Original Research

Role of Oxidative Stress and Genetic Polymorphism of Matrix Metalloproteinase-2 and Tissue Inhibitor of Metalloproteinase-2 in COPD

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Running Head:

Abbreviations:
COPD - chronic obstructive pulmonary disease
MMP - Matrix metalloproteinase
ECM - Extracellular matrix
IL - Interleukin
TIMP - Tissue inhibitor of metalloproteinase
NO - Nitric oxide
MDA – Malondialdehyde

Keywords: COPD; MMP2; TIMP2; SNP; oxidative stress

Funding Support:

Date of Acceptance: July 29, 2023 | ***Publication Online Date***: August 3, 2023

Citation: Sangwan P, Quasimi H, Sharma JG, Habib MA, Alam MI. Role of oxidative stress and genetic polymorphism of matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-2 in COPD. *Chronic Obstr Pulm Dis.* 2023; Published online August 3, 2023. https://doi.org/10.15326/jcopdf.2022.0370

ABSTRACT

Chronic obstructive pulmonary disease (COPD), a complaint described by progressive and inadequately reversible limitation in lungs with systemic inflammation, is largely current in India. There's no remedy available so far it is, thus, imperative to understand the underpinning pathogenesis of the complainant. A set of proteases known as Matrix metalloproteinase (MMPs) are especially involved in the process of alveolar destruction and mucus hypersecretion. There are responsible factors in an inheritable position to control COPD like MMPs and TIMPs (Tissue Inhibitor of Metalloproteinases). MMPs degrade extracellular matrix and lead to the pathogenesis of COPD [1]. TIMPs proteins that help to inhibit the Matrix metalloproteinases. [2]. This review summarizes the implicit part of crucial MMP-2 and TIMP-2 in COPD disease. Though the concept seems promising, limited knowledge about the exact functions of a particular MMP in COPD and the complications of MMP in substrate affinity makes this a grueling task. MMP2 and TIMP2 both are directly or indirectly regulated by oxidative stress and epigenetic mechanism which regulates their expressions. COPD is a seditious response to factors like dust, smoke, etc., and triggers extra-pulmonary goods which cause inflammation. [3]. This review explains the relationship between MMP2 and TIMP2 in COPD patients with oxidative stress, its impact on COPD pathogenesis, and gene expression of TIMP2 and MMP2 with their downstream effects. This also gives some insights into therapeutic interventions for targeting these enzymes. MMP2 and TIMP2 both play a role in the development of COPD and they need to be studied with the utmost focus.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

1. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major healthcare burden worldwide, although it manifests at an age generally after 40+ as part of multimorbidity, but there are evidences that there are events at an early stage of life that contribute to impaired lung functions in adults [4]. Lung tissue in COPD cases is characterized by variable and localized nonspecific changes such as habitual inflammation and tissue revamp with seditious cell infiltration, granulation tissue, thickened basement membrane and vascular wall, bronchial gland, and smooth muscle hyperplasia and hypertrophy, as well as fibrosis and epithelial metaplasia. Control lung tissue is characterized by overall low figures of cells immunoreactive for MMP-2 and TIMP-2 at different localizations. This indicates the stable, nonstop, and adaptive "birth" position of these tissue factors at a relative norm [5]. More pronounced findings of these factors in the bronchial and alveolar epithelium, and among alveolar macrophages are associated with the conservation of original lung homeostasis, including impunity and tissue reactivity; still, in hyaline cartilage, Malleability and the implicit compensatory response with the tissue increased. Significantly advanced figures of MMP-2 and TIMP-2 immunoreactive cells in COPD affected lung tissue compared to the control group indicate the part of these factors in tissue remodeling procedures. Small ascendance of TIMP-2 may limit tissue destruction processes [6].

COPD becomes worse when the walls of alveolar tissues start to damage because of its progression into Emphysema and Bronchitis and the capacity diminishes for the production of ECM [7-8]. MMP2 and TIMP2, both are very important for this progression because TIMP2 controls MMP2.

Although COPD is more common in men than women in developed countries, COPD is very prevalent in women because of high smoking rate and indoor air pollution due to biomass fuel in

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

developing countries [9]. High prevalence rate of COPD leads to high mortality and morbidity all over the world includes especially two diseases ischemic heart disease and cerebrovascular disease. However, considering that a third of COPD patients die of ischemic heart disease and a third of patients die of cardio-vascular disease with airflow limitation [10-12]. COPD disease progress includes loss of elasticity of alveoli and parenchymatous tissues leading to a decrease in Forced expiratory volume in 1 sec (FEV1) and subsequently leading to hyperinflation [13]. In addition to external factors, Genetic factors are also responsible for COPD like chronic bronchitis, loss of lung function, lung development, etc. [14, 15]. Since COPD is an inflammatory disease, it also damages DNA by Oxidative stress which leads to disturb equilibrium between tissue repair and cell proliferation [16]. Fig (1) shows that smoking leads to oxidative stress which damages DNA in COPD patients.

Smoking cessation is one kind of treatment that can be done to lower decline in lung function as well as exacerbations [17]. Patients who are addicted to tobacco, it is very hard for them to quit tobacco [18]. Arbitrations like intensive counselling, nicotine replacement therapy, and treatment with Varenicline helps to quit smoking and also much cost effective [19].

There are various genes like MMPs and TIMPs, which affects lung function and TIMPs inhibits MMPs. MMPs (Matrix Metalloproteinases) are a family of Calcium and Zinc dependent proteolytic enzymes degrades extracellular matrix which are derived from macrophages and neutrophils. Normally MMPs helps in remodeling of extracellular matrix, facilitates cell migration and are involved in immune responses by cleaving inactive forms of cytokines and chemokine's [20]. In pathologic conditions, a change in MMP expression and activity occurs which leads to destruction of extracellular matrix or tissue destruction and lung inflammation [21, 22].

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

TIMPs are Tissue Inhibitor of metalloproteinases which inhibits the activity or expression of MMPs. Basically, there are four TIMPs (TIMP1, TIMP2, TIMP3 and TIMP4) that inhibits active forms of MMPs. Although all are inhibitors of MMPs but they have different affinity for different MMPs as like TIMP2 is a potent inhibitor of MMP2 gene [23]. Distinct findings of tissue factors in bronchial epithelium, connective tissue, and blood vessels prove the involvement and significance of these structures in the morpho-pathogenesis of COPD. In general, COPD morpho-pathogenesis is characterized by increased MMP-2, TIMP-2 still, reduced Hsp-70 and hBD-4 expression that indicates high exertion of patient complex cytokine network, tissue remodeling, and overall tissue antimicrobial protection [24]. In COPD, bronchoscopically determined hypertrophy and habitual bronchitis are associated with the presence of MMP-2 in COPD-affected lung. Epithelial metaplasia and fibrosis in the histological sections are associated with worse functional parameters MMP-2. Also, the finding of granulation tissue is associated with a longer history of smoking and more pronounced tissue damage. Aging changes in the lungs at relative health are individual, indeed different for Individualities in colorful age groups, also, are substantially characterized by a lack of inflammation and variability, indeed a drop, in the number of vulnerable cells. In turn, in the case of COPD, aging is associated with worsening of the course of the complaint, as well as continuity of seditious cytokines and altered revamp with else typical COPD events. Fig 1 showing how free radicals leads to oxidative stress and DNA damage.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

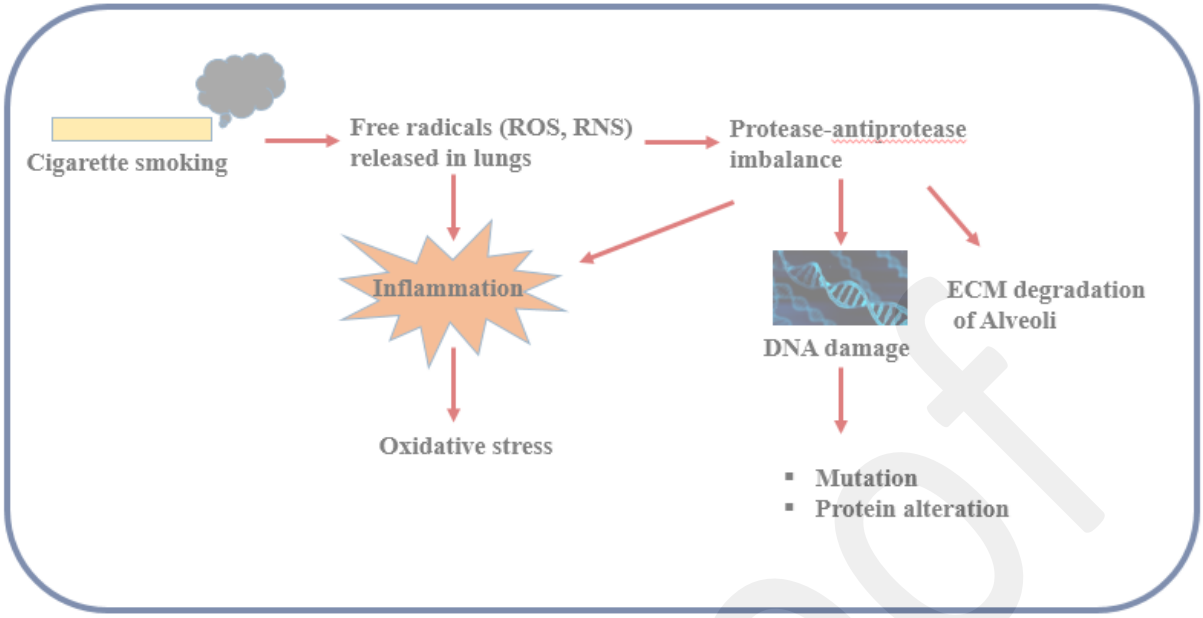

Fig 1: Cigarette smoking releases ROS and RNS in the lungs which creates proteases-antiproteases imbalance and leads to oxidative stress and DNA damage in COPD;ECM- Extracellular matrix, ROS- Reactive Oxygen species, and RNS- Reactive Nitrogen species

2. OXIDATIVE STRESS AND COPD

Oxidative stress is a potentially harmful imbalance in the oxidant–antioxidant balance in favor of the former [25]. This imbalance is because of the reactive molecules which include free radicals and non-radical reactive species. Generally, ROS includes superoxide anion and hydroxyl radical, they have unpaired electrons and their unstable nature triggers them to transfer electrons to other molecules via oxidation that leads to damage or inactivation. Lungs are vulnerable to damage by oxidative stress because of high concentrations of oxygen, increased blood supply, and exposure

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

to environmental toxins and pathogens. Cigarette and biomass smoke adds to this burden. A single puff of cigarette smoke contains 1 x 10^{15} oxidants molecules [26].

An important link between oxidative stress and the pathogenesis of COPD would come from the demonstration of the response of ROS with target lung motes and the presence of these oxidatively modified motes in increased quantities in the lungs of smokers, particularly those who develop COPD. ROS similar to O2 and OH, generated and released by activated vulnerable and seditious cells that are largely reactive and when generated close to cell membranes oxidize membrane phospholipids (Lipid peroxidation), a process that may continue as a chain response [27].

ROS directly targets proteins, DNA, and lipids which causes lung injury or generates several cellular responses via secondary metabolites or cytokines, or inflammatory molecules [28]. Fig 2 shows the different effects caused by oxidative stress.

