
**ESTIMATION OF ITERLEUKIN-8 AS A BIOMARKER
OF AIR POLUTION IN BRONCHIAL LAVAGE OF
ASTHMATIC PATIENTS**

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ABSTRACT

Bronchial asthma is regarded as an allergic inflammation of the airways. Air pollutants have been shown to enhance allergic responses and previous studies have demonstrated that air pollutant stimulate airway epithelial cells to produce various cytokines including inerleukin-8. Broncho alveolar lavage will be done to asthmatic patients to assess the level of interleukin 8 as a biomarker of air pollution trying to find a correlation between the level of the interleukin 8 and the spirometric parameters of the study group. There is a correlation between the level of interleukin-8 and the decreased FEV1 and FEV1/FVC parameter of the study group. The more the increase in the level of the interleukin-8 the less the value of FEV1 FEV1/FVC measured for the study group. There was a significant negative relation of between the levels of IL-8 and the FEV1 were the p value was 0.006. There was a significant negative relation between the levels of IL-8 and the values of FEV1/FVC were the P values was 0.000.

Air pollution is an exacerbating factor for asthma as detected by increased level of its biomarker (interleukin-8) in the lavage of the asthmatics.

Key words: Asthma - Pollution - interleukin-8

INTRODUCTION

The respiratory system is one of the primary interfaces with the external environment and faces unique demands to handle and detoxify inhaled gases and particles. It must both control and express inflammatory pathways in ways that preserve the primary functions of the respiratory system while protecting it from invasion by foreign infective agents or antigens. The respiratory system has an extraordinary surface exposed to the external environment, with the lower respiratory system having a surface area of approximately 100 m² in the adult human (Mercer *et al.*, 1994).

Bronchial asthma is regarded as an allergic inflammation of the airways. Air pollutants have been shown to enhance allergic responses and previous studies have demonstrated that air pollutant including DEPs stimulate airway epithelial cells to produce various cytokines including interleukin-8 (Sundeeep *et al.*, 2000).

Diesel Exhaust exposure induce an increase in interleukin-8 production in the human airways associated with neutrophilic and lymphocytic infiltration of the airways mucosa. These findings together suggest a possible mechanism for the association observed between increased ambient particulate matter concentrations and asthma exacerbations. (Sundeeep *et al.*, 2000).

Epidemiologic studies have shown associations between ambient ozone concentrations as a part of the air pollutant and asthma exacerbations (Thurston *et al.*, 1992).

The mechanisms leading to such exacerbations are likely related to airways inflammation, however it is unknown by which cell type it is driven. Neutrophils may play a pivotal role because their influx into the airways is prominent and maximal between 6 and 24 h after exposure to ozone (Koren *et al.*, 1989).

Exposure to outdoor ambient levels of PM_{2.5}, NO₂, and O₃ has been associated with increased asthma and respiratory symptoms in children (Albino Barraza-Villarreal *et al.*, 2008).

Bronchial lavage(BAL) is considered as the “gold standard” to assess airways inflammation. (Albino Barraza-Villarreal *et al.*, 2008).

Some studies compared the level of interleukin-8 in lavage and in sputum and found that interleukin-8, measured in sputum reflect the inflammatory responses in the lower airways of asthmatics, and may provide a noninvasive tool in epidemiologic studies on air pollution and asthma. (Koren *et al.*, 1989).

Some studies found that the size of the Particular Maters seems to determine the potential for cytokine induction; that is, the coarse PM is in general a more potent inducer of interleukin-6 and interleukin-8 than is the fine or the ultrafine PM. PM < 10 µm in diameter is more likely to enter the lung during inhalation. (Becker *et al.*, 2005).

AIM OF THE STUDY

To assess the impact of air pollution on the asthmatic patients and if there any connection between the air pollution and the asthmatic attacks.

To study the correlation between air pollution and interleukin-8 in bronchoalveolar lavage among the asthmatic patients

METHODOLOGY

Study site: The study was carried out in the Imbaba National Institute for Asthma and Chest Diseases .The investigator will contact the asthma patients at the asthma clinic.

