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POSTGRADUATE RESEARCH PROPOSAL

Proposal for PhD Student Application

Role of Immune and Inflammatory Responses in Induction of Airway Remodeling in Patients with Allergic Asthma

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Background

Asthma is a common worldwide disease with a prevalence of 5 to 10% [1]. A health condition that is characterized by chronic course of remission and exacerbation. Asthma is a heterogeneous respiratory inflammatory disease driven by immune responses and with different phenotypes and endotypes [2, 3]. Asthma is not a single entity and it is a heterogenic syndrome [4].Although, an extensive studies performed to illustrate the role of different cytokines and chemokines in induction of asthma pathogenesis; however, still there is uncovered sites in asthma pathogenesis. The outcomes of previous studies indicated that a large network of cytokines and chemokines are involved in inflammatory and immunologic responses [5, 6] in asthma with subsequent airway remodeling [7].

The scientific domination of thought that asthma is a Th2 cell mediated immunity to allergens changed to a concept that asthma is a much more complex syndrome with various phenotypes and endotypes [3,8,9]. The innate type 2 immunity [10, 11], and adaptive type 2 immunity [12] are involved in asthma pathogenesis. However, there are many cells that play a role in asthma pathogenesis through induction and production of various cytokines and chemokines. These cell types include T helper cells family epithelial cells, fibroblasts cells, eosinophils, neutrophils, macrophages, mast cells, basophils, and B lymphocytes [13-23].

In asthma biomarkers using for prediction of asthma therapy and outcomes is with great clinical significance [6]. Although, recent studies [24] provided findings that form a basis for asthma subgrouping in to phenotypes/endotypes, however, some studies results [25, 26] highlighted the need for finding a better indicator for asthma phenotypes and endotypes. There is a potential need for performing studies that clarify a biomarker as asthma progression or disease consequences indicator. Personalized therapy and precision medicine are the major goals for chronic inflammation such as asthma [24, 27-29].

Eosinophilic airway inflammation characterizes the clinical phenotype of early-onset allergic asthma, as well as the occurrence of late-onset non allergic asthma [30]. Eosinophils

play a potential role in asthma pathogenesis via production of variable cytokines, chemokines and specific inflammation granules [31]. Major basic protein (MBP), eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), and eosinophil peroxidase (EPX) are five basic proteins found within secondary eosinophil granule [32]. Eosinophil cationic protein also known as RNase3, and it is a single chain protein with apparent molecular masses ranging from (15.7 to 22 kDa) and is stored in the granules as non-toxic protein [17,33].

ECP was the most widely used marker from the eosinophil basic proteins in order to monitor asthma severity and response to treatment. The majority of previous studies indicated that ECP levels provide useful information allowing disease monitoring and treatment stratification [34, 35]. Serum ECP levels was higher than in plasma, since ECP released by eosinophil during the coagulation process [36]. In patient with eosinophilia, administration of anti interleukin 5 antibody induced a decline in serum ECP level with correlation to eosinophil number [37]. Plasma ECP level likely reflect the blood ECP concentration at the time of sampling thus plasma ECP level is a better marker that reflect the eosinophil activation intensity than ECP serum level, however, no data available to confirm such hypotheses [31]. The intensity of eosinophilic inflammation was reflected in concentrations of ECP in serum plasma and other body fluid.

Serum ECP level was significantly higher in asthma patients than in control and the level is declined significantly following corticosteroid therapy and ECP serum level determination is helpful for monitoring inhaled corticosteroid therapy in bronchial asthma [38]. Previous studies indicated that serum ECP is correlated with disease severity [39-41]. Thus we proposed that serum ECP level could be serving as a potential biomarker for evaluation asthma inflammation severity.

Mast cells activation as a response to IgE antigen binding receptor with Th2 cell activation initiates the chronic inflammation in asthma [42]. Mast cells contain high affinity IgE receptors and localised in connective tissue and mucosal membrane and blood vessels [43]. Mast cells play a potential key role in induction of inflammation and modulation of production of mediators and activated by cytokines, viral and bacterial antigens, hormones and growth factors [44]. Local production of monocytes/mast cells (MCP-1 and RANTES) chemotactic factors lead to accumulation of mast cells in inflamed tissues [45].

