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ORIGINAL ARTICLE

Demographic and Laboratory Parameters among Covid-19 Outpatients Versus and Hospitalized Patients

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ABSTRACT

The present study addresses the impact of age and gender on poor diagnosis COVID-19 disease and monitoring the levels of the serum biomarkers: LDH, D-dimer, CRP, TGF-B, IL-6 among COVID-19 patients. The study included 100 COVID-19 patients, recruited from hospitals in Baghdad governorate from February 2020 to May 2020 of both genders with media range of age of 38-59 years. Data revealed no significant impact of gender on COVID-19 disease outcome. Whilst age of participants was significantly higher (P<0.001) in needed ICU/admission died patients(59.0±12.67 years) compared to that of well -discharged patients (38.83±11.74 years). Age had a positive significant correlation with D-dimer (r= 0.276, p= 0.006), LDH (r= 0.318, p= 0.003), ferritin (r= 0.307, p= 0.005), CRP (r= 0.470 p<0.001), and WBC count (r= 0.410, p<0.001). D-dimer, LDH, ferrtin, and CRP levels were significantly higher (P<0.001) in needed ICU/admission died patients 'group compared to those recorded in well-discharged patients' group.The sensitivity and specificity of the tests at P<0.001 at cut off value of D-dimer= 0.56mg/ml, 285.7 U/L, 387.97 ng/ml, and 36.3 mg/Lwere (81 and 94%), (71 and 76%), (86 and 84%), and (91 and 84%), for D-dimer, LDH, ferritin, and CRP, respectively.

Keywords: COVID-19; age, gender; D-dimer; LDH; CRP; ferritin; IL-6; TGF-B

INTRODUCTION

Acute respiratory distress syndrome (ARDS) is the leading cause of death in COVID-19 (1). The actual reason for this being a prevalent immunopathological event for SARS-CoV-2, SARS-CoV, and MERS-CoV infections is unknown, however it is most likely due to a cytokine storm (1-4).Differentially expressed cytokines are produced at sites of tissue inflammation and released into the blood by a variety of cell types, including macrophages, lymphocytes, endothelial cells, epithelial cells, and fibroblasts, and are involved in the pathophysiology of COVID-19 (2). IL-6 is a significant pro-inflammatory mediator (3) with a chief role in the host's defense against contagions and tissue destruction in SARS-CoV-2 infection. Significant beneficial impacts of IL-6 blockade therapy using a humanized anti-IL-6 receptor antibody, tocilizumab was recently reported in cases with cytokine release syndrome (4).

Transforming growth factor beta 1 (TGF-β1), a member of the transforming growth factor beta superfamily of cytokines, [5]is a soluble cytokine secreted mostly by inflammatory cellsand a well-known regulator of immune responses. As a consequence of TGF-β activation pathways the triggered inflammation, apoptosis, and fibrosis, would lead to harshlydestructiveinfluences in the lungs and other tissues [5, 6].

In vitro study conducted by β, Boumazaet al. revealed the infection of monocytes and macrophages by SARS-CoV-2 result in TGF-β secretion [7]. Only one reports stated the increased TGF-β1 levels in two different subsets of $CD4^+$ immune cells of the COVID-19 patient [8].

SARS-CoV-2 is considered a dual disease: respiratory disease and a hematologic disease. Blood clots exist in COVID-19 patients and involve in deep venous thrombosis in lower extremities [9]. TGF-β is known to elicitthe production of Factor XII (FXII), stands at the onset of the coagulation signaling of the intrinsic cascade [10]. Thrombinmediates the cleavage of fibrinogen to fibrin and results in mature TGF-β1, creating a positive feedback loop [11].

As an inflammatory consequence induced by bacterial/viral infection or tissue destruction, the liver normally synthesizes the C-reactive protein (CRP) within 6-10 hrs of any tissue damage. Since COVID-19 infection is a status with hyper inflammatory response with a pathological dysfunction of innate host defense mechanisms accompanied by multiple organ failure and cytokines storm [12]. Hence, the CRP level during COVID-19 infection is a good indicative about the acuteness status of the illness and is a strong prognostic marker for COVD-19 poor outcome [13].

