Original Article

Study of Serum Adenosine Deaminase and IFN-γ Levels: A Biphasic Immune Response Model in COVID-19 Patients

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Abstract

Background: SARS CoV-2 (severe acute respiratory syndrome coronavirus 2) infection spread worldwide, leading to the covid-19 pandemic between 2019 and 2021. There is an increase in pro-inflammatory cytokines and chemokines. As a counterimmune response, there is activation of type 2 helper T cells (IL-4 & IL-10) to suppress inflammation. Initiating an abnormal excessive immune response is associated with a cytokine storm. The aim and objective is to estimate serum ADA and interferon-γ (IFN-γ) levels in covid-19 patients. To compare ADA and interferon-γ between ICU and non-ICU patients. To find correlation between ADA and interferon-γ. **Material and Methods:** The study group comprised ILI, SARI and pneumonia cases. A total of 79 patients were included in the study group. Patients were separated into ICU and non-ICU groups according to the severity of their conditions at the time of admission. There were 25 ICU patients and 54 non-ICU patients. **Results:** Serum ADA level was raised compared to the normal range. There was a significant elevation in ADA levels in non-ICU admitted patients compared to ICU patients. ($p<0.05$). Findings of our study revealed an increase in IFN-γ levels in covid-19 patients. The rise in ICU patients was more than non-ICU patients. **Conclusion:** In ICU-admitted covid-19 patients IFN-γ levels were elevated, indicating a response to immune activation. IFN-γ levels can be useful in diagnosing severe covid-19. A raised serum ADA level of more than 24 U/L indicates an adequate immunological response. ADA levels can be used to monitor patients response to treatment.

Keywords: covid-19, ADA, IFN-γ

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Introduction

Coronavirus disease 2019 (covid-19) caused a pandemic in India and worldwide between 2019 and 2021. In late December 2019, patients presented with viral pneumonia due to an unknown microbial agent in Wuhan, China. There were similarities in clinical features with β-coronavirus infections. It was named the 2019 novel coronavirus (2019-nCoV).[1] Coronaviruses are RNA viruses belonging to the subfamily of Coronavirinae (spherical shape) and the genus Betacoronavirus. The name refers to the fringe of surface projections surrounding the virus, resembling the solar corona.[2,3]

The virus showed similarities to SARS-CoV and MERS-CoV (Middle East respiratory syndrome coronavirus). SARS-associated coronavirus was established as the cause of severe acute respiratory syndrome (SARS). It was named SARS-CoV-2.[4] SARS is the human form of bird flu, or avian influenza. Humans do not have immune protection against avian viruses.[5]

2019-nCoV binds to the angiotensin converting enzyme 2 (ACE-2) receptor in humans.[6] The hallmark of severe covid-19 is the hyperinflammatory host response due to cytokine storm. Massive numbers of cytokines and chemokines are released as a result of an unchecked, dysregulated, and overwhelming pro-inflammatory immune response. These proinflammatory factors increase vascular permeability, resulting in alveolar damage.[7] A cytokine storm further initiates a chain of events: the immune system attacking the body, acute respiratory distress syndrome (ARDS), and multiorgan failure.[8] Therefore, we decided to study markers of inflammation in covid-19 patients.

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Adenosine deaminase (ADA) is an enzyme for purine metabolism and is required for normal development of the immune system. It enhances T-cell activation and proliferation. It can also modulate dendritic cell maturation.[9]

ADA has two isoforms ADA1 and ADA2. Ecto-ADA1 binds to CD26 and helps in T-cell proliferation. The function of ADA1 is to reduce the intracellular level of adenosine and prevent the death of lymphocytes.[10,11]

ADA2 has cytokine-like growth factor activity and helps in signal transduction. It induces the differentiation of monocytes into macrophages. It stimulates proliferation of macrophages and CD4+ T-cells. ADA2 is high in monocytes-macrophages. An increase in ADA2 occurs when these cells are infected by intracellular microorganisms. It helps in the upregulation of 2'-deoxyadenosine, which is toxic. Activated CD4+ T-cells secrete interferon γ. It in turn regulates macrophages' secretion of ADA2.[10,11]

Particular antigens or mitogens, particularly T cells and NK cells, activate lymphocytes, which in turn create IFN-γ. When IFN-γ binds to the receptor, it triggers the JAK/STAT1 signaling pathways, which results in the processing and presentation of major histocompatibility complex (MHC) class I antigen. IFN-γ is linked to an immunological response mediated by cytotoxic T lymphocytes (CTLs). IFN-γ exhibits anti-fibrotic qualities in addition to its antiviral effect.[12]

Hence, the study of ADA and IFN-γ in covid-19 levels may may help in detecting the severity of the disease and predicting the prognosis. There are no studies on the effect of covid-19 on serum ADA levels. Studies on IFN-γ in covid-19 are few and unclear.