Mast cells with two phenotypes, the mucosal mast cells and tissue mast cells [46]. Tissue mast cells contain chymase and tryptase, while mucosal mast cells contain tryptase only, additionally; both phenotypes were differing in their number, secretary granules type and stimuli responsiveness [46]. Mucosal mast cells contain more chondroitin, while tissue mast cells contain heparin and responded to neuropeptide while mucosal mat cells not respond [46]. Mast cells released mediators that were classified into cytokines/ chemokines, newly synthesized lipid mediators, and preformed mediators [47], however, this categorization not absolute as that TNF-α included in newly synthesized and preformed mediators. The preformed mediators include tryptase, chymase, histamine, proteoglycans (heparin and chondroitin sulphates)

carboxypeptidase A and their storage was in cytoplasmic granules [47]. These mediators play a role in the pathophysiology of asthma [31].

The human Tryptase locus on chromosome 16 includes genes that encode alpha and beta Tryptase [48], which are major protein products of human mast cells, much lesser amount being expressed by basophils [49]. Alpha and beta Protryptase are processed to maturity by cathepsin B and L, while Beta-Protryptase also can be sequentially processed by autocatalysis and cathepsin C [50, 51]. Mature beta tryptase is proteolytically active as a homotetramer; wheras mature alpha tryptase is not, despite being 93% identical in amino acid sequence. Protryptase are constitutively secreted by resting mast cells, whereas mature Tryptase which are stored in secretory granules are only secreted activated mast cells. The pro-and mature Tryptase baseline serum level ranged from 1to 11 ng/ml in healthy individual which reflecting primarily their genetic background [52].

Previous studies indicated Tryptase potential importance in inflammatory disease specially asthma. Being released by activated mast cells at the inflammatory sites causing bronchial hyperresponsiveness and local infiltration with eosinophil and neutrophil [53]. Tryptase induces bronchial constriction by mechanisms that involve mediator such as bradykinine, histamine and others. In addition, Tryptase act as potent mitogen for epithelial cells and airway smooth muscle cells, which suggest its potential role in airway hyperplasia and subsequent remodelling induction [54]. Histamine bronchial responsiveness in children with mild to moderately severe asthma highly correlated to mast cells Tryptase levels [55].

Gao et al [56] reported that serum baseline tryptase levels in children with mild and moderate to severe persistent asthma show significant increase in serum baseline tryptase as compared to controls and those with mild intermittent asthma. Additionally, serum baseline tryptase level is specific diagnostic tool to distinguish between asthmatic and healthy controls, and with good accuracy in differentiation of various asthma subgroups. Serum levels correlated with markers of disease severity. Shareef and Amin [39] in a recent study reported that serum tryptase is significantly accurate marker for the prediction of allergic asthma. If we consider that tryptase as key biomarker of mast cell activation, thus it may be hypothesised that there is an association between baseline serum tryptase and the development of allergy induced inflammation [56].

Role of tryptase in allergic inflammation induction and prognosis studied extensively in anaphylaxis, food allergy, venom allergy [57-59]. However, in asthma still there is a need of studies to overcome the underlying role of tryptase in asthma pathogenesis. Bronchial hyperresponsiveness is one of the findings that reflect asthma severity [60, 61]. Previous studies indicated an association between AHR and mast cells number in bronchial airway [62,63].In obese asthmatic children, serum tryptase was significantly higher in asthmatic children than in control and higher in obese than in non-obese asthmatic. There was negative correlation between serum tryptase and FEV1% and positive correlation with BMI [64]. Determination of serum tryptase is a marker that supports diagnosis of asthma in children who are unable to perform

pulmonary function test [65]. Limited available studies on the role of tryptase in asthmatic patients and most of the studies study population are children, thus induction of studies in adults Asthmatic to clarify the role of tryptase in asthma is warranted.

Asthma is a chronic inflammatory disease characterised by reversible airflow limitation and AHR, however, persistent inflammation in tissue of the airway may contribute to structural changes known as airway remodelling and subsequent airway obstruction that is not fully reversible and progressive loss of lung function over time [66]. The airway remodelling induced by many mechanisms which include, epithelial damage, repair and goblet cell hyperplasia [67-74]; subepithelial fibrosis [75-90]; increase in airway smooth muscle mass [91]; Myokine hyperplasia [92-97]; Myocyte hypertrophy [98,99]; smooth muscle migration [100,101]; inflammatory cell infiltration [102-117]; bronchial neovascularisation [118-122]; and cytokines induction [123-126].