Selection criteria: Clear history of asthmatic attacks. Reversible pulmonary airway obstruction of 15%after 20 min from the use of a bronchodilator. Preferably a middle age Asthmatics are subjected to air pollution either by living in polluted area or working under polluted conditions.

Exclusion Criteria: x-ray shadows Past history of any kind of chest diseases that may affect the lung permanently like old TB, lung fibrosis, bronchiectasis, chronic lung abscess, to exclude anything that may cause a wheeze on osculation and can be confused with the asthma wheeze.

STUDY DESIGN

All subjects chosen were subjected to the following:

Clinical examination: Were done with special emphasis on chest examination

Chest x-ray: X-ray were done to exclude any other chest problem apart from asthma.

Pulmonary function: Zhan body box pulmonary function instrument were used to do the following:

- 1- Vital capacity.
- 2- Forced vital capacity.
- 3- FVC/FEV1.
- 4- Detect the difference between the restrictive and the obstructive pattern of the lung function.
- 5- Forced Expiratory Volume in the first second.
- 6- Forced expiratory flow 25-75 to detect obstruction in small airways.
- 7- FEV1 reversibility pre and 20 min following 2 puffs of inhaled salbutamol .improvement of more than 15% is considered a reversible obstruction.

Reversibility in pulmonary function is diagnostic to asthma condition

Serum IgE: This is an IgE enzyme immunoassay. Patients with atopic allergic diseases such as atopic asthma , atopic dermatitis , and hay fever have been shown to exhibit increased total immunoglobulin E

levels in blood IgE quantitative test is based on solid phase enzyme-linked immunosorbent assay.

The assay system utilizes one mono clonal anti-IgE antibody. Blood sample will be collected from the study subjects is added to the IgE antibody coated microtiter wells and incubated with zero buffer at room temperature for 30 minutes. If human IgE is present in the specimen , it will combine with the antibody on the well .

Blood picture:

With special emphasis on the eosinophilic count

Fiberoptic bronchoscope

Conducting fiberoptic bronchoscope using a fiberoptic pentax, FB-18RX will be performed to all patients with the following steps:

- The patients were in the sitting position.
- I.V. route is established for any emergency medication
- The route of introduction of the bronchoscope were the nasal route.
- Premedication with a sedative (Medazolam 5 mg IV) to assure comfort to the subjects.
- Atropine sulfate one ampoule IM 30 min before the procedure to prevent the vaso-vagal attack secretion direness
- Topical anesthesia were used with xylocain 10% to achieve comfort and cough control of the subjects.
- The tip of the bronchoscope was introduced via the nose and will be wedged into the middle lobe of the lung.

- 100cc of warm saline to the body temperature to avoid thermal induced bronchoconstriction were injected in the middle lobe in aliquots of 20ml .

Detecting the BAL level of interleukin-8:

Interleukin-8 immuno-tech enzyme immunoassay kit were used to detect the level of interleukin-8 in the bronchial lavage of the asthmatic subjects.

Statistical analysis:

Analysis of the results were done to know the significance of the mean of interleukin-8 in BAL of the asthmatic patients and the relation to all variables of the study.

RESULTS

Table(1): Demographic data of the study population

Character		frequency	Percent	Total
Sex	Male	12	40%	30
	Female	18	60%	
Hazardous Occupation	Yes	14	46.7%	30
	No	16	53.3%	
Highly Polluted Area of Residence	Yes	23	76.7	30
	No	7	23.3	

Table(2): Mean value of the age and BMI among the study population

character	No	Mean ±SD
Age	30	44.9±14.19
BMI*	30	30.23±6.86

*BMI: Body Mass Index

Table(3): Mean value of IgE& IL-8 Among the Study Population

character	No	Mean ±SD
IgE	30	199.51 ±409.156
IL-8	30	97.385 ±59.573

Table (4): Correlation between mean value of IL-8 and characteristics of study population

character		IL-8
Age	Pearson correlation	0.162
	P value	0.394
	No	30
BMI	Pearson correlation	-0.236
	P Value	0.21
	No	30
Highly polluted area of Residence	Pearson correlation	0.337
	P value	0.069
	No	30
Hazardous Occupation	Pearson correlation	0.408
	P value	0.025
	No	30