The objectives of our study were to estimate serum ADA and IFN-γ levels in covid-19 patients admitted to ICU and those admitted to non-ICU settings. We aimed to compare the serum levels of ADA and IFN-γ between covid-19 patients admitted to the ICU and those admitted to non-ICU settings. To find the correlation between serum ADA and IFN-γ.

Materials and Methods

The study design was an analytical cross-sectional study. The study group comprised ILI (influenza-like illness), SARI (Severe Acute Respiratory Illness) and pneumonia cases admitted to the covid-19 center of a tertiary care hospital in North Karnataka, India. Diagnosis was confirmed by RT-PCR. Only RT-PCR positive patients for covid-19 were considered for sample collection. The study was approved by the Institutional Ethics Committee. The study included 79 patients with covid-19. Covid-19 patients with a history of active tuberculosis and patients with other associated bacterial, viral and parasitic infections were excluded from the study group.

The study was conducted from September 2021 to April 2022 for 8 months. After obtaining informed and written consent, 10 ml of venous blood samples were collected from covid-19 patients under aseptic precautions. Serum was separated and stored in a refrigerator at 2 to 8˚C. Serum was used for estimation of ADA and interferon γ. Adenosine deaminase concentration in serum was estimated on an autoanalyzer Siemens Dimension EXL 200, using ready-to-use kits from Bio-Systems. It is based on the principle of deamination of adenosine to inosine and ammonia. α-ketoglutarate present in the reagent combines with ammonia in the presence of NADH and the enzyme glutamate dehydrogenase (GLDH). ADA concentration in serum is determined using the rate of decrease in NADH absorbance, measured at 340 nm.[13]

The IFN-γ level was estimated using the human IFN-γ ELISA kits with catalogue no. KB1053 provided by KRISHGEN BioSystems. The BioTek Epoch microplate ELISA reader, an automated instrument, was used for obtaining results.[14]

Statistical Analysis: Non-parametric tests were applied after quantitative parameters were tested for normality by the Kolmogorov-Smirnov test. The proportions of males and females in the ICU and non-ICU groups were cross-tabulated, and Fisher's exact test was used for comparison. The Mann-Whitney sum rank test was used for comparison of study parameters between two groups. An unpaired t-test was done to compare the ages of patients in the ICU and non-ICU groups. P<0.05 was considered statistically significant. Correlation between different parameters was calculated using Spearman's correlation. SPSS software was used for statistical analysis.

Results

[Table 1] shows demographic data for the covid-19 patients included in the study. The study included 25 patients admitted to the ICU and 54 non-ICU admitted patients. The study categorized the patients based on the severity of their disease at the time of admission. ICU patients had oxygen saturation (SpO2) <94% on room air. The mean age of patients admitted to ICU was 59.3 years, and for non-ICU patients, it was 49.2 years. This shows an increase in age is associated with a severe form of disease, which may be due to associated co-morbidities like diabetes mellitus, hypertension, chronic bronchitis, ischemic heart disease, and chronic kidney disease.

Table 1: Demographic characteristics of study population

Parameters	Cases	ICU patients	Non-ICU patients	P-value
Cases	79	25	54	
Males (%)	46 (58.2%)	13 (52%)	33 (61.1%)	0.47
Females (%)	33 (41.2%)	12 (48%)	21 (38.9%)	0.47
Age (years) Mean (Range)	52.43 (19, 90)	59.32 (22, 88)	49.24 (19, 90)	0.038

Values are expressed as percentages and means. Fisher's exact test and unpaired t-test were performed to assess the difference between the means. A p-value < 0.05 is considered for significance.

[Table 2] shows ADA and IFNγ levels in ICU- and non-ICU-admitted patients. In our study, ADA level was more in non-ICU patients compared to ICU patients [Table 2, Figure 1]. The difference was significant (P<0.05). Findings of our study revealed an increase in IFN-γ levels in covid-19 patients [Figure 2]. The rise in ICU patients was more than

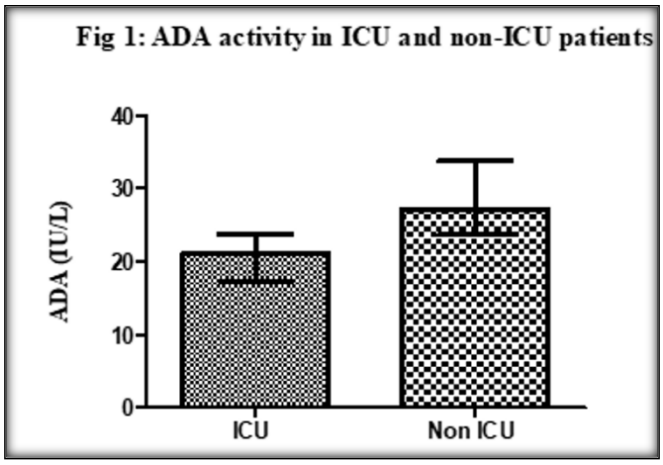
non-ICU patients, but the difference was not significant [Table 2], (P > 0.05).