Fibroblast growth factor, MMPs, tryptase, and actin, play a potential role in airway remodelling in asthma through three integrated and dynamic processes. The modelling initiated by epithelia cells, followed by amplification by immune cells and mesenchymal effectors functions [127].Recent findings of studies reported that animal models and in vitro studies have confirmed the involvement of airway epithelium, airway smooth muscle, and extracellular matrix components in Asthma- related airway remodelling. Eosinophils and mast cells involved in asthma related airway remodelling [128]. However, the American Thoracic Society task force stated the need for more research on airway remodelling.

The effective outcome of anti-IgE drug such as omalizumab in the treatment of moderate-to-severe asthma suggests that airway remodelling was mediated by IgE [129]. Contraction of bronchial smooth muscle contributes to airway narrowing. Also ASM involved in induction of bronchial inflammation through secretion of many mediators, inflammatory cells recruitment and activation (T-lymphocytes and mast cells) [130]. ASM is considered as inflammatory cells [131] and can contribute to an auto-activation loop involving mast cells and subsequent cytokines production [132]. Stimulation of ASM lead to production of a wide range of chemokines and cytokines, which participate in auto-activation loop [133,134], results in mast cells attraction by ASM [135,136]. Mast cells adhere to ASM [137-139]; promote mast cells survival and proliferation [140]. The activation and degranulation of mast cells are none allergen and allergen dependent [141-144], and lead to extracellular deposition of inflammatory product which contribute to ASM mass increase and AHR [131,145,146]. Chronic inflammation in bronchial asthma causes tissue injury with subsequent repetitive processes and remodelling thought to be the result of incomplete process [147]. The process early onset [148,149], which sometimes before eosinophilic inflammation [150] suggests that bronchial inflammation and remodelling may occur simultaneously in asthma [130]. ASM remodelling is characterised by increased extracellular matrix proteins in and around ASM bundles, increase in ASM size or hypertrophy, and increased ASM cell number or hyperplasia [130]. Bronchial airway remodelling characterised by increased number of my fibroblasts, which is a mesenchymal cells that due to

their phenotype, are described vas cross between fibroblast band smooth muscle cells [151]. Myofirbroblasts synthesize alpha smooth muscle actin (SMA) as Myocyte and extracellular matrix proteins as fibroblasts [151].

The extracellular matrix (ECM) is a macromolecules network, in which most abundant molecule is collagen which is only degraded by matrix metalloproteinases (MMPs) [152]. MMPs main biologic functions is the degradation of extracellular matrix proteins and glycoproteins, cytokines, membrane receptors, and growth factors [153-158].MMPs involved in many biologic processes such as angiogenesis, tissue repair and remodulation, embryogenesis, cellular differentiation, cell proliferation, morphogenesis, wound healing, cell migration and mobility, apoptosis, endometrial proliferation and ovulation [152-154, 156,157]. MMPs activity deregulation contribute to development of many pathologies that may be grouped in: fibrosis, tissue destruction and matrix weakening [153,156,157]. MMPs grouped into; collagenases type, gelatinases type, stromelysin types, matrilysin types, membrane-type metalloproteinases, and others [152, 153,157]. MMP-9 belong to gelatinases types, while MMP-16 belong to membrane-type metalloproteinases.

MMPs are enzymes family that can breakdown extracellular matrix proteins and thus lead to inflammation pathological processes, fibrosis and wound healing [159]. MMPs play a role in airway diseases and their levels increased in sputum, serum and bronchial lavage fluid in asthma patients [160-162].MMP-9 deficient animals could inhibit airway inflammation and MMP-9 immunoreactivity was correlated with disease severity [163]. However, high inflammatory responses in MMP-9 deficient mice suggest that MMP-9 exerted a defense function in asthma [164,165]. Thus MMP-9 is important factor in asthma pathogenesis, but still its role not fully understood [166].

