A randomized clinical trial study on the efficacy and safety of adalimumab and methylprednisolone pulse therapy in the treatment of COVID-19 patients with acute respiratory distress syndrome

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Abstract

Introduction: Adalimumab reduces the expression of the angiotensin-converting enzyme (ACE2) receptor at the cell surface, therefore it is thought to be effective in treating patients with COVID-19.

Objectives: The present study was conducted to evaluate the effectiveness of adalimumab and pulsed corticosteroids in treating patients with severe acute respiratory failure due to COVID-19.

Patients and Methods: The present double-blind clinical trial study was carried out on patients with COVID-19 referred to Imam Reza hospital, Tehran. Patients were randomly divided into two groups of intervention (patients under standard treatment according to the national protocol of Iran + methylprednisolone + adalimumab) and control (patients under standard treatment according to the national protocol of Iran + methylprednisolone).

Results: The patients’ hospitalization information shows that the duration of patients’ hospitalization in the intervention group was significantly shorter than their counterparts in the control group (∆P=0.041). Serum levels of total bilirubin on the ninth day (∆P=0.043) and GCS (Glasgow coma scale) on the ninth day (∆P=0.041) and tenth (∆P=0.039) in the adalimumab group were significantly increased compared to the control group. However, the direct bilirubin value on the eighth day (∆P=0.031), serum creatinine on the 8th (∆P=0.047), 9th (∆P=0.047) and 10th (∆P=0.047) days and also PEEF (pericarditis/pericardial effusion) on the tenth day were significantly lower in the intervention group than the control group.

Conclusion: The administration of adalimumab significantly increases the GCS of COVID-19 patients and reduces the length of hospital stay.

Trial Registration: This study is designed as a double-blind clinical trial (identifier: IRCT20200406046963N2, https://www.irct.ir/trial/55011), and has been approved by the ethics committee in biomedical research of AJA University of Medical Sciences (#IR.AJAUMS.REC.1400.032).

Introduction

With the outbreak of the novel coronavirus in 2019, the resulting infection has become a global health challenge (1-3). What is observed in most studies is the importance of immune system hyperactivity in the severity of symptoms and processes of multiple organ failure in patients with COVID-19 (4,5). An increase in tumor necrosis factor-alpha (TNF-α) may be associated with severe cases of COVID-19 (4-7). TNF-α inhibitors are effective in preventing lung damage in animal models (8). Therefore, it can be argued that blocking the TNF-α may play a reasonable role in correcting COVID-19. Inflammatory cytokines are mainly secreted by the macrophages. These cytokines are involved in acute phase reactions (4,8). Serum levels of these cytokines are increased in patients with COVID-19 (6,7,9). Overproduction and secretion of TNF-α and other inflammatory factors might suppress the immune system. According to some experimental reports,
anti-TNF-α therapy improves the disease in mice infected with the flu or respiratory syncytial virus (10,11). Therefore, TNF-α inhibitors may help to improve the inflammatory response in human lung disease (12). It is believed that the administration of a TNF-α inhibitor may reduce the pathological immune response in COVID-19 by reducing inflammatory mediators (13). Various documents have identified corticosteroids as a possible treatment for acute respiratory failure due to COVID-19 (14,15). One of these drugs is also adalimumab, which reduces COVID-19 receptors by decreasing the expression of the angiotensin-converting enzyme (ACE2) receptor on the cell surface (16,17).

**Objectives**

So far, no specific amount and dose have been introduced in previous studies for adalimumab. Due to few related studies, the guidelines still do not clearly state the effectiveness and safety of the proposed treatment (18-20). Therefore, we intended to evaluate the effectiveness of adalimumab and pulse corticosteroids in treating patients with acute respiratory failure due to COVID-19 in the intensive care unit (ICU).