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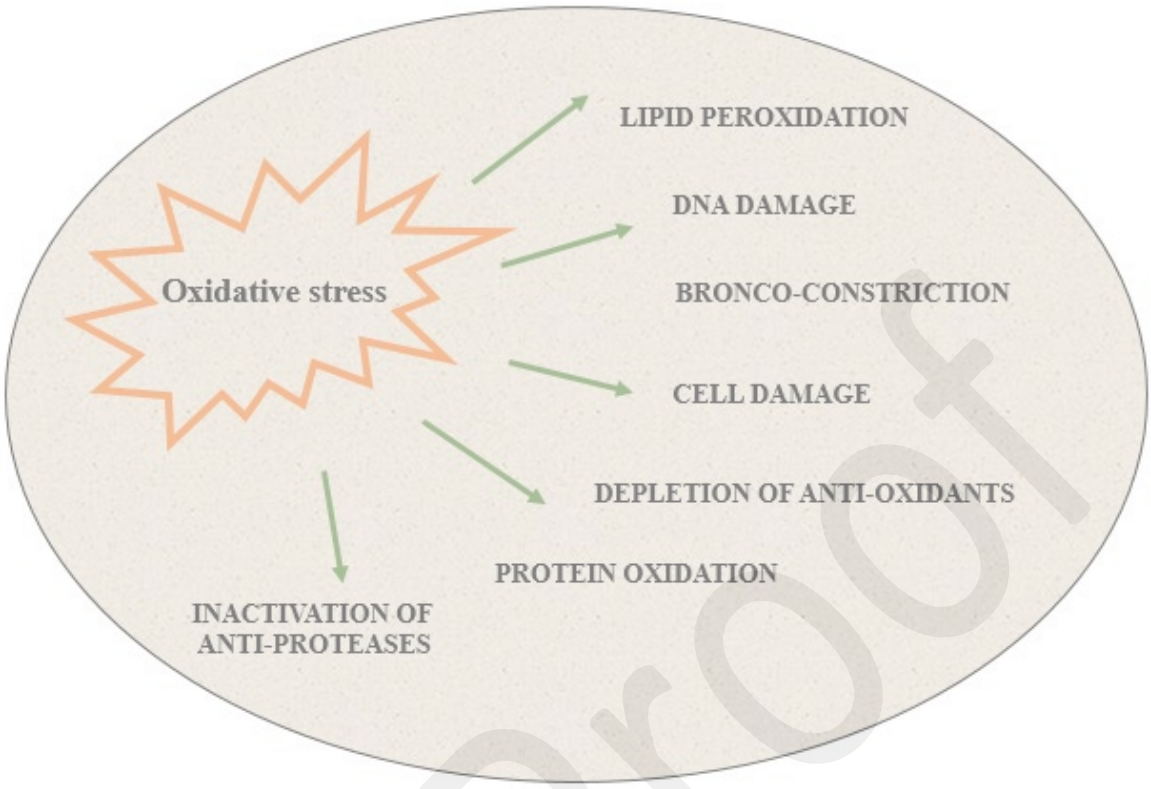

Fig 2: Different effects caused by Oxidative stress in COPD patient

Numerous effects of ROS in airways may be intermediated by the secondary release of seditious lipid intercessors similar to 4 hydroxy-2-nominal 4-(HNE), a footmark of oxidative stress/ lipid peroxidation. 4-HNE, a largely reactive diffusible end product of lipid peroxidation, is known toregulate various cellular events, similar to proliferation, apoptosis, and activation of MAPK signaling pathways [29]. 4-HNE has a high affinity towards cysteine, histidine, and lysine remainders. It forms adducts with proteins, altering their function. It also acts as a chemo-attractant for neutrophils in vitro and in vivo. Recent data indicate increased 4-HNE-modified protein situations in the airway and alveolar epithelial cells, endothelial cells, and neutrophils in subjects with airway inhibition, compared with subjects without airway inhibition [30]. Increased situations

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of 4-HNE adducts in the lungs of cases with COPD, later showed that cigarette smoking enhanced the situations of 4-HNE adducts in bronchiolar epithelial and alveolar type II cells in mice [31].

2.1 MDA

Malondialdehyde (MDA) is a reactive organic molecule found among eukaryotes and is made up of three carbon molecules with two aldehyde groups at the C- 1 and C- 3 positions. MDA formation is induced non-enzymatically by ROS and enzymatically by lipoxygenase [32]. However, due to their instability and reactivity in both instances, the measurement of primary lipid hydroperoxide products involves a laborious task. And that is why, the quantification of lipid peroxidation is done by estimating the concentration of secondary oxidation products derived from the primary products, which are mostly aldehydes like MDA [33]. Fig 3 shows the formation of MDA from polyunsaturated fatty acids.

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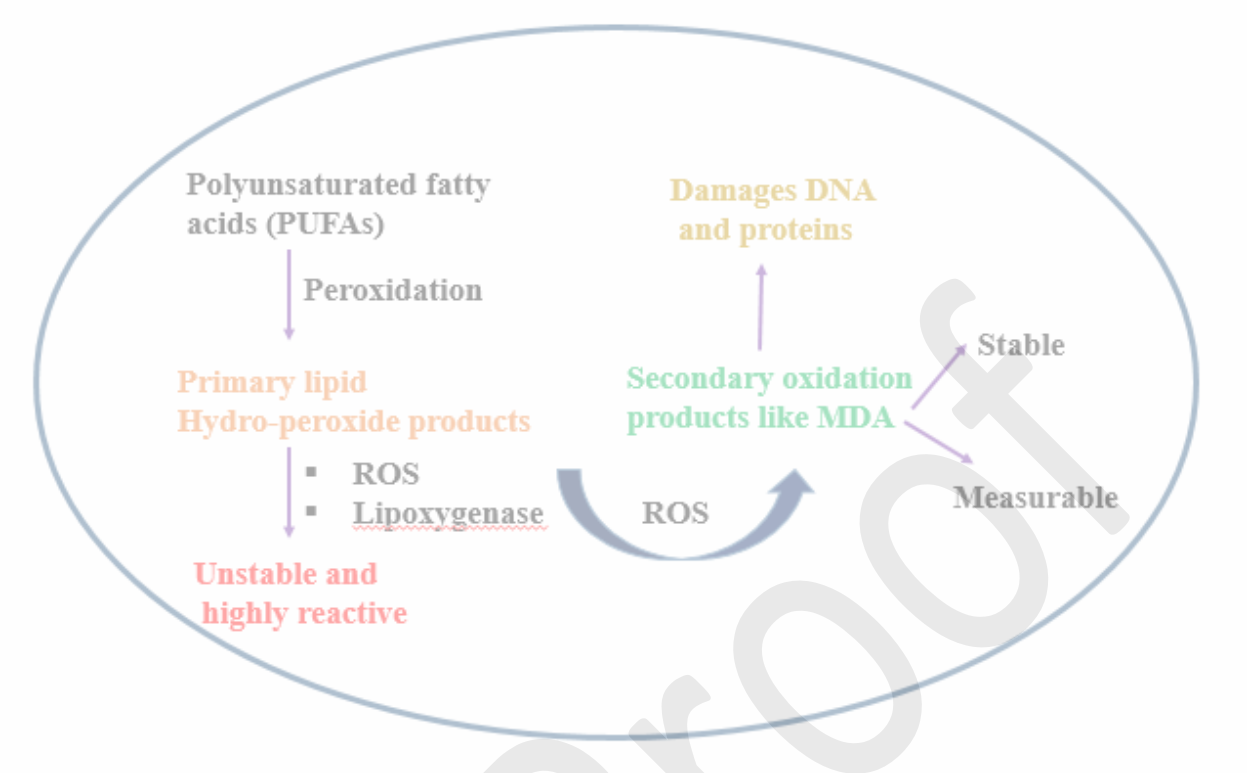

Fig-3: Lipid peroxidation from polyunsaturated fatty acids because of the high level of free radicals released in the body

MDA is widely used as a biomarker for lipid peroxidation in oxidative stress in COPD patients, in this a reaction of MDA with Thiobarbituric acid (TBA) makes MDA-TBA conjugate that absorbs light at 532 nm and gives red-pink color [34-36]. TBARS assay is considered as a good indicator of oxidative stress in biological samples [37]. Under acidic conditions (pH = 4) at 95°C, MDA bis-dimethyl acetal yields MDA. MDA and other chemicals (similar to MDA) reacts with two molecules of TBA to generate TBARS (Thio-barbituric acid reactive substances) which gives red-pink color at 532 nm [38, 39]. Generally, MDA level is elevated in COPD patients due to lipid peroxidation that further damages proteins, DNA, etc.

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2.2 GLUTATHIONE

Glutathione (GSH) is an important enzyme found in the lungs that protects our body. It is an antioxidant system. But its level is gradually reduce with continuous exposure to smoke in smokers. A high level of GSH is found in the epithelial lining fluid of cigarette smokers [40-41]. Plasma GSH level is determined using plasma, precipitated by Sulfo-salicylic acid in a 1:1 ratio. Then samples were kept at 4°C for one hour, then centrifuged at 3500 rpm for 15 min and after which the supernatant is collected. The mixture that is obtained contains 50 µl of supernatant, 50 µl of PBS (0.1 M, pH 7.4), and 10 µl of 0.4 % DTNB (5, 5′-dithiobis-(2-nitrobenzoic acid**)**. Then the chemical reaction gives yellow color which is measured at 412 nm and expressed in nm/ml of plasma [42].

2.3 SOD

SOD is an antioxidant enzyme that catalyzes the dismutation of superoxide anion to hydrogen peroxide, which is further detoxified into oxygen and water by the catalase enzyme in our body [43]. In COPD patients, the level of SOD is markedly decreased. The superoxide dismutase assay developed by Marklund and Marklund spectrophotometrically determines the SOD level in samples by checking the percent inhibition of auto-oxidation of Pyrogallol at 420 nm. SOD inhibits the auto-oxidation of Pyrogallol at an alkaline pH, with the increase in pH percentage inhibition also increases to a certain extent and SOD activity is thereby determined which is expressed in units per milliliter. Pyrogallol auto-oxidizes rapidly especially in alkaline solution. The reaction is inhibited by SOD to 99% at pH 7.9, indicating the total dependence on the participation of superoxide anion radical in this reaction. At pH 9.1 the reaction is still inhibited to over 90% by

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SOD. But at such higher alkalinity superoxide anion radical independent mechanism rapidly becomes dominant [44]. A unit of enzyme is defined as the amount of enzyme which inhibits the reaction by 50% [45].

2.4 Nitric Oxide

Nitric Oxide (NO) is released endogenously in the lungs and acts as a dilator of bronchial and vascular smooth muscles, a neurotransmitter, and an immune response mediator [46]. NO is a marker of airway inflammation, and its level is increased in patients with COPD. Cigarette smoke increases the formation of RNS (Reactive Nitrogen species) and leads to nitration and oxidation of plasma proteins [47]. Nitric oxide is generated from L-arginine and the reaction is catalyzed with the nitric oxide synthase (NOS) enzyme. NOS is normally found in endothelium and neurons, while inducible NOS (iNOS) is primarily occurs in macrophages. NOS catalyzes the conversion of L-arginine into L-citrulline and nitric oxide.

Corradi and colleagues reported that exhaled NO is increased in stable COPD and correlated positively with lung function as assessed by forced expiratory volume in 1 sec (FEV1) [48]. Rutgers and team found that NO metabolism was not affected in stable COPD patients [49]. Another study suggested that there was no difference between COPD and healthy controls and an inverse correlation was found between FEV1 and NO levels in COPD [50]. NO was assayed using Griess reagent method in plasma samples colorimetrically. Griess reagent reacts with nitrite to form pink to a dark pink color after incubation. The absorbance of samples is determined at 540 nm and NO level is expressed in picomole/milligram of protein using $NaNO_2$ as the standard [51]. Therefore, substantiation is accumulating that cigarette bank triggers oxidative stress, leading to inflammation in the lungs of smokers and cases with COPD. Fig 4 showing that cigarette smoke

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responsible for releasing free radicals and leads to inflammation in lungs and causes imbalance of MDA, NO, SOD and GSH etc in human body and it ultimately develops in COPD.

