



1. MBBS, M.Phil  
Associate Professor  
Institute of Molecular Biology and  
Biotechnology (IMBB),  
The University of Lahore, Lahore-  
Pakistan.
2. MBBS, M.Phil  
Assistant Professor  
Department of Physiology,  
AllamaIqbal Medical College  
Lahore-Pakistan.
3. MBBS, M.Phil  
Assistant Professor  
Department of Physiology,  
Shalamar Medical and Dental  
College.
4. BS, M.Phil  
Research Associate  
Institute of Molecular Biology and  
Biotechnology (IMBB),  
The University of Lahore, Lahore-  
Pakistan.
5. BS, M.Phil, PhD  
Associate Professor  
Department of Biochemistry,  
Independent Medical College  
Faisalabad.
6. BS, M.Phil, PhD  
Professor  
Institute of Molecular Biology and  
Biotechnology (IMBB),  
The University of Lahore, Lahore-  
Pakistan.

**Correspondence Address:**

Arif Malik (PhD)  
Institute of Molecular Biology and  
Biotechnology (IMBB),  
The University of Lahore, Lahore-  
Pakistan.  
arifuaaf@yahoo.com

**Article received on:**

18/04/2017

**Accepted for publication:**

15/12/2017

**Received after proof reading:**

05/04/2018

## INTRODUCTION

The prevalence of asthma is higher among women of reproductive age than in general population. Pregnancy is understood as major challenge in the management of asthma because it alters the course of asthma severity and its management which in turn affect pregnancy outcome such as low birth weight, prematurity and small for gestational age. Asthma although undoubtedly characterized by chronic inflammation, but this inflammatory response is quite complex. It may lead to remodeling response in the airway which resulted in structural alterations. These structural changes play a pivotal role in physiological dysregulation and clinical manifestations seen

## ASTHMATIC PREGNANT FEMALES; EXPRESSION OF MATRIX METALLOPROTEINASES (MMPs)

**Abdul Basit Ali Khan<sup>1</sup>, Madiha Ashraf<sup>2</sup>, Uzma Jamil<sup>3</sup>, Sulayman Waquar<sup>4</sup>, Nighat Aslam<sup>5</sup>, Arif Malik<sup>6</sup>**

**ABSTRACT... Objectives:** To evaluate the expression of MMPs and oxidative stress markers in pregnant Asthmatic females. **Period:** July 2015 to Dec 2015. **Design:** Cross sectional. **Setting:** Jinnah Hospital Lahore. **Background:** Maternal asthma throughout pregnancy unfavorably alters pregnancy outcomes but evaluation of the causes, and the ability to assess interventions, is restricted having inadequate knowledge of complex interactions between underlying inflammation, oxidativestress and structural changes in asthmatic airways. **Methods:** Total 100 patients were included in study. Fifty ascontrols. Clinically diagnosed asthmatic pregnant females coming to GyneaUnit of Jinnah hospital were included. Females on any medication or suffering from any congenital disease were excluded out of the current study. Matrix metalloproteinase's, TNF- $\alpha$ , 8OHdG, 4-HNE and Isoprostanes are measured by ELISA. **Results:** Results shows that MMP- 7 and MMP-9 have positive correlation with proinflammatory cytokine TNF- $\alpha$  but MMP- 8 have significantly positive correlation with it (TNF- $\alpha$  Vs. MMP-8  $r=0.946$ . On the other hand MMP-7 has significant correlation besides other MMPS (MMP-7 Vs.8OHdG  $r=0.719$ ) indicating a positive correlation between proteinases and marker of DNA damage indicating epithelial shedding in case of Asthma. **Conclusion:** The sole objective of effective asthma management in pregnancy is to prevent exacerbations. Antioxidants should be added in diet so to avoid oxidative stress caused to airway epithelial and structural cells and MMP inhibitors may be used as therapeutic agents as well as measuring MMP levels as diagnostic or prognostic markers.

