Original Article

Interleukin-33 and Soluble ST2 as Potential Biomarkers of Cancer in Opium Users: A Nested Case-Control Study

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What's Known

- Opium consumption increases the risk of developing various cancers.
- Interleukin-33 and soluble ST2 play critical roles in cancer development.

What's New

- Serum levels of Interleukin-33 are significantly higher and soluble ST2 levels are significantly lower in opium users than non-opium users.
- Opium users diagnosed with cancer exhibited significantly higher levels of Interleukin-33 and lower levels of soluble ST2 than the opium users, who were not diagnosed with cancer.

Abstract

Background: Opium abuse is one of the social hazards in the Middle Eastern countries. Opium consumption attributes to various malignancies. However, the exact molecular mechanism of this correlation still remains unclear. Cancer and inflammation are closely correlated. Interleukin-33 (IL-33) and its receptors, transmembrane ST2 (ST2L) and soluble ST2 (sST2), have been significantly associated with tumorigenicity. The present study aimed to investigate whether IL-33 and sST2 levels serve as cancer biomarkers in opium users.

Methods: Serum samples were collected from 100 opium users and 100 healthy non-opium users in a nested case-control design. The subjects with over five years of history of opium abuse were enrolled. To assess the incidence of malignancies, the opium users were followed up from 2014 to 2019. Serum levels of IL-33 and sST2 were measured using an ELISA kit. For comparison of IL-33 and sST2 levels between the groups, two-tailed Student's *t* test and Mann–Whitney U test were utilized, accordingly. Logistic regression analysis was performed to evaluate the influence of confounders on the incidence of cancer.

Results: During the five-year follow-up, eight opium users were diagnosed with cancer. Cancer was developed by 9.3 folds in the individuals abusing opium compared to that in the non-opium users (P=0.040, OR=9.3; 95%CI [1.1-79.4]). Serum levels of IL-33 were found to be significantly higher in the opium users than those in the healthy control group (P=0.001). The sST2 levels were significantly lower in the opium users (P=0.001). The opium users with cancer exhibited significantly higher levels of IL-33 and lower levels of sST2 than the cancer-free ones (P=0.001).

Conclusion: Decline in sST2 levels and rise in the level of IL-33 are valuable biomarkers in predicting cancers. Regarding the significant alterations in the levels of these biomarkers in the opium users, as well as those in the opium users diagnosed with cancer, IL-33 and sST2 may serve as potential biomarkers in the early prediction of cancer.

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Keywords ● Opium ● Opium dependence ● Interleukin-33 ● Neoplasms ● Inflammation ● Biomarkers ● Tumor

Introduction

Opium and opioid abuse are the major economic and health threats worldwide, especially in Asia and the Middle East.

The highest rate of addiction to opium in the world has been reported to belong to Iran. Opioid addiction census has been reported to be 2.8% of the population over the age of 15, which accounts for 1.12 million adults, while the world consumption rate is about 0.5%.[1] Among the health hazards of opium, the carcinogenic properties of this drug abuse have attracted a great deal of attention over the past decade. The association between opium use and several malignancies has been frequently reported.[2-4]

Opioids trigger numerous receptors in various organs. One of the systems that are affected by opioids is the immune system. There are three kinds of opioid receptors: μ, δ, and κ. The immune cells express many of these receptors. The immune function is regulated by the opioid peptides, which are secreted by the central nervous system. Furthermore, the opioid peptides produced by immunocytes may regulate the neuroendocrine system.[5] A previous study showed that subjects abusing opiates are more prone to various types of infection, since they confront weak innate immunity.[6] Since many years ago, it has been believed that opioids suppress immune responses. However, many reports have supported the multi-effect of opioids on the immune system, such as modulatory, suppressive, and binary influences. As immune function is vital for the body on account of its effect on inflammation, tumor cells, and infectious diseases, the evaluation of opioids effects on the immune responses is of great importance.[5]