**Patients and Methods**

**Study design**

Patients with the novel coronavirus (COVID-19) referred to Imam Reza hospital in Tehran were included in the study if they had the inclusion and excluded criteria. Inclusion criteria included the COVID-19 patient with moderate to severe severity of hospitalization in the ICU, needing respiratory support, PaO2/FiO2 <300 mm Hg, lactate dehydrogenase (LDH) >450 U/L, progressive course of COVID-19 disease and failure to respond to treatment according to the approved protocol, possibility of intubation 24 hours later. Excluded criteria included uncontrolled diabetes, active bacterial, viral and fungal infections procalcitonin positive and more than 0.5 μg/L or bacterial and fungal culture-positive of each body sample, severe electrolyte disturbance, history of a severe allergy to steroid products, active gastrointestinal bleeding. The research physicians were blinded to the patient group and the patients were blinded to the injected drug (double-blind). A total of 76 patients with COVID-19 were included in the study. Patients were then randomly divided into two groups of intervention (patients under standard treatment according to the national protocol of Iran + methylprednisolone+adalimumab and control (patients under standard treatment according to the national protocol of Iran+ methylprednisolone). All drugs and services provided in this study are provided free of charge to patients, and the costs are covered by the project budget.

In both groups, patients were treated according to Iran's national protocol and received 500 mg of methylprednisolone for three days. In the intervention group, the adalimumab 40 mg (Cinnora brand and Biotechnology Company CinnaGen product) was administered to patients only once on the first day of treatment as a subcutaneous pulse. The variables such as oxygen saturation (SaO2), bilirubin (Bil), lymphocyte, LDH, platelet (Plt), Glasgow Coma Scale (GCS) and spontaneous bacterial peritonitis (SBP) were then measured during the study.

**Data analysis**

Mean, or median was employed to describe quantitative variables according to the conditions and frequency report was applied for qualitative variables. Independent t test or Mann-Whitney U test was employed to compare quantitative outcomes between the two groups. An independent t-test or Wilcoxon test was used to compare the results before and after the intervention within each group. Statistical analysis were conducted with Statistical Package for the Social Sciences (SPSS), version 16.0 software package. Probability (P value) values of less than 0.05 were considered significant.

**Results**

A total of 94 participants responded to the survey between April 20 and June 20, 2020, with 76 participants with an average age of 66.43 years, providing complete data on the variables in the present analysis (Figure 1). According to Table 1, 36 (47.36%) of all individuals were men and 40 (52.63%) were women. Accordingly, 36 (47.36%) patients had a history of diabetes. However, hypertension with 34 (44.73%) cases and ischemic heart disease (IHD) with 27 (35.52 %) were the following most common underlying diseases; however, there was no significant difference between the two groups.

Table 1 shows that the duration of hospitalization of patients in the intervention group was significantly shorter than their counterparts in the control group (P = 0.041).

According to the results of Table 2, the passage of time has significantly changed in some indicators. Serum levels of total bilirubin on the ninth day (P = 0.043) and GCS on the ninth (P = 0.041) and tenth days (P = 0.039) in the adalimumab group were significantly increased compared to the control group. However, the direct bilirubin value on the eighth day (P = 0.031), serum creatinine on the 8th (P = 0.047), 9th (P = 0.047) and 10th (P = 0.047) days and also PEEF (pericarditis/periocardial effusion) on the tenth day were significantly lower in the intervention group than the control group.

Examination of the changes in the studied indices during ten days showed that the numerical value of total bilirubin had the most fluctuation between the two groups, while the serum creatine kinase (CPK) level and the numerical value of urine out in the intervention group increased appropriately compared to the control group (Figure 2).
Efficacy of adalimumab in COVID-19

Discussion
The immune structure of patients and their cellular immune responses determine the severity of COVID-19 (3). Meanwhile, most patients with a mild form of COVID-19 show a natural immune response to effectively eradicate the virus (5). Therefore, many previous studies have reported that the management of these immune responses can reduce the complications of COVID-19.

As a monoclonal immunoglobulin antibody, adalimumab inhibits TNF-α bioactivity by inhibiting TNF-α interaction with cell surface TNF receptors. This, in turn, inhibits interleukin 6 release, reduction, C-reactive protein, matrix metalloproteinases and adhesion molecules responsible for leukocyte migration (21,22). In this way, it can improve patients with coronavirus by reducing the stressful conditions caused by immune attacks.

The results also showed that the administration of adalimumab in patients with moderate to severe COVID-19 is effective on some clinical and laboratory indicators and leads to significant improvement.