**Key words:** Matrixmetaloproteinases (MMPs), Tumor Necrosis Factor-alpha (TNF- $\alpha$ ), 4-hydroxynonenal (4-HNE), 8-hydroxy-2-deoxyguanosine (8OHdG).

**Article Citation:** Khan ABA, Ashraf M, Jamil U, Waquar S, Aslam N, Malik A. Asthmatic pregnant females; expression of matrix metalloproteinases (MMPs). Professional Med J 2018; 25(4):582-588. DOI:10.29309/TPMJ/18.4571

in asthma.<sup>1</sup> Matrix metalloproteinase's (MMPs) also called matrixins playing a central role in these alterations in structural cells and tissues in asthmatics. MMPs are calcium dependent zinc containing endopeptidases belong to metzincin superfamily. At present in humans there are 28 members of MMP family known to occur in almost all organs and tissues.<sup>2</sup> They have a broad substrate specificity and play a crucial role in diverse biological and pathological processes such as cancer fibrosis and inflammation.<sup>3</sup> It is due to their ability to degrade extracellular matrix (ECM), proteolytic modulation of biologically active proteins and cell migration.<sup>4,5</sup> The family of MMPs is categorized into three groups:

collagenases (MMP1, MMP8, and MMP13) substrate of which is interstitial collagen type I, II, and III, (MMP2 and MMP9) are included in second type gelatinases degrading gelatins and basement membranes collagen such as collagen IV as well as stromelysins (MMP-3 and MMP-7) which degrade a wide variety of substrates such as proteoglycans, collagen, laminin and fibronectin.<sup>6,7</sup> The interstitial collagenase subfamily of MMPs MMP-1, -8, -13, and -14 are key proteinases that degrade interstitial collagen.<sup>8</sup>

TIMPs are the major endogenous regulator of MMP activities in the tissues. Its family includes four members, TIMP 1-4, that are structurally related.<sup>9</sup> For maintaining the structural integrity of tissues, the essential factor is a fine balanced equilibrium between deposition and degradation of extracellular matrix. In the development of pulmonary disease like Asthma the disturbance of balance between MMPs and their inhibitors TIMPs may be considered to be core event in whole process.<sup>5</sup> Several types of lung cells can release MMPs after exposure to smoke, exposure to second hand smoke, chemicals used in farming, hairdressing, manufacturing, fumes, certain microbes and parasites, growth factors and cytokines. MMP-9 was the first MMP to be studied in detail in pathogenesis of asthma. Evidence has found which revealed that bronchial epithelium also express MMP-9 which in turn behaves as an important player to promote infiltration of eosinophils into the airways of asthmatics.<sup>10</sup> Some other scientists have proposed that eosinophils which are the major cells in asthma related airway inflammation and not the neutrophils are the major factories for enhanced secretion of MMP-9 in asthma.<sup>11</sup> Likewise in asthmatics, an increase in the expression of MMP-8 has also been documented in biopsy samples from bronchi and in bronchoalveolar lavage which ascertains that this metalloproteinase also plays a significant role in the pathology of asthma.<sup>12,13</sup> MMP-7 was originally known as putative uterine metalloproteinases lead to increase invasiveness by activating MMP-2 and MMP-9.<sup>14</sup> Authors revealed that MMP-7 releases HBEGF which promotes cellular proliferation and tumor growth.<sup>15</sup> MMP-3 also releases HB-EGF

from extracellular matrix. MMP-3 and MMP-9 are able to release TGF- $\beta$  by cleaving GAGs side chains from proteoglycans and activated TGF- $\beta$  regulates the balance of ECM remodeling by regulating the expressions of MMPs and their tissue inhibitors TIMPs.<sup>16,17</sup>