Table (5): The correlation between mean value of interleukin -8 and spirometric parameter among the study group

Character		IL-8
FEV1	Pearson correlation	-0.490
	P value	0.006
	No	30
FVC	Pearson correlation	-0.328
	P value	0.077
	No	30
FEV1/FVC	Pearson correlation	-0.608
	P value	0.000
	No	30

The results in this study are shown in tables (1-5). Table 1 shows the demographic data among the study group. Total of 30 According to sex With 12 male (40%) and 18 female (60%) According to occupation. With 14(46.7%) of the total with a hazardous occupation with exposure to pollution like taxi drivers. Industrial workers in smelters Kiosk owners in polluted areas And 16 (53.3%) of the total with no history of hazardous occupation According to residence 23 of the total (76.7%) living in a highly polluted area in Cairo and the other 7 (23.3%) are living in rural areas near Cairo.

Table 2 shows the mean value of the age among the study group which is 44.9 and the mean BMI of the study population which is 30.2.

Table 3 shows Mean value of IgE which is 199.51 & the mean value of IL-8 which is 97.385. Among the Study Population

Table 4 shows the Correlation between mean value of IL-8 and characteristics of study population were there was insignificant relation between IL-8 and age were the P value was 0.394. There was insignificant relation between the IL-8 and the BMI were the P value was 0.21. There was significant relation between occupation and levels of IL-8 were the P value was 0.025. There was insignificant relation between residence and levels of IL-8 were the P value was 0.069.

Table 5 shows the Correlation between mean value of IL-8 and spirometric Parameter of the study group. There was a significant negative relation of between the levels of IL-8 and the FEV1 were the p value was 0.006

Which means the more level of IL-8 detected the lower the value of the FEV1 measured in the subjectspirometric study. There was a significant negative relation between the levels of IL-8 and the values of FEV1/FVC were the P values was 0.000. There was an insignificant relation between the value of FVC and the levels of IL-8 were the P value was 0.077

DISCUSSION

Epidemiological studies have revealed an association between pollution and allergic respiratory diseases. The main pollutants in this sense are nitric oxide, ozone, and particulate matter. Technical and industrial development has led to environmental pollution problems, with deleterious effects upon health. Epidemiological studies have revealed a statistical association between the levels of a pollutant or series of pollutants and the exacerbation of certain allergic respiratory diseases, among other processes. (Dávila *et al.*, 2007).

Our aim in this study is to assess the impact of air pollution on the asthmatic patients and if there any connection between the air pollution and the asthmatic attacks. And to study the correlation between air pollution and interleukin-8 in broncho-alveolar lavage (BAL) among the asthmatic patients. Our study group were selected according to the following clear history of asthmatic attacks.

Reversible pulmonary airway obstruction of 15% after 20 min from the use of a bronchodilator. Preferably a middle age asthmatics whom were subjected to air pollution either by living in polluted area or

working under polluted conditions. The result of the study was according to the following, There was insignificant relation between IL-8 and age There was insignificant relation between the IL-8 and the BMI There was significant relation between occupation and levels of IL-8 . There was insignificant relation between residence and levels of IL-8 There was a significant negative relation between the levels of IL-8 and the FEV1 . There was a significant negative relation between the levels of IL-8 and the values of FEV1/FVC. There was an insignificant relation between the value of FVC and the levels of IL-8

While Carlson revealed in his studies that High traffic exposure at the residential address was associated with lower than predicted FEV1 and FVC lung function compared with living further away in a large general population cohort. There were particular effects on women and individuals with obstructive disease. (Carlson *et al.*, 2015). which goes with our results in this study

In another study Norzila found that levels of IL-8 and IL-5 were elevated during the acute exacerbation and IL-8 concentrations decreased at resolution. Airway inflammation is present during an acute exacerbation of asthma, and is characterized by infiltration and activation of both eosinophils and neutrophils. The heterogeneity of airway inflammation in acute asthma may influence response to corticosteroid therapy. (Norzila *et al.*, 2000). which second our results in this study were levels of IL-8 were found elevated during the exacerbation.