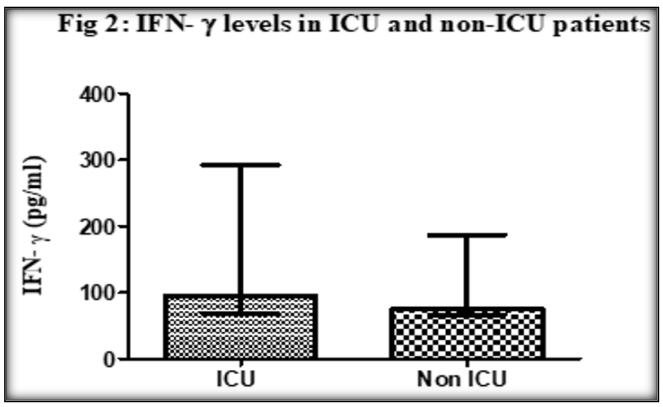
Table 2: Shows ADA and IFN γ levels in ICU and non-ICU patients

Parameters	ICU patients Median (IQR)	Non-ICU patients Median (IQR)	P-value
ADA (U/L)	21.10 (17.37, 23.74)	27.14 (23.66, 33.79)	0.0008
IFN γ (pg/ml)	95.56 (67.89, 292.8)	74.92 (66.54, 187.1)	0.38

Values are expressed as Median (IQR). The Mann-Whitney sum rank test was used to compare the difference between the means. A p-value < 0.05 is considered for significance.

[Figure 1&2] Shows median & IQR of ADA and IFN γ levels in ICU and non-ICU patients





[Figure 1&2] Shows ADA and IFN γ levels in ICU and non-ICU patients.
[Figure 1] ADA, Fig 2: IFN γ)

Influence of age on ADA levels: Since there is a baseline difference in the age of patients between the two groups, we present an adjusted analysis for the serum ADA comparison. We performed an analysis of co-variance by taking age as a co-variable. Results remain significant even after adjusted analysis.

[Figure 3] shows receiver operating characteristic curves (ROC) for serum ADA. Area under the curve (AUC) for serum ADA is 0.772, P<0.0001. At a cutoff value of 24 U/L of serum, ADA has a specificity of 74% and a sensitivity of 80%. A serum ADA level greater than 24 U/L indicates an adequate immunological response.