Given the importance of inflammation in various cancers, numerous studies have been conducted on the role of different interleukins (ILs), especially IL-33 and ST2, in tumor genesis and metastasis. The family of IL-1 is a major part of the immune system. When inflammation occurs in the body, it is led by the family of IL-1 signaling pathway.[7, 8] IL-33 is a novel member of the IL-1 family. ST2 is known as a receptor for IL-33.[9] Soluble IL-33 binds to soluble ST2 (sST2) in the blood.[10] IL-33 stimulates the cells of innate immunity to produce and release Th_2 cytokines, such as IL-4, IL-5, and IL-13.[9] Additionally, IL-33 stimulates Th_1 immune responses by inducing the release of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α).[11] Moreover, IL-33 is engaged in the maturation of dendritic and mast cells.[12] It is also involved in controlling the accumulation of regulatory T cell, which is mediated by activation of the lymphoid cells.[13, 14] Thus, IL-33 is crucial to both innate and adaptive immune responses.[15] It has the potential to activate the immune system under certain pathophysiological conditions, in addition to a dual role of both anti-inflammatory and pro-inflammatory in certain disorders.[16] The increased circulating IL-33 levels observed in gastric and lung cancer patients may be related to cancer progression through the ability of IL-33 to promote Th2 immune responses, thereby creating the microenvironment for tumor growth and progression.[17, 18] Elevated levels of IL-33 were observed in patients with gastric and lung cancer as well as hepatocellular carcinoma.[19]

In this study, we assessed the effect of opium on serum levels of IL-33 and sST2 in order to speculate on the possible carcinogenic mechanism of opium.

Patients and Methods

The protocol of this study was reviewed and approved by the Ethics Committee of Shiraz University of Medical Sciences under the ethical code IR.SUMS.REC.1397.178. Each patient signed an informed consent form approved by the local institutional Review Board.

Collection of Human Blood Samples

Whole blood samples were obtained from the Fasa Cohort Study (FACS), which is a population-based cohort study enrolling 10157 participants within the age range of 35 to 70 in a region in southern Iran with high rates of opium use (24.1%). The details of the enrollment protocol and the cohort profile are described elsewhere.[20-22] In summary, a complete history of medical conditions and physical examination of the participants was registered and a battery of laboratory tests at the enrollment time was obtained. The validated questionnaires, inquiring about physical activity, nutrition, social life, and other aspects of the participants' daily activities, were filled by trained personnel at the time of enrollment. Biobanks consisting of whole blood and serum of all the participants were stored in cold rooms. The participants were being followed regularly for 10 years.[22, 23] The daily use of different types of opium products was asked and registered per a validated questionnaire in FACS.[21] The samples were maintained at -70 ˚C in freezer. In a nested case-control design, 100 regular opium users were selected based on their opium use for over one year.

The subjects with over five years of history of opium abuse were enrolled in this study and followed up from 2014 to 2019. Opium users abused only opium and no other addictive substances. The individuals abusing opium with a history of any type of malignancy or those abusing substances besides opium were excluded from the study.

Herein, 100 controls were enrolled. The subjects' characteristics were adjusted for their demographic data, such as sex, age, and place of residence, as well as their anthropometric properties. Moreover, history of illnesses (heart diseases, stroke, diabetes mellitus, different malignancies, and endocrine diseases) were adjusted between the groups and were extracted from the same cohort (FACS). Blood samples were extracted from the biobank and defrosted for biochemical analyses.

Serum Detection of IL-33 and sST2

IL-33 and sST2 were measured via commercial ELISA kit (IL-33 human ELISA kit (ab119547-IL-33, Abcam, USA, and human sST2 ELISA kit, Elabscience, USA) according to the manufacturer's instructions, as described previously.[24] Measurement of both sST2 and IL-33 was carried out based on the Sandwich-ELISA principle. For serum sST2 assessment, after adding the samples and standards to the micro ELISA plate wells that were pre-coated with Human sST2 antibody and combined with the specific antibody, Avidin-Horseradish Peroxidase (HRP) was conjugated and a biotinylated detection antibody were added successively and then were incubated. Afterward, the substrate solution was added to each well. The wells containing Human sST2, biotinylated detection antibody, and Avidin-HRP conjugate were blue in color. Stop solution was added to each well to terminate the enzyme-substrate reaction. The optical density (OD) of the produced yellow color was measured with a UV spectrophotometer at a wavelength of 450 nm.

For the IL-33 assay, after adding the samples and standards to each well pre-coated with IL-33 specific antibodies, the plates were incubated at the room temperature. A Biotin-conjugated anti-Human IL-33 detection antibody was added to each plate after washing them; the plates were incubated once again at the room temperature. Subsequently, Streptavidin-HRP conjugate was added to each well and incubated at the room temperature. Ultimately, TMB was added to each well in order to be catalyzed with HRP, so that we could obtain a product in blue color. The addition of the acidic stop solution changed its color into yellow, which was detected at a wavelength of 450 nm read with a UV-spectrophotometer.