The enzymatic activity of MMPs inhibited by tissue inhibitors of matrix metalloproteinase (TIMPs) [167,168]. TIMP-1 secretion is associated with MMP-9 and TIMP-1secretion may lead to basement membrane thickening in asthma [169]. Thus MMP and TIMP imbalance may influence the clinical differences in chronic airway diseases [170,171]. MMP-9/TIMP-1 ratio in sputum of asthma patients decreased after recovery from acute asthma exacerbation indication a negative correlation between MMP-9 and TIMP-1 [172]. Additionally, decreased MMP-9/TIMP-1 ratio in chronic asthma contribute to airway obstruction [166,173]. Sputum MMP-9/TIMP-1 ratio is positively correlated with FEV1% in asthmatic patients [171]. Previous study showed that low serum MMP-9/TIMP-1ratio was found in asthmatic patients who demonstrate little improvement in FEV1% with treatment of corticosteroid [174]. However other studies not found a correlation between MMP-9/TIMP-1 and lung function [175] or positive correlation among MMP-9/TIMP-1 and lung function [166,176,177].

MMP-9 degrades collagen for which is the major components of the airway sub-epithelial basement membrane [178] and thus it may altered ECM within the smooth muscle layer of the airway. MMP-9 releases the active form of TGF beta [179] and may lead to increase of TGF beta in asthmatic patient airway, specifically in bronchial smooth muscle [132,180]. Also through

MMP-9 dependent mechanisms bronchial epithelium modulate bronchial smooth muscle proliferation[181]. In addition injury of epithelium increases the release of MMP-9 suggesting that epithelium and bronchial smooth muscle interact in asthma [130]. The increase in MMP-9 and MMP-9/TIMP-1 in airway and alveolar macrophage could be indicators of chronic airway inflammation and contribute to a greater decline in lung function in patients with chronic asthma [166].

MMP-9 was the first MMP to be investigated in depth for it is role in asthma pathology and was highly expressed in the BAL fluid and sputum from patients with asthma [171,182]. Asthma severity was associated with expression of high level of MMP-9 in bronchial biopsies from asthmatic patients [183,184]. MMP-9 high level expression in bronchial biopsies was associated with number of macrophage and neutrophil [185]. MMP-9 disrupt basement membrane through degradation of elastin and collagen type VI [186] and contribute to sub epithelial fibrosis, ECM deposition and thickness of airway wall [187]. Airway remodeling related to sub-basement membrane thickness [188] has been linked to AHR of the airway and subsequently lead to fixed airway limitation development and decline in lung function with asthmatic with time [189]. Recent studies revealed that alveolar macrophage secreted excessive MMP-9 over TIMP-1, which was associated with increased AHR and was a predictor of lung function decline acceleration [170].

High dose of corticosteroid did not change the levels of TIMP-1 and MMP-9 in induced sputum [190]; however others reported a significant decreased in MMP-9 in bronchial biopsies of asthmatics treated with inhaled steroids [191]. However Chung et al [166] reported that inhaled corticosteroids do not modified TIMP-1 and MMP-9 expression in alveolar macrophage from patients with chronic asthma who show rapid decline in FEV-1 suggesting that inhaled corticosteroid cannot inhibit the airway remodeling in chronic asthma. Previous studies showed that MMP-9 increased in asthma [192,193] and that it may be the cause airflow obstruction through induction of structural changes of the airway [194]. Persistent MMP-9 high level indicates that airway remodeling may be initiated at the beginning of the asthma development and may be exhibited as severe asthma [166]. In double knocked out mice model MMP-9 is the dominant airway MMP controlling in inflammatory egression cell [195]. Hong et al reported that serum MMP-9 was significantly higher in children with asthma as compared to control.

Matrix metalloproteinases (MMPs) play potential roles have in normal and pathologic processes such as wound healing, tissue remodeling, tumor invasion and angiogenesis [196]. Matrix metalloproteinase-16 (MMP16; MEROPS identifier M10.016), also called MT3-MMP, degrades various components of the extracellular matrix, including fibronectin and collagen type III without any effect on collagen I, II, IV and V collagen [197**].**

MMP-16 can not only directly degrade some matrix molecules, but can also activate pro-MMP-2 (gelatinase A), one of the most important MMPs in tissue remodeling and cell migration [198,199]. Matrix metalloproteinases (MMPs) are zinc-dependent and calcium-dependent proteases that cleave within a polypeptide (end peptidases). They degrade most

components of the extracellular matrix (such as growth factors, their binding proteins, and other bioactive molecules, as well as binding sites for cell-surface molecules) and some non-extracellular-matrix molecules [200].

