

RESEARCH ARTICLE**Are interleukin 6 and LTB₄ measurements in exhaled breath of COVID-19 and post-COVID-19 lung fibrosis patients easy, sensitive and useful indicators of the intensity of airway inflammation and fibrosis?****Authors**

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Abstract:

Many studies pointed to il-6 as hall marker for severity and prognosis of covid-19 patients and represent the most important cornerstone at the discovery of many drugs for inhibition of it for switch-off of cytokine storm which is the concern of all scientists and patients together and a new disease at future medicine as covid-19 begin at last of December 2019. The sources of il-6 after viral stimulation are from macrophages and monocytes at many inflammatory sites or from stimulation of the HPA axis (endocrine il-6 ACTH stimulated) or respiratory il-6 stimulated by strenuous resistive breathing because contracting of respiratory muscles is considered like exercise and produce cytokines due to glycogen depletion

The Discovery of a non-invasive and easy method to examine il-6 which is the most important marker for severity and cytokine storm represent a new horizon for future viruses and modern medicine and that accelerates the intervention and depression of cytokine storm as early as possible and open the door for the discovery of many drugs work on this exhaled il-6 so our method for examination of il-6 at exhaled of covid and postcovid-19 patients help doctors for rapid categorization of patients and suspicion of severity. So, our study is a novel method for examination of il-6 Herein we intend to investigate the level of IL-6 in a breath of COVID-19 patients for the diagnosis of the early stages of lung inflammation and fibrosis. LTB₄ is a potent chemoattractant and stimulator of neutrophils, without any significant effect on airway muscle. And increased in exhaled of children with mild and moderate to severe persistent asthma.

Introduction:

Coronavirus has arisen as a crisis. It primarily appears as inflammation in the lungs (pneumonia), which can progress to the extreme fiasco of the respiratory system. The significant sign of sickness is the fundamental incendiary insusceptible reaction portrayed by cytokine storm (CS). CS is set apart by increased values of provocative cytokines, primarily interleukin-6 (IL-6), IL-8, IL-10, tumor necrosis factor- α (TNF- α), and interferon- γ (IFN- γ). Of these, IL-6 is viewed as fundamentally connected with an elevated death rate. IL-6 is also a vigorous indicator for anticipating illness prognosis and weakening the patient profile. (1)

IL-6 was recognizable in the condensate of the breath of all healthful individuals who did not smoke; however, it was fundamentally elevated in COPD cases. Breathed-out condensate is nonmetastatic and distinctly well tolerated by individuals. There is no influence of the gathering process on the aviation route role or aggravation, and there is developing proof that anomalies in the condensate content might represent biochemical alterations in the fluid lining the aviation route. Numerous inflammatory indicators in asthmatic and COPD cases have been effectively investigated using this strategy in earlier studies. (2)

Alveolar macrophages, interstitial fibroblasts, and large-vessel and bronchial epithelial cells all create IL-6 within the lung. Values of IL-6 are elevated in

persistent inflammatory lung cases, for example, those because of bleomycin-instigated fibrosis, allogeneic transplantation, and a range of pulmonary interstitial illnesses in humans. Significant values of IL-6 have been observed in the instigated phlegm of sick people with COPD, especially during aggravation. Park et al. tracked down the expanded IL-6 values in the bronchioalveolar washout liquid of sick people with vague interstitial pneumonia/fibrosis and certain cases with interstitial pneumonia. (3)

Dowlati et al. discovered elevated values of IL-6 in BAL fluid and serum from lung cancer cases. (4) In the latest research, Bhowmik et al. discovered elevated IL-8 and IL-6 values in the phlegm of sick people with COPD who experienced repeated aggravations(5) .

In another investigation, leukotriene B₄ and interleukin-6 values were also found to be significant nonmetastatic markers for inflammation of aviation routes in people who smoke cigarettes(6) .