Table 1. Demographic and hospitalization information of patients in the two groups

| Indexes                        | Intervention group (n=36) | Control group (n=40) | P value |
|--------------------------------|--------------------------|----------------------|---------|
| Age (Mean ± SD) (year)         | 61.40±12.94              | 71.47±13.07          | 0.081   |
| Gender                         |                          |                      |         |
| Male (N (%))                   | 16 (44.4%)               | 20 (50.0%)           | 0.093   |
| Female (N (%))                 | 20 (55.5%)               | 20 (50.0%)           | 0.998   |
| Diabetes (N (%))               | 14 (38.8%)               | 12 (30.0%)           | 0.107   |
| CVA (N (%))                    | 4 (11.1%)                | 5 (12.5%)            | 0.351   |
| Asthma (N (%))                 | 1 (2.7%)                 | 2 (5.0%)             | 0.073   |
| HTN (N (%))                    | 17 (47.2%)               | 17 (42.5%)           | 0.618   |
| HE (N (%))                     | 1 (2.7%)                 | 0 (0.0%)             | 0.069   |
| AF (N (%))                     | 2 (5.4%)                 | 1 (2.5%)             | 0.069   |
| IHD (N (%))                    | 12 (33.3%)               | 15 (37.5%)           | 0.100   |
| Hypothyroid (N (%))            | 7 (19.4%)                | 2 (5.0%)             | 0.058   |
| Days of mechanical ventilation (Mean ± SD) | 6.69 ±5.54              | 8.19 ±11.23          | 0.041*  |
| Length in ICU (Mean ± SD)      | 10.5 ±5.9                | 9.67 ±9.22           | 0.098   |
| Length in hospital (Mean ± SD) | 12.43±6.4                | 12.19±10.15          | 0.074   |

CVA: cerebrovascular accident, HTN: Hypertension, HE: Hepatic encephalopathy, AF: Atrial fibrillation, IHD: Ischemic heart disease.
Table 2. Evaluation and comparison of clinical and laboratory factors of patients in the intervention group (sample group) compared to the control group

| Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| **SBP (mm Hg)** |       |       |       |       |       |       |       |       |        |
| Intervention | 134.6 ± 16.3 | 133.14 ± 14.9 | 138.46 ± 19.3 | 133.0 ± 23.6 | 130.8 ± 14.9 | 135.8 ± 15.7 | 132.0 ± 15.9 | 131.0 ± 19.6 | 124.5 ± 20.6 |
| Control | 129.7 ± 38.4 | 128.1 ± 39.6 | 126.8 ± 38.5 | 121.7 ± 38.3 | 123.1 ± 39.6 | 120.6 ± 45.9 | 113.0 ± 46.2 | 110.8 ± 50.9 | 121.2 ± 37.1 |
| \(P\) value | 0.176 | 0.180 | 0.164 | 0.103 | 0.109 | 0.096 | 0.082 | 0.097 | 0.107 |

| **DBP (mm Hg)** |       |       |       |       |       |       |       |       |        |
| Intervention | 79.8 ± 11.3 | 79.6 ± 8.4 | 81.6 ± 11.3 | 80.7 ± 10.5 | 82.3 ± 10.6 | 82.0 ± 10.6 | 77.6 ± 10.9 | 79.7 ± 11.1 | 77.2 ± 9.6 |
| Control | 71.4 ± 21.5 | 74.6 ± 22.3 | 72.2 ± 22.0 | 72.9 ± 23.4 | 73.7 ± 24.4 | 72.3 ± 26.8 | 66.7 ± 27.2 | 66.0 ± 30.1 | 75.8 ± 21.3 |
| \(P\) value | 0.091 | 0.086 | 0.084 | 0.087 | 0.083 | 0.081 | 0.090 | 0.061 | 0.491 |

| **FiO2 (mL)** |       |       |       |       |       |       |       |       |        |
| Intervention | 49.5 ± 20.2 | 51.5 ± 21.9 | 54.0 ± 24.8 | 55.9 ± 24.7 | 55.2 ± 19.0 | 53.0 ± 18.7 | 53.8 ± 21.8 | 58.9 ± 19.4 | 58.4 ± 18.8 |
| Control | 58.7 ± 23.9 | 62.2 ± 24.2 | 65.1 ± 25.3 | 60.48 ± 24.4 | 63.46 ± 24.9 | 67.8 ± 23.2 | 65.87 ± 24.9 | 69.15 ± 28.4 | 69.9 ± 29.15 |
| \(P\) value | 0.094 | 0.084 | 0.073 | 0.087 | 0.068 | 0.106 | 0.087 | 0.148 | 0.073 |