In animal model (*MMP-16*-deficient mice) study reveals a novel mechanism of extracellular matrix remodeling which is prerequisite for proper function of mesenchymal cells. In the absence of MT3-MMP, mice display growth inhibition due to decreased viability of mesenchymal cells in skeletal tissues. The inhibition of mesenchymal cell proliferation and migration was caused by the lack of cleavage of high-density fibrillar collagen [201]. The physiological significance of MT3-MMP (MMP-16) was further verified in mice that are double deficient for MT1-MMP and MT3-MMP. Double deficiency transcends the combined effects of the individual single deficiencies and leads to severe embryonic defects in palatogenesis and bone formation, which are incompatible with life. These defects are directly tied to loss of indispensable collagenolytic activities required in collagen-rich mesenchymal tissues for ECM remodelling and cell proliferation during embryogenesis [201].

Matrix metalloproteinase (MMP)-16 is a membrane-type metalloprotease and associated with proliferation, migration and invasion [202,203]. MMP-16 functions in cell development of some diseases by activating proMMP-2 (gelatinase A) [204,205]. MMP-2 is demonstrated to participate in the airway remodeling in bronchial asthma [206]. In addition, autophagy also plays an important role in airway remodeling and inflammation of asthma [207,208]. Loua et al [209] found that that miR-192-5p is predicted to bind to MMP-16 and autophagyrelated7(ATG7) and thus they suggest that miR-192-5p may take part in asthma via regulating MMP-16 and ATG7. Their finding suggested a potential role of MMP-16 in promoting cell proliferation and thus it was a vital target for asthma treatment which mainly via alleviation of airway inflammation and airway remodeling [210]. In literature limited reported studies on the role of MMP-16 in asthma pathogenesis, therefore, it is essential to conduct study to clarify its role in asthma.

Inflammation responses induction and subsequent airway remodeling are associated with high expression of growth factors, matricellular proteins and chemokines which contribute airway hyperresponsiveness and to irreversible bronchial obstruction, mainly in severe cases of asthma regardless of steroid treatment [7]. Transforming growth factor-β1 production by epithelial and inflammatory cells influence inflammation and remodeling of severe asthma cases. This cytokine is significantly higher in sputum of moderate asthmatic patients as compared to controls [211]. TGF-β1 attracts and activates wide range of inflammatory cells, airway smooth muscle alteration and epithelial cells transformation [212]. TGF-β with Periostin is with potential role for induction of airways structural changes in severe asthmatic with persistent bronchial obstruction [213]. Periostin is a matricellular protein that involved in airway remodeling and eosinophilic inflammation [214]. Periostin sputum levels is increased in patients with asthma and inversely correlated to FEV1% and suggested as risk factor in patients with long term steroids treatment [215-217]. In addition, fibroblast growth factor-2 (FGF-2) levels in bronchial biopsies and sputum from patients with asthma are inversely with pulmonary function [218]. In contrast,

recent studies reported a protective role of FGF-2 in pulmonary disease by enhancement of regeneration of epithelial cells [219,220] and TGF-β remodeling effects antagonism [221].

Tan et al recent review [17], on the role of FGF2 as an immunomodulatory factor in asthma suggest that FGF2 is potentially involved in inflammation regulation in asthma and may be a therapeutic target for treatment of asthma.Reffat et al [222], reported that sputum Periostin is correlated with asthma severity and eosinophilic asthma.

