A Case-Control Study of Comparison of Plasma Levels of Matrix Metalloproteinase-9 in Inhaled Opium Addicts and Clinically Healthy Persons

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Original Article

Abstract

Background: Metalloproteinase-9 exists in the basement membrane of normal tissues and is a major factor in cancer invasion and lymphatic metastasis. Smoking has been reported to increase the metalloproteinase level, but the role of opium consumption in metalloproteinase level has not yet been examined. The current research intended to examine the impacts of opium consumption on the serum levels of metalloproteinase.

Methods: This case-control research was conducted in Kerman (in the southeast of Iran), after getting medical approve by the ethics committee. Case group of 33 non-smokers with no active inflammatory diseases who had the experience of inhaled opium and its derivatives were compared with a control group of 40 non-smokers with no active inflammatory disease and no experience of inhaled opium and its derivatives. Student's t-test, mean, and chi-square test were employed to determine the correlation between the variables.

Findings: No statistically meaningful variation was detected in plasma metalloproteinase concentration between the case and control groups (P = 0.160). Also, there was no significant relation between the plasma metalloproteinase concentration and urinary morphine in case groups (P = 0.410), but a statistically significant correlation was found between gender and metalloproteinase in both the case and control groups (P = 0.003).

Conclusion: According to the possible role of opioid drugs directly on cancer and the prognosis metalloproteinase and cancer, further studies are suggested.

Keywords: Metalloproteinase; Opium; Case-control studies; Iran

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Introduction

Extracellular matrix metalloproteinases (MMPs) are metal-dependent end peptidases involved in proteolysis' activities for which they need zinc ions. MMPs are a major class of enzymes related to the degradation of extracellular matrix elements. The MMP family is classified into six subgroups, including stromelysins, matrilysin, gelatinizes, collagenases, membrane and other MMPs.[1,2] Twenty-four types of MMPs have been so far identified, 23 of which are found in humans.

MMPs are involved in different physiological and pathophysiological processes, such as growth and differentiation of the embryo and fetus, ovulation, wound healing, heart failure, arthritis, atherosclerosis, diseases of the teeth, bone remodeling, cancers, etc. Evidence suggests that MMPs are involved in all stages of cancer progression, including proliferation, apoptosis, and angiogenesis.[3] However, some studies have reported a relationship between metalloproteinase and diagnosis and poor prognosis of some cancers, such as lung, breast, gastric cancers, and melanoma. In some other studies, MMPs have been recognized to be effective in developing new treatments for cancers.[4]

So far, some researches were carried out to evaluate the impacts of tobacco products on cancer. They showed the serum level of metalloproteinase in smokers, diabetes mellitus, hypertension, infectious, and inflammatory diseases, and any cancer type, as well as those who had the experience of using steroids and non-steroid anti-inflammatory drugs, smoking, and oral use of opium.

Methods

A fasting sample of 5 ml blood was taken from each person in both groups to determine the serum level of matrix metalloproteinase-9 (MMP-9). Then, the samples were sent to a laboratory for liver function tests, fasting blood sugar, blood urea nitrogen (BUN) levels, and erythrocyte sedimentation rate (ESR). The levels of MMP-9 in patients were measured according to the human MMP-9 Enzyme-linked immunosorbent assay (ELISA) Kit Protocol. Values below the cut-off point of 900 ng/ml were considered negative, and greater than or equal to it were found to be positive. In addition to taking blood samples from patients addicted to opium by

inhalation, their urinary morphine levels were measured and according to the cut-off point of 2000 ng/ml, values between 0-1999 were considered negative, and greater than or equal to 2000 were considered positive. The present research was confirmed by the ethics committee of Kerman University of Medical Sciences, Iran. Oral informed consent was obtained from the participants and the tests were carried out.

The standard deviation, mean, and frequency were employed in both groups to express the qualitative and quantitative parameters. The chi-square test and Student's t-test were employed to evaluate the qualitative parameters among the groups and to compare the quantitative variables, respectively. The data were analyzed using Stata Statistical Software (Release 11.0, Stata Corporation, College Station, TX, USA). The P-value less than 0.05 was considered statistically significant.

Results

In the current research, there were 25 males (56.8%) and eight females (27.6%) in the case group and 19 men (43.1%) and 21 women (72.4%) in the control group. The average age was 47.2 and 44.6 years in the case and control groups respectively. Fourteen people (42.4%) in the case group and 16 people (40.0%) in the control group were ≤ 40 years of age. In the 41-60 age groups, there were 14 and 19 people (42.4% and 47.5%) in the case and control groups, respectively. In the age group over 60 years, five people (15.2%) were in the case group and 27 (67.5%) in the control group. The average serum levels of MMP-9 were 905 in the case group and 1481 in the control group. Six subjects of the case group (18.2%) and 13 of the control group (32.5%) were positive for serum levels of MMP-9. Twenty-seven subjects of the control group (81.8%) and 27 of the case group (67.5%) were negative for serum levels of MMP-9.