Lactate dehydrogenase (LDH), an intracellular enzyme found in all tissue,does catalyze the conversion of pyruvate to lactate and NADH and NAD^+. High serum levels of LDH in any individual is an indicative of cytoplasmic cell's membrane damage induced by viral infection [14,15]. Elevated levels of LDH in COVID-19 patients are expected as long as the LDH is already exists in lung tissue. Additionally, inthrombotic microangiopathy, which is correlated withto renal failure and cardiac damage, LDH levels are high [16,17].

D-dimer, a plasmin cleavage product, is made up of two adjacent fibrin 'D' domains and released as a single molecule [18]. In SARS-CoV correlated coagulopathy, the high D-dimer levels might result from upregulated urokinase-type plasminogen activator [19].High levels of D-dimer (>1 µg/mL) were reportedly to play a crucial role in poor prognosis in COVID-19 patients [20,21].

Ferritin levels on admission in COVID-19 patients were 1.5 to 5.3 times higher in patients with severe disease compared to those with less-severe disease[22,23].

The aim of the current study is monitoring the levels of some serum biomarkers like LDH, D-dimer, IL-6, LDH, and TGF-B in COVID-19 patients in Iraq.

MATERIALS AND METHODS

Study cohort: The study cohort encompassed 100 Iraqi COVID-19 patients (21 to 81 years, both genders), recruited from hospitals in Baghdad governorate; Ibn-Al Qiph hospital and Al-Kindi Teaching hospital from February 2020 to May 2020.All participants signed the ethical consent settled by the Institutional Review Board (IRB) of Al-Nahrain University.

Blood sampling: Around three ml of venous blood were collected from each patient in a sterile plane tube. After 15 min to allow clotting, the blood sample was centrifuged at 4,000 rpm for 15 min. The resultant serum was kept at -20 ºC until being processed.

Quantification of IL-6,TGF- B1, D-dimer, LDH, and Ferritin serum level: The levels of Interleukin-6 (IL-6) and TGF- B1 in serum samples wereestimated using Sandwish-ELISA method using Horseradish Peroxidase (HRP)-conjugated antibody specific for IL-6and TGF-B1 ELISA was performed in Microelisastripplateaccording to the instructions of the manufacturer.

The level of D-Dimer in serum samples was estimated using antigen –antibody reaction using anti-human D-Dimer antibodies coated on the latex particles according to a previously reported procedure (Poudel et al., 2021). LDH, ferritin, and CRP were

measured in fully automated analyzercalledBeckman Coulter Analyzers,

Statistical analysis: SPSS software version 25.0 was used to analyse all of the data (SPSS, Chicago). The normality of continuous data was tested (Shapiro Wilk test). The mean and standard deviation of normally distributed data were calculated and examined using the Student t-test. The Mann Whitney U test was used to assess data having non-normal distributions, which were reported as median and range. The Chi-square test was used to examine categorical variables that were expressed as numbers and percentages.The numerous markers were evaluated using a receiver operating characteristic curve (ROC) to predict the COVID-19 severity. The possible link of several indicators with age and duration severity of COVID-19 was investigated using Pearson's correlation test and binary logistic regression to calculate ratios (OR) and their associated 95 percent confidence intervals (CI). A statistically significant difference was defined as a p-value less than 0.05.

RESULTS

Correlatonof Demographic Characteristics with COVID-19Outcome: The mean age of the required ICU/died patients (59.0±12.67 years) was significantly higher (P<0.001) than that of discharged patients (38.83±11.74 years)(Table 3-1). Conversely, no significant difference (P=0.217) was evidenced among the two groups of patients regarding the gender.