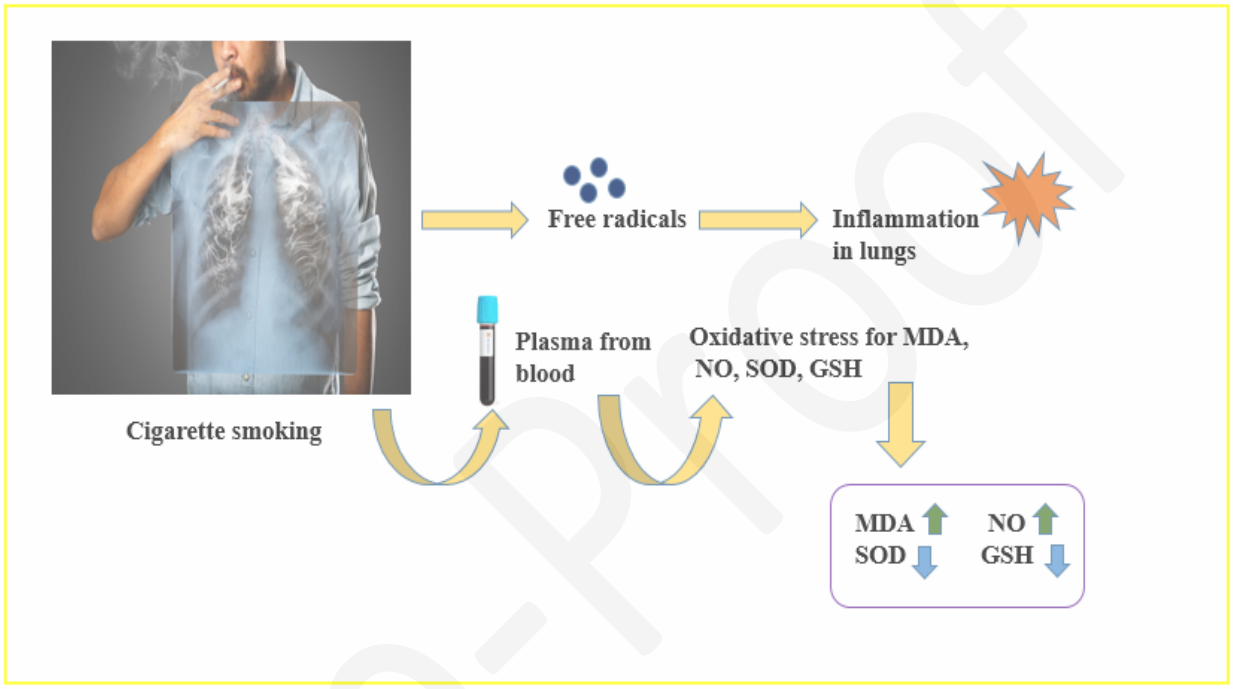

Fig 4: Oxidative stress leads to inflammation and changes the levels of MDA, SOD, GSH and Nitric oxide in human plasma

2.5 Impact of oxidative stress on COPD pathogenesis:

Oxidative stress contributes to the pathogenesis of COPD by inactivation of antiproteases, elevation of bronchial inflammation by redox; activation of neutrophils, macrophages, and fibroblasts; abnormal airway T cell population, etc. [52, 53]. Free radicals like superoxide ($^{\circ}O_2$),

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sulfite ions, nitrogen monoxide or nitrogen dioxide radicals, hydroxyl radicals (°OH), etc are responsible for many changes in lung airways or affect different mechanisms which lead to the development of COPD. ROS may remodel ECM and blood vessels, regulates or stimulates the secretion of mucus, inactivate antiproteases, activation of transcription factors in thelungs.[54-57]. Cigarette smoking is also a crucial factor in COPD pathogenesis as the smoke contains high concentration of free radicals that accumulates neutrophils and macrophages into the lungs further leading to COPD exacerbation [58, 59]. Free radicals released from cigarette smoke cause pulmonary dysfunction [60]. Smoking also enhances 4 hydroxy-2-nonenal (4-HNE), a highly reactive diffusible end product of lipid peroxidation, it forms adducts with proteins and alters their functions in COPD [61]. Reactive nitrogen species (RNS) cause an abnormal inflammatory response in COPD by increasing nitrated proteins [62]. The antioxidant capacity of COPD also lowers because of smoking and exacerbations. Glutathione maintains the integrity of the lung airspace epithelial barrier but decreases the level of GSH in the epithelial leads to loss of barrier function and permeability [63, 64].

Many signal transduction or mechanisms are affected by oxidative stress which plays an important role in COPD pathogenesis such as the inactivation of antitrypsin α1-AT by oxid ation of methionine in its active site of the protein, lead to the low level of protein and lung airways are damaged by proteases [65].

3. MMP2 and TIMP2

MMP2 is a type of Gelatinase A, 72 KDa protein released by smooth muscle cells and epithelial cells in the lungs as well as by lymphocytes and macrophages by infiltrating the tissue [66].It helps in vascularization, endometrial menstrual breakdown, and inflammatory activities [67].

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Extracellular matrix (ECM) provides mechanical support to cells and is a complex of different proteins like collagen, laminins, cytokines, fibronectins, and growth factors but MMPs proteolytic activity degrades ECM and also provides signals to embedded cells to react to stimuli [68]. MMP2 also cleaves non-ECM molecules like pro-TNF-α, pro-IL-1β, pro-IL8, MCP-3, etc. and can also activates various MMP Zymogens as pro-MMP-1, pro-MMP-2, pro-MMP-9 and pro-MMP-13 [69-72].

MMP2 contains polymorphic sequences in its promoter site like Sp-1 binding site. Sp-1 binding site determines the basal activity of MMP2 promoter [73]. It has been proved that the polymorphism in the promoter site of MMP2 results in change in expression of MMP2 gene [74].

TIMPs (Tissue inhibitor of Metalloproteinase) is present in four active forms (TIMP1, TIMP2, TIMP3 and TIMP4) which inhibits the activities of MMPs by binding to their active sites [75]. TIMP2 is a potent inhibitor of MMP2. It was also seen that TIMP2 is involved in pulmonary disease characterized by abnormal tissue remodeling of alveolar structure in lungs like emphysema, fibrosis, acute respiratory distress syndrome and lung cancer [76]. TIMP2 promotes cell growth and protects cell from apoptosis. TIMPs also shape and organize appropriate ECM environment and architecture [77, 78]. Fig 5 depicts MMP2-TIMP2 regulation and degradation of ECM by MMP2. .

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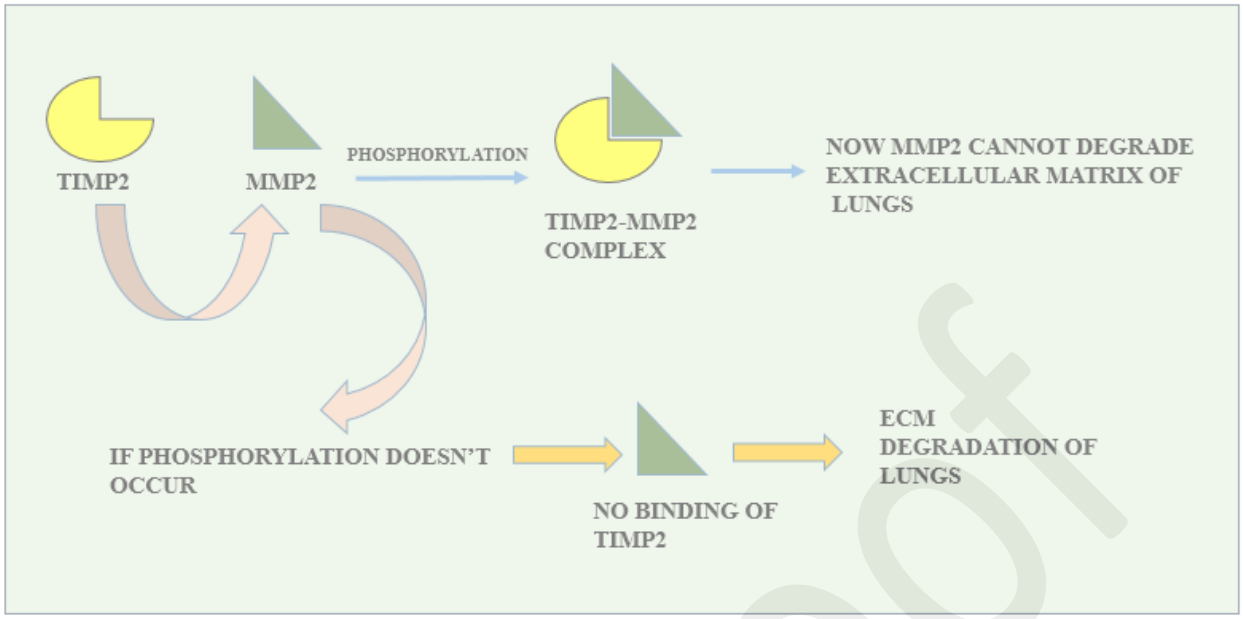

Fig-5: Regulation of MMP2 by TIMP2 as TIMP2 inhibits MMP2 by phosphorylation but without its binding, MMP2 degrading ECM of lungs;TIMP2- Tissue Inhibitor of Metalloproteinase 2, MMP2- Matrix Metalloproteinase 2

Both Bronchial epithelial cells and airway muscle cells secrete MMP2, and smoke or dust trigger the up-regulation of MMP2 protein in COPD cases [79]. TIMP2 is a potent asset of MMP2 and it binds with its active site after phosphorylation on three different sites by the c-Src kinase enzyme. After phosphorylation, TIMP2 exclusively binds with MMP2 and inhibits its exertion [80].

3.1 Importance of TIMP2 and MMP2 in COPD:

MMP2 is a Gelatinase A enzyme responsible for COPD's pathogenesis. It is released from macrophage and bronchiolar epithelial cells and requires TIMP2 for its activation [81, 22]. MMP2 causes pathological changes in the pulmonary interstitium and lung function of COPD [83] and is

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

also involved in ECM remodeling and interstitial fibrosis [84]. TIMP2 inhibition by of MMP2, is involved in abnormal ECM accumulation [85, 86]. TIMP2 binds non-covalently to MMP2 with the help of MMP14 and regulates their activities. TIMP2 binds pro-MMP2 molecule (inactive) with MMP14 molecule. The N-terminal of TIMP2 binds with the active site of MMP2 and inactivating it by forming an inhibitory complex. The binding of TIMP2 to MMP14 allows the cleavage of pro-MMP2 by a free MMP14 molecule, as an active MMP2 molecule [87-89]. Loss of TIMP2 leads to a decrease in the activation of pro-MMP2 [90]. TIMP2 is inhibited when it undergoes a mutation or when free radicals are produced. And because it is in charge of regulating MMP2 production, MMP2 will become greatly elevated, begin digesting the ECM in the lungs, and cause COPD. The remodeling of the lung matrix, which is important for the pathogenesis of COPD, is carried out by MMP2 and TIMP2. [91, 92].

4. MMP2 and TIMP2 at Global level:

MMP2 Single nucleotide polymorphisms (SNPs) have been seen among various populations with TIMP2 SNPs worldwide. Different SNPs have been studied all these years.

MMP2 -1306 at promoter site (rs243865) in central Bulgaria has been studied and that included 71 individuals.. This study showed that the genotype but not allele frequency was different between COPD patients and controls. The carriers of T alleles were at higher risk for developing COPD [93]. MMP2 rs243864, rs3918253, and rs11646643 SNPs were associated with a high risk of COPD in the Mexican population [94]. MMP2 rs2285053 and rs243865 SNPs were also found

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

to be associated with the development of COPD or lung carcinoma in Asians but not the Caucasians population [95].

TIMP2 gene polymorphism at two different positions +853 G/A and -418 G/C showed a down-regulation of TIMP2 in COPD patients than controls in the Japanese population which lead to the up-regulation of MMP2 in patients for degradation of ECM in COPD patients. G/A nucleotide substitution at +853 in exon 3 and G/C substitution at -418 in Sp1 binding site in the Japanese population, showed elevated genotypic and allelic frequency at +853 position and higher allele frequency at -418 position between COPD patients and the control group [96].

But these two SNPs differed in the Egyptian population, +853 G/A showed significant genotype difference in controls and COPD patients but there was no difference between controls and COPD patients in -418 G/C SNP [97]. Table 1 summarizes different SNPs of TIMP2 and MMP2 genes in different regions worldwide studied in COPD patients. MMP2 and TIMP2 were also found to be associated with chronic bronchitis in Tatar children [98].