LTB₄ is a potent chemoattractant and stimulator of neutrophils, without any significant effect on airway muscle. And increased in exhaled of children with mild and moderate to severe persistent asthma. (7)

The sources of il-6 after viral stimulation are from macrophages and monocytes at many inflammatory sites or from stimulation of the HPA axis (endocrine il-6 ACTH stimulated) or respiratory il-6 stimulated by strenuous resistive

breathing because contracting of respiratory muscles is considered like exercise and produce cytokines due to glycogen depletion. IL-6 may originate from the exercising muscles themselves (8) this needs more clinical studies on the measurement of il-6 as a result of excessive strenuous resistive breathing during covid-19 and stimulation of il-6

Following clinical trials, our research and our unique technique may pave the way for future therapeutics for COVID-19 and post-COVID-19 lung fibrosis using inhaler transport medications as a new challenge for overcoming pandemic sequelae. This provided a novel method for measuring exhaled IL-6. We investigated whether IL-6 generated by airways, endocrine IL-6, or immunological IL-6 is dangerous. We can characterize IL-6 as a storm of cytokines under obsessive conditions to three main sources: resistant IL-6 delivered from the respiratory framework, such as interstitial fibroblasts and bronchial epithelial cells and fibroblasts; second endocrine IL-6 created from the zona glomerulosa of adrenal organs invigorated by ACTH; and invulnerable IL-6 from macrophages and other insusceptible cells.

From past examinations, we truly want a method OK to patients and simple, noninvasive, touchy so we plan for clinical preliminary enrolled at clinicaltrials.gov about the estimation of interleukin-6 at breathed out condensate of Coronavirus patients and post-19 Coronavirus patients with lung fibrosis might be a helpful apparatus, simple, delicate for early

intercession with hostile to il6 drugs and decide the level of seriousness by fiery markers for the power of aviation route irritation.

Methods:

At our study are registered at ClinicalTrials.gov ID: NCT05157204 will do the following steps

1- Exhaled breath condensate the breath condensate samples will be collected using a specially designed condensing chamber (Eco screen; TurboDECCS device for exhaled breath condensate (EBC) collection (Medivac SRL, Italy)

2. IL-6 assay IL-6 concentrations in the breath condensate will be measured using a specific enzyme immunoassay kit (EIA) (Cayman Chemical, Ann Arbor, USA). The assay will directly be validated using gas chromatography/mass spectrometry. The intra-assay and inter-assay variability will be 10% or less. The detection limit of the assay will be 1.5 pg/ml after a 2-h development period. The reproducibility of repeat IL-6 measurements will be assessed by the Bland and Altman method and the coefficient of variation.

3. Statistical analysis Unpaired t-test was used to compare the two groups, and correlations between variables were performed using Spearman's rank correlation test, $P < 0.05$ being considered significant.

Conclusion: This is the first study to examine IL-6 levels as a noninvasive easy method in the exhaled breath condensate

of patients with COVID-19. According to the addressed information in our protocol, the anticipated results are expected to be significant and present more novelty in the era of COVID-19 treatment and cytokine storm diagnosis via detection of the causing factor which is IL-6 and LTB4 and as a marker for prognosis of the case and long-term postCovid-19 lung fibrosis and may be an indicator for other complications as smell dysfunctions and brain fog or menstrual dysfunctions and this study open the window to apply many therapeutics for inhibition of exhaled il-6

Abbreviations:

IL-6: interleukin 6

BAL: bronchoalveolar lavage

Ltb4: leukotriene b4

ACTH Adrenocorticotropic hormone

HPA hypothalamic–pituitary–adrenal axis

Highlights:

1-This is the first and novel study for measurement of il-6 and LTB4 at the exhaled breath of covid-19 patients

2-Positive results of this study will open the new challenge in diagnosis and prognosis of covid-19 patients and acceleration the treatment

3-The extra use of respiratory muscles by severe cases of covid-19 may be due to the production of il-6 from muscles itself or monocyte stimulation (respiratory il-6) as results by previous studies

4-Covid-19 may change the game as we need to recategorize the types of il-6 as there are many sources for il-6 production like endocrine il-6 ACTH stimulated, immune il-6 stimulated by ill1 beta and angiotensin II, and respiratory il-6 stimulated by strenuous resistive breathing and may produce by respiratory muscles due to glycogen depletion as rapid breath during covid-19

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