Association of miR-155, miR-187 and Inflammatory Cytokines Il-10 and TNF-α in Chronic Opiate Abusers

PURVI PUROHIT (dr.purvipurohit@gmail.com)
All India Institute of Medical Sciences Jodphur https://orcid.org/0000-0001-8559-2911
Dipayan Roy
All India Institute of Medical Sciences Jodhpur
Shailender Dwivedi
All India Institute of Medical Sciences Gorakhpur
Naresh Nebhinani
All India Institute of Medical Sciences Jodhpur
Praveen Sharma
All India Institute of Medical Sciences Jodhpur

Research Article

Keywords: MicroRNA, Opiate, IL-10, IL-6, TNF-α

Posted Date: May 13th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-486700/v1

Abstract

The current study determined levels of inflammatory cytokines TNF-α, IL-6, IL-10 and immune-regulatory miR-155 and miR-187 expressions in chronic opiate abuse. Adults (n = 48), meeting the 5th Edition of the DSM criteria regarding opioid use disorder, and healthy controls (n = 46) were included in the study. Serum samples were analysed for inflammatory cytokines IL-10, IL-6, TNF-α using ELISA and PBMCs processed for miRNA expression using SybrGreen chemistry. Cases showed significantly raised IL-10 and TNF-α and reduced IL-6. Using RNU6 for normalization, dose-dependent corresponding upregulation of miR-155-5p and downregulation of miR-187-5p were evident at opiate dose > 1500 gm/day, with a corresponding increase of TNF-α and IL-10. MiR-155 showed a significant negative correlation with IL-6 and TNF-α; miR-187 showed a significant positive association with TNF-α at > 1000 g/day consumption. Therefore, increasing consumption of opium probably enhances inflammation leading to immunomodulation and aberrant expression of has-miR-155-5p and has-miR-187-5p in opiate abusers.

1. Introduction

Opium is the second most commonly abused substance after tobacco in developing countries of the Middle East and Asian regions. In India, the number of opiate abusers is increasing every year with a great number residing in Western Rajasthan[1]. Opioids can interfere with the immune system by participating in the function of the immune cells and causing modulation of the innate and acquired immune responses. The traditional notion of opioids is immunosuppressive[2]. However, recent studies indicate that the role of opioid receptors working through various mechanisms. Since the immune system plays a critical role in various physiological and pathophysiological processes, including inflammation, tumour growth and metastasis and drug abuse, therefore it is important to elucidate the relationship between opioids and immune function [2]. Cytokines are an important weapon in the armoury of inflammatory reactions. They are produced by the immune cells and play a significant role in regulating the activity of the immune system. Cytokine production is stimulated by several stimulants (e.g. levetiracetam, topiramate, and carbamazepine) [3]; and opium derivatives have also been shown to affect cytokine production[4]. Furthermore, there are conflicting reports on the levels of pro- and anti-inflammatory cytokines in opiate addiction [5,6]. IL-10 acts as an anti-inflammatory cytokine, with reports of its higher levels in opium abusers as compared to the control group [5] and IL-10 negatively regulates acute and chronic inflammation by blocking or reducing the output of pro-inflammatory cytokines from immune cells, including TNF-α, IL-6, and IL-12 has been reported. IL-10 suppresses cytokines primarily at the level of gene transcription but also influences post-transcriptional events [7]. MicroRNAs (miRNAs) are evolutionarily conserved small, noncoding RNAs that post-transcriptionally regulate gene expression by targeting specific messenger RNAs (mRNAs) for degradation or translational repression. Emerging evidence suggests that miRNAs play a key role in the regulation of immunological functions including innate and adaptive immune responses, development and differentiation of immune cells, and prevention of autoimmunity [8]. Studies are now revealing that miRNAs modulate the concentration of key target proteins over a narrow range in a dose-dependent manner, i.e. the cellular concentration of a miRNA dictates the protein output of its target genes and is therefore of key importance in miRNA-mediated

control. Small changes in the concentration of key cellular proteins being affected by miRNA control, can have significant biological consequences, in line with many examples of pathogenic hemizygous null mutations in man and mouse [9]. Therefore, miRNAs play a crucial role in the regulation of the immune system.