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Gene	SNPs	Location	Substitution	References
TIMP2	+853	Exon 3	G/A	[97]
	-418	Sp1 binding site	G/C	[97]
MMP2	rs243865	-1306 Promoter	C/T	[74]
	rs243839	Intron	G/A	[94]
	rs243835	Intron	C/T	[94]
	rs243864	-790 Promoter	T/G	[94]
	rs11646643	Intron	A/G	[94]
	rs2285053	735 Promoter	C/T	[95, 98]

Table 1: Different SNPs of TIMP2 and MMP2

COPD, a common respiratory complaint, is one of the leading causes of morbidity and mortality

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among smokers and some non-smokers [99]. India contributes a significant and raising chance of COPD mortality which is evaluated to exist among the loftiest in the people; crude assessments indicate there are 30 million COPD cases in India [100].

MMP-2, an important modulator of cell proliferation, is buried by both, bronchial epithelial cells and airway smooth muscle cells. Increased vulnerable reactivity for MMP-2 in COPD lungs , substantially in alveolar macrophages and airway epithelial cells was observed [101]. Increased MMP-2 exertion in mice exposed to long-term cigarette banks was also reported. MMP-2 mRNA up-regulation and increased protein in lavage and lung towel was also linked to emphysema convinced by the wood bank [102]. In discrepancy, a study using ray prisoner micro-dissected samples of mortal lung parenchyma has shown that MMP-2 gene expression situations drop with the inflexibility of the patient [103].

However , TIMPs haven't been considered as remedial targets for COPD due to their variation in affinity for different proteases. Though the part of TIMPs in cranking pro-MMPs is well accepted, several studies live that reported the MMP-limited function of TIMPs, similar to inhibition of the mitogenic response of mortal endothelial cells to growth factors and creation of apoptosis [104-106].

5. Impact of oxidative stress on the gene expression or the genetic mutations of TIMP2 and MMP2:

Oxidative stress because of cigarette smoking or biomass fuel affects the genetic makeup of the MMP2 and TIMP2 gene which alters their protein product and is responsible for COPD pathogenesis. According to a study conducted on the Japanese population, the polymorphism of +853 G/A and -418 G/C SNPs in the TIMP2 gene was higher in smokers. Smoking destabilizes

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the TIMP2 gene and causes its low production [107, 108]. The same polymorphism of +853 SNP was seen in an Egyptian population associated with COPD in smokers [109].

MMP2 is mainly expressed in mesenchymal cells (mainly fibroblasts) during development and tissue regeneration. Due to airway remodeling, there are structural changes with an inflammatory response. There are many promoter polymorphisms in the MMP2 gene that alter their expressions such as -1306 C>T SNP. This gene expression is altered because smoking and oxidative stress lead to inflammation and ECM degradation which affects this gene at the genetic level [110]. Smoking causes the release of various oxidative factors or imbalances oxidant or antioxidant, or proteinase or anti-proteinase enzymes which alters the genetic makeup of the MMP2 or TIMP2 gene. This causes a change in their downstream products at the transcriptional or translational level and changing their functions and leading to the development of COPD.

Most of the MMPs share common cis-elements in their promoters, which are regulated by various cytokines like (IL-1b, TNF-α, TGF-β), growth factors (EGF, VEGF, bFGF), oxidative stress factors, xenobiotics [111, 112]. This stimulus induces signal transduction pathways leading to the activation of transcriptional factors such as NF-κb, STATs, ETS factors, AP-1, etc [111, 113]. In this respect, the naturally occurring DNA sequence changes in the regulatory regions especially promoters of the genes encoding MMPs affect the transcriptional activity and influence the balance between MMPs and TIMPs thus causing development and progression of COPD [114].

6. The impact of oxidants on MMP2 and TIMP2:

Oxidants are free radicals (reactive forms of oxygen and nitrogen species) that are highly unstable, reactive, and tend to make reactions with different molecules, for example, hydroxyl radical OH°, superoxide, hydrogen peroxide, nitric oxide, etc. Nitric oxide (°NO_2), a reactive free radical cause

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epithelial damage and induce inflammation in the airways which ultimately affects the MMP2 secretion that is upregulated due to the inflammation and downregulates TIMP2 in the airways. Reactive Oxygen Species (ROS) react with thiol groups that preserve MMP latency (115) thus activating MMPs. ROS enhances the expression of MMP2 via xanthine oxidase (116, 117) and via mechanical stretch-induced vascular NADPH oxidase (118). $^{-}$OONO (produced by the reaction of nitric oxide and superoxide) produces posttranslational modifications of the MMP2 structure, thus resulting in an active full-length MMP2 enzyme (119, 120). Radiation produces ROS including $O_2^{\circ-}$ and H_2O_2 activates MMP2 (121). H_2O_2 also activates the Ca^{2+} ATPase activity of MMP2 (as MMP2 is Ca dependent enzyme) in smooth muscle membranes with Vitamin E preventing the increase in activity of MMP2 and Ca^{2+} ATPase activity (122).

ROS generated by xanthine and xanthine oxidase system (XOD) reduces TIMP2 mRNA level, inhibited by catalase scavenging. ROS especially affects the stability of mRNA of MMP2 and TIMP2 (123). There are various other ROS which affects TIMP2 at transcriptional or translational level.

7. Impact and downstream effects of the TIMP2 and MMP2 gene polymorphism:

The expression levels of MMPs are affected by polymorphisms in promoter regions of genes encoding these enzymes. MMP2 is a type IV collagen, and can also degrade elastin molecules. There are three polymorphisms in the promoter region of this gene. Two of them are at -1306 and -735 positions and the third SNP is at -1575 position. The previous two SNPs cause disruption of the Sp1 regulatory element and strikingly decreased promoter activity in macrophages and

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

epithelial cells. The third SNP at -1575 is located immediately 5' to an estrogen receptor (ER) binding site and it functions as an enhancer and leads to increased transcriptional activity and also showed linkage disequilibrium and demonstrated an additive reduction in estrogen-dependent reporter activity [124]. MMP2 -1306TT genotype showed to have excess FEV1 decline and a lower mean of FEV1% compared to the wild-type carriers [125]. The extent of inflammation correlated with the reduction in FEV1 and FEV1/FVC ratio, and probably with the accelerated decline in FEV1 characteristics of COPD [126]. The lower promoter activity of the T allele may lead to increased deposition of connective tissue in the lungs and the development of airflow obstruction, which may be the reason for an increased risk for the development of COPD.

The genetic polymorphism of the TIMP2 gene at +853 G/A (at exon 3 in the TIMP2 gene) and -418 G/C (in the promoter region of TIMP2) SNPs supports the possibility of the development of COPD in different populations. This +853 SNP can down-regulate the TIMP2 activity by changing the secondary structure of the mRNA which inhibits the ribosomal binding and/or decreases the mRNA stability [127 , 128]. MMP2/ TIMP2 system plays an important role in the formation of pulmonary emphysema. So, the mutations of the TIMP2 gene down-regulate its own activity which results in the progression of the breakdown of the lung matrix [129].

Another nucleotide substitution at the -418 position, located in the consensus sequence for the Sp1 binding site in the promoter region. There may be the possibility that this substitution results in the downregulation of the transcriptional activity of the TIMP2 gene and increased activity of MMP2 which results in the development of COPD [130].

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8. Mechanism:

Cigarette smoke is responsible for the neutrophil and macrophage influx to the airways in the lungs for NETosis (Neutrophil Extracellular Trap formation) [131-133]. Neutrophil extracellular traps are neutrophils extrude webs of decondensed chromatin with histones, neutrophil elastase, EN-RAGE (Extracellular newly identified receptor for advanced glycation end products binding protein), and myeloperoxidase [134 -136]. NETs were observed in stable and severe COPD patients and correlated with disease severity as well [137 , 138]. EN-RAGE is responsible for the downstream signaling cascade for the pro-inflammatory cytokines in this disorder [139]. Lytic NETosis induces the MAPK/ERK pathway which leads to the production of NADPH and Hydrogen peroxide, it causes the production of Neutrophil Elastase which ultimately leads to the production of ROS and RNS [135]. Leukotriene B4 (LTB4) triggers influx of neutrophils in airways and cause exacerbation in COPD patients and disease progression [140]. Macrophage and neutrophil influx increase in the pulmonary sections.

Early growth response gene product 1 (EGR1) in endothelial cells, binds to the MM14 promoter and it induces its expression. In turn, MMP14 activates MMP2 and upregulates its production [141]. Also, some transcription factors like GATA1 (Globin transcription factor 1), AP1 (activator protein 1), STAT3C (Signal transducer and activator of transcription 3), and NFκB (Nuclear factor kappa B) regulate the expression of MMP2. Tumor necrosis factor alpha (TNFα), Interferon-gamma (IFN-γ), and Interleukin 1β (IL-1β) also triggers the upregulation of MMP2 in lungs [142

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]. IL-1β also causes pulmonary inflammation by triggering metalloproteinases in epithelial cells [143]. Also, IL13 is a pro-inflammatory cytokine that causes hyper-expression of mRNA of MMP2 in the lungs [144]. Reactive oxygen species (ROS) and RNS produced by cigarette smoke, and hydrogen peroxide in mitochondria cause tissue damage in the lungs and increase MMP2 gene expression. RNS leads to inflammation by increasing MMPs expression in the lungs [145-147]. So, all these factors causes inflammation in lungs and hyperexpression of MMP2 that primarily causing degradation of ECM of the lungs. Given that, TIMP2 is an inhibitor of MMP2. The expression of MMP2 if found higher than TIMP2, suggests that TIMP2 is inhibited or down-regulated, further indicating the rationale behind the damage of lungs ECM or alveoli or hyper mucous secretion. NETosis also causes MMP2 mutation which is responsible for various changes in gene expression and responsible for causing COPD in a particular population. Environmental factors like air pollution, dust or passive smoke, biomass fuel also causes the production of ROS/RNS in our body and ultimately leads to inflammation. Macrophage releases MIF (Migration inhibitory factor) cytokine which also causes inflammation in the lungs.

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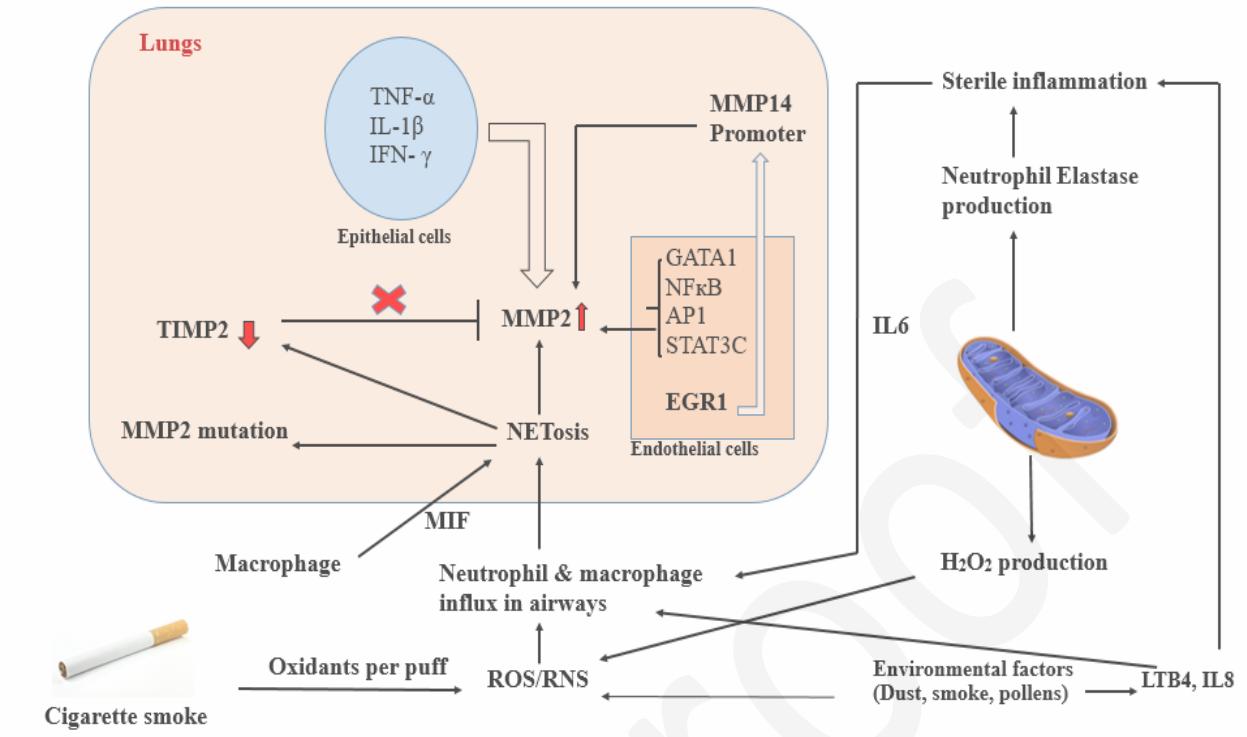

Fig-6: Mechanism showing different transcription factors, cytokines, and environmental factors causing damage in lungs or changes in the expression of MMP2. NETosis leads to mutation, inflammation in lungs which indirectly inhibits TIMP2 and upregulates MMP2.

