higher prevalence of post-operative respiratory, cardiovascular, systemic, and local problems.<sup>2</sup> Analgesics requirement during this period and hospitalization were also significantly higher as compared to controls. Incidence of the post-operative systemic difficulties is higher (36.6%) in opium addicts as compared to the control group which contains opiate withdrawal syndrome, septicemia, acute renal failure, shock, and cerebrovascular accidents.<sup>10</sup> It has been shown that mortality rate was 7 times higher in opium addicts during elective surgery as compared to non-addicts.<sup>2</sup> However, it was only marginally greater in opium addicts during emergency surgical procedures as compared to non-addicts. Hemodynamic, cardiovascular, pulmonary, and renal dysfunction were documented as well.<sup>12,16,17</sup> Furthermore, opium addiction can change endocrine hormones,<sup>18</sup> cytokines,<sup>19</sup> wound healing,<sup>20</sup> post-operative adhesion process,<sup>9</sup> and cause immunodeficiency.<sup>21</sup> Pain control with opioid or non-steroidal anti-inflammatory drugs analgesics can improve wound healing.<sup>22</sup>  $\beta$

endorphin, enkephalin, and morphine, all being opioid derivatives can increase IFN- $\gamma^{23}$  and can lead to patient's susceptibility to alveolar type-I/acute respiratory distress syndrome pathogenesis.<sup>24</sup>

### Conclusion

Results revealed that opium addicts had significantly higher levels of INF $\gamma$  and lower levels of IL-4. The increased level of pro-inflammatory cytokine INF $\gamma$  and decreased anti-inflammatory level of IL4 might lead to an

increased inflammatory response in opium addicted rats.

### Conflict of Interests

The Authors have no conflict of interest.

### Acknowledgements

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## اثر اعتیاد به مواد مخدر بر سطح سیتوکین‌های التهابی و ضد التهابی پس از جراحی

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### مقاله پژوهشی

### چکیده

**مقدمه:** اعتیاد به اوپیوم از طریق تغییر در ترشح سیتوکین‌ها، می‌تواند پاسخ‌های ایمنی به استرس‌هایی مانند تروما و جراحی را تغییر دهد. در مطالعه حاضر اثرات اعتیاد به اوپیوم بر سطح سیتوکین‌های  $TNF\alpha$  (Tumor necrosis factor)،  $INF\ \gamma$  (Interferon- $\gamma$ )،  $IL-4$  (Interleukin-4) و  $IL-10$  قبل و پس از جراحی بررسی شد.

**روش‌ها:** موش‌های صحرایی نر (۲۰ سر) به صورت تصادفی به دو گروه شاهد و معتاد به اوپیوئید (ارزیابی اعتیاد از طریق نالوکسان) تقسیم شدند. سپس سطح سیتوکین‌ها قبل، بلافاصله و ۲۴ ساعت پس از جراحی بررسی گردید.

**یافته‌ها:** سطح  $INF\ \gamma$  در همه فازهای مطالعه در گروه معتاد نسبت به گروه شاهد افزایش داشت. در گروه معتاد، میزان  $IL-4$  در فازهای بلافاصله و ۲۴ ساعت پس از جراحی نسبت به گروه شاهد، کاهش نشان داد. سطح  $IL-4$  در فاز پس از جراحی نسبت به قبل از جراحی کمتر بود. اختلاف معنی‌داری در سطح سیتوکین‌های  $TNF\alpha$  و  $IL-10$  در تمام فازهای مطالعه (قبل، ۶۰-۲۰ دقیقه و ۲۴ ساعت پس از جراحی) مشاهده نشد.

**نتیجه‌گیری:** اعتیاد به مواد مخدر باعث افزایش سطح  $INF\ \gamma$  و کاهش سطح پلاسمایی  $IL-4$  می‌شود. به نظر می‌رسد که موش‌های معتاد شده با اوپیوئید استعداد بیشتری جهت ابتلا به افزایش التهاب دارند.

**واژگان کلیدی:** سیتوکین‌ها؛ اینترلوکین‌ها؛ اعتیاد به اوپیوم؛ لاپاراتومی؛ موش صحرایی

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