There are no studies exploring the roles of microRNA in opiate dependents, however, microarray analysis in varied drug addictions have revealed altered expression of miRNA regulating both innate and adaptive immune systems like miR-155, miR-187, miR-190, and miR184 [10]. Besides, Rossato et al. (2012) [11] showed in human monocytes that miR187 is involved in the physiological regulation of IL-10–driven anti-inflammatory responses and demonstrated miRNA-mediated strategies controlling cytokine expression in human monocytes activated by a TLR4 agonist (LPS). Similarly, miR155 is a significant player in both innate and adaptive immunity and is upregulated in macrophages in alcohol abuse [12].
Although there is an elevated incidence of inflammatory disorders in drug abusers (alcohol, opioid) [13], the role of miRNA in the immune regulation of drug abusers and the functions of most miRNAs concerning drug abuse and immune diseases is still not clearly understood [14].

No studies have explored the dynamics of pro and anti-inflammatory cytokines in response to changing dose of stimulant, i.e. opiate and expression of their corresponding miRNAs, i.e. IL-10 for miR187 and TNF- α for miR155. We hypothesize that chronic opiate addiction triggers immune modulation leading to altered levels of IL-10, IL-6 and TNF- α and their targeted hsa-miR-155-5p and hsa-miR-187-5p. This will help understand the roles of these miRNAs concerning IL-10, IL-6 and TNF-α in opiate addiction. Since immunomodulation forms the basis of several disease conditions, this may go a long way in understanding immune diseases in such patients.

2. Methods

2.1 Study participants and inclusion and exclusion criteria

The study was conducted in the Department of Biochemistry and Psychiatry of a tertiary care centre for a duration of one and half year. Opiate dependent males were recruited from the outpatient departments of the de-addiction clinic of the Department of Psychiatry, and a non-government organization (NGO), Manklaav drug de-addiction and rehabilitation centre of the city reporting for de-addiction. The study was planned as a case–control study and was initiated after the Institutional Ethics Committee (IEC) approval. A total of 48 opiate abusers were recruited after informed consent. Adults, who met the Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria of the American Psychiatric Association regarding opioid use disorder (n = 48), and healthy controls (Control Group or CG, n = 46).

The inclusion criteria were to include male patients of the age group 18-50 years, who were chronic opiate abusers (not abusing other substances like cocaine, marijuana or amphetamine), for a minimum of one year. Female opiate abusers do not report to the de-addiction clinics due to the patriarchal social structure of the region. A convenience sampling method was employed at the de-addiction clinic. All opiate-dependent subjects consumed opium orally as pure opium (available in packets of 50 gms). The daily

dose consumed was reported by the attendants of the patients. A urine sample of the study participants was collected for the screening of opiate; those having a value >300 ng/ml was considered positive and were recruited to the study. Urinary Opiate was analysed using the Enzyme Immunoassay kit of Randox and the cut-off was taken as mentioned in the kit insert. The cases recruited showed no evidence of withdrawal symptoms (such as insomnia, nausea, vomiting, diarrhea, agitation, headaches, hallucinations and blurred vision) at the time of sampling. Multi-drug abuse was excluded and cases with only oral opium abuse were included. History of any other drug consumption which affects the immune response was taken to exclude those participants. Patients with any co-morbidity (Diabetes mellitus, Hypertension, Hypothyroidism, Coronary artery disease, Rheumatic disorders, HIV, HCV and other inflammatory disorders as well as smoking) were also excluded from the study.

The controls were individuals working in our healthcare setup, such as students and hospital staff who did not have any history of drug abuse or smoking and did not have any inflammatory disorder (rheumatoid arthritis, diabetes and asthma based on history and physical examination), and had no psychiatric ailment.

