The Role of IL6, IL8, TNF α, INF α and some of the positive acute-phase protein in the prognosis of SARS COVID-19

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Abstract
A case-control study was conducted in the AL HUSSAIN hospital in Thiqar governorate from February 2020 to July 2020. A total of 188 individuals involved in the current study, 94 of them with SARC COVID 19 infected patients, and 96 health as controls were enrolled in this study. Serum was collected from each of them to assess the levels of INFα, TNFa, ferritin, D-dimer, LDH, CR protein. Evaluation of cytokine concentrations in patients and control groups was done by ELISA kits according to the manufacture’s instruction. The concentration of serum interleukins, INFα, TNFa and the serum concentration of ferritin, D.dimer, LDH, CR protein were measured in patients and control groups. The mean of IL6, IL8, INFα, TNFa concentration were higher inpatients than the control group with statistically significant differences (P-value 0.00). The Serum concentrations of ferritin, D.dimer, LDH, CR protein (684.4, 0.9, 536.7, and 29.9) were higher than the normal range respectively.

Keywords: IL6, IL8, INFα, TNFα, SARS, COVID-19

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Introduction
COVID-19 is a novel β-coronavirus caused by infection with SARS-CoV-2. It is zoonotic disease infected humans [1-2]. The first case of infection of this virus was reported in a shellfish market in south Wuhan in December 2019 [3]. The infection with COVID-19 stimulates a strong immune response which involved cytokines. Cytokines are small proteins released by different cells that have a specific effect on the interactions and cell communications [4]. The Cytokines (proinflammatory) levels were increased in the most severe cases, clinical and laboratory parameters correlated with evocative of a cytokine storm [5-7]. COVID-19 increase macrophage activity and defects in lymphocyte cytolytic activity with uncontrolled immune system activation due to cytokine storm, acute respiratory syndrome and multiorgan failure [8-10]. This term (cytokine storm) refers to uncontrolled immune system production and generalized inflammatory response [11]. Cytokine storms characterize a wide spectrum of infectious and non-infectious diseases [12]. The increased circulating levels of pro-inflammatory cytokines and Chemokines in SARS patients are associated with pulmonary inflammation and extensive lung involvement in, similarly to what happens in MERS-CoV infection [13]. Recently reported that infected patients by COVID 19 also show high levels of pro-inflammatory cytokines and Chemokines [14] large amounts of cells release pro-inflammatory cytokines and Chemokines precipitates and sustains the aberrant systemic inflammatory response [13-16]. Acute respiratory distress syndrome and multiple organ failure readily followed by the immune system “attacking” the body, in turn, will cause, the final result being death, in the most severe cases of SARS-CoV-2 infection [17]. Interferon: cytokines play a central role in virus-directed innate immunity binds specific receptors and result in the expression of genes encoding a protein with anti-viral or immunomodulatory properties. Tumor necrosis factor α: It Is apyrogenic cytokine released from immune cells in the acute phase of inflammation and infection. It is a central cytokine in viral diseases and is associated with many chronic inflammatory and autoimmune diseases [18]. Interleukins: A family of cytokines plays an important role in the differentiation and activation of immune cells. Interleukins stimulation increases the acute phase signaling, production of secondary cytokines and activation of immune cells to the site of the infection [19]. Interleukin-6 (IL-6) deserves a more extensive effective involvement in the coronavirus-induced cytokine storm. IL-6 is crucially involved in acute inflammation due to its role in regulating the acute phase response. IL-6 secreted by most cells and lymphocytes, and other tumor cells [20]. The production of IL-6 is increased by tumor necrosis factor and interleukin-1β [21] positively increased levels of IL-6 in COVID-19 patients are...
related to disease severity and pathogenesis of the cytokine storm [22-23], for this reason, high serum IL-6 levels were suggested as predictors for disease severity [24-25].