In parallel to inflammatory and structural changes cells in pregnant asthmatics, cells are subject to increased oxidative stress, as stimulated inflammatory cells, such as macrophages and eosinophils, produce reactive oxygen and nitrogen species. Oxidative stress may play a significant role in pathophysiology of asthma and may be a final common pathway leading to tissue damage. The increased oxidative stress in asthma is demonstrated by the increased concentrations of 8-isoprostane (a product of oxidized arachidonic acid) in exhaled breath condensates.<sup>18</sup> *Malondialdehyde (MDA) a lipid peroxidation product were found to be elevated in plasma of asthmatics.*<sup>19</sup> 8-OHdG is prominent marker of DNA damage, released into the circulation system after the DNA repair.<sup>20</sup> 4-hydroxynonenal (4-HNE), are vital players in oxidative stress that is produced from oxidation of lipids containing omega-6 acyl groups, such as linoleic and arachidonic groups.<sup>21</sup> Alveolar macrophages can produce large amounts of TNF- $\alpha$  in the lung environment suggesting a vital role in the pathogenesis of asthma.<sup>22</sup> Both structural and inflammatory cells in the airways can release cytokines, such as tumor necrosis factor (TNF-alpha).<sup>23</sup> When patients with asthma are given inhalation of TNF-alpha, it causes airway hyperresponsiveness and significant increase in sputum neutrophilia and eosinophilia within 24 hours.<sup>24</sup>

## METHODOLOGY

### Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

Malondialdehyde (MDA) or lipid peroxidation in the blood sample of the patients is evaluated calorimetrically by the estimation of Thiobarbituric acid reactive substances (TBARS) according to the explained method of Ohkawa.<sup>25</sup> For the purpose about 200  $\mu$ l to 0.2ml of sample, 0.2ml of 8.1% Sodium dodecyl sulfate (SDS), 1.5 ml of 20%

acetic acid and 1.5 ml of 0.8% TBA will be added. After centrifugation at 3000 rpm for 10 min the upper organic layer will be taken and its OD will be read at 532 nm against an appropriate blank without the sample. Its levels were expressed in millimoles.

#### Determination of Tumor Necrosis Factor by ABCAM's TNF Alpha Human Elisa KIT

TNF alpha is estimated with the help of commercially available ELISA kit by Abcam. The following kit is designed for the quantitative measurement of TNF alpha in supernatants, serum, plasma samples and buffered solutions. For the purpose monoclonal antibody specific TNF alpha has been coated onto the wells of the microtiter strips provided. Samples are added and the reaction was initiated by adding the appropriate amounts of following reagents in these wells. When they were incubated for the first time monoclonal antibodies were incubated along with them. Afterwards they were washed and enzyme Streptavidin-HRP was added which binds and illuminates it was again incubated followed by washing. A substrate solution was added which acts on the bound enzyme to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of TNF alpha present in the samples. And finally absorbance was measured with the help of ELISA reader

#### Estimation of MMP-3, 7,8 ,9,11 and 13

Matrix metalloproteinase 3, 7, 8, 9,11 and 13 were estimated by using human ELISA kit (BioVendor).

#### Estimation of 8-Hydroxy Deoxyguanosine (8OHdG)

8- Hydroxy deoxyguanosine (8OHdG) were measured by the help of commercially available DNA damage diagnostic kit by Enzo Pharma USA.

#### Estimation of 4-Hydroxy Nonenal (4-HNE)

4-HNE was estimated with the help of commercially available ELIZA kit by Enzo Pharma USA.

#### Determination of Isoprostanes

Isoprostanes formed as a result of lipid

peroxidation was estimated with the help of commercially available ELIZA kit by EnzoPharma USA.