Statistical analysis indicated no difference in serum levels of MMP-9 among the case and the control groups (P = 0.14) (Table 1). Although a positive correlation was found between serum level of morphine and MMP-9 (Pearson χ^2 = 0.67), this association was not statistically significant (P = 0.410). There was a positive correlation between the gender and level of MMP-9 (P = 0.040), and the positive probability of the serum levels of MMP-9 was six times higher in female (Pearson χ^2 = 0.603) (Table 2).

Table 1. Demographic data of the case and control groups

Variables	Control group	Case group	P[*]
Gender [n (%)]			0.010
Male	19 (43.2)	25 (56.8)	
Female	21 (72.4)	8 (27.6)	
Age (year) (mean ±SD)	44.6 (1.93)	47.2 (2.47)	0.890
< 40	16 (40.0)	14 (42.4)	
41-60	19 (47.5)	14 (42.4)	
> 60	5 (12.5)	5 (15.2)	
MMP-9 (ng/ml) (mean ±SD)	1482 (396)	905 (237)	0.160
Positive	13 (32.5)	6 (18.2)	
Negative	27 (67.5)	27 (81.8)	

[*]Level of significance < 0.05
MMP-9: Matrix metalloproteinase-9; SD: Standard deviation

Table 2. The relationship of metalloproteinase with urinary morphine and gender

Variables	Pearson χ^2	P[*]
Morphine	0.67	0.41
Gender	0.603	0.003

[*]Level of significance < 0.05

Discussion

The current research was conducted to evaluate the impacts of opium consumption on the serum levels of MMP-9. The study of risk factors, early diagnosis, and cancer treatment methods have led researchers worldwide to apply appropriate strategies for reducing mortality caused by this disease and enhancing patient's quality of life by reducing risk factors, early diagnosis, and implementing an appropriate treatment method.

Lymphatic metastasis is a factor involved in the determination of the type of treatment and prognosis of cancer patients that reduces the patient survival by 50%.[5] The MMP-9 that exists in the basement membrane in normal tissues[6] is a major factor in cancer invasion and lymph node metastasis. Changes in the extracellular matrix and destruction of intermolecular barriers and increased secretion of the enzyme which leads to destruction of cell barriers can increase cancer metastases.

In this study, no significant variation was observed in the serum levels of MMP-9 among the opium users and the control group. This finding, at least in this study, suggests that the use of opioid drugs has no impact on the serum levels of MMP-9. As far as we know, no similar study has been performed to evaluate the impact of opium on the MMP-9. Torii et al. studied the relationship between MMP-9 and gastric cancer and reported that MMP-9 in human plasma has 96 percent

specificity and 56 percent sensitivity in gastric cancer evaluation.[7] So far, some studies have been conducted on the relationship between opioid drugs and its derivatives and the incidence of cancers, such as esophageal, bladder, laryngeal, and recently gastric cancers, but in most of them, the effects of age, gender, and other main confounding variables have not been well controlled. This study had a small sample size, and it may be the reason why the correlation between MMP and the use of opioid drugs was not statistically significant.

Smoking is one of the main factors that can increase MMP-9.[8] In a research performed by Lim et al.,[9] the balance among MMP-9 and tissue inhibitor of metlloproteinase-1 was examined in smokers, and it was revealed that their levels were higher in smokers that may lead to the destruction of lung tissue and eventually, emphysema in these people.

In a research carried out by Xu et al., the impacts of nicotine on differentiation, oxidation, bacterial killing, and secretion of MMP-9 by neutrophils was investigated. The results showed that nicotine increased the number of cells in the last phases of differentiation (metamyelocytes and segmented neutrophils) and in the neutrophils that were exposed to nicotine, oxidation and bacterial killing was reduced and the secretion of MMP-9 was increased.[10]

No studies have carried out so far to evaluate the impact of opium as an independent factor on the markers associated with cancer. However, according to a study conducted on 50000 Iranians in Golestan Province, Iran,[11] to examine the effect of opium on mortality, it was shown that the opium addicts had a higher risk of mortality compared

with the control group (hazard ratio: 1.81).

