The Role of Adenosine Deaminase Levels in The Diagnosis of COVID-19 Infection

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Abstract

Introduction and background: Adenosine deaminase (ADA) is an enzyme produced from body cells after infections due to activations of the immune response toward the microbes. The enzyme was previously used for the diagnosis of tuberculosis, lymphomas, sarcoidosis, lupus, and severe combined immunodeficiency (SCID). COVID-19 is a viral disease caused by Coronaviruses and infected the respiratory tract of patients mainly. The aim of this study is to measure and correlate the level of ADA with COVID-19 during the infection. patient and methods: Two methods; conventional enzymatic (Guisti and Galanti method) and ELISA methods were used to measure the level of ADA in 48 individuals, 12 were suffering from severe COVID-19, 12 has a moderate infection and 24 individuals were used as the control they are negative PCR- COVID-19 infections. Results: Both methods show that the level of ADA in patients suffering from COVID-19 infection is significantly higher in patients with severe infections compared with those in control and moderate infections' P <0.001, conclusion: This study concluded that the use of ADA levels in the serum of COVID-19 patients will be useful during the period of the disease.

Keywords: Adenosine deaminase, ADA, COVID-19, ELISA assay, Enzymatic assay

Introduction

Adenosine deaminase is an important enzyme in the body that is produced by cells after viral or bacterial infections. The level of the enzyme increases due to the activation of the immune response toward the invaders[1,2] . The white blood cells especially the lymphocytes were involved in the processes of the infection pathway[3] . Conditions that trigger the immune system, such as an infection by *Coronaviruses*, the virus that causes COVID-19, may cause increased amounts of ADA to be produced in the cells where the virus is present[4-6] .Globally covid-19 interferes with the health system and millions of patients around the world severing from fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, diarrhea[7,8] . Interstitial pneumonia that evolves in severe cases of COVID-19 [9] induced inflammatory cytokine storm which stimulates the cells to produce adenosine capable to restrain the acute inflammatory process and decreasing the damage caused by the immune system, also inhibiting the adenosine transporters to decrease platelet activation and thrombosis[10-12]. It's believed that the ADA test is not definitive, but it is a rapid test and may be elevated even when there are few viruses[4,13]. ADA results may be used to help guide treatment until results from a PCR are available. On another hand, the clinical laboratory does not depend on the test as a diagnostic test alone, but it may be used as a comparing the PCR or fast antibody test used for diagnosis of COVID-19 infection. The increased level of ADA in the serum of patients during Coronaviruses infection associated with the signs and symptoms of COVID-19 may help in confirming the diagnosis. This is especially true when there is a high prevalence of coronaviruses infections in the country where these patients are located. The researcher also found that ADA may be an elevation for other reasons, such as lymphomas, sarcoidosis, and lupus [14-15]. Currently, a few studies were published that have evaluated the ADA activity in the diagnosis and treatment of COVID-19 [4,5]. Therefore, this study aimed to measure the amount of ADA level in the serum of COVID-19 patients to aid in the diagnosis of the disease.

Patients and Methods

Patients' data: Forty-eight blood samples were collected from three groups of subjects: Group A; 12 patients suffering from severe COVID-19 and on venerator, Group B; 12 patients with moderate COVID-19 and Group C: 24 individuals healthy (Negative PCR as control).

patients admitted to the Governmental Hospital located in Jordan and analyzed. Group A (12 patients) regards as a severe case they use a ventilator and were confirmed as positive for COVID-19 based on a history of exposure to the virus, clinical manifestations, lung computed tomography (CT scan), and Nasopharyngeal swab samples were handled as per standard procedure and analyzed nucleic acid amplification test by reverse transcription-polymerase chain reaction (RT-PCR) according to the protocol of diagnosis and treatment [16]. Group B (12 patients) are suffering from moderate COVID-19 and they are positive for RT-PCR. Group C (24) are normal individuals negative for COVID-19 RT-PCR. All procedures performed in the present

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study were in accordance with the ethical standards of the national research committee The laboratory procedures according to the 1964 Helsinki Declaration and its later amendment's tests were performed in the medical laboratory located in the same Governmental Hospital accordingly to GLP guide[17]. This test was performed in a CLIA-certified laboratory and is intended for clinical purposes.

Collection of samples: Blood samples were collected from patients routinely in the hospital with the regulation of laboratory safety. Serum was obtained after centrifugation of blood at 1000Xg for 20 minutes. Blood collection tubes were disposable, non-pyrogenic, and non-endotoxin.

ADA assay: Two methods were used to determine the ADA in serum of all subjects involved in this study:

1. Enzymological method: The original method described by Guisti and Galanti [16] based on the Berthelot reaction was used, the reaction depends on the color of indophenol formation. The first step of the reaction is the deamination of adenosine and releasing of ammonia, then catalyzing the reaction by glutamate dehydrogenase a comping an allosteric activator. Absorption of light (A°) was determined spectrophotometrically at 340 nm, which has a direct relation to ADA activity. The calculation depends upon the unit of ADA, one unit equal to the amount of enzyme required to release 1 mmol of ammonia/min from adenosine at standard conditions.

Interpretation of results

Results were expressed as units per liter per minute (U/L/min). By this method, ADA activity can be measured up to 100 IU/L.

