Original Article

Serum level of substance P in patients with lung injuries due to sulfur mustard

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Abstract

Background: Chronic bronchiolitis is the most important problems of chemical victims of mustard gas. Diverse studies suggest that substance P (SP) as a member of tachykinin neuropeptides, has a significant role in the neurogenic inflammation processes of the airways and lungs. We aimed to determine the serum level of SP in chemical victims of mustard gas and compare it with normal subjects.

Materials and Methods: The chemical victims were divided into the 2 groups of 30:A group with mild to moderate pulmonary symptoms and other group with moderate to severe symptoms and compared with 3rd group as healthy controls. After preparing our samples and using the SP kit, final analysis was performed with enzyme-linked immunosorbent assay reader.

Results: The Concentration of circulatory SP levels in the chemical patients was 2.86 ± 1.47 ng/ml and had not a significant difference with the control group (3.15 ± 1.03 ng/ml) ($P > 0.05$). The circulatory SP levels were 2.48 ± 0.92 ng/ml and 3.28 ± 1.73 ng/ml in patients with moderate to severe symptoms and mild to moderates ($P < 0.05$) respectively.

Conclusion: The SP may have a role in pulmonary complications of mustard gas. The lower level of SP in the moderate to severe patients may be due to corticosteroid consumption in such severe cases. However, further studies are needed to clarify the roles and mechanism of SP in this setting.

Key Words: Lung injuries, substance P, sulfur mustard

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INTRODUCTION

Tachykinins are a group of neuropeptides, which regulate diverse biological responses, some of these responses contribute to pulmonary diseases such as chronic obstructive pulmonary disease (COPD), asthma, and bronchitis.[1] Substance P (SP), a peptide with 11 amino acid units that is a member of the family of tachykinin neuropeptides.[2,3] Afferent nerve fibers were the main source of SP for years because it acts as a sensory neurotransmitter. However, in recent years, it has become evident from various studies that immune cells are a major source of tachykinins, especially SP.[3-5] One of the significant functions of SP is a special effect on broncho-constriction induction.[6] Other actions of SP are mast cells degranulation,[4] chemotaxis and neutrophils adhesion especially to

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bronchial epithelial cells.[7] All these actions indicate the inflammatory potency of SP, which is as a mediator of neurogenic inflammation.[8] Moreover, the ability of SP in activation of fibroblasts and smooth muscles of airways, testifies the role of SP in remodeling of airway during chronic inflammatory processes such as COPD, bronchitis, and asthma.[2] Vasodilatation, increasing of vascular permeability, transmission of pain impulses, regulation of behavioral disorders, nausea, and vomiting are the other actions of SP.[9,10]

SP performs its biologic actions by stimulation of natural killer (NK)-1 receptor, a G-protein coupled receptor for SP bearing seven transmembrane domains. Binding of SP to NK-1 R triggers a signal transduction cascade by producing diacylglycerol and inositol triphosphate. These elements activate protein kinase C and lead to release of intracellular calcium. NK-1 R also activates the release of arachidonic acid, a compound for production of prostanoid mediators. These mediators elicit the manifestations of inflammation.[2]

There are evidences of the role of neurogenic inflammation in airways by mediation of SP and the changes of its serum and sputum levels.[11] Similar evidences exist in asthma and chronic bronchitis.[6] Furthermore, SP and some other neuropeptides effect on pulmonary alveolar macrophages, which indicates to the importance of SP in chronic inflammatory processes.[5]

Chronic pulmonary complications are the most complaint of the chemical victims of mustard gas who were exposed to sulfur mustard by Iraqis during Iran-Iraq war.[12] In a study in guinea-pigs was shown that sulfur mustard (SM) intoxication induces airway muscle hyper responsiveness to SP by reducing tracheal epithelial neutral endopeptidase activity and that glucocorticoids might inhibit this hyper responsiveness by increasing this activity.[13] Few previous studies are on skin lesions[14] and response to SP in animal fields. However, in human, the main pulmonary consequence of mustard gas is isolated bronchiolitis obliterans,[15-18] and the serum level of SP in such disorder has not been evaluated. For the first time, we aimed to study the relation between SP serum level and bronchiolitis obliterans in chemical victims.