## RESULTS

The role of metalloproteinases was established as prognostic and diagnostic variables and used to determine the severity of disease, stage and management protocols. The antioxidant profile of asthmatic pregnant females have crucial role in the pathogenesis and progression of disease as data shown in Table-I. Suggested level of malondialdehyde (MDA) differ significantly ( $p=0.018$ ) presented with higher level ( $5.19 \pm 0.31$ ) in disease group as compared to healthy controls ( $0.945 \pm .31$ ) respectively. The potential initiator of underlying inflammation in Asthmatic pregnant, TNF-a, higher levels were recorded  $9.19 \pm 1.09$  in asthmatics as compared to  $2.19 \pm 0.64$  in healthy controls. MMP-11 shows higher level in diseased ( $105.16 \pm 4.26$ ) as compared to control ( $28.19 \pm 3.09$ ). The level of MMP-7 differ significantly ( $P=0.013$ ) among disease group ( $115.26 \pm 4.16$ ) as compared to healthy controls ( $56.29 \pm 7.16$ ). The levels of MMP-3,8, 9,13 were recorded higher in asthmatic pregnant ( $41.19 \pm 3.09$ ), ( $110.54 \pm 7.18$ ), ( $245.26 \pm 4.16$ ) and ( $173.26 \pm 8.16$ ) relative to control ( $21.06 \pm 1.09$ ), ( $31.29 \pm 3.09$ ), ( $40.16 \pm 4.16$ ) and ( $37.19 \pm 4.16$ ). 8OHdG (ng/ml) an indicator of DNA damage were recorded to be statistically significant ( $p=0.016$ ) higher among disease group ( $3.09 \pm 0.81$ ) than control ( $16.29 \pm 1.73$ ). 4-HNE, is a second toxic messenger of free radicals, its higher levels were observed ( $3.19 \pm 0.16$ ) as compared to healthy one  $0.23 \pm 0.09$ . Isoprostanes, a specific product of lipid peroxidation are statistically significant ( $p=0.019$ ) and  $41.19 \pm 3.19$  higher in asthmatic pregnant than that in healthy control ( $9.18 \pm 0.841$ ).

## DISCUSSION

Asthma can be ranked as one of most common respiratory diseases tending to convert a normal pregnancy into high risk pregnancy with severe implications on the development of fetal and infant immune system.

Variables	Control (n=50)	Subject (n=50)	P (≤0.05)
MDA (nmol/ml)	0.945±0.31	5.19±1.08	0.018
TNF-α (pg/ml)	2.19±0.64	9.19±1.09	0.011
MMP-11 (ng/ml)	28.19±3.09	105.16±4.26	0.004
MMP-7 (ng/ml)	56.29±7.16	115.26±4.16	0.013
MMP3 (ng/ml)	21.06±1.09	41.19±3.09	0.047
MMP-8 (ng/ml)	31.29±3.09	110.54±7.18	0.024
MMP-9 (ng/ml)	40.16±4.16	245.26±4.16	0.019
MMP-13 (ng/ml)	37.19±4.16	173.26±8.16	0.040
8OHdG (ng/ml)	3.09±0.81	16.29±1.73	0.016
4-HNE (mmol/L)	0.23±0.09	3.19±0.16	0.038
Isoprostanes (pg/ml)	9.18±0.841	41.19±3.19	0.019

**Table-I. Levels of circulating biochemical variables in asthmatic pregnant females**

Variables (Vs.)	(r)	P-Value
TNF-α Vs. 8OHdG	0.591**	0.001
TNF-α Vs. MMP-11	0.564*	0.031
TNF-α Vs. MMP-7	0.746**	0.005
TNF-α Vs. MMP-3	0.758*	0.019
TNF-α Vs. MMP-8	0.946*	0.013
TNF-α Vs. MMP-9	0.749*	0.032
TNF-α Vs. MMP-13	0.568*	0.028
MMP-11 Vs. 8OHdG	0.667**	0.009
MMP-7 Vs. 8OHdG	0.719***	0.000
MMP-3 Vs. 8OHdG	0.664*	0.041
MMP-8 Vs. 8OHdG	0.630*	0.019
MMP-9 Vs. 8OHdG	0.657*	0.016
MMP-13 Vs. 8OHdG	0.674*	0.034