2.2 Sample collection, RNA isolation, and reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR)

A venous blood sample collected on the day of their admission, with the last opiate dose taken within 24 hours of the sample collection. The venous blood sample of the study subjects was collected in erythrocyte lysis buffer (Qiagen). The PBMCs cell lysate was processed for extraction of miRNA (Qiagen miRNAeasy mini kit). The quantity and quality of the extracted miRNA were assessed using a Nanoquant plate (BioTek) at 260 nm and 280 nm, and those with a ratio greater than 1.8 were processed further for cDNA synthesis using cDNA kit specific for hsa-miR-155-5p and has-miR-187-5p (miScript II RT Kit by Qiagen). The cDNA samples were further analysed using the qPCR CFX96 of Bio-Rad using SybrGreen chemistry (Qiagen miScript SYBR Green PCR Kit). The PCR data was analysed using the CFX96 analysis software and expressed as delta Cq values. The fold change expression was analysed after normalization using RNU6.

2.3 Interleukin assay

The interleukins were analysed using ELISA kits from Finetest for IL-6 (range: 4.69-300pg/ml; CV: inter-assay <10% and intra-assay <8%), IL-10 (range: 7.813-500 pg/ml ; CV: inter-assay <10% and intra-assay <8%) and TNF-α (range: 15.6-1000 pg/ml; CV: inter-assay <10% and intra-assay <8%).

2.4 Statistical analysis

The statistical analysis was performed using SPSS21 for correlation analysis of biochemical parameters and PCR data. The continuous variables were analysed using descriptive statistics. Comparison between groups was carried out by one-way analysis of variance (ANOVA), and linear regression analysis was used to analyse the effects of variable opium consumption.

3. Results

The current case-control study demonstrated in chronic opiate abusers significantly raised IL-10, and TNF-α, a significantly reduced IL-6 (Table 1) as compared with healthy controls. Further, one way ANOVA for inflammatory cytokines showed a non-significant difference across the groups when categorised according to dose of opiate (oral consumption of opium), although the rise in TNF-α was much greater than the rise in anti-inflammatory IL-10 (Table 2). An analysis of fold change expression of hsa-miR155-5p and hsa-miR-187-5p showed downregulation (Table 3). This may be responsible for the higher expression of IL-10 and TNF-α in the cases as compared to controls. However, one-way ANOVA for miRNA expression with different amount of opium consumption (Table 3) showed that with an increasing dose, there was upregulation of miR-155-5p at a consumption of 750-1000 gm opium and >1500 gm opium, which possibly is due to the effect of raised TNF-α. At an opium consumption <1000 gm/day both the miRNAs showed no significant association with the cytokines (Table 4). Pearson's correlation analysis showed a significantly negative association of miR-155-5p with IL-6 and TNF-α and a positive association with IL-10 at opium consumption greater than 1000 gm/day. For miR-187-5p, there was a significantly positive association with TNF-α at a daily consumption >1000 gm/day (Table 5).

4. Discussion

Chronic opiate abuse is capable of significant immunomodulation and possibly forms the basis of various diseases associated with opiate abuse [15]. Regulation of inflammatory responses is ensured by coordinated control of gene expression of participating immune system proteins and cells. One group of gene expression regulators, functions of which have recently been started to be uncovered concerning any type of inflammatory condition, is a class of short single-stranded RNA molecules termed microRNAs (miRNAs). miRNAs function together with partner proteins and mainly cause gene silencing through degradation of target mRNAs or inhibition of translation [16]. The current study for the first time shows the effect of chronic opiate abuse, as well as its dose on the expression of inflammatory cytokines and their associated miRNAs (Tables 1 & 3). We have reported earlier in a prospective clinical study a strong association of low-grade inflammation with dependence years in pure opiate abusers [1]. The current study reports a dose-dependent increase in the serum levels of TNF-α, expression hsa-miR-155-5p, and hsa-miR-187-5p with increasing per day consumption. The significantly raised levels of TNF-α suggest a pro-inflammatory status.

The hsa-miR-155-5p is a pleiotropic molecule that regulates both central and peripheral inflammation [17] (Qayum et al., 2016). It has been reported to be induced by both IL-10 [16] and TNF-α [18]. We observed chronic opiate abuse induces an inflammatory response, wherein as the dose increases, the anti-inflammatory response of IL-10 is over-ridden as observed by its non-significant increase and a significant increase of TNF-α in cases as compared to controls.