| **Plt (10^8/µL)** |       |       |       |       |       |       |       |       |        |
| Intervention | 233.8 ± 89.2 | 209.4 ± 73.2 | 241.8 ± 118.6 | 245.5 ± 90.1 | 236.2 ± 76.1 | 216.5 ± 73.2 | 209.6 ± 69.6 | 209.6 ± 69.6 | 187.9 ± 67.9 |
| Control | 147.2 ± 70.9 | 216.7 ± 91.05 | 205.7 ± 84.9 | 199.8 ± 87.7 | 184.1 ± 90.9 | 186.1 ± 101.0 | 209.9 ± 94.5 | 186.5 ± 63.2 | 189.64 ± 64.3 |
| \(P\) value | 0.064 | 0.106 | 0.070 | 0.066 | 0.068 | 0.081 | 0.148 | 0.637 | 0.107 |

| **Bili-D (mg/dL)** |       |       |       |       |       |       |       |       |        |
| Intervention | 0.408 ± 0.19 | 0.380 ± 0.349 | 0.733 ± 0.408 | 0.400 ± 0.336 | 0.742 ± 0.489 | 0.975 ± 0.906 | 1.00 ± 0.882 | 1.10 ± 1.38 | 0.700 ± 0.141 |
| Control | 0.33 ± 0.12 | 0.32 ± 0.10 | 0.41 ± 0.25 | 0.38 ± 0.23 | 0.56 ± 0.40 | 0.54 ± 0.30 | 0.83 ± 1.07 | 3.00 ± 0.23 | 0.40 ± 0.18 |
| \(P\) value | 0.078 | 0.097 | 0.084 | 0.071 | 0.068 | 0.051 | 0.100 | 0.31* | 0.20 ± 0.00 |

| **Bili-T (mg/dL)** |       |       |       |       |       |       |       |       |        |
| Intervention | 1.233 ± 0.54 | 1.20 ± 0.604 | 1.10 ± 0.52 | 1.14 ± 0.51 | 1.61 ± 0.48 | 1.93 ± 1.27 | 1.72 ± 0.96 | 1.46 ± 1.93 | 2.05 ± 0.35 |
| Control | 0.92 ± 0.48 | 1.16 ± 0.56 | 1.35 ± 0.69 | 1.45 ± 0.64 | 1.42 ± 0.49 | 2.24 ± 2.15 | 1.18 ± 0.45 | 1.01 ± 0.54 | 1.06 ± 0.403 |
| \(P\) value | 0.087 | 0.417 | 0.308 | 0.291 | 0.094 | 0.087 | 0.182 | 0.094 | 0.043* |

| **GCS** |       |       |       |       |       |       |       |       |        |
| Intervention | 14.85 ± 0.60 | 14.9 ± 0.17 | 14.7 ± 0.82 | 14.5 ± 1.13 | 14.4 ± 1.94 | 14.4 ± 2.00 | 14.9 ± 0.426 | 14.5 ± 1.65 | 14.5 ± 1.73 |
| Control | 12.73 ± 3.02 | 12.61 ± 3.15 | 12.02 ± 2.27 | 12.10 ± 3.34 | 11.96 ± 3.55 | 11.47 ± 3.70 | 11.06 ± 3.88 | 10.38 ± 4.38 | 8.65 ± 4.54 |
| \(P\) value | 0.071 | 0.068 | 0.068 | 0.063 | 0.061 | 0.057 | 0.053 | 0.041* | 0.039* |

| **SrCr (mg/dL)** |       |       |       |       |       |       |       |       |        |
| Intervention | 1.21 ± 0.36 | 1.17 ± 0.36 | 1.10 ± 0.24 | 1.06 ± 0.18 | 1.03 ± 0.27 | 1.23 ± 1.30 | 0.99 ± 0.15 | 0.98 ± 0.204 | 1.00 ± 0.41 |
| Control | 1.59 ± 1.00 | 1.60 ± 1.14 | 1.64 ± 1.52 | 1.57 ± 1.37 | 1.57 ± 1.18 | 1.77 ± 1.75 | 1.78 ± 1.44 | 2.12 ± 2.24 | 2.07 ± 2.19 |
| \(P\) value | 0.063 | 0.079 | 0.061 | 0.059 | 0.058 | 0.064 | 0.054 | 0.047* | 0.047* |
Table 2. Continued