Statistical Analysis

SPSS® 21.0 for windows® was utilized for data analysis (SPSS Inc., Chicago, Illinois). Continuous variables are presented as mean±SD. The normality of the data was analyzed using the Kolmogorov-Smirnov test. IL-33 serum values passed Kolmogorov–Smirnov normality test and a two-tailed Student's *t* test was employed to compare this parameter between the groups. Since serum values of sST2 did not pass Kolmogorov–Smirnov normality test, Mann–Whitney U test was used to compare the level of sST2 between the groups. For the comparison of categorical parameters, Chi square test was used, with Yates or Fisher's corrections applied when needed. Moreover, logistic regression analysis was performed to assess the influence of confounders on the incidence of cancer. P values<0.05 were considered to be statistically significant.

Results

The demographic data of the enrolled subjects are demonstrated in table 1. No significant differences were observed between the two study groups regarding demographic and anthropometric or disease states.

Table 1: Demographic characteristics of the enrolled participants

Variables		Opium users (N=100)	Non-opium users (N=100)	P value
Sex	Male	77	71	0.42
	Female	23	29	
Age (year, mean±SD)		52.6±9.7	51.3±10.5	0.36
BMI (Kg/m^2, mean±SD)		22.4±3.5	23.1±2.6	0.11
Marital status	Married	63	66	0.66
	Single	37	34	
Alcohol consumption	Frequently	53	58	0.48
	Never	47	42	
Smokers		33	30	0.65
History of diabetes		13	11	0.66
History of stroke		5	3	0.47*
Endocrine diseases		9	5	0.27*

Student's *t* test was used to compare continuous variables. Chi square test was used to compare categorical values. *Fisher's exact test was used; P value<0.05 was considered to be statistically significant.

Table 2: Serum levels of IL-33 and sST2 in the non-opium users and opium users

Variable	Non-opium users (N=100) mean±SD	Opium users (N=100) mean±SD	P value
IL-33 (pg/mL)	2.67±0.33	3.43±0.44	0.001
sST2 (pg/mL)	33.50±7.92	11.50±2.34	0.001

Student's t-test was used to compare IL-33. Mann–Whitney U test was used to compare the level of sST2 between the groups. P value<0.05 was considered to be statistically significant.

Table 3: Serum levels of IL-33 and sST2 in the cancer-free opium users (Control) and opium users with cancer (Case)

Variable	Control (N=92) mean±SD	Case (N=8) mean±SD	P value
IL-33 (pg/mL)	3.16±0.26	3.9±0.22	0.001
sST2 (pg/mL)	27.08±20.57	412.44±220.04	0.001

Student's *t* test was used to compare IL-33. Mann–Whitney U test was used to compare the level of sST2 between the groups. P value<0.05 was considered to be statistically significant.

The factors influencing serum concentrations of IL-33 and sST2, such as alcohol consumption, cigarette smoking, diabetes mellitus, endocrine and autoimmune diseases, inflammatory diseases, like inflammatory bowel disease, and history of stroke did not vary significantly between the two study groups. Among the 200 studied individuals (100 non-opium users and 100 opium users), eight opium users were diagnosed with cancer through a five-year follow-up. Only one non-opium user was gotten cancer during this period. Using logistic regression analysis, the incidence rate was 9.3 folds higher in opium users (P=0.040, RR=9.3; 95%CI=1.1-79.4). The malignancies diagnosed in the opium users were as follows: three individuals were diagnosed with bladder cancer, two developed laryngeal cancer, one developed esophageal cancer, one was diagnosed with gastric cancer, and one with lung cancer. Hodgkin's lymphoma was diagnosed in one non-opium user. The demographic data of the subjects diagnosed with cancer were described previously.[25]

As shown in table 2, the serum level of IL-33 was significantly higher in the opium users than the healthy controls (P=0.001). Concerning the serum level of sST2, it was significantly lower in the subjects addicted to opium than that in the healthy volunteers (P=0.001). According to tables 2 and 3, the opium users diagnosed with cancer exhibited significantly higher serum concentrations of IL-33 and lower serum concentrations of sST2 than the cancer-free opium users (P=0.001).

Discussion

Our results revealed a significant increase in IL-33 serum concentration along with a remarkable decrease in sST2 serum concentration in the opium users than the control group. Additionally, higher levels of IL-33 and lower levels of sST2 were observed in the opium users diagnosed with cancer than those who were cancer-free.