At lower doses of opium, there is downregulation of miR-155-5p and a rise in expression of IL-10, as seen in Tables 2 & 3. At lower doses of opium, miR-187-5p gradually upregulated and possibly induced IL-10,

as also observed by Rossato et al. (2012).[11] However, at opium consumption greater than 1500gm/day, there is upregulation of hsa-miR-155-5p which targets and inhibits IL-10 expression, thus showing a pro-inflammatory status acquisition with a very high dose of opium.

A higher expression of hsa-miR-155-5p causes inhibition of IL-10 and certain transcription factors like SHIP-1, SOCS-1 and BCL6 in macrophages and this relieves the inhibition on the expression of TNF-α [19]. Similar observations have been made in the current study since, with increased consumption of opium (750-1000 gm/day and >1500 gm/day), there was up-regulation of miR-155-5p and an increased level of TNF-α. Also, miR-155-5p and TNF-α axis exists in an animal model of temporal lobe epilepsy and is responsible for neuro-inflammation in epilepsy in both human and animal model [20]. A similar axis may be responsible for the dose-dependent immunomodulation to enhance a pro-inflammatory cytokine response of chronic opium abusers.

IL-10 negatively regulates acute and chronic inflammation by blocking or reducing the output from immune cells of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-12. [10] Since the amplitude and duration of the inflammatory response are regulated precisely by IL-10, we analysed the plasma levels of IL-10 at various doses of opium abuse and it was observed that there was a gradual but non-significant increase in the plasma levels of IL-10 with increasing dose of opium (Table 2). Further, Rossato et al. (2012) reported in an in vitro model, induction of the miR-187 in an IL-10 dependent manner, which further causes a transcriptional inhibition of mRNA of TNFα and IL-6 (via Ikβζ).[11] Thus suggesting an anti-inflammatory role of IL-10 and hsa-miR-187-5p. Correspondingly in the current study, with increasing dose, there was a downregulation of hsa-miR187-5p but non-significant an increasing level of IL-10. An up-regulation of miR-187 at higher doses was still not sufficient to inhibit the expression of TNF-α and IL-6 mRNA. Thus, showing that a compensatory increase of pro-inflammatory cytokines may occur in response to rising IL-10.

At the cellular level, the hsa-miR-155-5p expression is strongly induced by different TLR ligands including LPS and consistent with the pro-inflammatory properties of hsa-miR-155-5p, TNF-α translation is enhanced by the presence of hsa-miR-155-5p via increasing mRNA stability [21]. Therefore, our reports of a dose-dependent significant increase in fold change expression of hsa-miR-155-5p (Table 3), and TNF-α levels are possibly due to chronic high levels of opium inducing the overexpression of hsa-miR-155-5p.

Clinical or recreational use/abuse of morphine has also been shown to be associated with sustained immune activation and promotes sepsis and septic shock, thus reinforcing the inflammatory effect of chronic exposure to opiates[22]. Further, a Pearson's correlation analysis (Table 5) showed miR-155-5p to be significantly negatively associated with IL-6 and TNF-α and miR-187-5p showed significantly positively associated with TNF-α at >1000 gm/day consumption. Also, linear regression analysis showed TNF-α to be an independent significant predictor of miR-187 expression (Table 6). Since miR187 regulates TNF-α expression via IL-10, the down-regulation of miR187 promotes the expression of TNF-α and inflammation. Also, TNF-α regulates the expression of miR-155-5p via IL-6, thus leading to an upregulation of miR-155-5p. Overall, with increasing opium consumption, there is a co-dependent regulatory network of cytokines

and miRNAs which develop an inflammatory environment and thus showing a state of low-grade inflammation to be present in opiate addicts.

The small sample size is a limitation for the study and further larger prospective studies involving human participants along with the route of opiate abuse would be crucial to unravel the mechanistic details of altered dynamics of pro- and anti-inflammatory miRNAs. Another limitation is that a strict control on the dose of oral opium was reported by the attendants of the abusers; a better dose and response effect can be validated by using in-vitro models like cell lines or use of animal models.