**Aim of study**
To evolution the role of IL6 IL8 TNF α INF α and some of the positive acute-phase protein in the prognosis of SARS COVID-19

**Material and methods**

**Population and study design**
A case-control study involved all ages and gender was admissions from Imam AL Hussain Hospital in Thi-Qar Governorate during the period from February 2019 to July 2019. All patients’ data were recorded clinical presenting features, patient history, chronic disease, sign, and symptom. In this study 188 cases from controls and patients groups 94 of them infected with SARS-COVID 19 Their ages were between (1 month-90 years) had been already diagnostic depended on The clinical manifestations of COVID-19 appear after an incubation period of around 5–6 days and most frequently include fever, coughing, and fatigue, with the possible onset of sputum production, headache, diarrhea, dyspnea, and/or lymphopenia, among others. Computed tomography images of patients with severe complications of COVID-19 reveal the presence of pneumonia although with abnormal characteristics, including evidence of pulmonary ground-glass opacities. In some patients, ground-glass opacities were detected in sub pleural regions in both lungs, in addition to a positive nasal swab of Real-time PCR.

**Materials and Equipment**

**Equipment**
The types of equipment used in the current study are shown in Table (1).

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Company</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elisa reader</td>
<td>Human</td>
<td>Germany</td>
</tr>
<tr>
<td>Elisa washer</td>
<td>Human</td>
<td>Germany</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>Sigma</td>
<td>Germany</td>
</tr>
<tr>
<td>Incubator</td>
<td>Carbolite</td>
<td>Turkey</td>
</tr>
<tr>
<td>Micropipette (automatic) 1000 – 500</td>
<td>Eppendorf</td>
<td>Germany</td>
</tr>
<tr>
<td>Micropipette (automatic 10 -50 – 100)</td>
<td>Slaed</td>
<td>Germany</td>
</tr>
<tr>
<td>Micro centrifuge</td>
<td>Beckman</td>
<td>Germany</td>
</tr>
<tr>
<td>Shaker</td>
<td>Velbscientifica</td>
<td>Europe</td>
</tr>
<tr>
<td>Cobbas 411e</td>
<td>HITACHI</td>
<td>USA</td>
</tr>
</tbody>
</table>

**Diagnostic kits**
Kits and reagents that were used in this study are shown in Table (2).

<table>
<thead>
<tr>
<th>Kits</th>
<th>Company</th>
<th>Country of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin</td>
<td>Hitachi</td>
<td>Germany &amp; Japan</td>
</tr>
<tr>
<td>D.dimer</td>
<td>Genuri</td>
<td>China</td>
</tr>
<tr>
<td>LDH</td>
<td>Biolabo</td>
<td>France</td>
</tr>
<tr>
<td>CR-Protein</td>
<td></td>
<td>China</td>
</tr>
<tr>
<td>Human IL6 ELISA Kit</td>
<td>Biobase</td>
<td>China</td>
</tr>
<tr>
<td>Human IL8 ELISA Kit</td>
<td>Biobase</td>
<td>China</td>
</tr>
<tr>
<td>Human TNFα ELISA Kit</td>
<td>Biobase</td>
<td>China</td>
</tr>
<tr>
<td>Human INFα ELISA Kit</td>
<td>Biobase</td>
<td>China</td>
</tr>
</tbody>
</table>

**Collection of blood samples**
A total of five ml of blood was drawn from control and patient groups. Blood samples were centrifuged at 3000 r.p.m and sera were stored at -20 C° for measuring the level of (Ferritin, LDH, CR-protein, D.dimer, IL-6, IL-8, and TNF α, INF α assay)

**Evaluation of the cytokines level**
Enzyme-linked technology (kits of Immunosorbent assay) provided by the Biobase biotechnology company was used to detect the level of certain cytokines in a blood sample of both groups.
ELISA Test (Sandwich-ELISA)

Principle
1. For the specific cytokines to be an evaluation, the relevant micro plate utilized was supplied with a coated of a specific antibody to (IL-6, IL8, TNFα, INFα) pre-applied by the manufactures.
2. Serum samples must come into contact with the respective Elisa Micro plate, thus combining with the target antibody.
3. Before incubating the micro plates, based on the particular cytokine type, the biotinylated antibody was provided together with Avidin-Horseradish peroxidase (HPR) was added to each well, in this manner, the micro plate formed a conjugated then incubated.
4. Uncombined parts were washed away, and then the substrate solution was added to each well to reveal the blue color.
5. To cut off the reaction between enzyme and substrate, sulphuric acid was utilized and the solution displayed a yellow tint.
6. The spectrophotometer was used to measure optical density (OD), 450 ± 2-nanometer wave lengths are exhibited.
7. The optical density of the samples was compared against the optical density of the standard curve, the value of optical density is directed related to the concentration of cytokines and influenced in proportion to their concentration in solution.