Conflicting results of different studies [7,223, 224] in which some suggest the use of FGF2 as target for treatment, while the others recommend its use as drug for treatment warranted the study of serum levels of FGF2, TFG-β1 and Periostin in different asthma clinical settings. FGF9 stimulate mesenchymal proliferation and knocking out FGF9 disrupts the mesenchymal-epithelial signaling loop [225], and contribute to lung hypoplasia [226]. In addition, Loffrendo et al [227], in a gene network analysis reported a dysfunction of epithelial barrier in mild, moderate and severe asthma. FGF 9 increased in biopsies of eosinophilic oesophagitis patients and induces proliferative responses to eosinophilic inflammation in oesophagitis [228] and these findings may suggest that FGF9 could play a role in bronchial asthma.

The major constituent of the ASM contractile apparatus is alpha smooth muscle actin (α-SMA), a protein that forms the filament lattice to which the phosphorylated myosin heads attach to shorten the muscle [229]. This protein is often used as a marker for mature, contractile smooth muscle cells [230] but is also known to play a role in fibroblast contractility [231]. It has been postulated that increased expression of α-SMA or cytoskeletal actins may contribute to the lack of effect of deep inspiration seen in asthmatics [232] by increasing stiffness and making the muscle more resistant to the force reduction caused by oscillating strain [233,234]. However, to date no difference in the expression of α-SMA in ASM from asthmatics and non-asthmatics has been reported [235,236]. Woodman et al. have shown that human ASM cells increase their expression of α-SMA when co-cultured with mast cells or when mast cell-derived -tryptase was added [237]. Tryptases are the most abundant serine-proteinase found in the secretory granules of mast cells, with -tryptase being the major isoform expressed. -tryptases have been implicated in the pathogenesis of allergic and inflammatory diseases such as asthma. Woodman et al. also found that ASM-derived TGF-1 secretion and agonist-provoked contraction were increased in the presence of -tryptase [237]. Overall, it has been suggested that -tryptase increases TFG-1 secretion in ASM, which then up regulates contractile protein expression in ASM via autocrine stimulation.

Bronchospasm induced in non-asthmatic human subjects can be easily reversed by a deep inspiration (DI) whereas bronchospasm that occurs spontaneously in asthmatic subjects cannot [238]. This physiological effect of a DI has been attributed to the manner in which a DI causes airway smooth muscle (ASM) cells to stretch, but underlying molecular mechanisms–and their failure in asthma–remain obscure [239-246]. Of all known bronchodilators, among the most effective are the deep inspirations (DIs) and sighs that occur spontaneously in humans roughly

once every 6 minutes [239-246]. In the human, moreover, DIs suppress agonist-induced bronchospasm as well as exercise-induced bronchospasm [240,247-249]. In the living guinea pig, similarly, imposed DIs suppress hyperpnea-induced bonchospasm [250]. In addition to dilating bronchospastic airways, DIs taken before administration of a constricting agonist attenuate subsequent airway narrowing, a phenomenon termed DI-induced bronchoprotection [251]. Simply put, in the healthy lung breathing facilitates breathing [252]. During a spontaneous asthmatic attack, however, this favorable dynamic becomes substantially attenuated or even reversed [239, 240, 241, 252,253]. To explain the salutary effects of a DI a variety of reductionist approaches have been undertaken using both theory and in vitro experimentation. To simulate a DI, investigators typically impose a tissue stretch that is comparable in magnitude and in timing to that expected physiologically in vivo. These approaches have spanned the levels of the molecular acto-myosin interaction [254-258], the isolated airway smooth muscle (ASM) cell [259, 260], the isolated ASM strip [244,255, 261,262], and the small airway integrated within the precision cut lung slice [247,263-268]. These approaches have yielded results that are largely consistent with one another and also compatible with observations made in intact animals and humans in vivo. By contrast, others have used the experimental preparation of the isolated segment of central airway to show little or no bronchodilatory effect in response to imposed ASM stretch [269 -271]; the reasons remain unclear for this striking discrepancy between results obtained using this particular preparation versus the body of literature described above. Nevertheless, the overwhelming weight of evidence in vivo, in vitro, and in silico supports the proposition that during induced bronchospasm a DI produces marked bronchodilation that increases with increasing magnitude of the applied stretch, and that this bronchodilation is attributable to stretch-induced reduction in contractile forces generated by airway smooth muscle. During the spontaneous bronchospasm that is a cardinal feature of asthma, a DI typically fails to produce bronchodilation, however [239-241]. The mechanisms accounting for this failure remain unclear. This failure has been attributed in varying degrees to a wide variety of sources including: decreased tethering of the airway wall to the surrounding lung parenchyma [261, 272,273], thickened connective tissue layers [272,273] and increased ASM tissue mass [252,261], all of which are thought to protect the ASM from stretch during a DI and thus permit ASM to remain in a shortened and stiffened contractile state. Additional hypotheses to explain the observations include ASM adaptation [274, 275]; increased ASM shortening velocity or passive stiffness [276,277]; and a defective response to stretch at the level of asthmatic airway smooth muscle itself [278]. Zyxin has never before been implicated in airway dysfunction, but is known to be a highly dynamic and mechano-responsive regulator of the actin cytoskeleton that localizes to focal adhesions and accumulates rapidly at sites of spontaneous acute stress fiber strain [279-281]. Zyxin-mediated stress fiber repair occurs on a timescale comparable to that of re-contraction of ASM after stretch [259,281] suggesting that zyxin might play a role in regulating key mechanical properties of ASM and its response to a DI. This issue was studied by Rosner et al [238] and they found that in response to a transient stretch of physiologic magnitude