**Table-I. Pearson s’ correlation coefficients of prognostic variables in the development of asthmatic pregnant females**

Since the prevalence of asthma is on an increasing trend in the general population, therefore its incidence is also increasing in pregnant females hence posing an increased threat to normal gestational process. asthma exacerbations can be referred as one of the most significant risk factor towards the development of lethal outcome of pregnancy like increase risk of fetal morbidity and mortality. Underlying mechanism of inflammation and structural changes in extracellular matrix involves complex interplay of MMPs, cytokines, growth factors and reactive oxygen and nitrogen species. There is increased production of several inflammatory proteins due to increased transcription of selected genes by DNA binding factors and transcription factor NF-κB, which is activated by TNF-α and oxidants that are released from inflammatory cells during the phenomenon of “respiratory burst”. The inflammatory cells have released higher amounts

of reactive oxygen species that may leak into surrounding cells resulting in greater quantities of free radicals in tissues of airway. As a result of these oxidants the bronchial epithelium shows evidence of damage seen in bronchoscopy with loss of epithelial columnar cells.<sup>26</sup> Evidence for this is the increased expression of the epidermal growth factor receptor (EGFR, HER1).<sup>27</sup> Following epithelial insult during asthmatic attack, the epithelium becomes an important source of mediators, growth factors and chemokines that add to prevalent inflammation and fibroblast proliferation, leading to airway remodeling.<sup>28</sup> TNF-α mediated NF-κB pathway enhances the expression of a variety of genes like VCAM and encoding intercellular adhesion molecule-1 ICAM-1.<sup>29</sup> These adhesion molecules are responsible for recruitment of eosinophils from circulation. Blockade of TNF-α also decreased production of Th2 cytokine, levels of serum IgE, goblet cell

hyperplasia and peri-bronchial fibrosis.<sup>22</sup> In Table-II, show a positive correlation between TNF- $\alpha$  and MMP-3,7, 8,11, 13. TNF- $\alpha$  Vs. MMP-8,  $r=0.948$ , TNF- $\alpha$  Vs. MMP -3,  $r=.758$ , TNF- $\alpha$  Vs. MMP-7,  $r=0.746$ , TNF- $\alpha$  Vs. MMP-9,  $r=0.749$ , these findings show that TNF- $\alpha$  released by inflammatory cells increases the expression of metalloproteinases by activation of NF $\kappa$ B and through MAPK/ERK pathways promoting diverse structural changes in the airway from degradation of extracellular matrix, proteolytic modulation of biologically active proteins including other MMPs and also active form of TNF-alpha leading to preterm delivery in asthmatic pregnancy. When membrane-bound TNF- $\alpha$  is converted to a soluble active form, this enhancement of its biological activity is being mediated by MMP-7 and MMP14.<sup>30</sup> TNF- $\alpha$  amplifies the expression of various MMPs that is Stromelysins like MMP-3, MMP-7 and collagenases such as, MMP-1, MMP-8, MMP-9 in pulmonary fibroblasts.<sup>31</sup> TNF- $\alpha$  is widely accepted as main initiator and regulator of inflammation and is upregulated in the asthma biopsies<sup>32</sup> and persistently high TNF- $\alpha$  is significant independent risk factor for Asthma.<sup>33</sup> TNF-alpha had been linked with increased expression of adhesion molecules such as VCAM, ICAM and E-Selectin resulting in increased migration of eosinophils and neutrophils.<sup>34</sup>