Table 1: Demographic characteristics of COVID-19 cohort participants (n=100)

Variable	Discharged well (n=**77**)	Need ICU admission/died (n=**23**)	p-value
Age, years Mean±SD	38.83±11.74	59.0±12.67	<0.001†
Gender Male Female	46(59.74%) 31(40.26%)	17(73.91%) 6(26.09%)	0.217 ‡

† Student t-test

‡ Chi square /exact Fisher tests

Correlation of Age and Duration with Different Serum Biomarkers: Spearman's correlation test was used to explore the possible correlation of ageand duration with different biomarkers. Age had a positive significant correlation with D-dimer (r= 0.276, p= 0.006), LDH (r= 0.318, p= 0.003), ferritin (r= 0.307, p= 0.005), CRP (r= 0.470 p<0.001), and WBC count (r= 0.410, p<0.001) (Table 3-2). Transforming growth factor- β showed a negative significant correlation with viral load (r= -0.262, p= 0.015). However, disease duration showed a positive significant correlation with D-dimer (r= 0.319, p=0.002), LDH (0.265, p= 0.025), ferritin (r= 0.283, p= 0.008), and CRP (r= 0.349, p<0.001) (Table 3-2,Figures 3.1,3.2,3.3).

Table 2: Spearman's correlation of age and duration with different serum biomarkers

Variable	Age		Duration	
	r	p-value	r	p-value
D-dimer	0.276	0.006	0.319	0.002
LDH	0.318	0.003	0.265	0.025
Ferritin	0.307	0.005	0.283	0.008
CRP	0.470	<0.001	0.349	<0.001
IL-6	0.022	0.828	-0.016	0.880
TGF-β	0.145	0.180	-0.054	0.622
WBC	0.410	<0.001	0.148	0.215

Association of serum Biomarkers with Patients' Outcome: Data regarding laboratory parameters were found to be non-normally distributed. Accordingly, Mann Whitney U-test were used to compare these parameters between discharged well and required ICU/died patients.Only, fourserum biomarkers (D-dimer, LDH, CRP, and ferritin) showed a significant association (P<0.001) with the patient's outcome (Table 3-3). (???)_The above-mentioned four serum biomarkers exhibited higher levels than the normal levels in both patients' groups. The levels of these serum biomarkers were significantly higher (P<0.001) in the required ICU/died patients than those observed in the well-discharged patients' group. No significant difference was evidenced regarding IL-6 and TGF-B among the two patients' groups.

Table 3: Levels of serum biomarkers in COVID-19 patients.

Variables	Discharged well (n=77)	Need ICU admission/died(n=23)	p-value‡
D-dimer, ng/ml Median Range	0.2 0.02-1.56	1.55 0.18-8.44	<0.001
CRP, mg/L Mean±SD Range	18.2 0-119.65	58.0 7.44-17.4	<0.001
LDH, U/L Mean±SD Range	211.35 110-1808	601.0 117.0-1850	0.001
Ferritin, ng/ml Median Range	147.3 4.71-1000	706 227.8-1000	<0.001
IL-6, pg/ml Median Range	12.31 0.44-244.13	11.65 4.61-234.89	0.701
TGF-β, pg/ml Median Range	31.22 0.37-243.66	6.45 2.11-78.6	0.182

‡ Mann Whitney U test, P value significant at <0.05.