5. Conclusion

The study results show that chronic opium abuse causes altered expression of hsa-miR187-5p, hsa-miR155-5p and may-be responsible for increasing serum TNF-α, lowering of IL-10. The altered dynamics of the two miRNA are affected by the daily consumption of opium, leading to the altered immune response observed with chronic opium abuse. Further, the characterization of these miRNAs in in-vitro models can explain the mechanistic details of immunomodulation observed in our study. We conclude that increasing consumption of opium enhanced inflammation by up-regulation of miR-155-5p and relative down-regulation of miR-187-5p, inhibiting IL-10, promoting IL-6 and relieving inhibition on TNF-α. Thus, opium dose may be responsible for the immunomodulation in opium abusers, leading to the aberrant expression of miRNA.

Future studies exploring the effect of chronic opiate dependence in association with duration of dependence can prove to be beneficial for better management of these patients.

Declarations

Conflict of Interest Statement: The authors declare no conflict of interests.

Acknowledgement: None.

Ethics: The study commenced after institutional ethics permission for the use of biological samples from study participants.

Informed consent- All the study participants were informed about the objective of the study, the nature of study and were given free will to withdraw from the study at any time.

Ethics approval and consent to participate: The study was approved by the institutional ethics committee (IEC) before commencement.

Consent for publication: The study was approved for publication according to the IEC

Availability of data and materials: Not applicable

Competing interests: There were no competing interests.

Funding: This study was funded by an internal grant from the Institutional Review and Ethics Board, AIIMS Jodhpur [AIIMS/Res(02)/2015-16/308]

Authors' contributions:

Purvi Purohit: Grant awarded, Study concept, study design, sample analysis, data collection and analysis, interpretation, drafting manuscript.

Dipayan Roy: Data collection and analysis, drafting manuscript.

Shailender Dwivedi: study design, sample analysis, manuscript editing.

Naresh Nebhinani: Patient recruitment, manuscript editing.

Praveen Sharma: Data analysis, Manuscript editing.

Acknowledgements: None

References

1. Purohit, P., Nebhinani, N., & Sharma, P. (2017). Evaluation of Antioxidant Status, High Sensitivity C-reactive Protein, and Insulin Resistance in Male Chronic Opiate Users Without Comorbidities. Indian journal of psychological medicine, 39(3), 299–305. https://doi.org/10.4103/0253-7176.207330
2. Liang, X., Liu, R., Chen, C., Ji, F., & Li, T. (2016). Opioid System Modulates the Immune Function: A Review. Translational perioperative and pain medicine, 1(1), 5–13.
3. Himmerich, H., Bartsch, S., Hamer, H., Mergl, R., Schönherr, J., Petersein, C., Munzer, A., Kirkby, K. C., Bauer, K., & Sack, U. (2014). Modulation of cytokine production by drugs with antiepileptic or mood stabilizer properties in anti-CD3- and anti-Cd40-stimulated blood in vitro. Oxidative medicine and cellular longevity, 2014, 806162. https://doi.org/10.1155/2014/806162
4. Asadikaram, G., Igder, S., Jamali, Z., Shahrokhi, N., Najafipour, H., Shokoohi, M., Jafarzadeh, A., & Kazemi-Arababadi, M. (2015). Effects of Different Concentrations of Opium on the Secretion of Interleukin-6, Interferon-γ and Transforming Growth Factor Beta Cytokines from Jurkat Cells. Addiction & health, 7(1-2), 47–53.
5. Ghazavi, A., Mosayebi, G., Solhi, H., Rafiei, M., & Moazzeni, S. M. (2013). Serum markers of inflammation and oxidative stress in chronic opium (Taryak) smokers. Immunology letters, 153(1-2), 22–26. https://doi.org/10.1016/j.imlet.2013.07.001
6. Kuang, Y. M., Zhu, Y. C., Kuang, Y., Sun, Y., Hua, C., & He, W. Y. (2007). Xi bao yu fen zi mian yi xue za zhi = Chinese journal of cellular and molecular immunology, 23(9), 821–823
7. Shao, Y., Cheng, Z., Li, X., Chernaya, V., Wang, H., & Yang, X. F. (2014). Immunosuppressive/anti-inflammatory cytokines directly and indirectly inhibit endothelial dysfunction–a novel mechanism for maintaining vascular function. Journal of hematology & oncology, 7, 80. https://doi.org/10.1186/s13045-014-0080-6