Preparation of Reagents
All reagents were brought to room temperature (18-25c°) proceeding use.

Wash Buffer
Thirty milliliters of the concentration wash buffer were diluted into 750 ml by using deionized or distilled water. The unused solution should be returned to the refrigerator where the temperature is 4C°. When crystals had been shaped in the concentration, they were warmed with a 40C° water path, mixing gently until the crystals had totally broken up.

Standard
The standard was prepared within 15 minutes directly used.

Biotinylated detected Ab
Biotinylated detected Ab was diluted using a specific diluents to get the concentration 1:100 (100-micron liter was required)

Concentration horseradish peroxidase Conjugation
The method of preparation was similar to what was conducted in the preparation of Biotinylated detected Ab.

Substrate Reagent
The reagent was introduced to the micro plate through the means of sterilized tips.

Washing by Automated Washer
Wash buffer (350µl) was added to every well, the defined length of time between injection and aspiration was about 60sec.

Procedure:
1. One hundred Microtiter (standard, Blank, or samples) was added per well. The reference standard and sample diluted was added to the well as blank in the bottom of ELISA plate wells, all of those before mentioned solutions were accumulated and incubated at 37c°90 minutes.
2. The solution from each well was removed, and (100 µl) biotinylated detected Ab working solution was added to each well and incubated at 37c° for one hour.
3. All wells were aspirated and washed by over flooding them with around 350 µL of wash buffer. The liquid was drained completely with each subsequent step. When it comes to the last wash remaining wash buffer was aspired and a plate was left to rest upside down against special paper after shaking them to slide off a remaining droplet
4. The work solution, which is determined to be horseradish peroxidase with the volume of (100 µl) was added in each well and before incubator for 30 minutes at 37 c° the were sealed with plate sealer.
5. The wash process was repeated five times as performed previously.
6. Then each will receive 90 ml of the substrate solution, incubated at 37C° for about 15 minutes, another sealer for the plate was applied. The reaction was observed within 30 minutes.
7. Subsequently, every well-received (50µl) of stop solution so immediate shifting to yellow tint color.
8. Reader for micro plates was used for evaluation optical density for each well immediately, 450 nm is the setting of the micro plate reader.

Calculation of results
Average for double reading was measured for each sample and standard, the average zero was considered as standard optical density. Graphing software was used to calculate and plot the results, 2 axes were used for graphing:
1. Concentration
2. Optical density values and a curve are graphed.

By using the concentration of standard samples, it will result in standard curve best equation; another matter to note is in the case of the diluted sample, those values to be multiplied by a factor of dilution after standard curve determination. Re-testing of samples with proper dilution agent is performed when the optical density value is found to be even higher than the standard curve upper limit.

Statistical analysis
The data were analyzed using description statistic (mean and standard deviation) independent sample $t$-test the level significant was set at $p < 0.05$ SPSS (Statistical Packing for Social Sciences) version 20.

**RESULTS**

The age group of the infected patient was included in the study.

All age groups and both gender included in the current study were 94. They were classified according to their age group as shown in tables (3-4). The highest percentage in the case study was 24.4% within the age (50-59 years), followed by (30-39 years) which was shown to be 20.2%, age group between (1month-90 yrs) showed the lowest percentage 1.06%.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Frequency</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month-9 years</td>
<td>1</td>
<td>1.06%</td>
</tr>
<tr>
<td>10-19 years</td>
<td>2</td>
<td>2.1%</td>
</tr>
<tr>
<td>20-29 years</td>
<td>8</td>
<td>8.5%</td>
</tr>
<tr>
<td>30-39 years</td>
<td>19</td>
<td>20.2%</td>
</tr>
<tr>
<td>40-49 years</td>
<td>13</td>
<td>13.8%</td>
</tr>
<tr>
<td>50-59 years</td>
<td>23</td>
<td>24.4%</td>
</tr>
<tr>
<td>60-69 years</td>
<td>14</td>
<td>14.8%</td>
</tr>
<tr>
<td>70-79 years</td>
<td>8</td>
<td>8.5%</td>
</tr>
<tr>
<td>80-90 years</td>
<td>6</td>
<td>6.3%</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No</th>
<th>IL6 Pg/ml</th>
<th>IL8 Pg/ml</th>
<th>TNFα Pg/ml</th>
<th>INFα Pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>94</td>
<td>48.84±12.98</td>
<td>59.32±10.88</td>
<td>31.45±9.11</td>
</tr>
</tbody>
</table>