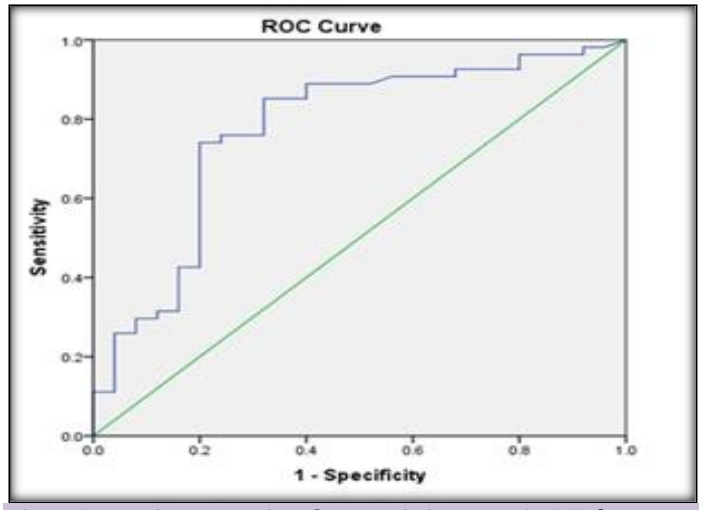


Figure 3: Receiver operating characteristic curves (ROC) for serum ADA

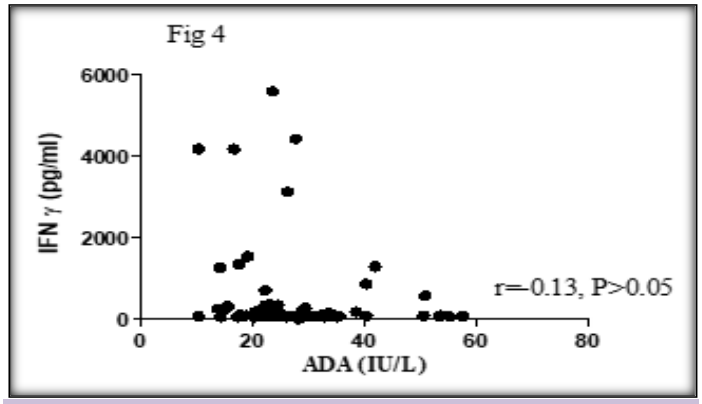


Figure 4: Correlation graph between ADA & IFN γ

[Table 3 and Figure 4] show the correlation between ADA and IFN-γ. In our study, there was no correlation found between IFN-γ and ADA (r = -0.13, p > 0.05). The cause may be due to different stimuli and different sources required for the synthesis and release of each parameter being studied.

Table 3: Table shows correlation between different parameters

Parameter	r	P-Value
IFN-γ vs ADA	-0.13	0.24 (>0.05)

Spearman's correlation was used to see the correlation between ADA and IFN γ.
A p-value < 0.05 is considered for significance.

Discussion

In our study, the increase in ADA level was greater in non-ICU patients compared to ICU patients [Figure 1]. The difference was significant [Table 2]. A rise in ADA may be due to the presence of live virus inside lymphocytes and macrophages. There is increased production of cAMP and adenosine inside the cells secondary to activation by IL-6 and TNF-α. As a counter action ADA production is increased. ADA1 isoenzyme converts adenosine to inosine and ammonia and binds to CD26 and adenosine receptors. ADA2 isoenzyme binds to adenosine and proteoglycan receptors of CD4+ T-cells and promotes cell proliferation and differentiation.[15] There are only a few studies on the role of ADA in covid-19 positive cases. Findings of our study were similar to Lorena Franco-Martinez et al. He reported that values of salivary tADA and its isoenzymes were found to increase in covid-19 positive cases and convalescent patients when compared with healthy controls.[16] Kelvin Hei-yeung Chiu et al. performed RT-PCR to look for mRNA (messenger RNA) expression of ADA, TNFα, and IL-6. Results showed an increase in nasopharyngeal ADA, TNFα, and IL-6 mRNA expression in covid-19 patients.[17]

Findings of our study revealed an increase in IFN-γ levels in ICU patients more than non-ICU patients, but the difference was not significant p=0.77(>0.05) [Figure 2 & Table 2]. G. Chen et al.'s investigation revealed that patients with severe forms of COVID-19 had higher IFN-γ levels than those with milder forms of the virus, which is similar to the results of the present study.[18] Hu ZJ et al. reported that in the SARS-CoV-2 infection, lower circulating levels of IFN-γ are associated with progression to lung fibrosis.[19] Yang et al., in his study, proposed that the initially low IFN-γ levels contribute to an increase in viral load and cause tissue damage. This increase in IFN-γ levels leads to an exacerbated inflammatory response. Hence there is increase in levels of IFN-γ in severe cases.[20]

According to a study by Skaria SD et al., inhaled IFN-γ was administered for 80 weeks to individuals with idiopathic pulmonary fibrosis. There was significant improvement in lung capacity and diffusion capacity. Hence, elevated interferon-gamma levels may have a role in conditioning the lungs in infections and further prevent progression to fibrosis.[21]

Nina Le Bert et al., in their study, reported a fine balance between antiviral and inflammatory responses in asymptomatic positive patients. Thus, virus-specific cellular immune responses help in eliminating pathogens. In symptomatic patients, there was a disproportionate secretion of inflammatory cytokines, similar to the findings of our study.[22]

Results of the present study showed a rise in serum IFN-γ levels in ICU-admitted patients when compared to non-ICU-admitted patients. Hence, it indicates an association between a rise in cytokine levels and the severity of the disease. Further studies with a larger sample size are needed to confirm the association. The rise in ADA levels was greater in non-ICU admitted patients. This indicates an immunological response to increased intracellular viremia and cell lysis. In the future, the present study will help work on the possible role of adenosine receptor activators in the prevention of fibrosis and inflammation.

Conclusion

In severe covid-19 patients infection with SARS-Cov-2 caused a disproportionate immune response. Elevated IFN-γ levels in patients admitted to the ICU confirm immune activation. Hence, raised IFN-γ levels can be used as a marker for early diagnosis of the onset of cytokine storm and initiation of treatment. The study also proposes that a baseline serum ADA level of more than 24 U/L is essential for an adequate immunological response. This further associates with the availability of a significant number of functional CD4+ T-cells. Thus, ADA levels can be used to monitor patients responses to treatment.

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Conflicts of interest

There are no conflicts of interest.

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