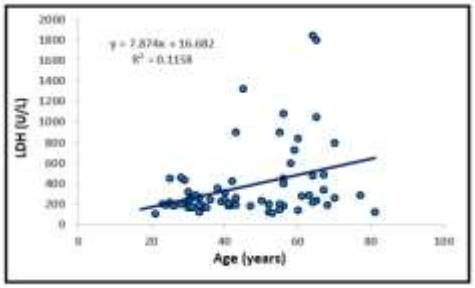

Figure 1: Scatter plot and regression line between age and LDH

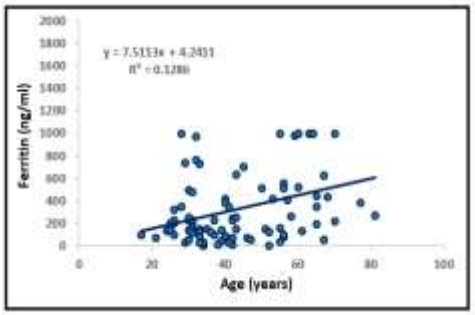

Figure 2: Scatter plot and regression line between age and ferritin

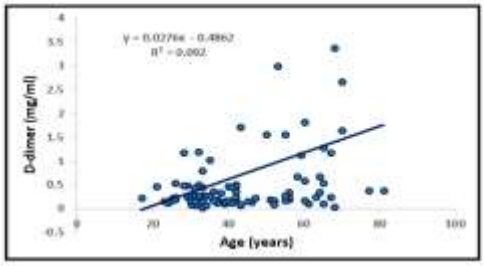

Figure 3: Scatter plot and regression line between age and D-dimer

Predictive Value of serum biomarkers: Receiver operating characteristic (ROC) curve was used to evaluate the predictive value of serum biomarkers with significant rise in patients with worse outcome (Figure 3-2).

For D-dimer, The sensitivity and specificity of the test at cut off value of D-dimer= 0.56mg/mlwas81% and 94%, respectivelyat P<0.001.

For LDH, the sensitivity and specificity of the test at cut off value of LDH= 285.7 U/Lwas 71% and 76%, respectivelyat P<0.001.

For ferritin, the sensitivity and specificity of the test at cut off value of ferritin= 387.97 ng/mlwas 86% and 84%, respectivelyat P<0.001.

For CRP, the sensitivity and specificity of the test at cut off value of CRP= 36.3 mg/Lwas91% and 84%, respectivelyat P<0.001.

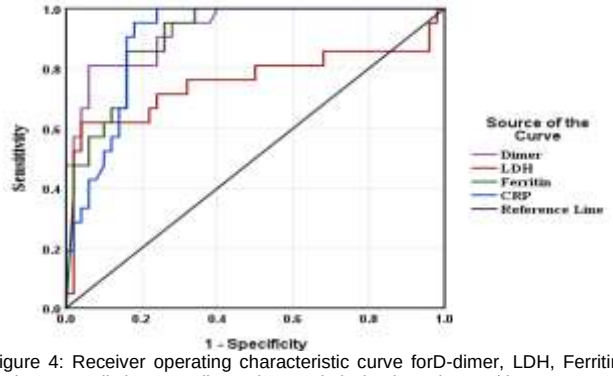

Figure 4: Receiver operating characteristic curve forD-dimer, LDH, Ferritin and CRP predicting mortality and ICU admission in patients with COVID-19

DISCUSSION

Our data revealed no significant impact (P>0.05) of the gender regarding the outcome of COVID-19 disease. The literature of review showed contradictory findings regarding the influence of gender on COVID-19 outcome. For example, in China, a previous study inferred a mortality rate of 2.8 and 1.7% among men and females COVID-19 patients, respectively [24]. Another previous study conducted in China revealed a low mortality rate in women (0.4%) compared to that in men (7.2%) COVID-19 patients [25]. A previous meta-analysis study carried out on5057 COVID-19 patients, revealed the high mortality rate among the hospitalized male group (26). In the meta-analysis study of Biswas and co-workers conducted on 64,676 coronavirus cases, it was revealed that COVID-19 male patients were significantly correlated with elevated risk of mortality compared to COVID-19 female patients (P<0.00001) (27). Consistent to our data, Al-Bari, et al. inferred no significant difference between the death rate among male and females COVID-19 patients in Bangaladesh (28).

Our data revealed that elderly COVID-19 aged of 59 years were the most affected patients. . Similarly, a previous study conducted in Iraq showed that the highest date rate of COVID-19 patients was among elderly category of 60-69 years(29). Likewise, the study of Saeed et al., conferred that the most affected group of COVID-19 patients aged from 30-39 years (30).The meta-analysis of Nasiri conducted with 5057 patients revealed that the most affected COVID-19 patients aged 49 years (3). In Bangladesh, the death rate in the total infected COVID-19 cases was 50.2% among elderly patients of over 60-years (28). The meta-analysis study of Biswas et al., demonstrated that COVID-19patients with age ≥50 years had a significant escalated risk of mortality compared to cases <50 years (4). A previous study showed that COVID-19 patients aged ⩾60 years demonstrated disease severity and long-term disease course compared to cases <60 years (31).In China, the case-fatality profile among COVID-19 patients of different ages was in the following order: people>80 years> 70-79 years > 60-69 years > 50-59 years > below 50 years (32).Likewise in Italy, the highest case-fatality rate was observed among COVID-19 patients of 81-90 years (33). In England and Wales the highest deaths of COVID-19 patients were among patients aged ≥65 years (34).