Over the last decade, evidence has clearly indicated that inflammation plays a crucial role in tumor genesis, and that an inflammatory microenvironment is a vital element for the growth of all tumors;[26] however, the exact causal molecular mechanisms still remain elusive.[27]

Among the health hazards of opium, the carcinogenic properties of this drug abuse have attracted a lot of attention over the past decade. Yet, the molecular mechanism of the relationship between opioids and cancer is obscure. Emphasizing the role of opioid receptors in cancer, the expression of μ-opioid receptor genes (MOR) has been observed in human lung, colon, and prostate cells leading to tumor growth and metastasis.[28-30] As questioned by Vallejo and colleagues, a clinically relevant dilemma is whether the immunosuppressive property of opioids, such as opium, is effective in the development of cancer. Opioids may modulate both cell-mediated and humoral immune responses.[31] There is a great body of evidence suggesting an increased expression of opioid receptors on lymphocytes and mononuclear phagocytes, which may modulate immune responses.[32, 33] On the other hand, exogenous opioids affect receptor expression and secretion of inflammatory cytokines.[31]

IL-33, a member of the IL-1 family, is an important mediator for the innate immune pathway, signaling Th-2 responses. IL-33 seems to have a double-action. Firstly, as a normal cytokine, it makes a complex by binding to its transmembrane receptor, ST2L; and secondly, as an intracellular nuclear factor, it can regulate gene transcription.[26] Nonetheless, the soluble form of ST2 receptor, sST2, seems not to have a role in IL-33 signaling. It binds to IL-33 and inhibits its binding to ST2L. IL-33 is thought to function as an "alarmin" released after cell

necrosis alerting the immune system of tissue damage or stress. It is clear that the strong stimulatory effects of IL-33 on some immune cell types are likely to influence inflammatory diseases.[34] However, the precise role of IL-33 and IL-33/ST2 signaling pathways in cancer is still not well understood.

Elevated serum levels of IL-33 have been demonstrated in several types of cancer. Activation of ST2 receptor by IL-33 stimulates the growth and metastasis of various cancers,[35-37] and inhibits the anti-tumor immunity.[38] Therefore, it has been proposed as an important effector in tumor progression. By binding to the sST2 receptor, IL-33 activates the ST2–ERK1/2 pathway and consequently upregulates the production of two inflammatory mediators, namely MMP-3 and IL-6, which may contribute to the pathophysiologic effects of IL-33 in gastric cancer.[39] Interestingly, *in vitro* data has demonstrated that knocking down IL-33 decreased the invasion and metastatic capacity in esophageal cancer cells, while overexpression of IL-33 showed a contradictory effect.[40] Regarding the signaling of IL-33/sST2 in cancer, IL-33 recruits IL-1R-associated kinase 1 and 4 to the receptor complex in the cytoplasmic region of ST2 by binding to the IL-33 receptor, triggering several signaling downstream molecules, such as NF-κB. This process enhances the expression of different proteins, which could lead to inflammation and promote metastasis.[41, 42]

Furthermore, the over-expression of IL-33 causes the production of the immature dendritic cell. These cells are responsible for the generation of T_{reg} and thus, tumor progression and metastasis.[38]

It has been stated that ST2 deficiency leads to an increase in pro-inflammatory cytokines, such as TNF-α and IL-17,[38] which are believed to play a pivotal role in cancer promotion and progression.[43, 44]

The ability of IL-33 to activate the cells of both the innate and adaptive immune system makes IL-33 an important cytokine in the initiation and spread of inflammation.[45] Given the significant role of IL-33 in tumor progression and metastasis,[17, 46] this cytokine could be a good candidate as a prognostic biomarker. Since in the course of inflammation, sST2 binds to IL-33 in the blood, the harmful effects of IL-33 in the body are reduced.[47, 48] Therefore, the obtained results herein, showing lower levels of sST2 and as a result, higher free serum levels of IL-33 in the opium users may propose a potential biomarker property for IL-33 and sST2 in the early detection of cancer in opium users. For note, the major limitation of our study is the small sample size. It could be suggested that the signaling of IL-33/sST2 be studied in detail in opium users in future research.

Conclusion

Our findings suggested a predictive value of serum IL-33/sST2 levels in opium users for the development of cancer. It may be postulated that manipulating the IL-33/sST2 pathway has preventive and/or therapeutic implications in cancers, especially in opium users.

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Authors' Contribution

N.F: conception and design of the work, acquisition, analysis and interpretation of data for the work, drafting and revising the manuscript critically for important intellectual content; K.J: Acquisition, analysis and interpretation of data for the work; drafting and revising the manuscript critically for important intellectual content; A.D: Interpretation of data for the work; revising the manuscript critically for important intellectual content; All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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