In the present study there is a positive correlation between TNF- $\alpha$  Vs. 8OHdG  $r=0.591$  suggesting an association between proinflammatory cytokine TNF- $\alpha$  and 8-OHdG which is the indicator of DNA damage in airway epithelial cells. Reactive oxygen species and nitrogen species can induce cytokine and chemokine production through induction of the oxidative stress-sensitive transcription of NF- $\kappa$ B in bronchial epithelial cells.<sup>35</sup> In Table-II, there is a positive correlation of MMP-7 Vs.8OHdG  $r=0.719^{***}$  suggesting that various oxidants lead to inflammation in the airways by activation of production of proinflammatory proteins ultimately resulting in increased expression of MMPs in various inflammatory and structural cells. The increase in matrix leads to protease/antiprotease imbalance resulting in loss of integrity of extracellular matrix and production of factors like TGF- $\beta$ , IGF, PDGF and HB-EGF. These

growth factors by various pathways like MAPK/ERK and AP-1 leads to tissue damage and tissue remodeling with loss of columnar cells known as epithelial shedding evidenced by presence of mucosa in sputum of pregnant asthmatics. Certain hazardous pregnancy outcomes have been associated with maternal asthma such as preeclampsia, intrauterine growth retardation, preterm delivery.<sup>36</sup> Abruptio placentae, antenatal or postnatal haemorrhage.<sup>37</sup> All these tissues may mediate cooperative or divergent signals that lead to preterm or term parturition by triggering the onset of labor comprises a fetal endocrine cascade involving the fetal HPA-axis which, in most species, leads to an increase in estrogen and decrease in progesterone in maternal plasma. Mazoret al.,<sup>38</sup> determined that median plasma 17 beta-estradiol is significantly higher in women with preterm birth and significantly lower progesterone/17 beta-estradiol ratios than those women who are delivering at term. The resultant oxidative insult leads to elevated cortisol and oxytocin levels and along with it there is increased expression of the oxytocin receptors.<sup>39</sup> These two effects leads to commencement of phosphoinositol pathway due to which calcium bind with calmodulin, this complex in turn mediate uterine contractions by enhancement of actin myosin interactions, ultimately leading to premature rupture of membrane heading to preterm labour.<sup>40</sup>

## CONCLUSION

The objective of effective asthma management in pregnancy is to sustain the finest possible asthma control and avoid exacerbations, preventing day and night symptoms and maintaining lung function and normal activity. Antioxidants should be added in diet so to avoid oxidative stress caused to airway epithelial and structural cells and minimize the complex interplay between oxidative burden and airway remodeling ultimately leading to prematurity. The excessive airway remodeling as a result of an imbalance in the equilibrium of the normal process of synthesis and degradation of ECM could substantiate antiprotease treatment as MMP inhibitors may be used as therapeutic agents. MMP levels may be utilized as diagnostic or prognostic markers. Other possible approaches

may be tissue targeted gene therapy with TIMPs or TIMP variants that selectively inhibits specific matrix to prevent excessive proteolysis damage to the tissues.

## CONFLICT OF INTEREST

Authors declares no conflict of interest

Copyright© 15 Dec, 2017.