9 Other mechanisms affecting TIMP2 and MMP2 expressions such as epigenetic changes:

Epigenetic changes are stable changes in cell function or environmental behavior that affect the way our gene works but without the change in their genetic sequence. MMP2 activity is regulated at the transcriptional level by methylation, histone modification, non-coding RNA regulation, and chromatin remodeling [148]. There are various factors like smoke, dust, pollution, biomass fuel, etc. which directly or indirectly cause epigenetic changes in various cytokines like TNF-α, IL8, HNE, NO, etc. [149 , 150] at the transcriptional or translational level by acetylation, methylation, or phosphorylation which might be responsible for the changes in MMP2 or TIMP2 expression

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but currently there are no such reports that present any particular changes targeting these two enzymes in COPD.

10. Possible potential therapeutic intervention targeting TIMP2 or MMP2 nowadays or in the future:

Over or under-expression, Imbalance between TIMP2 & MMP2 may be corrected by usage of drugs. . Synthetic MMP inhibitors were used in preclinical and clinical studies. MMP inhibitors (Batimastat, Marimastat, Prinomastat, Neovastat, CMT-3, BMS-275291) were found efficacious against MMP-1, 2, 3, 9, 12 in experimental models of lung, breast, and colon cancer with reduced tumor growth by blocking tumor angiogenesis [151].

Simvastatin modulated the expression of MMP2 in lung cancer. It plays a key role in the prevention and treatment of lung cancer in COPD [152]. Compounds 25 and 26 can be considered good therapeutic agents for treating COPD emphysema. Inhibitors targeting specific proteases (MMPs) or protease inhibitors (TIMPs) can be used to treat COPD. These inhibitors can be used in combination with the conventional COPD drugs like ICS, LABA, Tiotropium.. MMP2 or TIMP2 can also be targeted by using specific antibodies or targeted potent inhibitors but there are no evidences that targets specifically these enzymes as yet. Currently, There are not much information present regarding targeting of MMP2 or TIMP2 enzymes in treating COPD.

11. Future prospects:

COPD and protease-antiprotease imbalance is very well known. Proteases degrades lung tissues and leads to emphysema or chronic bronchitis. TIMP2 inhibits MMP2 so that it cannot degrades extracellular matrix of lungs but when it fails because of the oxidative stress or any other genetic

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

reason, it leads to activation of MMP2. Numerous studies reported the relation of MMP2 and TIMP2 in different populations but , it has not been reported in North-Indian population till date. Do you think, Is this the same scenario in Indian population also? Many studies have been performed in the Indian population, but till date nobody correlated MMP2-TIMP2 at genetic level. So, will it give the same result? Also NET traps causing inflammation in lungs of COPD and increases exacerbation also responsible for severity of disease. So, NET traps might be a factor causing this illness. So we can also target to prevent NET traps in lungs or develop a medicine to treat these traps which is not available till date.

12 . DISCUSSION

COPD is an inflammatory disease, it has various responsible factors like dust, smoke, genetic makeup, etc. COPD is directly or indirectly affected by the imbalance created in the human body because of these inflammatory markers and leads to oxidative stress. Oxidative stress is created generally because of the consumption of cigarette by men and biomass fuel by women in villages or local areas where hukkas and chullas are used. In our body, free radicals leads to release some inflammatory molecules like IL8, IL6, TNF-alpha, etc. cytokines which ultimately causes the imbalance between oxidants and anti-oxidants which disturbs body's equilibrium and push our body system towards oxidative stress and responsible for the low level of SOD, GSH, NO and

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

high level of MDA in COPD patients but there are few reports which suggested that they have found high level of SOD and NO in COPD patients [70, 74]. Biomass fuel is also responsible for the COPD in females in villages and small towns in India. Oxidative stress is also a key factor in DNA damage. DNA damage caused by enzymes leads to mutation in DNA and protein change.

This is well known fact that matrix-metalloproteinases degrades the lung epithelial cells until or unless they are inhibited by tissue inhibitors like TIMPs. So, TIMPs and MMPs are closely related with each other. TIMPs inhibits the MMPs enzymes but in diseases like COPD, these MMPs are inhibited by TIMPs as well as the inflammation created in human physiological system, so they are hyper-regulated and starts to eat lung's walls. It degrades extracellular matrix of lungs. Various SNPs were studied in different regions like Asians, Egyptians, etc. and found different results for polymorphism of these SNPs in COPD and control groups. Different SNPs were studied of MMP2 and TIMP2 genes, where -1306 MMP2 SNP was having different genotype in Central Bulgaria population but not different allele frequency but MMP2 rs243864, rs3918253, rs11646643 SNPs and MMP2 rs2285053 and rs243865 were associated with the risk of COPD in Mexican and Asian population respectively. TIMP2 +853 and -418 SNPs studied in Japanese population, showed the low level of TIMP2 protein in COPD patients. Also there was difference in Egyptian population, where +853 SNP in TIMP2 was showing differences in COPD and Control group but not in -418 SNP. Mechanism showing different possible pathways which could be a triggering point in severity of disease or cause of disease. All these factors are very important which we can study or do some experiments for treating COPD.

So, there are various views regarding these genotype or allelic differences in COPD and control groups of different populations all over the world. Many have seen differences but few have not.

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We are going to correlate the MMP2 and TIMP2 genetic differences in COPD and control groups of the North Indian population for the very first time. Till date, to the best of our knowledge, it is not reported anywhere.

13. CONCLUSION

COPD is a respiratory disease which is caused by smoke, dust, biomass fuel, cigarette, etc. and also due to oxidative stress which is triggered by the inflammatory molecules. These inflammatory molecules lead to severity of disease in patients. Proteases-antiproteases imbalance also plays a key role in COPD development. MMP2-TIMP2 are related to each other because TIMP2 is an inhibitor of MMP2 and in case of COPD, when TIMP2 is down-regulated, MMP2 starts to digest the extracellular matrix and leads to respiratory dis-comfort in patient. Recapitulating, MMP-2 appears to play an important part in the airway ECM remodeling in COPD. It appears that numerous MMPs are over-regulated in alveolar macrophages, both in humans with emphysema and in cigarette bank-convinced mouse and guinea gormandizer models; and over-expression of some MMPs is significant. Since MMPs retain an active catalytic point and natural *in vivo* impediments, they incontinently become therapeutic targets. A wide range of MMP inhibition may be useful for minimal inhibition of ECM declination. Monoclonal antibodies against MMPs, targeting the substrate of MMPs, can alike be capitalized to clock productive MMP inhibition. Combinatorial therapy , similar to an MMP asset plus a β2 agonist or low-cure steroids could be another strategy for remedial operation of the complaint. Despite rigorous scientific trouble, the discovery of applicable MMP assets for COPD remains a challenge. It is not easy to develop MMP

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

impediments that are largely picky and specific thereby targeting only applicable MMPs. It's pivotal to understand the successional expression and individual contribution of each MMPs in the initiation and progression of COPD to allow for the development of a picky and specific target. Also, systemic toxin, lack of correlation between the exertion of the asset and MMP situations in the tube, and poor efficacy are some of the issues which need attention. Defensive impunity and towel form should also be a matter of concern while considering MMP inhibitory therapeutic strategy.

DECLARATION OF INTEREST:

We hereby declare that the manuscript has not been previously published in any language anywhere and that is not under simultaneous consideration by another journal. None of the authors have any conflict of interest or any financial ties to disclose.

AUTHORS CONTRIBUTION:

PS and HQ contributed to the study conception and design; data acquisition, analysis, and interpretation; drafting of the manuscript and final approval of the version to be published. JGS, AH and MIA contributed to the study conception and design; data acquisition, analysis, and interpretation; drafting of the manuscript; critical revision of the manuscript for important intellectual content; and final approval of the version to be published.

References:

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

1. Shapiro S D. The macrophage in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999; 160:S29-32.
2. Murphy G. Tissue inhibitors of metalloproteinases. Genome Biol 2011; 12, 233.
3. Markewitz B A, Owens M W, Payne D K. The pathogenesis of the chronic obstructive pulmonary disease. Am J Med Sci 1999; 318:74-8.
4. Martinez FD. Early-life origins of chronic obstructive pulmonary disease. New England Journal of Medicine. 2016 Sep 1; 375(9):871-78.
5. Fischer B.M., Pavlisko E., Voynow J.A. (2011). Pathogenic triad in COPD: oxidative stress, protease-antiprotease imbalance, and inflammation. Int J Chron Obstruct Pulmon Dis, 6, 413–421. https://doi.org/10.2147/COPD.S10770
6. Srivastava P.K., Dastidar S.G., Ray A. (2007). Chronic obstructive pulmonary disease: role of matrix metalloproteases and future challenges of drug therapy. Expert Opin Investig Drugs, 16, 7, 1069–1078. https://doi.org/10.1517/13543784.16.7.1069
7. Harju T, Kinnula VL, Paakko P et al (2010) Variability in the precursor proteins of collagen I and III in different stages of COPD. Respir Res 11:165.
8. Vestbo J, Hurd SS, Rodriguez-Roisin R (2012) The 2011 revision of the global strategy for the diagnosis, management and prevention of COPD (GOLD)—why and what? Clin Respir J 6:208–214.
9. Adeloye D, Chua S, Lee C, et al. Global and regional estimates of COPD prevalence: systematic review and meta-analysis. J Glob Health 2015; 5: 020415.
10. Anthonisen NR, Skeans MA, Wise RA, Manfreda J, Kanner RE, Connett JE. The effects of a smoking cessation intervention on 14.5-year mortality: a randomized clinical trial. Ann Intern Med 2005; 142: 233–39.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

11. Calverley PM, Anderson JA, Celli B, et al. Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. N Engl J Med 2007; 356: 775–89.

12. Franssen FM, Soriano JB, Roche N, et al. Lung function abnormalities in smokers with ischemic heart disease. Am J Respir Crit Care Med 2016; 194: 568–76.

13. O'Donnell DE. Hyperinflation, dyspnea, and exercise intolerance in chronic obstructive pulmonary disease. Proc Am Thorac Soc 2006; 3: 180–84.

14. Obeidat M, Hao K, Bosse Y, et al. Molecular mechanisms underlying variations in lung function: a systems genetics analysis. Lancet Respir Med 2015; 3: 782–95.

15. Wain LV, Shrine N, Miller S, et al. Novel insights into the genetics of smoking behaviour, lung function, and chronic obstructive pulmonary disease (UK BiLEVE): a genetic association study in UK Biobank. Lancet Respir Med 2015; 3: 769–81.