|                   | Day 1                | Day 2                | Day 3                | Day 4                | Day 5                | Day 6                | Day 7                | Day 8                | Day 9                | Day 10               |
|-------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Urine-out (mL)    | Intervention         | 1760 ± 863           | 2140 ± 970           | 2509 ± 1296          | 2303 ± 745           | 2502 ± 770           | 2501 ± 980           | 3152 ± 1060          | 2551 ± 777           | 3048 ± 1425          | 2963 ± 1012          |
|                   | Control              | 1661.5 ± 808         | 2005 ± 1056          | 2126 ± 1072          | 2031 ± 1078          | 2129 ± 1314          | 1921 ± 1173          | 1996 ± 1147          | 1883 ± 912           | 1620 ± 830           | 1624 ± 1085          |
| P value           | 0.321                | 0.209                | 0.210                | 0.244                | 0.182                | 0.103                | 0.063                | 0.190                | 0.100                | 0.057                |
| O₂-saturation (%) | Intervention         | 87.71 ± 15.89        | 86.1 ± 15.6          | 85.5 ± 15.7          | 86.5 ± 16.3          | 87.2 ± 16.8          | 86.1 ± 17.9          | 86.5 ± 18.9          | 85.5 ± 21.7          | 83.8 ± 23.18         |
|                   | Control              | 89.39 ± 7.68         | 89.78 ± 7.22         | 89.7 ± 7.68          | 90.4 ± 8.25          | 89.19 ± 7.10         | 88.5 ± 9.05          | 89.19 ± 7.30         | 88.94 ± 8.04         | 89.29 ± 4.73         | 85.50 ± 12.09        |
| P value           | 0.110                | 0.100                | 0.094                | 0.098                | 0.097                | 0.144                | 0.098                | 0.095                | 0.055                | 0.059                |
| PEEF              | Intervention         | 8.00 ± 0.00          | 8.66 ± 1.15          | 7.80 ± 1.78          | 8.00 ± 1.06          | 7.62 ± 1.50          | 7.33 ± 1.65          | 7.10 ± 0.37          | 6.75 ± 0.49          | 6.37 ± 0.49          |
|                   | Control              | 9.09 ± 3.20          | 10.91 ± 3.31         | 11.25 ± 3.19         | 9.35 ± 3.31          | 9.90 ± 3.33          | 10.1 ± 3.92          | 10.75 ± 2.78         | 10.50 ± 3.33         | 11.00 ± 4.41         | 12.75 ± 5.12         |
| P value           | 0.090                | 0.081                | 0.079                | 0.094                | 0.077                | 0.082                | 0.083                | 0.083                | 0.054                | 0.023*               |
| BG (%)            | Intervention         | 182.7 ± 1.67         | 191.4 ± 11.04        | 215.1 ± 14.58        | 214.4 ± 61.15        | 204.58 ± 55.75       | 311.3 ± 530.4        | 259.0 ± 333.2        | 205.7 ± 90.8         | 211.8 ± 75.5         | 192.1 ± 60.69        |
|                   | Control              | 189.72 ± 68.2        | 255.1 ± 296.3        | 212.0 ± 78.75        | 206.9 ± 78.15        | 223.8 ± 88.8         | 203.3 ± 72.4         | 188.4 ± 64.1         | 172.0 ± 55.2         | 172.7 ± 62.4         | 167.3 ± 55.0         |
| P value           | 0.257                | 0.099                | 0.951                | 0.901                | 0.764                | 0.052                | 0.067                | 0.095                | 0.081                | 0.106                |
| CPK (U/L)         | Intervention         | 359.2 ± 385.8        | 276.5 ± 362.8        | 241.4 ± 260.7        | 210.9 ± 153.1        | 248.9 ± 313.7        | 240.8 ± 234.7        | 282.5 ± 331.9        | 334.8 ± 487.6        | 404.6 ± 581.1        | 376.4 ± 301.7        |
|                   | Control              | 320.5 ± 717.6        | 396.3 ± 710.1        | 513.1 ± 850.7        | 398.0 ± 560.9        | 307.6 ± 465.7        | 278.0 ± 400.7        | 205.0 ± 237.3        | 191.5 ± 224.8        | 238.0 ± 205.8        | 259.0 ± 205.8        |
| P value           | 0.177                | 0.065                | 0.045*               | 0.55                 | 0.110                | 0.371                | 0.102                | 0.080                | 0.083                | 0.0134               |
| LDH (U/L)         | Intervention         | 831.1 ± 273.5        | 943.58 ± 494.9       | 997.9 ± 429.8        | 1047 ± 349.8         | 1082.9 ± 371.6       | 1177.6 ± 439.3       | 1139.5 ± 468.5       | 1064 ± 348.1         | 1162.1 ± 429.2       | 1098.4 ± 345.9       |
|                   | Control              | 734.6 ± 306.8        | 952.0 ± 351.4        | 1072 ± 571.4         | 1090.8 ± 574         | 1093.8 ± 953         | 847.7 ± 389          | 933.4 ± 131.8        | 860.2 ± 407.7        | 901.7 ± 391.9        | 776.4 ± 409.2        |
| P value           | 0.109                | 0.344                | 0.310                | 0.739                | 0.809                | 0.180                | 0.200                | 0.157                | 0.190                | 0.100                |