Masjedi et al.[12] also examined the effect of opium as an independent parameter for lung cancer and found that opium increases the danger of lung cancer to an odds ratio of 3.1. Naghibzadeh Tahami et al.[13] conducted research on the upper gastrointestinal cancers (stomach, esophagus, liver, and pancreas) in Kerman. They indicated that the use of opium and its derivatives increased the risk of these cancers by four times, and the increased use of opioid drugs had a direct correlation with the risk of these cancers.[13]

All studies have shown that the growing use of opioid drugs increase the risk of cancers, and the repeatability of the results confirms causality. Therefore, according to studies, it seems that opium consumption can increase mortality rate and the risk of cancers, especially lung cancer. In the present research, a positive correlation was detected among the consumption of opioid drugs and the increased level of MMP-9, as a predictive factor for diagnosing cancers and the lymphatic metastases. However, probably due to the small sample size, this correlation was not statistically significant. Studies on the effect of opium on the markers associated with cancer are not available, but some studies have been conducted on markers that are related to cancer. For example, a survey carried out on 101 patients to examine the effect of focal adhesion kinase (FAK) and E-cadherins in cervical metastases in supraglottic cancer, and it was found that the decreased E-cadherin and increased FAK were associated with a higher risk of metastasis.[14]

Schneider et al. [15] conducted a study to evaluate the impact of FAK e on the invasion of tumor cells of the oral cavity, and they concluded that FAK expression was higher in squamous cell carcinoma (SCC25) cells which are more invasive than SCC15 cells.

Field et al.[16] conducted a study to examine the effect of p53 on cases with head and neck SCC who had the history of smoking. They found that p53 was increased in 67% of 73 patients studied. Also, p53 was more positive in those who were heavy smokers.

Christopoulos et al.[17] found that in laryngeal SCC, as the stage of cancer advances, the CD44 hyaluronic receptor is expressed more than the hyaluronidase.

Hashimoto et al.[18] investigated the link between MMP-1 and MMP-3 and the head and neck SCC. There was a significant positive relation between the serum levels of metalloproteinase and prognosis of cancer and all have indicated that the serum level of metalloproteinase is a good predictive factor for the course of the cancer. As mentioned, some studies have recently reported the relationship between the use of opium and its derivatives and the diffusion of cancer.

Conclusion

In our study, no significant difference was observed in plasma concentrations of MMP-9 between addicts and healthy controls. Also, no correlation was found between the urinary morphine measured and MMP-9. Further studies are required in this regard with a larger sample size and stronger methodology such as prospective studies, and also other markers are recommended to be examined, including FAK, E-cadherin, CD44, hyaluronidase, Test lamp-Multipurpose (TL-MP), membrane type-1 MMP, MMP-1, etc.

Conflict of Interests

The Authors have no conflict of interest.

Acknowledgements

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مطالعه مورد– شاهدی سطح پلاسمایی ماتریکس متالوپروتئیناز–۹ (MMP-9) در افراد معتاد به تریاک استنشاقی و افراد سالم

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مقاله پژوهشی

چکیده

مقدمه: متالوپروتئیناز–۹ در غشای پایه بافت‌های طبیعی وجود دارد و عامل اصلی در تهاجم سرطان و متاستاز لنفاوی به شمار می‌رود. تاکنون نقش مصرف سیگار در افزایش میزان متالوپروتئیناز مشخص شده، اما نقش مصرف اپیوم بر روی میزان متالوپروتئیناز بررسی نشده است. مطالعه حاضر جهت بررسی تأثیر مصرف اپیوم بر روی میزان متالوپروتئیناز سرمی صورت گرفت.

روش‌ها: در این مطالعه مورد– شاهدی که در شهر کرمان (واقع در جنوب شرقی ایران) انجام گردید، گروه مورد شامل ۳۳ نفر فرد غیر سیگاری و بدون بیماری فعال التهابی و دارای سابقه مصرف استنشاقی اپیوم و مشتقات آن، با ۴۰ نفر گروه شاهد غیر سیگاری و بدون بیماری فعال التهابی که سابقه مصرف استنشاقی اپیوم و مشتقات آن را نداشتند، مقایسه شدند. با استفاده از آزمون‌های t و χ^2، میانگین و نسبت بین متغیرها مورد بررسی قرار گرفت.

یافته‌ها: بین نسبت غلظت پلاسمایی متالوپروتئیناز در دو گروه مورد و شاهد هیچ‌گونه تفاوت معنی‌داری وجود نداشت (P = ۰/۱۶۰). همچنین، ارتباط معنی‌داری بین نسبت غلظت پلاسمایی متالوپروتئیناز و مورفین ادرار در دو گروه مورد و شاهد مشاهده نشد (P = ۰/۴۱۰). از طرف دیگر، ارتباط بین جنسیت و متالوپروتئیناز در دو گروه مورد و شاهد معنی‌دار بود (P = ۰/۰۰۳).

نتیجه‌گیری: با توجه به نقش احتمالی مواد اپیوئیدی بر روی سرطان و ارتباط مستقیم متالوپروتئیناز با پیش‌آگهی و پیشرفت سرطان‌ها، انجام مطالعات بیشتر پیشنهاد می‌شود.

واژگان کلیدی: متالوپروتئیناز، اپیوم، مطالعات مورد– شاهدی، ایران

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