The current study reveals a significant correlation (P<0.05) between the duration time and the clinical presentations of COVID-19 disease. A previous study stated that the real time -PCR test positivity and duration of the symptom duration associated significantly with initial viral load (35). The elevation of viral load is very possibly to impose overburden to body's immune response accompanied by severe illness (36). The study conducted by Wenyu et al., revealed negative significant correlation between the viral load of COVID-19 and the lymphocyte count and distinct positive correlation between the viral load and the neutrophil count and the CRP.Obviously, the correlation between the viral load of COVID-19 and the illness severity is still not fully understood. This would oblige the indispensable need to conduct further studies for further exploration and unveiling the molecular mechanisms behind this confounder.

Previous research revealed that the CRP level is positively correlatedwith the diameter of lesions existed in the lung with likelihood of reflection of illness severity among COVID-19 patients (37). It was reported that the elevationof neutrophil, CRP, D-dimer, and LDH might point out the progression of COVID-19 and the reduction in lymphocytecount (23). Additionally, Wenyu et al. discovered a positive linear correlation between the high viral load and the high CRP level (36).

Reportedly, D-dimer elevates in COVID-19 cases, associates with illness severity, and is trustworthy prognostic marker for mortality rate. Our finding revealed a significant difference (P<0.001)among the two stratified enrolled groups in the cohort study: well discharged group and ICU admitted /died group. Likewise, Yao et al., inferred that average D-dimer level in non-survivors was significantly higher than in survivors (38). Other previous study conferred that D-dimer >1 µg/ml is considered one of factors imposing risks for the death among adult COVID-19 inpatients on admission (39.Yu et al., concludedthat acute cases of COVID-19 display a higher level of D-dimer than those with non-acute clinical presentations, and D-dimer > than 0.5 µg/ml is correlated with acuteCOVID-19 infection (40). A recent meta-analysis and meta-regression study has revealed that D-dimer could forecast acute and fatal consequences in patients with COVID-19 with mild sensitivity and specificity, and diagnose venous thromboembolism (VTE) with high sensitivity but low specificity (41). Conversely, previous studies revealed coagulopathy and D-dimer elevations in 3.75–68.0% of the COVID-19 inpatients and outpatients (42,43). Reportedly, the elevation of D-Dimer is restricted not only to the viral infection like COVID-19 but also to any pathologic or non-pathologic process that would raise the production of fibrin (44). Moreover, some studies verified the non-usage of D-Dimer in the context of biomarker for viral pneumonia (45).A retrospective study conducted by Yao et al, defined cut off value >2.14 mg/ml of D-dimer upon admission forecastingmortality in inpatients with 88.2% and 71.3% of sensitivity and specificity, respectively (38).

Our finding revealed a significant increase in the level of LDH in the ICU admitted /died group compared to the well discharged group. LDH rise in COVID19 patientsevidences injuries in lung and tissue and is associated with poor diagnosis at a level >250 U/L (46). Some studies demonstrating that LDH is not correlated with poor prognosis (46). However, a systematic review and meta-analysis study including 10399 from 21 studies conferred the association of LDH with poor diagnosis in COVID-19 patients (47). Our finding showed a positive significant correlation between the elevation of LDH and the age. However, Martha et al., revealed no association between the elevation of LDH and the age (47). The heterogeneity could be attributed to various cut-off points, lab references and tools employed in diagnosis.

Our finding revealed that the level of ferritin is significantly higher in ICU admitted/died COVID19 patients when compared to

the well discharged group. The magnitude of inflammation existed at admission of COVID-19 patients, demonstrated by high levels of ferritin, is autonomously predictive of mortality in COVID19 inpatients (48). Vargas-Vargas and Cortés-Rojocited (49) and Bianchini et al(50) recommended the usage of the serum ferritin as one of the indicators of death in patients with COVID-19.