16. Houghton AM, Mouded M, Shapiro SD. Common origins of lung cancer and COPD. Nat Med 2008; 14: 1023–24.

17. Anthonisen NR, Skeans MA, Wise RA, Manfreda J, Kanner RE, Connett JE. The effects of a smoking cessation intervention on 14.5-year mortality: a randomized clinical trial. Ann Intern Med 2005; 142: 233–39.

18. Jimenez-Ruiz CA, Andreas S, Lewis KE, et al. Statement on smoking cessation in COPD and other pulmonary diseases and in smokers with comorbidities who find it difficult to quit. Eur Respir J 2015; 46: 61–79.

19. Hoogendoorn M, Feenstra TL, Hoogenveen RT, et. al. Long-term effectiveness and costeffectiveness of smoking cessation interventions in patients with COPD. Thorax 2010; 65: 711–18.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

20. Elkington PTF, Friedland JS. Matrix metalloproteinases in destructive pulmonary pathology. Thorax 2006; 61:259 –266.

21. Gueders MM, Foidart JM, Noel A, et al. Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in the respiratory tract: potential implications in asthma and other lung diseases. Eur J Pharmacol 2006; 533:133–144.

22. Shapiro SD. The macrophage in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999; 160:S29-S32.

23. Howard EW, Bullen EC, Banda MJ: Preferential inhibition of 72- and 92-kDa gelatinases by tissue inhibitor of metalloproteinases-2. J Biol Chem 1991, 266(20):13070-13075.

24. Vitenberga, Zane et al. (2017) "COPD affected lung tissue remodelling due to the local distribution of MMP-2, TIMP-2, TGF-β1 and HSP-70. Papers on Anthropology XXVI/2, 2017, pp. 157–167. DOI: 10.12697/poa.2017.26.2.16.

25. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol. 1997 Mar; 82(2):291-5. doi: 10.1113/expphysiol.1997.sp004024. PMID: 9129943.

26. Pryor, W.A.; Stone, K. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. Ann. N. Y. Acad. Sci. 1993, 686, 12–27.

27. Ahmad A, Shameem M, Husain Q. Altered oxidant-antioxidant levels in the disease prognosis of chronic obstructive pulmonary disease. Int J Tuberc Lung Dis. 2013 Aug; 17(8):1104-9. doi: 10.5588/ijtld.12.0512. PMID: 23827037.

28. Rahman I, MacNee W. Role of transcription factors in inflammatory lung diseases. Thorax. 1998 Jul; 53(7):601-12. doi: 10.1136/thx.53.7.601. PMID: 9797762; PMCID: PMC1745272.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

29. Uchida K, Shiraishi M, Naito Y,et. al. Activation of stress signaling pathways by the end product of lipid peroxidation. 4-hydroxy-2-nonenal is a potential inducer of intracellular peroxide production. J Biol Chem. 1999 Jan 22; 274(4):2234-42. doi: 10.1074/jbc.274.4.2234. PMID: 9890986.

30. Rahman I, van Schadewijk AA, Crowther AJ,et. al. 4-Hydroxy-2-nonenal, a specific lipid peroxidation product, is elevated in lungs of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2002 Aug 15; 166(4):490-5. doi: 10.1164/rccm.2110101. PMID: 12186826.

31. Aoshiba K, Koinuma M, Yokohori N, Nagai A. Immunohistochemical evaluation of oxidative stress in murine lungs after cigarette smoke exposure. Inhal Toxicol. 2003 Sep; 15(10):1029-38. doi: 10.1080/08958370390226431. PMID: 12928978.

32. Farmer EE, Mueller MJ. ROS-mediated lipid peroxidation and RES-activated signaling. Annu Rev Plant Biol. 2013; 64:429-50. doi: 10.1146/annurev-arplant-050312-120132. Epub 2013 Feb 28. PMID: 23451784.

33. Davey MW, Stals E, Panis B, et. al. High-throughput determination of malondialdehyde in plant tissues. Anal Biochem. 2005 Dec 15; 347(2):201-7. doi: 10.1016/j.ab.2005.09.041. Epub 2005 Oct 21. PMID: 16289006.

34. Ohkawa H, Ohishi N, Yagi K. Reaction of linoleic acid hydroperoxide with thiobarbituric acid. J Lipid Res. 1978 Nov; 19(8):1053-7. PMID: 103988.

35. Khoubnasabjafari M, Soleymani J, Jouyban A. Avoid using spectrophotometric determination of malondialdehyde as a biomarker of oxidative stress. Biomark Med. 2018 Jun;12(6):551-554. doi: 10.2217/bmm-2017-0437. Epub 2018 Jun 6. PMID: 29873547.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

36. Morales M, Munné-Bosch S. Malondialdehyde: Facts and Artifacts. Plant Physiol. 2019 Jul; 180(3):1246-1250. doi: 10.1104/pp.19.00405. PMID: 31253746; PMCID: PMC6752910.

37. Dasgupta, A & Klein, K. (2014). Methods for Measuring Oxidative Stress in the Laboratory. 10.1016/B978-0-12-405872-9.00002-1.

38. Shibata, T., et al. Identification of a lipid peroxidation product as a potential trigger of the p53 pathway. Journal of Biological Chemistry. 281(2), 1196-1204 (2006).

39. Skoog, D.A., West, D. M., Holler, F.J., and Crouch , S.R. Sampling, Standardization, and Calibration. In Fundamentals of Analytical Chemistry. 9th ed., Ch. 7, 153-196, Cengage-Brooks/Cole, Belmont, CA, (2014).

40. Rahman I, Li XY, Donaldson K,et. al. Glutathione homeostasis in alveolar epithelial cells in vitro and lung in vivo under oxidative stress. Am J Physiol. 1995 Sep; 269(3 Pt 1):L285-92. doi: 10.1152/ajplung.1995.269.3.L285. PMID: 7573460.

41. Cantin AM, North SL, Hubbard RC, et. al. Normal alveolar epithelial lining fluid contains high levels of glutathione. J Appl Physiol (1985). 1987 Jul; 63(1):152-7. doi: 10.1152/jappl.1987.63.1.152. PMID: 3040659.

42. D.J. Jollow, J.R. Mitchell and N. Zampaglione et al. Bromobenzene-Induced Liver Necrosis. Protective Role of Glutathione and Evidence for 3,4-Bromobenzene Oxide as the Hepatotoxic Metabolite. *Pharmacology.* Vol. 11(3):151-169. DOI: 10.1159/000136485.

43. McCord JM, Fridovich I. The reduction of cytochrome c by milk xanthine oxidase. J Biol Chem. 1968 Nov 10; 243(21):5753-60. PMID: 4972775.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

44. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974 Sep 16; 47(3):469-74. doi: 10.1111/j.1432-1033.1974.tb03714.x. PMID: 4215654.

45. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972 May 25; 247(10):3170-5. PMID: 4623845.

46. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev. 1991 Jun; 43(2):109-42. PMID: 1852778.

47. Petruzzelli S, Puntoni R, Mimotti P, Pulerà N, Baliva F, Fornai E, Giuntini C. Plasma 3-nitrotyrosine in cigarette smokers. Am J Respir Crit Care Med. 1997 Dec; 156(6):1902-7. doi: 10.1164/ajrccm.156.6.9702075. PMID: 9412573.

48. Corradi M, Majori M, Cacciani GC, Consigli GF, de'Munari E, Pesci A. Increased exhaled nitric oxide in patients with stable chronic obstructive pulmonary disease. Thorax. 1999 Jul; 54(7):572-5. doi: 10.1136/thx.54.7.572. PMID: 10377199; PMCID: PMC1745512.

49. Rutgers SR, van der Mark TW, Coers W, Moshage H, Timens W, Kauffman HF, Koëter GH, Postma DS. Markers of nitric oxide metabolism in sputum and exhaled air are not increased in chronic obstructive pulmonary disease. Thorax. 1999 Jul; 54(7):576-80. doi: 10.1136/thx.54.7.576. PMID: 10377200; PMCID: PMC1745531.

50. Maziak W, Loukides S, Culpitt S, Sullivan P, Kharitonov SA, Barnes PJ. Exhaled nitric oxide in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1998 Mar; 157(3 Pt 1):998-1002. doi: 10.1164/ajrccm.157.3.97-05009. PMID: 9517624.

51. Arif E, Ahsan A, Vibhuti A, Rajput C, Deepak D, Athar M, Singh B, Pasha MA. Endothelial nitric oxide synthase gene variants contribute to oxidative stress in COPD.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

Biochem Biophys Res Commun. 2007 Sep 14; 361(1):182-8. doi: 10.1016/j.bbrc.2007.07.008. Epub 2007 Jul 16. PMID: 17658478.

52. MacNee W. Oxidants and COPD. Curr Drug Targets Inflamm Allergy. 2005 Dec; 4(6):627-41. doi: 10.2174/156801005774912815. PMID: 17305519.

53. Hansel TT, Barnes PJ. New drugs for exacerbations of chronic obstructive pulmonary disease. Lancet. 2009 Aug 29; 374(9691):744-55. doi: 10.1016/S0140-6736(09)61342-8. PMID: 19716967.

54. Loukides S, Bakakos P, Kostikas K. Oxidative stress in patients with COPD. Curr Drug Targets. 2011 Apr; 12(4):469-77. doi: 10.2174/138945011794751573. PMID: 21194408.

55. Rahman I, MacNee W. Role of oxidants/antioxidants in smoking-induced lung diseases. Free Radic Biol Med. 1996; 21(5):669-81. doi: 10.1016/0891-5849(96)00155-4. PMID: 8891669.

56. Rahman I, MacNee W. Lung glutathione and oxidative stress: implications in cigarette smoke-induced airway disease. Am J Physiol. 1999 Dec; 277(6):L1067-88. doi: 10.1152/ajplung.1999.277.6.L1067. PMID: 10600876.

57. Rahman I, MacNee W. Role of transcription factors in inflammatory lung diseases *Thorax* 1998; **53:**601-612.

58. Church DF, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect. 1985 Dec; 64:111-26. doi: 10.1289/ehp.8564111. PMID: 3007083; PMCID: PMC1568603.

59. Nakayama T, Church DF, Pryor WA. Quantitative analysis of the hydrogen peroxide formed in aqueous cigarette tar extracts. Free Radic Biol Med. 1989; 7(1):9-15. doi: 10.1016/0891-5849(89)90094-4. PMID: 2753397.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

60. Gompertz S, Bayley DL, Hill SL, Stockley RA. Relationship between airway inflammation and the frequency of exacerbations in patients with smoking related COPD. Thorax. 2001 Jan; 56(1):36-41. doi: 10.1136/thorax.56.1.36. PMID: 11120902; PMCID: PMC1745913.

61. Uchida K, Shiraishi M, Naito Y, Torii Y, Nakamura Y, Osawa T. Activation of stress signaling pathways by the end product of lipid peroxidation. 4-hydroxy-2-nonenal is a potential inducer of intracellular peroxide production. J Biol Chem. 1999 Jan 22; 274(4):2234-42. doi: 10.1074/jbc.274.4.2234. PMID: 9890986.

62. Petruzzelli S, Puntoni R, Mimotti P, Pulerà N, Baliva F, Fornai E, Giuntini C. Plasma 3-nitrotyrosine in cigarette smokers. Am J Respir Crit Care Med. 1997 Dec; 156(6):1902-7. doi: 10.1164/ajrccm.156.6.9702075. PMID: 9412573.

63. Morrison D, Rahman I, Lannan S, MacNee W. Epithelial permeability, inflammation, and oxidant stress in the air spaces of smokers. Am J Respir Crit Care Med. 1999 Feb; 159(2):473-9. doi: 10.1164/ajrccm.159.2.9804080. PMID: 9927360.

64. Rahman I, Li XY, Donaldson K, Harrison DJ, MacNee W. Glutathione homeostasis in alveolar epithelial cells in vitro and lung in vivo under oxidative stress. Am J Physiol. 1995 Sep; 269(3 Pt 1):L285-92. doi: 10.1152/ajplung.1995.269.3.L285. PMID: 7573460.