| P value | 0.000 | 0.000 | 0.000 | 0.000 |

Mean serum levels of IL6, IL8, TNFα, and INFα in controls, and patients group

The mean levels of serum cytokines TNF α, IL8, IL6, and INF α were measured in infected Covid-19 patients and control groups. IL6, IL8, TNF, INF were highly elevated in the infected group than controls (48.84±12.98 pg/ml), (59.32±10.88 pg/ml), (31.45±9.11 ng/ml), (21.67±6.31 pg/ml) respectively. The differences between patients and control groups were statistically significant (p-value <0.00)

Mean serum levels of ferritin, D.dimer, C.R Protein in patients group

The mean serum levels of ferritin, D.dimer, LDH, C.R Protein for infected Covid-19 patients (684.4), (0.9), (536.7), (29.9) respectively showed highly elevated than the normal value as showed in table (5).

<table>
<thead>
<tr>
<th>No</th>
<th>Ferritin</th>
<th>D.dimer</th>
<th>LDH</th>
<th>C.R Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal value</td>
<td>Newborns 25-200 ng/mL 6th-15 years 7-140 ng/mL Adult males 20-390 ng/mL Adult female 10-150 ng/mL Females &gt;49 Y 12-263 ng/mL</td>
<td>&lt;0.5 Mg/L</td>
<td>&lt;248 U/L</td>
<td>&lt;10 Mg/L</td>
</tr>
<tr>
<td>Patients</td>
<td>94</td>
<td>684.4</td>
<td>0.9</td>
<td>536.7</td>
</tr>
</tbody>
</table>

The mean ± SD levels of serum cytokines IL6, IL8, TNF alpha, INF alpha were measured in patients groups and control groups. IL6, IL8, TNF, INF were higher in patients age group (30–39 years) with serum level (63.35±16.36 pg/ml), (92.912±22.21 pg/ml), (36.09±9.405 pg/ml), (25.84±7.96 pg/ml) respectively than control groups. The differences between patients and control groups were statistically significant (p-value <0.00) Serum level of ferritin, LDH, D.dimer showed highly elevated than normal value while the age group (30 – 39 years) recorder highly result than other groups as shown in table 4. C.R. Protein showed to be highly elevated in the age group (40 – 49 years).
Table 6: Mean concentration of cytokines for different age groups