and duration, zyxin repairs cyto-architecture at localized regions of stress fiber fragmentation, facilitates recovery of contractile force generated by the isolated mouse ASM (MASM) cell, and slows dilation of the small airway embedded within a precision cut lung slice (PCLS). At the levels of cytoskeleton, isolated cell, and integrated airway tissue, zyxin is thus seen to stabilize ASM structure and contractile properties at current muscle length. To assess the relevance of this finding to humans, they then compared the expression of zyxin in ASM obtained from bronchi of non-asthmatic subjects to that from fatal and non-fatal asthmatic subjects. Importantly, we found increased abundance of zyxin in the ASM of fatal asthmatics. Assessment of the role of zyxin in the regulation of Zyxin in fatal asthma murine airway dynamics in the PCLS combined with the differential abundance of zyxin protein in fatal asthmatics points to pathological overexpression of zyxin as a potential explanation of the failure of a DI to dilate bronchial airways in fatal asthma. Consistent with that expectation, Chin et al. [278] showed that ASM from 6 asthmatics (4 fatal) versus 6 non-asthmatics has less relaxant response to a simulated DI and recovered faster following stretch. Ijpma et al.[282], by contrast, used a protocol similar to that of Chin et al.[278] but did not find a difference in the ASM response to stretch in asthmatics versus controls. However, 7 of 8 asthma cases in the study Ijpma et al were non-fatal [282] compared with 4 of 6 in the study of Chin et al. [278]. In the limited context of responses to stretch in ASM from non-fatal asthmatics, the observations reported here, by Chin et al. [278], and by Ijpma et al.[282] are mutually consistent. The changes in zyxin localization at the subcellular level are rapid and transient. Chronically or acutely stretched cells reinforce their cyto-architecture through zyxin recruitment, but this accumulation of zyxin dissipates rapidly once the actin structures had been reinforced. Biophysical observations at the levels of cytoskeletal structure, single cell mechanics, and intact airways embedded within lung slices clearly establish that zyxin stabilizes the ASM contractile apparatus, its cytoskeletal scaffolding, and thus sustains the effect of ASM contraction on airway narrowing. Observations in human tissue samples establish a clear increase in zyxin protein in the ASM of people who died as the result of an asthma attack. As such, zyxin is seen not only to reinforce airway smooth muscle but also to accumulate in airways of fatal asthmatics. Although these data implicate zyxin in the impaired ability of a DI to relax ASM in life threatening asthma [278], they do not determine whether high zyxin levels are a contributing cause or an effect of fatal asthma.

Koopman et al [286], using actin dynamic modulator (Wingless-integrase, WNT-5A) suggested that WNT-5A expression increased in asthmatic ASM and play an active role in maintaining contraction of ASM. Additionally, WNT-5A treatment drives actin polymerization and regulates TGF-ß1-induced expression of α-SMA through ROCK-mediated actin polymerization. Previous studies evaluated the effectiveness of different materials on airway remodeling in animal models of asthma and human indicated attenuation of airway modelling and suggested the role of SMA actin in this process [284-287]. Boser et al [288] found that in asthma there is a marked increase in α-SMA+myofibroblasts in the lung parenchyma.