MATERIALS AND METHODS

We carried a historical cohort study on the chemical victims of mustard gas with pulmonary symptoms after taking a written agreement from them in Baqiyatallah hospital.

Based on the "Global Initiative for Chronic Lung Diseases" guidelines, disease severity was categorized into mild, moderate and severe categories.[19] We divided our cases into 3 groups of 30; the group 1 with mild to moderate pulmonary symptoms, the group 2 with moderate to severe pulmonary symptoms and the group 3 with normal subjects as a control group.

The inclusion criteria were as follows: History of exposure to mustard gas, pulmonary problems in physical exam and pulmonary function testing (PFT), Age range is 20-70 years, voluntary participation. The study design was approved by Ethic board of Baqiyatallah University of Medical Sciences, Tehran, Iran. Written consent form was obtained from all participants.

The bronchogenic carcinoma, pneumonia or bacterial bronchitis in the recent month, cigarette smoking and addiction to other opioids were considered as exclusion criteria.

After routine physical examination and pulmonary function test, blood samples were taken with heparinized syringe and then centrifuged them.

Blood samples were centrifuged within 30 min at 4°C at 3,000 g for 10 min. Serum samples were stored at –80°C in the 1 ml tube until performance of the assay. SP level was measured by competitive enzyme immunoassay (SP Enzyme Immunoassay kit; Cayman Chemical; Ann Arbor, MI) with a working range of 3.9-500 pg/ml, typically with an IC_{50} (50% B/B_0) of ~30 pg/ml and IC_{80} (80% B/B_0) of ~8 pg/ml. The analysis was completed with enzyme-linked immunosorbent assay reader in the wave length of 405 nm (with reference of 620 nm).

All our data were analyzed by using the SPSS software version 13.

RESULTS

Altogether, 60 chemical patients, which 30 of them had mild to moderate pulmonary symptoms and 30 of them had moderate to severe pulmonary symptoms, and also 30 people as a control group was studied.

The mean ± SD of SP concentration was 2.86 ± 1.47 and 3.15 ± 1.03 pg/ml in chemical exposed patients and the control group, respectively. There was no significant difference concerning SP concentration in the chemical exposed patients and the control group [$P = 0.38$; Figure 1].

Furthermore, the study of chemical exposed patients with moderate to severe symptoms revealed that SP

mean concentration (2.48 ± 0.92 pg/ml) was significantly lower than serum concentration in patients with mild to moderate symptom (3.28 ± 1.73 pg/ml) and the control group (3.15 ± 1.03 pg/ml). (P = 0.003). The Figure 2 shows the differences.

DISCUSSION

We found that the mean serum level of SP in the chemical victims had never significant difference with the control group. However, the mean serum level of SP in moderate to severe patients was significantly lower than mild to moderate patients, but there was no significant difference between the mild to moderate patients and the control group.

An animal study has been shown that elevation of SP circulatory level has a vital role in the inflammation of the lung, which was induced by hydrogen sulfide.[20] Other animal study revealed that SP and some other neuropeptides have a role in the mucus secretion and bronchial inflammation of sulfur-Dioxide induced airways lesions in rats.[21] Tian *et al*. found significant elevation of SP concentration in sputum and serum of COPD patients in comparison with normal subjects. The main point in this study was that patients have never used any drugs during the research process.[11]

Furthermore, it was shown that elevation of SP and another tachiykinin named NK-A in bronchoalveolar lavage fluid can make hyper stimulation of airways in the inflammation process.[20] Accordingly, NK-A, a member of tachykinin family, could be considered as another important target in the future studies of chemical victims. Other studies have been demonstrated significant increased SP levels in induced-sputum of no-smoker asthmatic patients and also in the chronic bronchitis patients in comparison with normal ones.[2,22,23]

Grissell *et al*. showed that reduced SP gene expression is associated with bacterial colonization in the kid's airways, which indicates the anti-bacterial role of SP.[24] De Vries *et al*. study on the guinea-pigs revealed that SP is a notable agent in the induction of airways hyper responsiveness in the inflammation process of asthma.[25] The latter animal study suggests that SP likely makes this effect by mediation of nerve growth factor (NGF). The NGF can be a new target for future studies on these victims.