<table>
<thead>
<tr>
<th>AGE GROUPS</th>
<th>Ferritin (20-200)</th>
<th>LDH &lt;248 U/L</th>
<th>D.dimer &lt;0.5</th>
<th>C.R Protein &lt;10mg/L</th>
<th>IL6 Pg/ml</th>
<th>IL8 Pg/ml</th>
<th>TNFα Pg/ml</th>
<th>INFα Pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>NV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month – 9 year</td>
<td>189</td>
<td>179</td>
<td>&lt;0.5</td>
<td>21</td>
<td>17.38±4.473</td>
<td>27.56±6.70</td>
<td>11.01±2.531</td>
<td>12.32±3.75</td>
</tr>
<tr>
<td>10-19</td>
<td>200</td>
<td>180</td>
<td>&lt;0.5</td>
<td>27</td>
<td>28.98±7.368</td>
<td>31.4±7.75</td>
<td>21.4±5.114</td>
<td>19.35±5.80</td>
</tr>
<tr>
<td>20 – 29 yrs</td>
<td>470</td>
<td>321</td>
<td>0.7</td>
<td>31.3</td>
<td>47.02±12.62</td>
<td>56.76±13.33</td>
<td>31.84±7.365</td>
<td>23.65±6.94</td>
</tr>
<tr>
<td>30 – 39 yrs</td>
<td>1760</td>
<td>1210</td>
<td>1.7</td>
<td>42</td>
<td>63.35±16.36</td>
<td>92.91±22.2</td>
<td>36.09±9.405</td>
<td>25.84±7.96</td>
</tr>
<tr>
<td>40 – 49 yrs</td>
<td>1540</td>
<td>976</td>
<td>1.2</td>
<td>47</td>
<td>60.925±15.7</td>
<td>87.27±19.69</td>
<td>48.71±12.84</td>
<td>26.147±8.5</td>
</tr>
<tr>
<td>50-59</td>
<td>870</td>
<td>760</td>
<td>1.3</td>
<td>22</td>
<td>53.4±13.10</td>
<td>64.78±14.22</td>
<td>33.04±8.24</td>
<td>22.87±6.82</td>
</tr>
<tr>
<td>60-69</td>
<td>363</td>
<td>678</td>
<td>1.1</td>
<td>31</td>
<td>50.32±13.15</td>
<td>60.02±13.33</td>
<td>29.32±7.25</td>
<td>20.43±5.98</td>
</tr>
<tr>
<td>70-79</td>
<td>467</td>
<td>311</td>
<td>0.7</td>
<td>26</td>
<td>51.02±13.31</td>
<td>53.98±11.77</td>
<td>28.91±7.11</td>
<td>20.11±5.88</td>
</tr>
<tr>
<td>80-90</td>
<td>301</td>
<td>216</td>
<td>1.02</td>
<td>22</td>
<td>56.01±14.7</td>
<td>54.00±12.10</td>
<td>30.65±7.55</td>
<td>21.65±6.08</td>
</tr>
</tbody>
</table>

Discussions
SARS COVID-19 is still a public health problem; Research has opened the probability of immune response to such infected patients complications with special emphasis on the effects of pro-inflammatory and anti-inflammatory cytokines in the role of cytokines storm in addition to the level of D.dimer.

**Interleukin 6**
Mean levels of IL6 in patients infected by the coronavirus were higher than the control group and this difference was highly statistically significant. This finding was in agreement with findings reported by Al-Samkari et al. (2018) [9], and Huang et al. (2020) [14], elevated IL-6 levels have been observed in SARS cases and related to the severity of symptoms [26-28] increased level of IL-6 is associated with the severity of COVID-19 [14]. Recent study reported elevation of IL-6 levels in relationship with symptoms severity [29-30]. Cytokine storm and progressive disease demonstrated in high expression of IL-6 in patients with COVID-19 [31]. Cardiac damage has also been observed with elevated levels of IL-6, in these patients [32]. IL-6 in the Sievier SARS-CoV-2-patient induced cytokine storm and [33-35], inflammation, and pulmonary fibrosis [36-37]

**Interleukin 8**
The mean levels IL8 for COVID 19 were higher than control groups and show statistically significant and this finding was disagreement with Gong J et al. (2020) [42] that showed no change in the level of IL-8 levels in mild and severe patients, proinflammatory cytokines including IL-8 and another contribution to the occurrence of acute respiratory disease syndrome [38].

**Tumor necrosis factor-alpha**
The result of the current study showed the mean level of tumor necrosis factor-alpha was higher in patients with COVID-19 compared to the control group. The differences were highly statistically significant and this finding was agreed with Huang et al. (2020), Liu Y et al. (2020) and Chen G et al. (2019) [29], that show elevated serum level of TNF-α in patients with SARS COVID-19 are higher with more severe disease. TNF-α was one of the cytokines whose overproduction was related to a poor prognosis in patients with SARS-CoV (39-40.41).The concentration of TNFα is correlated with disease severity [42].

**Interferon-alpha**
The mean level of serum INFα in patients was high level than the controls. To the best of our knowledge, there are no published studies between the relationships of INFα with a patient with COVID-19

**Ferritin**
Serum ferritin levels elevated more than the normal range and this funding was in agreement with study Zhou F et al. (2020) [6] that showed the level of ferritin in patients SARS COVID-19, were higher.pro-inflammatory effects immune dysregulation, suggesting that ferritin levels increased and non-stop, due to contributing to the cytokine storm [43] hyperferritinemia might be a critical factor influencing the severity of COVID-19.