8. Pauley, K. M., & Chan, E. K. (2008). MicroRNAs and their emerging roles in immunology. Annals of the New York Academy of Sciences, 1143, 226–239. https://doi.org/10.1196/annals.1443.009
9. Xiao, C., & Rajewsky, K. (2009). MicroRNA control in the immune system: basic principles. Cell, 136(1), 26–36. https://doi.org/10.1016/j.cell.2008.12.027
10. Zhang, K., Jing, X., & Wang, G. (2016). MicroRNAs as regulators of drug abuse and immunity. Central-European journal of immunology, 41(4), 426–434. https://doi.org/10.5114/ceji.2016.65142
11. Rossato, M., Curtale, G., Tamassia, N., Castellucci, M., Mori, L., Gasperini, S., Mariotti, B., De Luca, M., Mirolo, M., Cassatella, M. A., Locati, M., & Bazzoni, F. (2012). IL-10-induced microRNA-187 negatively regulates TNF-α, IL-6, and IL-12p40 production in TLR4-stimulated monocytes. Proceedings of the National Academy of Sciences of the United States of America, 109(45), E3101–E3110. https://doi.org/10.1073/pnas.1209100109
12. Asquith, M., Pasala, S., Engelmann, F., Haberthur, K., Meyer, C., Park, B., Grant, K. A., & Messaoudi, I. (2014). Chronic ethanol consumption modulates growth factor release, mucosal cytokine production, and microRNA expression in nonhuman primates. Alcoholism, clinical and experimental research, 38(4), 980–993. https://doi.org/10.1111/acer.12325
13. Loftis, J. M., & Huckans, M. (2013). Substance use disorders: psychoneuroimmunological mechanisms and new targets for therapy. Pharmacology & therapeutics, 139(2), 289–300. https://doi.org/10.1016/j.pharmthera.2013.04.011
14. Friedman, H., Newton, C., & Klein, T. W. (2003). Microbial infections, immunomodulation, and drugs of abuse. Clinical microbiology reviews, 16(2), 209–219. https://doi.org/10.1128/cmr.16.2.209-219.2003
15. Rebane, A., & Akdis, C. A. (2013). MicroRNAs: Essential players in the regulation of inflammation. The Journal of allergy and clinical immunology, 132(1), 15–26. https://doi.org/10.1016/j.jaci.2013.04.011
16. Woodbury, M. E., Freilich, R. W., Cheng, C. J., Asai, H., Ikezu, S., Boucher, J. D., Slack, F., & Ikezu, T. (2015). miR-155 Is Essential for Inflammation-Induced Hippocampal Neurogenic Dysfunction. The Journal of neuroscience: the official journal of the Society for Neuroscience, 35(26), 9764–9781. https://doi.org/10.1523/JNEUROSCI.4790-14.2015
17. Qayum, A. A., Paranjape, A., Abebayehu, D., Kolawole, E. M., Haque, T. T., McLeod, J. J., Spence, A. J., Caslin, H. L., Taruselli, M. T., Chumanevich, A. P., Baker, B., Oskeritzian, C. A., & Ryan, J. J. (2016). IL-10-Induced miR-155 Targets SOCS1 To Enhance IgE-Mediated Mast Cell Function. Journal of immunology (Baltimore, Md.: 1950), 196(11), 4457–4467. https://doi.org/10.4049/jimmunol.1502240
18. Yee, D., Shah, K. M., Coles, M. C., Sharp, T. V., & Lagos, D. (2017). MicroRNA-155 induction via TNF-α and IFN-γ suppresses expression of programmed death ligand-1 (PD-L1) in human primary cells. The Journal of biological chemistry, 292(50), 20683–20693. https://doi.org/10.1074/jbc.M117.809053
19. Alivernini, S., Gremese, E., McSharry, C., Tolusso, B., Ferraccioli, G., McInnes, I. B., & Kurowska-Stolarska, M. (2018). MicroRNA-155-at the Critical Interface of Innate and Adaptive Immunity in

Arthritis. Frontiers in immunology, 8, 1932. https://doi.org/10.3389/fimmu.2017.01932

