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ORIGINAL ARTICLE

Study the level of sputum matrix metalloproteinase-9 and tissue inhibitor metaloprotienase-1 in patients with interstitial lung diseases

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KEYWORDS

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Abstract *Background:* Pulmonary fibrosis, the final result of a large variety of interstitial lung diseases, is characterized by an aberrant remodeling of extracellular matrix (ECM) with a profound disturbance of the normal lung architecture. This remodeling includes the exaggerated accumulation of ECM components in the interstitial and alveolar spaces and the disruption of the basement membranes. It has long been accepted that MMPs play an important role in the pathogenesis of pulmonary fibrosis, but the exact mechanisms are not well characterized. There are several interrelated processes—such as ECM remodeling, basement-membrane disruption, epithelial-cell apoptosis, cell migration, and angiogenesis—in which MMPs may play a central role, either by ECM direct cleavage or by generating bioactive mediators. TIMPs can modulate cellular processes such as cell growth, apoptosis and migration, and can be both anti- and pro-tumorigenic. This study aimed to examine the changes in induced sputum as regards MMP-9, TIMP-1 and levels of inflammatory cells in ILD patients compared with sputum of healthy non smokers.

Subjects and methods: Thirty subjects were included in this study and were classified into the following two groups: Group I included twenty patients diagnosed clinically, radiologically and physiologically as interstitial lung diseases. Group II included ten healthy non smoker subjects. Sputum induction was done and processed to assess matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1(TIMP-1) and cytological examination with cellular count.

Results: In this study, we have demonstrated that levels of sputum MMP-9 and TIMP-1 were significantly increased in patients with interstitial lung diseases than normal persons with highly significant statistical differences ($p = 0.001$). MMP-9 was positively correlated with number of neutrophils in the airway with highly significant statistical difference ($p = 0.001$).

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Background

The interstitial lung diseases are a clinically challenging and diverse group of over 150 disorders characterized by varying degrees of fibrosis and inflammation of the lung parenchyma or interstitium. The interstitium of the lung spans the region between alveolar epithelium and pulmonary vascular endothelium. This region includes a variety of cell types (fibroblasts, myofibroblasts, and macrophages) and matrix components (collagens, elastin, and proteoglycans) [1]. Pulmonary fibrosis, the final result of a large variety of interstitial lung diseases, is characterized by an aberrant remodeling of extracellular matrix (ECM) with a profound disturbance of the normal lung architecture. This remodeling includes the exaggerated accumulation of ECM components in the interstitial and alveolar spaces and the disruption of the basement membranes [2]. The pathologic findings in pulmonary fibrosis (excessive accumulation of ECM and remodeling of the lung architecture) are a consequence of disturbances in two physiologically balanced processes: proliferation and apoptosis of fibroblasts and accumulation and breakdown of ECM. When the normal balance between ECM deposition and turnover is shifted toward deposition or away from breakdown, excessive ECM accumulates [3]. Several possible origins of ECM producing mesenchymal cells have been described, and have included accumulation of resident lung fibroblasts, homing and fibroblastic differentiation of bone marrow- derived cells such as circulating fibrocytes or monocytes [4–7], or epithelial-mesenchymal transition (EMT) [8]. In addition to altered mesenchymal cells, abnormalities of the alveolar epithelium in patients with pulmonary fibrosis have been noted from the earliest descriptions of the disease process [9,10].

Matrix metalloproteinases (MMPs) are a family of zinc containing endopeptidases, which is a subset of the metzincin superfamily of metalloproteinases. These regulatory proteases are the extracellular matrix (ECM) remodelers characterized by their substrate specificity to degrade ECM proteins [11,12]. MMPs can be separated into 6 main classes according to their substrate specificity, cellular location and primary structure: collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs and others [13–15]. Recently, a new classification system has been proposed based on MMP structure rather than on their substrate specificity: archetypal MMPs, matrilysins, gelatinases, and furin activable MMPs [16,17]. In general, MMPs levels are usually low in normal adult resting tissues, and with some exceptions, their production and activity are maintained at virtually undetectable levels. By contrast, their expression becomes elevated when there is a challenge to the system, such as wound healing, repair or remodeling processes, in diseased tissues and even in several cell types grown in culture [18].

It has long been accepted that MMPs play an important role in the pathogenesis of pulmonary fibrosis, but the exact mechanisms are not well characterized. There are several interrelated processes—such as ECM remodeling, basement-membrane disruption, epithelial-cell apoptosis, cell migration, and angiogenesis—in which MMPs may play a central role, either by ECM direct cleavage or by generating bioactive mediators [2]. The participation of MMPs in lung fibrosis has been analyzed in several interstitial lung diseases in humans and in experimental models such as those provoked by bleomycin, paraquat plus hyperoxia and silica [19]. MMP-9, also called gelatinase B, contains additionally a type V collagen like domain that is highly glycosylated, which has been suggested to have an effect on substrate specificity [20].

MMP-9 gene expression and protein have also been shown to be elevated in lungs and BAL fluids from patients with IPF [21–25]. The majority of MMPs are not expressed in normal healthy tissues but are expressed in diseased tissues that are inflamed or undergoing repair and remodeling [26,27]. Pulmonary epithelial cells may also be a significant source of MMPs as they express MMP-1, -2, -7 and -9. Intracellularly, MMP secretion is primarily regulated by the prostaglandin (PG) and mitogen activated protein kinase (MAPK) signal transduction pathways.

Tissue inhibitors of metalloproteinases (TIMP) are a family of secretory proteins that are able to inhibit matrix metalloproteinase activities through non-covalent binding of pre- or active forms of MMPs at molar equivalence. By inhibiting MMPs, TIMPs may also influence MMP-mediated processes such as processing of cytokines, degradation of growth factor binding proteins, and the release of ECM-bound growth factors [11,28–31]. TIMPs can modulate cellular processes such as cell growth, apoptosis and migration, and can be both anti- and pro-tumorigenic [11,13,32,33]. TIMP-1 works as a natural inhibitor of MMP-9 and is found in most tissues and body fluids. By inhibiting MMPs activities, TIMPs are involved in tissue remodeling and regulation of ECM metabolism. The TIMP family consists of four members sharing important structural features as well as the ability of MMP inhibition [11,13].

Induced sputum (IS) collection is a non-invasive method for assessment of airway inflammation in the airways [34,35]. This study aimed to examine the changes in induced sputum as regards MMP-9, TIMP-1 and levels of inflammatory cells in ILD patients compared with sputum of healthy non smokers.

Subjects and methods

Subjects

Thirty subjects were included in this study and were classified into the following two groups: Group I included twenty patients diagnosed clinically, radiologically and physiologically as interstitial lung diseases. Group II included ten healthy subjects. Patients with history of COPD, bronchial asthma or recent respiratory tract infection were excluded from this study.

Methods

A written informed consent was obtained from all subjects. The two groups underwent full history taking including history of smoking (current, ex, and non smoking), history of chest symptoms (cough, expectoration, wheezes and dyspnea) and history of any other co morbidities. Also full clinical examination (general, full local respiratory system examination including inspection, palpation, percussion and auscultation, with special regards to manifestations of right sided heart failure as lower limbs edema, congested neck veins, tenderness over right hypochondrium, and dullness on right parasternal area)