## REFERENCES

- Elias JA. **Airway remodeling in asthma. Unanswered questions.** Am. J. Respir. Crit. Care Med.2000; 161: S168-S171.
- Khokhar R, Murthy A, Weiss A. **Metalloproteinases and their natural inhibitors in inflammation and immunity.** Nat. Rev. Immunol.2013; 13(9):649-65.
- Vanlaerel, Libert C **Matrix metalloproteinase's as drug targets in infections caused by gram-negative bacteria and in septic shock.** Clin.Microbiol. Rev.2009; 22: 224-239.
- Yoon HK, Cho HY, Kleeberger SR. **Protective role of matrix metalloproteinase 9 in ozone-induced airway inflammation** Environ Health Perspect.2007; 115:1557-1563.
- Vandenbroucke RE, Dejonckheere E and Libert C. **A therapeutic role for matrix metalloproteinase inhibitors in lung diseases.** European Respir. J.2011; 38: 1200-1214.
- Graham HK, Horn M and Traord AW. **Extracellular matrix profiles in the progression to heart failure:** European young physiologists symposium keynote lecture-bratislava. Acta. Physiologica.2008; 194(1): 3-21.
- Spinale FG. **Myocardial matrix remodeling and the matrix metalloproteinases: influence on cardiac form and function.** Physiological Reviews. 2007; 87(4):1285-1342.
- Owen CA, Campbell EJ. **The cell biology of leucocytes mediated proteolysis.** Journal of leucocyte biology. 1999; 65(2):137-150.
- Murphy G, Docherty A. **The matrix metalloproteinases and their inhibitors.** A.J. Respir. Cell Mol. Bio 1992; 7: 120-125.
- HanZ, Junxu, Zhong N. **Expression of matrix metalloproteinases MMP-9 within the airways in asthma.** Respir Med. 2003; 97(5):563-7.
- Ohno I, H Ohtani, Y Nitta, J Suzuki, H Hoshi, M Honma, et al. **Eosinophils as a source of matrix metalloproteinase-9 in asthmatic airway inflammation.** Am. J. Respir. Cell Mol. Biol. 1997; 16: 212-219.
- Cataldo D, Munaut C, Noel A, Frankenne F, Bartsch P, Foidart JM, et al. **MMP-2-and MMP-9-linked gelatinolytic activity in the sputum from patients with asthma and chronic obstructive pulmonary disease.** Int. Arch. Allergy Immunol.2000; 123: 259-267.
- Prikk K, Maisi P, Pirila E, Reintam MA, Salo T, Sorsa T, et al. **Airway obstruction correlates with collagenase-2 (MMP8) expression and activation in bronchial asthma.** Lab. Invest.2002; 82: 1535-154.
- Wang FQ, So J, Reierstad S, Fishman DA. **Matrilysin (MMP-7) promotes invasion of ovarian cancer cells by activation of progelatinase.** Int. J. Cancer.2005; 114:19-31.
- Yu WH, Woessner JF, Mc Neish JD, Stamenkovic I. **CD44 anchors the assembly of matrilysin/ MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling.** Genes Dev.2002; 16:307-323.
- Kessenbrock K, Plaks V, Werb Z. **Matrix metalloproteinases: regulator soft he tumor microenvironment** Cel. 2010; 141(1):152-67.
- AnnesJP, Munger JS, Rifkin DB. **Making sense of latent TGF $\beta$  activation.** Journal of Cell Science. 2002; 116 (2):217-224.
- Montuschi P, Corradi M, Ciabattoni G, Nightingale J, Kharitonov SA, Barnes PJ. **Increased 8 isoprostane, a marker of oxidative stress, in exhaled condensation in asthma patients.** Am. J. Respir. Crit. Care Med.1999; 160:216-220.
- Rahman I, Morrison D, Donaldson K MacNee W. **Systemic oxidative stress in asthma, COPD and smokers.** Am. J. Respir. Crit. Care Med.1996; 154: 1055-1060.
- Kasai H, Crain PF, Kuchino Y. **Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair.** Carcinogenesis. 1986; 7:1849-51.
- HuiqinZ, Huiyong Y. **Role of lipid peroxidation derived 4-HNE in cancer focusing on Mitochondria.** Redox Biology. 2014; S2213-2317(14)00135-9.
- Kim JY, Sohn JH, Choi JM, Lee JH , HongCS, Park JW **Alveolar macrophages play a key role in cockroach-induced allergic inflammation via TNF- $\alpha$  pathway.** PLoS One. 2012; 7(10):47971.
- Barnes PJ. **Cytokine-directed therapies for the treatment of chronic airway diseases.** Cytokine Growth Factor Rev. 2013; 14:511-522.
- Thomas PS, Heywood G. **Effects of inhaled tumour necrosis factor alpha in subjects with mild asthma.**

Thorax. 2002; 57:774-778.

25. Ohkawa H, Ohishi N, Yagi K **Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction.** Anal Biochem. 1979; 95(2):351-8.

26. Montefort S, Roberts JA, Beasley R, ST Holgate ST, Roche WR. **The site of disruption of the bronchial epithelium in asthmatic and non-asthmatic subjects.** Thorax 1992; 47:499-503.