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, Plt: platelets, Bili: Bilirubin, GCS: Glasgow Coma Scale, SrCr: Scavenger Receptor Cysteine-Rich, BG: Blood gas, CPK: Creatine kinase, LDH: Lactate Dehydrogenase, PEEF: Pericarditis/pericardial effusion
can be clearly described is the effect of taking this drug for more than a week, since all the mentioned indicators showed significant changes after the eighth day. It should be noted that the administration of this drug significantly reduced the length of hospital stay of patients compared to the controls ($P<0.05$). It has previously been shown to be an effective treatment for the management of patients with COVID-19 (23-25). Based on a study, patients with rheumatic disorders who had previously received TNF-α inhibitors developed a mild form of COVID-19 (26). Therefore, it has been suggested that adalimumab, in addition to improving the symptoms of COVID-19, may also be useful in treatment (27).

Conti et al. showed that patients with adalimumab-treated psoriasis had no respiratory infection symptoms despite close contact with patients (28). A case of psoriasis was previously reported, which was treated with adalimumab once every two weeks for a year and recovered rapidly from COVID-19. The patient in the authors’ ward also quickly recovered (30). In our opinion adalimumab is a promising agent in controlling COVID-19 inflammation.

**Conclusion**

In this study, administration of adalimumab in patients with COVID 19 admitted to the ICU was associated with a significant increase in GCS patients and a decrease in hospitalization in long-term.

**Limitations of the study**

One of the limitations of this study was the death of patients during the study, in which case the person was excluded from the study.

**Authors’ contribution**

Conceptualization: RHF, RM, ANA, OY.
Methodology: OY and EH.
Validation: OY and EH.
Formal analysis: RHF, RM, ANA.
Investigation: OY and EH.
Resources: OY and EH.
Data curation: OY, EH, ANA.
Writing—original draft: RHF, RM, ANA, OY.
Writing—review and editing: ANA, EH, OY.

**Visualization:** OY, EH.
**Supervision:** OY, EH.
**Project administration:** EH.

**Conflicts of interest**

The authors declare there is no conflict of interest.

**Ethical issues**

The present study was conducted according to the Declaration of Helsinki and with the approval of the ethics committee of AJA University of Medical Sciences. Thus, the Ethics Committee in Biomedical Research of AJA University of Medical Sciences reviewed the implementation process of this study and declared it applicable following its approved protocols (IR.AJAUMS.REC.1400.032). According to the structure defined for the study (randomized clinical trial), informed consent was obtained from all participants before the intervention, and the whole process of treatment and intervention was free in the form of research. The trial protocol was approved by the Iranian Clinical Trial Registry (identifier: IRCT20200406046963N2, [https://www.irct.ir/trial/55011](https://www.irct.ir/trial/55011)). Besides, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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