The last point is that devastation of SP – producing sensory fibers by "capsaicin" – the irritant substance in hot pepper – can significantly inhibit harmful effects of cigarette smoke on the lung tissues.[26]

However, our moderate to severe patients were treated with corticosteroids during the study and it was ethically impossible to avoid drugs consumption. As a result, the anti-inflammatory effects of corticosteroid drugs could lead to decline SP serum level in these patients.[23] In a study by Semple *et al*. on 197 infants admitted to hospital with human respiratory syncytial virus bronchiolitis they found that severe bronchiolitis in infants was associated with reduced airway interferon gamma and SP.[27] Consequently, lower SP levels can explain our finding in these patients because one of the main complications of chemical victims is severe bronchiolitis.[12-14, 28] In addition, it can be assumed that mustard gas exposure could induce some mediators that ablate sensory fibers, which produce SP in lung tissue.

CONCLUSION

There were not considerable differences in SP levels between chemical exposed patients and healthy controls. The exact mechanism(s) is not clear; however, corticosteroids consumption may cause lower SP

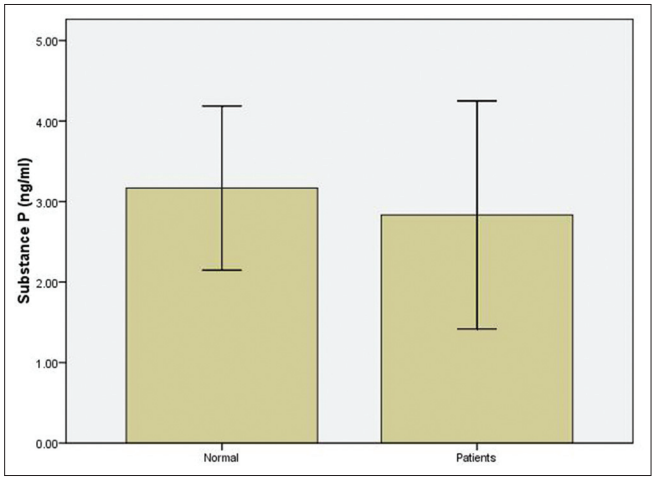

Figure 1: The serum levels of substance P in chemical exposed patients and healthy subjects

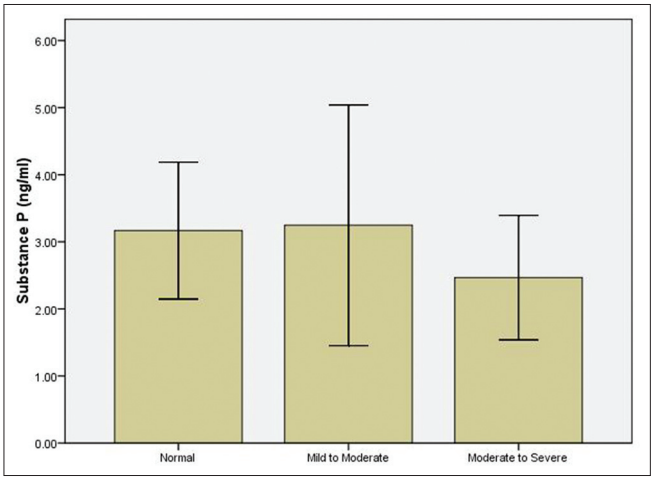

Figure 2: The circulatory levels of substance P in the mild to moderate, moderate to severe patients and control group

concentration in victims with severe symptoms than the mild ones. Evaluation of this hypothesis will need to broad human and also animal studies in the future.

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