65. Carp H, Miller F, Hoidal JR, Janoff A. Potential mechanism of emphysema: alpha 1-proteinase inhibitor recovered from lungs of cigarette smokers contains oxidized methionine and has decreased elastase inhibitory capacity. Proc Natl Acad Sci U S A. 1982 Mar; 79(6):2041-5. doi: 10.1073/pnas.79.6.2041. PMID: 6979049; PMCID: PMC346118.

66. Baraldo S, Bazzan E, Zanin ME, Turato G, Garbisa S, Maestrelli P, Papi A, Miniati M, Fabbri LM, Zuin R, Saetta M. Matrix metalloproteinase-2 protein in lung periphery is related to COPD progression. Chest. 2007 Dec 1;132(6):1733-40.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

67. Devarajan P, Johnston JJ, Ginsberg SS, Van Wart HE, Berliner N. Structure and expression of neutrophil gelatinase cDNA. Identity with type IV collagenase from HT1080 cells. J Biol Chem 1992;267: 25228-32.

68. Mott JD, Werb Z. Regulation of matrix biology by matrix metalloproteinases. Curr Opin Cell Biol 2004;16:558-64.

69. Amalinei C, Caruntu ID, Giusca SE, Balan RA. Matrix metalloproteinases involvement in pathologic conditions. Rom J Morphol Embryol. 2010;51:215-228.

70. Tacheva T, Dimov D, Vlaykova T. Gelatinases in COPD and bronchial asthma. Sci Technol Med. 2012; 2(1):255-260.

71. Fanjul-Fernandez M, Folgueras AR, Cabrera S, LopezOtin C. Matrix metalloproteinases: evolution, gene regulation and functional analysis in mouse models. Biochim Biophys Acta. 2010; 1803:3-19.

72. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res. 2003; 92:827-839.

73. Qin H, Sun Y, Benveniste EN. The transcription factors Sp1, Sp3, and AP-2 are required for constitutive matrix metalloproteinase-2 gene expression in astroglioma cells. J Biol Chem. 1999; 274:29130-29137.

74. Tacheva, T., Chelenkova, P., Dimov, D., et. al. (2015). Frequency of the common promoter polymorphism MMP2− 1306 C>T in a population from central Bulgaria. *Biotechnology & Biotechnological Equipment*, *29* (2), 351-356.

75. Greene J, Wang M, Liu YE, et. al. Molecular cloning and characterization of human tissue inhibitor of metalloproteinase 4. J Biol Chem 1996; 271: 30375–30380.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

76. Ohnishi K, Takagi M, Kurokawa Y, Satomi S, Konttinen YJ. Matrix metalloproteinase-mediated extracellular matrix protein degradation in human pulmonary emphysema. Lab Invest 1998; 78: 1077– 1087.

77. Fischer B.M., Pavlisko E., Voynow J.A. (2011). Pathogenic triad in COPD: oxidative stress, protease-antiprotease imbalance, and inflammation. Int J Chron Obstruct Pulmon Dis, 6, 413–421.

78. Ghanei M., Ghalejooghi N.A., Nourani M.R., Harandi A.A., Fooladi A.A. (2010). Effect of TGFß1 and TIMP2 on disease activity in asthma and COPD. Iran J Allergy Asthma Immunol, 9, 2, 79–86.

79. Ramos C, Cisneros J, Gonzalez-Avila G, Becerril C, Ruiz Vc, Montaño M. Increase of Matrix Metalloproteinases in Woodsmoke-Induced Lung Emphysema in Guinea Pigs. Inhalation Toxicology 2009; 21:119-32.

80. Bakowska J, Adamson IY. Collagenase and gelatinase activities in bronchoalveolar lavage fluids during bleomycin-induced lung injury. J Pathol. 1998 Jul; 185(3):319-23. doi: 10.1002/(SICI)1096-9896(199807)185:3<319::AID-PATH114>3.0.CO;2-L. PMID: 9771487.

81. Ohnishi K, Takagi M, Kurokawa Y, Satomi S, Konttinen YT. Matrix metalloproteinase-mediated extracellular matrix protein degradation in human pulmonary emphysema. Lab Invest. 1998 Sep; 78(9):1077-87. PMID: 9759652.

82. Zhang Y, Li Y, Ye Z, Ma H. Expression of Matrix Metalloproteinase-2, Matrix Metalloproteinase-9, Tissue Inhibitor of Metalloproteinase-1, and Changes in Alveolar Septa in Patients with Chronic Obstructive Pulmonary Disease. Med Sci Monit. 2020 Oct 18; 26: e925278. Doi : 10.12659/MSM.925278. PMID: 33070147; PMCID: PMC7580176.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

83. Deb, Nilanjana & Mallick, Sudipta & Jaiswal, Ashish & Manna, Anirban & Mabalirajan, Ulaganathan & Swarnakar, Snehasikta. (2019). Role of MMPs and Oxidants in Lung Diseases. 10.1007/978-981-13-8413-4_8.

84. Ratajczak-Wielgomas K, Gosk J, Rabczyński J, Augoff K, Podhorska-Okołów M, Gamian A, Rutowski R. Expression of MMP-2, TIMP-2, TGF-β1, and decorin in Dupuytren's contracture. Connect Tissue Res. 2012; 53(6):469-77. doi: 10.3109/03008207.2012.686542. Epub 2012 Jul 24. PMID: 22512703.

85. Vitenberga-Verza, Zane & Pilmane, Māra & Babjoniseva, Aurika. (2017). COPD affected lung tissue remodelling due to the local distribution of MMP-2, TIMP-2, TGF-β1 and HSP-70. Papers on Anthropology. 26. 157. 10.12697/poa.2017.26.2.16.

86. Strongin AY, Collier I, Bannikov G, Marmer BL, Grant GA, Goldberg GI. Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. J Biol Chem. 1995 Mar 10; 270(10):5331-8. doi: 10.1074/jbc.270.10.5331. PMID: 7890645.

87. Butler GS, Butler MJ, Atkinson SJ, Will H, Tamura T, Schade van Westrum S, Crabbe T, Clements J, d'Ortho MP, Murphy G. The TIMP2 membrane type 1 metalloproteinase "receptor" regulates the concentration and efficient activation of progelatinase A. A kinetic study. J Biol Chem. 1998 Jan 9; 273(2):871-80. doi: 10.1074/jbc.273.2.871. PMID: 9422744.

88. Peeney D, Liu Y, Lazaroff C, Gurung S, Stetler-Stevenson WG. Unravelling the distinct biological functions and potential therapeutic applications of TIMP2 in cancer. Carcinogenesis. 2022 Jun 4; 43(5):405-418. doi: 10.1093/carcin/bgac037. PMID: 35436325; PMCID: PMC9167030.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

89. Wang Z, Juttermann R, Soloway PD. TIMP-2 is required for efficient activation of proMMP-2 in vivo. J Biol Chem. 2000 Aug 25; 275(34):26411-5. doi: 10.1074/jbc.M001270200. PMID: 10827175; PMCID: PMC2683068.

90. Strongin AY, Collier I, Bannikov G, Marmer BL, Grant GA, Goldberg GI. Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. J Biol Chem. 1995 Mar 10; 270(10):5331-8. doi: 10.1074/jbc.270.10.5331. PMID: 7890645.

91. Lähteenvuo J, Rosenzweig A. Effects of aging on angiogenesis. Circ Res. 2012 Apr 27; 110(9):1252-64. doi: 10.1161/CIRCRESAHA.111.246116. PMID: 22539758; PMCID: PMC4101916.

92. Wang, Z., Juttermann, R., and Soloway, P.D. TIMP2 is required for efficient activation of proMMP2 in vivo. J. Biol. Chem. 2000; 275, 26411-26415.

93. Tacheva T, Chelenkova P, Dimov D, Petkova R, Chakarov S, Vlaykova T. Frequency of the common promoter polymorphism MMP2− 1306 C> T in a population from central Bulgaria. Biotechnology & Biotechnological Equipment. 2015 Mar 4; 29(2):351-6.

94. Montoya J, Ramos J, Montaño M, Ramírez-Venegas A, Sansores RH, Pérez-Rubio G, Velázquez-Uncal M, Camarena A, Ramos C, Falfán-Valencia R. Genetic polymorphisms of matrix metalloproteinases and protein levels in chronic obstructive pulmonary disease in a Mexican population. Biomark Med. 2015; 9(10):979-88. doi: 10.2217/bmm.15.75. Epub 2015 Oct 6. PMID: 26439471.

95. Guo XT, Wang JF, Zhang LY, Xu GQ. Quantitative assessment of the effects of MMP-2 polymorphisms on lung carcinoma risk. Asian Pac J Cancer Prev. 2012; 13(6):2853-6. doi: 10.7314/apjcp.2012.13.6.2853. PMID: 22938472.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

96. Hirano K, Sakamoto T, Uchida Y, Morishima Y, Masuyama K, Ishii Y, Nomura A, Ohtsuka M, Sekizawa K. Tissue inhibitor of metalloproteinases-; 2 gene polymorphisms in chronic obstructive pulmonary disease. European Respiratory Journal. 2001 Nov 1; 18(5):748-52.

97. Hegab AE, Sakamoto T, Uchida Y, Nomura A, Ishii Y, Morishima Y, Mochizuki M, Kimura T, Saitoh W, Kiwamoto T, Iizuka T. Association analysis of tissue inhibitor of metalloproteinase2 gene polymorphisms with COPD in Egyptians. Respiratory medicine. 2005 Jan 1; 99(1):107-10.

98. Korytina GF, Akhmadishina LZ, Viktorova EV, Tselousova OS, Danilko KV, Kochetova OV, Viktorova TV. Extracellular matrix remodeling genes polymorphisms and risk of chronic bronchitis and recurrent pneumonia in children. J Hum Genet. 2013 Jul; 58(7):467-74. doi: 10.1038/jhg.2013.24. Epub 2013 Apr 11. PMID: 23575435.

99. Global Initiative for Chronic Obstructive Lung Disease (GOLD): GOLD 2017 global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease, 2017 report. [https://goldcopd.org/gold-2017-global-strategy-diagnosis-management-%20prevention-copd/]

100. Koul P A. Chronic obstructive pulmonary disease: Indian guidelines and the road ahead. Lung India 2013; 30:175-7.

101. Gueders M M, Foidart J M, Noel A, Cataldo D D. Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in the respiratory tract: potential implications in asthma and other lung diseases. Eur J Pharmacol 2006; 533:133-44.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

102. Ramos C, Cisneros J, Gonzalez-Avila G, Becerril C, Ruiz Vc, Montaño M. Increase of Matrix Metalloproteinases in Woodsmoke-Induced Lung Emphysema in Guinea Pigs. Inhalation Toxicology 2009; 21:119-32.

103. Wills-Karp M. Interleukin-13 in asthma pathogenesis. Immunol Rev 2004; 202:175-90.

104. Owen C A, Hu Z, Barrick B, Shapiro S D. Inducible expression of tissue inhibitor of metalloproteinases-resistant matrix metalloproteinase-9 on the cell surface of neutrophils. Am J Respir Cell Mol Biol 2003; 29:283-94.

105. Culpitt S V, Rogers D F, Traves S L, Barnes P J, Donnelly L E. Sputum matrix metalloproteases: comparison between chronic obstructive pulmonary disease and asthma. Respiratory Medicine 2005; 99:703-10.

106. Docherty A J, Lyons A, Smith B J, Wright E M, Stephens P E, Harris T J et al. Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity. Nature 1985; 318:66-9.

107. Hirano K, Sakamoto T, Uchida Y, Morishima Y, Masuyama K, Ishii Y, Nomura A, Ohtsuka M, Sekizawa K. Tissue inhibitor of metalloproteinases-2 gene polymorphisms in chronic obstructive pulmonary disease. Eur Respir J. 2001 Nov; 18(5):748-52. doi: 10.1183/09031936.01.00102101. PMID: 11757622.