27. Puddicombe SM, R Polosa, A Richter, MT Krishna, PH Howarth, ST. Holgate, et al. **The involvement of the epidermal growth factor receptor in epithelial repair in asthma.** FASEB J. 2000; 14:1362-137.

28. Holgate ST, DE Davies, PM Lackie, SJ Wilson, SM Puddicombe, JL Lordan. **Epithelial-mesenchymal interactions in the pathogenesis of asthma.** J. Allergy Clin. Immunol. 2000 ;105:193-204.

29. Sugimoto H , Kataoka T, Garashi M, Hamada M, Takeuchi T, K Nagai. **E-73, an Acetoxyl analogue of cycloheximide, blocks the tumor necrosis factor-induced NF-κB signaling pathway.** BBRC.2000; Vol 277(2): 330-333.

30. Gearing AJ, Beckett P, Christodoulou M. **Matrix metalloproteinases and processing of pro-TNF-α.** J. of Leukocyte Bio. 1995; 57(5):774-777.

31. Lindner D, Zietsch C, Becher PM, Schulze K, Schultheiss HP, Westerman D. **Differential expression of matrix metalloproteases in human fibroblasts with different origin.** 2012. Bio. Research Int.

32. Bradding P, Roberts JA, Britten KM, S Montefort, Djukanovic R, R Mueller, et al. **Interleukin-4, -5, and -6 and tumor necrosis factor-alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines.** Am J. Respir. Cell Mol. Biol. 1994; 10 (5):471-80.

33. Halonen M, Lohman IC, Stern DA, Ellis WL , Rothers J, Wright AL. **Perinatal tumor necrosis factor-α production, influenced by maternal pregnancy weight gain, predicts childhood asthma.** Am J Respir Crit Care Med. 2013 Jul 1; 188(1): 3541.

34. **Godding V, Stark J, Sedgwick J. Adhesion of activated eosinophils is enhanced by tumor necrosis factor alpha and interleukin-1 beta.** Am. J. Respir. Crit. Care Med. 1995; **13:555-62.**

35. Biagioli MC, Kaul P, Singh I, Turner RB. **The role of oxidative stress in rhinovirus induced elaboration of IL-8 by respiratory epithelial cells.** Free Rad. Biol. Med. 1999; **26: 454-4**

36. Murphy V. **A meta-analysis of adverse perinatal outcomes in women with asthma.** BJOG; 2011; 118:1314-23.

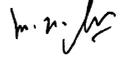
37. Clifton VL, Engel P, Smith R, Gibson P, Brinsmead M, Giles WB. **Maternal and neonatal outcomes of pregnancies complicated by asthma in an Australian population.** Aust N Z J Obstet Gynaecol. 2009; 49: 619-626.

38. Mazor M, Hershkovitz R, Chaim W, Levy J, Sharony Y, Leiberman JR, Glezerman M. **Human preterm birth is associated with systemic and local changes in progesterone/17 beta-estradiol ratios.** Am. J. Obstet. Gynecol. 1994; 171(1):231-6.

39. Robinson RS, Mann GE, Jamming GE, Wathes DC. **Expression of oxytocin, estrogen and progesterone receptors in uterine biopsy samples throughout the oestrous cycle and early pregnancy in cows.** Reproduction. 2001; 122(6):965-79.

40. Arthur P, Taggart MJ, Mitchell BF. **Oxytocin and parturition; a role for increased myometrial calcium and calcium sensitization.** Front Biosci. 2007; 12:619-33.

**AUTHORSHIP AND CONTRIBUTION DECLARATION**

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Abdul Basit Ali Khan	Reviewing	
2	Madiha Ashraf	Editing	
3	Uzma Jamil	Reading	
4	Sulayman Waquar	Writing, Statistics	
5	Nighat Aslam	Reading	
6	Arif Malik	Conceptulization, Methodology	