20. Li TR, Jia YJ, Wang Q, Shao XQ, Zhang P, Lv RJ. Correlation between tumor necrosis factor alpha mRNA and microRNA-155 expression in rat models and patients with temporal lobe epilepsy. Brain Res. 2018 Dec 1;1700:56-65. doi: 10.1016/j.brainres.2018.07.013. Epub 2018 Jul 11. PMID: 30006293.
21. Cheung ST, So EY, Chang D, Ming-Lum A, Mui AL. Interleukin-10 Inhibits Lipopolysaccharide Induced miR155 Precursor Stability and Maturation. PLOS ONE ; 2013:8(8): e71336
22. Banerjee, S., Meng, J., Das, S., Krishnan, A., Haworth, J., Charboneau, R., Zeng, Y., Ramakrishnan, S., & Roy, S. (2013). Morphine induced exacerbation of sepsis is mediated by tempering endotoxin tolerance through modulation of miR-146a. Scientific reports, 3, 1977. https://doi.org/10.1038/srep01977

Tables

Table 1
Basic demographic profile of the study population

Parameters	Control (n = 46)	Opiate abusers (n = 48)	p-value (Student's t-test)
Age	33.00 ± 7.34	32 ± 9.0	0.574
Opiate dependence (Months)	-	85.13 ± 8.295	-
SBP (mm of Hg)	121.73 ± 6.35	119.37 ± 14.35	0.43
DBP (mm of Hg)	81.87 ± 3.30	78.13 ± 5.70	**< 0.001**
IL-6 (pg/ml)	18.36 ± 2.143	4.391 ± 0.8153	**< 0.0001**
IL-10 (pg/ml)	13.54 ± 1.978	30.42 ± 3.76	**< 0.0001**
TNF-α (pg/ml)	97.741 ± 8.252	157.270 ± 10.882	**0.0121**

Table 2
One-way ANOVA for dose-dependent analysis of IL-10, IL-6 and TNF-α

Dose	<=100 gm/day	<=1000 gm/day	>=1500gm/day	ANOVA (*p*-value)
IL-10	30.305 ± 4.16	29.833 ± 3.81	31.094 ± 3.54	F = 0.50; p = 0.607
IL-6	2.226 ± 2.06	4.994 ± 1.13	5.231 ± 0.70	F = 0.55; p = 0.57
TNF-α	120.799 ± 8.77	170.571 ± 14.73	199.533 ± 39.66	F = 2.09; p = 0.13

Table 3
One way ANOVA showing variance in the levels of hsa-miR155-5p and miR-187-5p with variation in dose of opiate per day

Group	N	RNU6	Cq miR-187-5p	ΔΔCq	FCE	ANOVA
Control	46	18.511 ± 0.321	31.696 ± 5.576		0.0243	F = 9.0792
Group I (Opiate intake15-30 gram/month)	14	18.412 ± 0.227	34.626 ± 2.053	5.36		*p* < 0.0001
Group II (Opiate intake 150–450 gram/month)	9	18.491 ± 0.241	36.425 ± 1.120	4.55	0.0428	
Group III (Opiate intake 750-1000gram/month)	10	18.389 ± 0.1992	32.004 ± 1.426	-1.05	**2.07**	
Group IV (Opiate intake 1500-6000gram /month)	15	18.478 ± 0.293	32.685 ± 2.178	-0.42	**1.366**	
Group	**N**	**RNU6**	**Cq miR-155-5p**	**ΔΔCq**	**FCE**	**ANOVA**
Control	46	18.511 ± 0.321	31.696 ± 5.576		0.0007	F = 1.6540
Group I (Opiate intake15-30 gram/month)	14	18.429 ± 0.231	32.335 ± 8.555	10.40		*p* = 0.1915
Group II (Opiate intake 150–450 gram/month)	9	18.491 ± 0.241	28.354 ± 10.767	8.79	0.0022	
Group III (Opiate intake 750-1000gram/month)	10	18.389 ± 0.199	32.870 ± 6.020	0.07	0.9532	
Group IV (Opiate intake 1500-6000gram /month)	15	18.478 ± 0.293	29.044 ± 2.095	-1.53	**2.89**	