108. Barnes PJ. Chronic Obstructive Pulmonary Disease. N Engl J Med 2000; 343:269-280. DOI: 10.1056/NEJM20000727343040.

109. Hegab AE, Sakamoto T, Uchida Y, Nomura A, Ishii Y, Morishima Y, Mochizuki M, Kimura T, Saitoh W, Kiwamoto T, Iizuka T, Massoud HH, Massoud HM, Hassanein KM, Sekizawa K. Association analysis of tissue inhibitor of metalloproteinase2 gene

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

polymorphisms with COPD in Egyptians. Respir Med. 2005 Jan; 99(1):107-10. doi: 10.1016/j.rmed.2004.05.017. PMID: 15672858.

110. Tanya Tacheva, Dimo Dimov, Pavlina Chelenkova, Rumena Petkova, Krasimir D imov, Stoyan Chakarov, Tatyana Vlaykova. Eur Respir J. 2015 46: PA861; **DOI:** 10.1183/13993003.congress-2015.PA861.

111. Ishida M, Mikami S, Kikuchi E, Kosaka T, Miyajima A, Nakagawa K, Mukai M, Okada Y, Oya M. Activation of the aryl hydrocarbon receptor pathway enhances cancer cell invasion by upregulating the MMP expression and is associated with poor prognosis in upper urinary tract urothelial cancer. Carcinogenesis. 2010 Feb 1; 31(2):287-95.

112. John A, Tuszynski G. The role of matrix metalloproteinases in tumor angiogenesis and tumor metastasis. Pathol Oncol Res. 2001; 7(1):14-23. doi: 10.1007/BF03032599. PMID: 11349215.

113. Ala-aho R, Kähäri VM. Collagenases in cancer. Biochimie. 2005 Mar-Apr; 87(3-4):273-86. doi: 10.1016/j.biochi.2004.12.009. PMID: 15781314.

114. Ye S. Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. Matrix Biol. 2000 Dec; 19(7):623-9. doi: 10.1016/s0945-053x(00)00102-5. PMID: 11102751.

115. H.E. Van Wart, H. Birkedal-Hansen. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc. Natl. Acad. Sci. USA, 87 (1990), pp. 5578-5582.

116. S. Rajagopalan, X.P. Meng, S. Ramasamy, D.G. Harrison, Z.S. Galis. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro: implications for atherosclerotic plaque stability. J. Clin. Invest., 98 (1996), pp. 2572-2579.

117. F. Valentin, J.L. Bueb, P. Kieffer, E. Tschirhart, J. Atkinson. Oxidative stress activates MMP-2 in cultured human coronary smooth muscle cells. Fundam. Clin. Pharmacol., 19 (2005), pp. 661-667.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

118. K. Grote, I. Flach, M. Luchtefeld, E. Akin, S.M. Holland, H. Drexler, B. Schieffer . Mechanical stretch enhances mRNA expression and proenzyme release of matrix metalloproteinase-2 (MMP-2) via NAD(P)H oxidase-derived reactive oxygen species. Circ. Res., 92 (2003), pp. e80-e86.

119. R. Schulz. Intracellular targets of matrix metalloproteinase-2 in cardiac disease: rationale and therapeutic approaches. Annu. Rev. Pharmacol. Toxicol., 47 (2007), pp. 211-242.

120. S. Viappiani, A.C. Nicolescu, A. Holt, G. Sawicki, B.D. Crawford, H. Leon, T. va n Mulligen, R. Schulz. Activation and modulation of 72 kDa matrix metalloproteinase-2 by peroxynitrite and glutathione. Biochem. Pharmacol., 77 (2008), pp. 826-834.

121. Zhang HJ, Zhao W, Venkataraman S, Robbins ME, Buettner GR, Kregel KC, Oberley LW: Activation of matrix metalloproteinase-2 by overexpression of manganese superoxide dismutase in human breast cancer MCF-7 cells involves reactive oxygen species. *J Biol Chem* 2002, 277(23):20919-20926. 10.1074/jbc.M109801200.

122. ManDal, M., Das, S., Chakraborti, T. *et al.* Role of matrix metalloprotease-2 in oxidant activation of Ca^{2+}ATPase by hydrogen peroxide in pulmonary vascular smooth muscle plasma membrane. *J Biosci* **28**, 205–213 (2003). https://doi.org/10.1007/BF02706220.

123. Kawaguchi, Y., Tanaka, H., Okada, T. *et al.* The effects of ultraviolet A and reactive oxygen species on the mRNA expression of 72-kDa type IV collagenase and its tissue inhibitor in cultured human dermal fibroblasts. *Arch Dermatol Res* **288**, 39–44 (1996). https://doi.org/10.1007/BF02505041.

124. Hashimoto T, Uchida K, Okayama N, Imate Y, Suehiro Y, Hamanaka Y, Ueyama Y, Shinozaki F, Yamashita H, Hinoda Y. Association of matrix metalloproteinase (MMP)-1 promoter polymorphism with head and neck squamous cell carcinoma, Cancer Letters, Vol 211(1), 2004,19-24.

125. Van Diemen, C., Postma, D., Siedlinski, M. *et al.* Genetic variation in *TIMP1* but not *MMPs* predict excess FEV_1 decline in two general population-based cohorts. *Respir Res* **12**, 57 (2011). https://doi.org/10.1186/1465-9921-12-57.

126. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, Zielinski J; Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

2007 Sep 15; 176(6):532-55. doi: 10.1164/rccm.200703-456SO. Epub 2007 May 16. PMID: 17507545.

127. Kim CH, Oh Y, Lee TH. Codon optimization for high-level expression of human erythropoietin (EPO) in mammalian cells. Gene. 1997 Oct 15; 199(1-2):293-301. doi: 10.1016/s0378-1119(97)00384-3. PMID: 9358069.

128. Ross J. mRNA stability in mammalian cells. Microbiol Rev. 1995 Sep; 59(3):423-50. doi: 10.1128/mr.59.3.423-450.1995. PMID: 7565413; PMCID: PMC239368.

129. Ohnishi K, Takagi M, Kurokawa Y, Satomi S, Konttinen YT. Matrix metalloproteinase-mediated extracellular matrix protein degradation in human pulmonary emphysema. Lab Invest. 1998 Sep; 78(9):1077-87. PMID: 9759652.

130. De Clerck YA, Darville MI, Eeckhout Y, Rousseau GG. Characterization of the promoter of the gene encoding human tissue inhibitor of metalloproteinases-2 (TIMP-2). Gene. 1994 Feb 25; 139(2):185-91. doi: 10.1016/0378-1119(94)90753-6. PMID: 8112602.

131. Willemse BWM, ten Hacken NHT, Rutgers B, Lesman-Leegte I, Postma DS, Timens W. Effect of 1-year smoking cessation on airway inflammation in COPD and asymptomatic smokers. European Respiratory Journal. 2005 Nov; 26(5):835-45.

132. Morris A, Beck JM, Schloss PD, Campbell TB, Crothers K, Curtis JL, et al. Comparison of the respiratory microbiome in healthy nonsmokers and smokers. Am J Respir Crit Care Med. 2013 May 15; 187(10):1067-75.

133. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004 Mar 5; 303(5663):1532-5.

134. Yipp BG, Kubes P. NETosis: how vital is it? Blood. 2013 Oct 17;122(16):2784-94

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

135. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. J Cell Biol. 2010 Nov 1; 191(3):677-91.

136. Grabcanovic-Musija F, Obermayer A, Stoiber W, Krautgartner WD, Steinbacher P, Winterberg N, et al. Neutrophil extracellular trap (NET) formation characterises stable and exacerbated COPD and correlates with airflow limitation. Respir Res. 2015; 16:59.

137. Obermayer A, Stoiber W, Krautgartner W-D, Klappacher M, Kofler B, Steinbacher P, et al. New Aspects on the Structure of Neutrophil Extracellular Traps from Chronic Obstructive Pulmonary Disease and In Vitro Generation. Plos One. 2014 15/05/2014; 9(5).

138. Pedersen F, Marwitz S, Holz O, Kirsten A, Bahmer T, Waschki B, et al. Neutrophil extracellular trap formation and extracellular DNA in sputum of stable COPD patients. Respir Med. 2015 Oct; 109(10):1360-2.

139. Foell D, Wittkowski H, Vogl T, Roth J. S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. J Leukoc Biol. 2007; 81:28–37.

140. Crooks SW, Bayley DL, Hill SL, et al. Bronchial inflammation in acute bacterial exacerbations of chronic bronchitis: the role of leukotriene B4. Eur Respir J 2000; 15: 274–280.

141. Haas TL, Stitelman D, Davis SJ, Apte SS, Madri JA. Egr-1 mediates extracellular matrix-driven transcription of membrane type 1 matrix metalloproteinase in endothelium. J Biol Chem 1999; 274:22679–22685.

142. Ning W, Li CJ, Kaminski N, Feghali-Bostwick CA, Alber SM, Di YP, Otterbein SL, Song R, Hayashi S, Zhou Z, Pinsky DJ, Watkins SC, Pilewski JM, Sciurba FC, Peters

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

DG, Hogg JC, Choi AM. Comprehensive gene expression profiles reveal pathways related to the pathogenesis of chronic obstructive pulmonary disease. Proc Natl Acad Sci USA 2004; 101:14895–14900.

143. Lappalainen U, Whitsett JA, Wert SE, Tichelaar JW, Bry K. Interleukin-1beta causes pulmonary inflammation, emphysema, and airway remodeling in the adult murine lung. Am J Respir Cell Mol Biol 2005; 32:311– 318.

144. Corry DB, Rishi K, Kanellis J, Kiss A, Song Lz LZ, Xu J, Feng L, Werb Z, Kheradmand F. Decreased allergic lung inflammatory cell egression and increased susceptibility to asphyxiation in MMP2- deficiency. Nat Immunol 2002; 3:347–353.

145. Fink MP. Role of reactive oxygen and nitrogen species in acute respiratory distress syndrome. Curr Opin Crit Care 2002; 8:6–11.

146. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; 408:239– 247.

147. Lass A, Suessenbacher A, Wolkart G, Mayer B, Brunner F. Functional and analytical evidence for scavenging of oxygen radicals by L-arginine. Mol Pharmacol 2002; 61:1081–1088.

148. Nicoglou A, Merlin F. Epigenetics: A way to bridge the gap between biological fields. Stud Hist Philos Biol Biomed Sci. 2017 Dec; 66:73-82. doi: 10.1016/j.shpsc.2017.10.002. Epub 2017 Oct 13. PMID: 29033228.

149. Rahman I, Marwick J, Kirkham P. Redox modulation of chromatin remodeling: impact on histone acetylation and deacetylation, NF-kB and pro-inflammatory gene expression. Biochem Pharmacol. 2004 Sep 15; 68(6):1255-67. doi: 10.1016/j.bcp.2004.05.042. PMID: 15313424.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370

150. Marwick JA, Kirkham PA, Stevenson CS, Danahay H, Giddings J, Butler K, Donaldson K, Macnee W, Rahman I. Cigarette smoke alters chromatin remodeling and induces proinflammatory genes in rat lungs. Am J Respir Cell Mol Biol. 2004 Dec; 31(6):633-42. doi: 10.1165/rcmb.2004-0006OC. Epub 2004 Aug 27. PMID: 15333327.

151. Hoekstra R, Eskens FA, Verweij J. Matrix metalloproteinase inhibitors: current developments and future perspectives. Oncologist. 2001; 6(5):415-27. doi: 10.1634/theoncologist.6-5-415. PMID: 11675519.

152. Falcone D, Gallelli L, Di Virgilio A, Tucci L, Scaramuzzino M, Terracciano R, Pelaia G, Savino R. Effects of simvastatin and rosuvastatin on RAS protein, matrix metalloproteinases and NF-κB in lung cancer and in normal pulmonary tissues. Cell Prolif. 2013 Apr; 46(2):172-82. doi: 10.1111/cpr.12018. PMID: 23510472; PMCID: PMC6495807.

Published online August 3, 2023 https://doi.org/10.15326/jcopdf.2022.0370