2. Quantitative sandwich Enzyme Immunoassay: Human Adenosine Deaminase (ADA) ELISA Kit[18] (Catalog Number. MBS700350, My BioSource, com) was used for the quantitative determination of ADA concentrations in serum samples ((CD creative Diagnostics. User manual,2020). This assay used the quantitative sandwich enzyme immunoassay technique according to instructions of the manufacturing company; briefly: The Antibody specific for ADA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ADA present is bound by the immobilized antibody. After removing any unbound substances by washing, a biotin-conjugated antibody specific to ADA was added to the wells and washed again. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells, and color developed in proportion to the amount of ADA bound in the initial step. The color development was stopped, and the intensity of the color was measured by an ELISA reader. A standard curve was plotted according to instructions. The curve was plotted as OD 450 verse ng/ml of ADA activity.

Statistical Analysis:

Chi-square and one-way ANOVA tests were used to determine the significate of the results [19](Al-Murrani,2021) (SPSS 26, *p* value <0.005 considered significant.

Results

The enzymological methods show that the ADA level was significantly highest in group A (severe COVID-19), compared with groups B & C(. the mean value being 23.85,11.25 & 3.2 U/L respectively (P value < 0.001) (**Table 1**). Out of 48 individuals, 26 patients belonged to groups of study have a mean ADA level of >10 U/L distributed as 10 patients belonged to group A (severe COVID-19) and 9 and 7 patients belonged to groups B and C (moderate and negative COVID-19) and 22 patients < 10 U/L belonged to group B and C (**Table2**). The serum level of ADA was highest in the patients with severe COVID-19 (group A), compared with the other groups. In the Enzyme-linked immunosorbent assay (ELISA) for measuring the level of ADA in the serum as detection enzymes, the measurement of enzyme in the serum samples was determined from the standard curve plotted prior to the calculation; the ADA activity was corresponding to 1 ng/ml equal to 3.25 IU/L according to standard curve plotted. **Table 3** shows the mean ADA level measured by the ELISA method; group A measured 29 group B 24 and group C 8 ng/ml which was significantly highest in group A compared to group B and C (P value < 0.001). The conversion of these values to U/L to compare the measurements in both methods will be equal to 37.38, 29.9, and 19.90 U/L respectively. The variation in the measurements of serum ADA levels between both methods may be related to sensitivity and specificity. The sensitivity of both methods was 100%, and the specificity of the enzymatic method less than the ELISA method.

Table 1. Mean serum ADA level in the elements of study

Group	Mean ADA level (U/L) (min-max)
Group A (sever COVID-19)	23.85 (4.55-70.55)
Group B (moderate COVID-19)	11.25 (1.45-44.45)
Group C (Negative COVID-19)	3.2(0.25-10.50)

Table 2. Serum ADA level at 10 U/L in the elements of study

Serum ADA level	Number of cases	Group A	Group B	Group C
>10	26	10	9	7
<10	22	2	3	17
Total	48			
P value < 0.001 (statistically significant) Sensitivity = 26/26 *100 = 100% Specificity = 26/48 * 100 = 54.16%				

Table 3. Determination of serum ADA level by ELISA of subject study	
Group*	ADA level (ng/ml) (min-max)
A	29 (3.44-66.6)
B	24 (1.4-26.8)
C	8 (0.34-15.8)
*Group A (severe COVID-19 patients, Group B (moderate COVID-19) patients, and Group C (negative PCR for COVID-19 individuals).	

Discussion

The Adenosine deaminase (ADA) activity has been used as a diagnostic tool to confirmation of tuberculosis infections[20,22,23] , cancer [15] and as a way to propose treatment of COVID-19 [4,5,24,6]. Few studies used ADA level as a diagnostic procedure for COVID-19 [3,4] and that encourages us to design an experiment to investigate if the serum ADA level aid in the diagnosis of COVID-19, since the data and patients available in the local hospital. A team of researchers in the same hospital investigated the relation of CBC in COVID-19 patients[17], and they concluded that there is severe inflammation in patients accompanied by cytokine storms and reduced activation of the platelet.[3] mentioned that the level of ADA will be increased in advanced respiratory distress syndrome and inhibition of adenosine transporters reduce platelet activation. The important role of ADA in pulmonary infections was useful for the diagnosis of diseases affecting the respiratory tract [4,6,21-23]because the Adenosine plays important role in activation and regulation of immune response [3]. During the inflammation of the lungs due to bacterial or viral infections, the adenosine was accumulated in the tissues at the site of inflammation and ligand to the receptors expressed by lymphocytes to activate signals[25]. There are two important adenosine deaminases (ADA1 & ADA2) found in human serum and other biological fluids [1,15]. The ADA 1 is important in the cells because it's breakdown the harmful adenosine derivatives to the immune system, and mutation in this enzyme leads to immunodeficiency [3]. The level of ADA will increase in different pathological conditions, during COVID-19; researchers around the world try to correlate the markers and signals to help in the diagnosis and treatment of the disease. The result of this study reveals that the measurements of ADA levels in the serum of COVID-19 patients were very useful in the diagnosis of the disease in a sensitive, cheapest, and non-invasive method. The high level of ADA found in patients suffering from COVID-19 due to the release of the enzyme from dying cells in the lung is associated with the release of immunological mediators such as cytokines as compared with the control non-infected patients or even with the patients with moderate infections. No significant differences were found between the conventional and ELISA methods for measurements of the level of ADA in the serum of patients.

Conclusions

The current study concludes that the use of measurement of ADA levels in patients' serum was useful and can be used in the diagnosis of COVID-19.

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