CONCLUSIONS

Air pollution is a major threat associated with the environment around the world because everyone needs to breathe to survive. We breathe in almost anything that is in the air, which includes particles from air pollutants. Depending on the size of the particles, some of the particles could deposit anywhere along the respiratory tract or, worse, penetrate deep into the gas exchange region (*J. Zhao et al 2013*). Inflammation is the first responder of immune response to infection or injury in the body. Inflammation is detected by an increased concentration of biomarkers in the human body. This inflammatory action is meant to take place in a short time. When the response proceeds for a longer period, it is probable to damage the body. Unfortunately, the defined mechanisms underlying the association between air particles and an increased risk of respiratory symptoms are still unclear. Inflammatory response is connoted as a biologic tool that links air particles with health effects. (*Gallagher et al., 2010*). Interleukin-8 levels in BAL has a negative significant correlation with the FEV1, FEV1/FVC among the study group. Interleukin-8 can be used as a biomarker for airway pollution effect on the airways .

RECOMMENDATION

Asthmatic patients should not be exposed to air pollution either in areas of residency or at work. Enforcement of low for environmental regulation specially low (NO 4 / year 1994) which regulate all aspect of environmental pollution in EGYPT. Regular inspection of levels of air pollutant matters in areas known to be a high risk. Regular inspection of traffic vehicles for gas emissions. Enforcement of Filters use by plants with high industrial emissions.

Municipal control on presence of workshops among residential areas.

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تحديد مستوى الأنترلوكين (٨) في الغسيل الشعبي الحويطلي كمؤشر لتلوث الهواء في مرضى حساسية الصدر

[٨]

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المستخلص

تعتبر حساسية الشعب الهوائية والربو وهوعبارة عن التهاب مناعى للشعب الهوائية. وقد أظهرت الدراسات المختلفة ان الملوثات الهوائية تعزز حساسية الشعب الهوائية وأظهرت دراسات سابقة أن ملوثات الهواءمثل خوراج عادم وقود الديزل تحفز الخلايا لاننتاج السيوتوكينز مثل الانترلوكين الثامن، ومن هذه الملاحظات نجد انه توجد علاقة بين ملوثات الهواء وزيادة انتاج الانترلوكين الثامن فى الشعب الهوائية وتزامن هذا مع تفاقم أعراض حساسية الشعب الهوائي. ان الهدف من الدراسة إيجاد صلة قوية بين تلوث الهواء ممثلة فى مستوى الانترلوكين الثامن فى الغسيل الرئوى لمرضى حساسي الشعب الهوائية. طريقة البحث: تاريخ مرضى واضح من المصابين بالربو، الأشعة السينية، الفحص السريري العام، وظائف للرئة، نسبة ال IgE فى الدم، المنظار الليفى الضوئى الشعبى، يتم بعد ذلك قياس نسبة الانترلوكين الثامن فى الغسيل الرئوى عن طريق استخدام الكاشف المناسب بواسطة جهاز الايليزا وقد أثبت التحليل الإحصائي للنتائج وجود علاقة عكسية بين نسبة الانترلوكين الثامن فى الغسيل الرئوى لمرضى الربو الشعبى وتدهور وظائف الرئة الخاصة بالمرضى.

التوصيات: ابتعاد مرضى الحساسية عن الملوثات الهوائية. تفعيل قانون البيئة المصرى (قانون ٤ لسنة ١٩٩٤). مراقبة الانبعاثات الملوثة للهواء فى المناطق المعروفة بارتفاع نسبة ملوثات الجو بها تركيب فلاتر على مداخن المصانع. مراقبة الانبعاثات المضرة من وسائل النقل والمركبات المختلفة. مراقبة السلطات البلدية بعدم السماح بوجود ورش داخل المناطق السكنية.