**CR-protein**
CR-protein in our study was higher than the normal range (29.9 mg/L) this elevated increase of CRP due to COVID-19 infection related to acute inflammatory pathogenesis during which released of multiple cytokines and there was associated
with severity of disease COVID-19 patients in this study showed elevated CRP levels, which is in agreement with other studies done by Zhu Net al. (2020) and Young BE et al. (2020) [44]. The mortality rate of COVID-19 may be linked with altered levels of some blood markers. Serum C-reactive protein level significantly changes in severe patients with COVID-19, clinical parameters, has been found as an important marker for evaluation degree of disease. Wang G et al. (2020), and marker for monitoring disease severity (47) C-reactive protein binds to Phosphocholine expressed highly on the surface of damaged cells. (48) C-reactive protein concentration falls up to 86% in inflammation and or tissue damage is resolved, making it a useful in severe COVID-19 patients (49-50) in severe cases CRP level elevated more than mild or non-severe infection.

**D.dimer**

D-dimer is produced during fibrin breakdown and serves as a marker of fibrinolysis activity. In the current study observed that the D-dimer was elevated more than the normal range in sever COVID-19 patients; D-dimer levels were positively correlated with levels of CRP, LDH, and ferritin. Significantly higher D-dimer levels in patients with COVID-19 because the secondary infection of bacterial pneumonia, that activation of the coagulation system [51]. Prognosis of patients with COVID significantly increased in D-dimer level Yin et al. 2020, Zhang et al 2020 [52-53] another reported suggested a relationship between IL6, IL8, TNFalpha, INFalpha and activation of the coagulation cascade, involved D-dimer in critical cases [55] fibrinolysis inhibition and activate coagulation process observed in patients with severe sepsis because the role of endothelial injury due to activity of pro-inflammatory cytokines (56) Another study done by Yu et al (2020) [57] suggested alterations in haemostatic systemic balance during infection due to dysregulation of the urokinase pathway and contributes to lung pathology.

**Lactate dehydrogenase**

LDH is an intracellular enzyme found in cells in most organic systems, which catalyzes the inter conversion of pyruvate and lactate, with concomitant inter conversion of NADH and NAD⁺ (58) Lactate dehydrogenase in our study showed elevated level more than the normal range. Up regulation of the glycolysis pathway due to decreased oxygenation, acidic extracellular pH and multiple organ injury increase level of LDH in addition to activation of metalloprotease and stimulation macrophage mediated-infection may cause mediated cytokine-tissue damage and LDH release [59].

**Conclusions and recommendation**

1. The present study revealed a significant indicator between cytokines level and SARC COVID19.
2. There is a significant association between IL-6, IL-8, TNFα and INFα levels of SARC COVID19 infection
3. The mean concentration of interleukin 6 (48.84±12.98Pg/ml) was higher in the study group than the control group (8.025±2.14 Pg/ml) used IL-6 receptor monoclonal antibody is recommended in the critical case.
4. The mean concentration of interleukin 8 (59.32±10.88 Pg/ml) was higher in the study group than the control group (21.5498±9.85 Pg/ml).
5. The mean concentration of tumor necrosis factor α (31.45±9.11 Pg/ml) was higher in the study group than the control group (8.5346±7.22Pg/ml)
6. Immunotherapy for IL-6, IL-8, Interferon-alpha, and TNFα may be potential targets of COVID-19 and decrease the event of the cytokine storm.
7. The mean concentration of interferon α (21.67±6.31 Pg/ml) was higher in the study group than the control group (6.240±2.18Pg/ml). Interferon-alpha the preferred solutions remain the better-evaluated, safer intravenous and subcutaneous routes
8. Mean concentration of ferritin (684.4 ng/ml) was higher in the study group than normal range. So A possible strategy to decrease ferritin levels might be the treatment with iron chelators. Deferoxamine may be a good candidate,
9. The mean concentration of D, dimer (0.9mg/L) was higher in the study group than the normal range
10. The mean concentration of C.R. protein (29.9 mg/L) was higher in the study group than the normal range.

**No any conflict of interest to declare**

**Reference**


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