Fibrocytes can differentiate into myofibroblasts as indicated by the expression of α-SMA. This phenomenon could be induced by TGF-β and is more marked in asthma patients with airway obstruction. Fibrocytes presence are confirmed in airway of asthmatic patients and more precisely within the bronchial smooth muscle bundles or close to the basement membrane. Additionally, allergen exposure contribute to fibrocyte-like cells accumulation within the bronchial mucosa of patients with allergic asthma. Fibroblasts cultured from BAL fluid in patients with mild asthma express fibrocyte proteins, suggesting that fibroblasts derive from circulating fibrocytes. The co-expression of α-SMA in fibrocyte-derived cells suggests that circulasting progenitor cells diffetrentiate into myofibroblast and then into bronchial smooth muscle cells. Also, bronchial smooth muscle cells themselves may promote fibrocytes migration, which is partially mediated by the production of platelet-derived growth factor [289].

Experimental studies showed that T cells adhere to airway smooth muscle via vascular cell adhesion molecule-1 and drive airway smooth muscle growth through direct contact between the T cells and smooth muscle α-actin (α-SMA)+ cells [289]. In bronchial biopsies from patients with asthma, T cells localize with proliferation of α-SMA+ cells in subepithelial location and within the airway smooth muscle. This finding confirmed the prior experimental demonstration that the involvement of T cells/α-SMA+ cell contact mechanism in airway smooth muscle heperplastic growth translates into an asthma disease mechanism [289]. Collectively, actin SMA may play a potential role in airway remodeling and asthma pathogenesis.

AIM :

This study aiming to elucidate mechanisms that control airway smooth muscle cell phenotype and function, and identify how these contribute the role of airway myocyte and fibroblasts in the pathogenesis of bronchial asthma.

OBJECTIVES TO :

1. Determine serum levels of FGF-18 and FGF-23that may play a role in asthma pathogenicity, and their relation to disease severity and phenotypes.
2. Investigate the distribution of FGF-18 and FGF-23 in airway biopsies compared to controls.
3. Investigate the role of MMP-16 and MMP-9 in asthma pathogenesis.
4. Illustrate the association between asthma severity and some biomarkers such as Fibroblast growth factors, MMPs, tryptase, actin, and ECP.
5. Estimate Complete Blood Count and total eosinophil account.
6. Determine total and specific serum IgElevel.
7. Determine the serum levels of eosinophil cationic protein(ECP) and Tryptase in bronchial asthma.
8. Clarify the role of Actin Smooth muscle in asthma pathogenesis.

Materials and methods

Study design

Case-control study

Study population

The study has to be carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human subjects. Subjects with allergic asthma are to be included in the study. Asthma diagnosis by a specialist physician based on the criteria of the Global Institute for Asthma (GINA). The study population is of two groups. Demographic and clinical characteristics to be collected by patient interview using a pre-designed questionnaire. Total and specific IgE to be determined using ELISA.

Group I.

This group included 50 asthmatic patients and 50 matched control recruited from any Allergy clinic. This group are subjected for immunohistochemistry study to detect ECP, Tryptase, FGF -18, MMP-16 and Actin-SMA.

Group II

This group included 50adult asthmatic patients (> 12 years old) with asthma attending asthma and allergy center. Control group (50 subjects) of apparently healthy age and sex matched individuals to be recruited on the basis of : no history of respiratory or other diseases that may interfere with targeted biomarkers estimation, base line forced expiratory volume in 1 second (FEV1) more than 80% and FEV1/ forced vital capacity (FVC) ratio of >0.7.

INCLUSION CRITERIA:

Cases are to be selected on inclusion criteria such as: positive for specific IgE, increased total IgE level, pulmonary function test, and a minimum of two asthma symptoms (wheeze, cough and dyspnea).

EXCLUSION CRITERIA

The exclusion criteria include:

1. Other lung-related or medical illnesses,
2. Respiratory infection
3. A known respiratory disorder other than asthma.
4. Gastroesophageal reflux disease.
5. Cardiac disease.
6. Those receiving systemic corticosteroids within 4 weeks before the study.
7. Smoker.
8. Pregnant women.

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