FCE = Fold change expression; ANOVA = analysis of variance

Table 4
Correlation analysis of chronic opiate abusers at dose < 1000 gm/day (n = 29)

		Cq187	IL-10	IL-6	TNF-α	dependence (months)
Pearson Correlation	**Cq187**	1.000	− .214	− .095	.236	.053
	IL10	− .214	1.000	.160	.226	− .134
	IL6	− .095	.160	1.000	− .158	.134
	TNF-α	.236	.226	− .158	1.000	− .183
	dependence (months)	.053	− .134	.134	− .183	1.000
Sig. (1-tailed)	Cq187	.	.133	.313	.109	.392
	IL10	.133	.	.204	.120	.244
	IL6	.313	.204	.	.207	.245
	TNF-α	.109	.120	.207	.	.171
	dependence (months)	.392	.244	.245	.171	.
		Cq155	IL-10	IL-6	TNF-α	dependence (months)
Pearson Correlation	**Cq155**	1.000	− .019	.030	− .194	− .235
	IL10	− .019	1.000	.160	.226	− .134
	IL6	.030	.160	1.000	− .158	.134
	TNF-α	− .194	.226	− .158	1.000	− .183
	dependence (months)	− .235	− .134	.134	− .183	1.000
Sig. (1-tailed)	Cq155	.	.461	.439	.156	.110
	IL10	.461	.	.204	.120	.244
	IL6	.439	.204	.	.207	.245
	TNF-α	.156	.120	.207	.	.171
	dependence (months)	.110	.244	.245	.171	.

Table 5
Correlation analysis of chronic opiate abusers at dose > 1000 gm/day

		Cq155	**IL-10**	**IL-6**	**TNF-α**	**dependence (months)**
Pearson Correlation	**Cq155**	1.000	.537	− .506	− .375	.045
	IL10	**.537**	1.000	− .363	− .116	.109
	IL6	**− .506**	− .363	1.000	.213	− .097
	TNF-α	**− .375**	− .116	.213	1.000	− .166
	dependence (months)	.045	.109	− .097	− .166	1.000
Sig. (1-tailed)	**Cq155**	.	.009	.014	.057	.427
	IL10	**.009**	.	.063	.318	.328
	IL6	**.014**	.063	.	.191	.347
	TNF-α	.057	.318	.191	.	.248
	dependence months	.427	.328	.347	.248	.
		Cq155	IL-10	IL-6	TNF-α	dependence (months)
Pearson Correlation	**Cq187**	1.000	− .171	.277	.724	− .176
	IL10	− .171	1.000	− .363	− .116	.109
	IL6	.277	− .363	1.000	.213	− .097
	TNF-α	**0.724**	− .116	.213	1.000	− .166
	dependence months	− .176	.109	− .097	− .166	1.000
Sig. (1-tailed)	**Cq187**	.	.242	.125	.000	.235
	IL10	.242	.	.063	.318	.328
	IL6	.125	.063	.	.191	.347
	TNF-α	**< .0001**	.318	.191	.	.248
	dependence months	.235	.328	.347	.248	.

Table 6
Linear regression analysis of chronic opiate abusers at dose > 1000 gm/day

Model		Unstandardized Coefficients		Standardized coefficients	t	Sig.	95% CI for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
155-5p	(Constant)	12.897	12.950		.996	.336	-14.878	40.671
	IL-10	.749	.390	.401	1.918	.076	− .088	1.585
	IL-6	− .169	.116	− .309	-1.456	.168	− .418	.080
	TNF-α	− .027	.020	− .275	-1.368	.193	− .070	.015
	Dependence (months)	− .005	.013	− .075	− .377	.712	− .033	.023
187-5p	(Constant)	30.200	3.261		9.260	.000	23.206	37.195
	IL10	− .023	.098	− .046	− .237	.816	− .234	.187
	IL6	.016	.029	.109	.555	.588	− .046	.079
	TNF-α	.018	.005	.688	3.681	**.002**	.008	.029
	Dependence (months)	− .001	.003	− .046	− .251	.805	− .008	.006

Only those cases for which dose was > = 1000 gm/day were considered for this model, which was significant at $p < 0.020$