The association between IL-6 and iron metabolism is thoroughly known (51), however, iron parameters are not yet regarded a key biomarker to examine septic evolution. In majority of recent standards for COVID-19 (52) or in studies concerning sepsis (53), there is no reference of ferritin or other iron parameters. Whilst, in a late prospective study (54), iron parameters like ferritin and transferrin saturation levels have correlated significantly (P=0.043 and 0.034, respectively) with SOFA score (Sequential Organ Failure Assessment).

In COVID19 hospitalized inpatients for acute abdomen or other surgical pathology, the acute inflammatory process has correlated with the key parameters but not with ferritin alteration. Conversely, in COVID patients, iron alteration looks to happen instantly (55). A recent meta-analysis study (56) showed that ferritin were regarded in 4out of 16 studies analyzed, nonetheless, ferritin levels could be used in stratification of COVID19 illness's severity. This indicates the reality that a very little bit studies regard iron metabolism in COVID and non-COVID patients at the time being.

Our study considered no significant difference in IL-6 levels between the two stratified enrolled groups: well discharged group and the ICU admitted /died group. The concentration of IL-6 > 24 pg/mL at beginning of evaluation did predict the progress of hypoxemia necessitating hospitalization with satisfactory sensitivity and specificity. IL-6 looks as a crucial predictor for the progress of the acute Covid-19 and couldassist for primary identification of patients who are in a great need of hospitalization (57).A vitalattribute of IL-6 up-regulation in Covid19 is that it leads the progress of severe lung injury implicating its usage as an initial biomarker of acute disease (58). Predominant hypothesisor speculation is that overexpression of IL-6 would play a key role in the stimulation and development of the alleged cytokine storm resulting in lung injury(59). It is assumed that IL-6 does increase the lung permeability of lung capillaries pushing the ARDS progress and furthermore does stimulate the pathway of coagulation imposingmicrothrombi in lung circulation and elevates the probability of risk of thrombotic event occurrence (60). The direct role of IL-6 in Covid-19 pathogenesis is verified by discoveries that IL-6 inhibition does improve the prognosis of acute Covid-19 (60,61).

Our finding revealed that there is no significant difference in the level of TGF-β among the two enrolled groups in this study: discharged well group and ICU admitted /died group. A recent study conducted by Ferreira-Gomes would suggest that in acute COVID-19 cases, SARS-CoV-2 would trigger a chronic immune reaction; instructed by TGF-β, and is distracted from itself (62).TGF-β, transforming growth factor -β ,is also recognized as an outstanding immune regulator (63), and it does promote fibrosis (64), a comorbidity of acute COVID-19 cases (65). Hence, therapeutic targeting of TGF-β could be a solution to improveacute COVID-19, particularly, when putting into considerationthe fibrosis-inducing capacity of TGF-β (66). The study of Shen et al., developed a practical model to identify the characteristics of cytokines storm, a signature remark in febrile and covid-19 diseases (67). This model is mainlyappropriate for recognizing febrile and infectious diseases such asCOVID-19. According to this model, characteristics of cytokine storm and pathogenesis of COVID-19 have been postulated to be a result of the disequilibrium in the cytokine network that occur as a consequence of the elevated biological activity of TGF-β. This would definitely impose distinct clinical presentations in the form of fever, fatigue, pneumonia, dry cough, and missing of olfactory in some cases. Research and clarification of the pathogenesis of COVID-19 will share to precision treatment.

CONCLUSIONS

In a sample of Iraqi COVID-19 patients (n=100), elderly people (≥59 years) was more susceptible for poor prognosis. Gender did not impose any significant consequence on disease outcome. The four serum biomarkers D-dimer, LDH, CRP, and ferritin might be used as indicative for poor COVID-19 disease outcome as they exhibited significant higher levels among required ICU admitted/died patients compared to their levels in the well-discharged patients. For further unveiling the molecular mechanisms behind the role of the aforementioned biomarkers in COVID-19 disease outcome, a large retrospective study would be recommended to be conducted in Iraq from the date of Coronavirus emergence till now.

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