

How to Cite:

Dhumad, G. D., Kadhim, H. S., Shamran, H. A. ., Abed, H. D., & Sheat, M. M. (2022). Outlook of probable association of TGF- β 1, viral load, with levels of some serum biomarkers and their role in the severity of COVID-19 in a sample of Iraqi population. *International Journal of Health Sciences*, 6(S5), 5547–5564. <https://doi.org/10.53730/ijhs.v6nS5.9927>

Outlook of probable association of TGF- β 1, viral load, with levels of some serum biomarkers and their role in the severity of COVID-19 in a sample of Iraqi population

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Abstract--The COVID-19 pandemic remains a rapidly spreading public health emergency with grave consequences. The disease is caused by infection with the severe acute respiratory syndrome coronavirus-2. The current study cohort included an Iraqi population with RT-PCR positive COVID-19 who were divided into two clinical groups: those who were discharged home well (n = 77) and those who required ICU admission/died (n = 23). Here we study, the age and sex on COVID-19 illness with biomarkers: TGF-B, Viral level, LDH, Oxygen prerequisite D-dimer, and CRP, among COVID-19 patients. Data revealed no basic influence of sex introduction on the result. Whereas age of individuals was basically higher $P < 0.001$ in (required ICU admission /died compared to that of well –discharge patients (38.83±11.74 a long time)). Level of TGF- β 1 exposed no significant difference between the two enrolled groups link with viral load and

duration. Period conversely had a positive noteworthy relationship with (D-dimer, CRP, LDH. Besides, (D-dimer, LDH, and CRP) levels were on the whole elevated on essentially ICU/admission compared to well-discharged patients group. The middle illness duration in well-released patients were 7.0 days (3-18 days) compared with nine days (6-14 days) in patients required ICU/died with remarkable distinction. Moreover, the tremendous larger part of patients required ICU/died (95.65%) required O₂ versus only 13% of well-released patients with a highly significant difference. Moreover, more than one-quarter (26.07%) of patients required ICU/died were ex/current smokers compared with only 9.09% of discharged well patients who had such habit with a significant difference.

Keywords---SARS-CoV-2, TGF- β 1, Viral load, LDH, O₂ requirement, D-dimer.

Introduction

The World Health Organization (WHO) pronounced the serious intense respiratory disorder coronavirus 2 episode widespread because of always expanding of the cases exterior China on March 11, 2020.(1) SARS-CoV-2 infected Patients can create crown virus illness (COVID-19), brought about high rates of hospitalization and (ICU) entrance (2). Among hospitalized patients with COVID-19 in China, the rate of patients who required ICU care has shifted from 5% to 32% (3). One of the properties of SARS-CoV-2 which makes it spread so effectively, is the heterogeneity of human safe reactions it triggers from asymptomatic to deadly . Be that as it may, an inclination for extreme COVID-19 is less likely to be fair related to the measurements of pathogen or to a particular course of contamination, but or maybe to progressed age, recommending that wasteful safe reactions may play an urgent part(4) In extraordinary COVID-19, SARS-CoV-2 in this way triggers a ceaseless secure reaction possessed from itself and instructing by TGF- β . TGF- β cytokine may be plays a basic parcel in tissue repair after harmed. Signaling pathway of TGF- β 1 is overwhelmingly included inside the fibrotic lung pathogenesis, since it incites fibroblast development and partition. reportedly that TGF- β 1 activates pulmonary fibrosis by sanctioning two pathway (SMAD-dependent ,SMAD-independent pathways) ,growing collagen declaration and ECM . (5) The SARS-CoV-2 virus uses angiotensin-converting enzyme 2/membrane protease Serine2 (ACE-2/TMPRSS2) as an entry point to infect the host. [6].

Expansion to this fundamental pathway, (SARS-CoV-2) can infect human cells by the endosomal [7]. Later reports have embroiled the ACE-2-rich lipid pontoons dependable for this endocytic handle [8]. Lipid flatboats, sequentially, are upgraded by the Caveolin-1 protein, a protein included within the negative direction of TGF- β 1 throughout the internalization of TGF- β film receptors [9] Due to hypercytokinemia, macrophages, neutrophils, and T cells go on at the location of infection , causing lung damage [10]. IL-4 activates the elective activation of M2 macrophages, intercedes the capacities of T- helper 2 cells. This would discharge TGF- β 1, fortifying fibroblast development. (11)

The receptors of TGF- β type I and II (TGFRI and II) order some signaling cascades intracellular, counting non- canonical PI3K/AKT and the canonical SMAD2/3-4 pathways. The complex SMAD2/3-4 triggers by TGFRI that ties profibrotic and fibrogenic proteins expression incites as: α -SMA and Collagen I (develop), coming about in acceptance myofibroblast actuation and extracellular matrix (ECM) testimony. (5) Besides, The PI3K/AKT pathway increments the battle of fibroblasts and multiplication of myofibroblasts. more to the point, once the complex of the CD44v6/HA is created, (PI3K/AKT pathway) leads to fibroblasts' resistance to apoptosis. So CD44v6/HA interaction increases production of(MMP-9), that leads to the performance of TGF- β 1 (Figure 1.1) (12,5) .

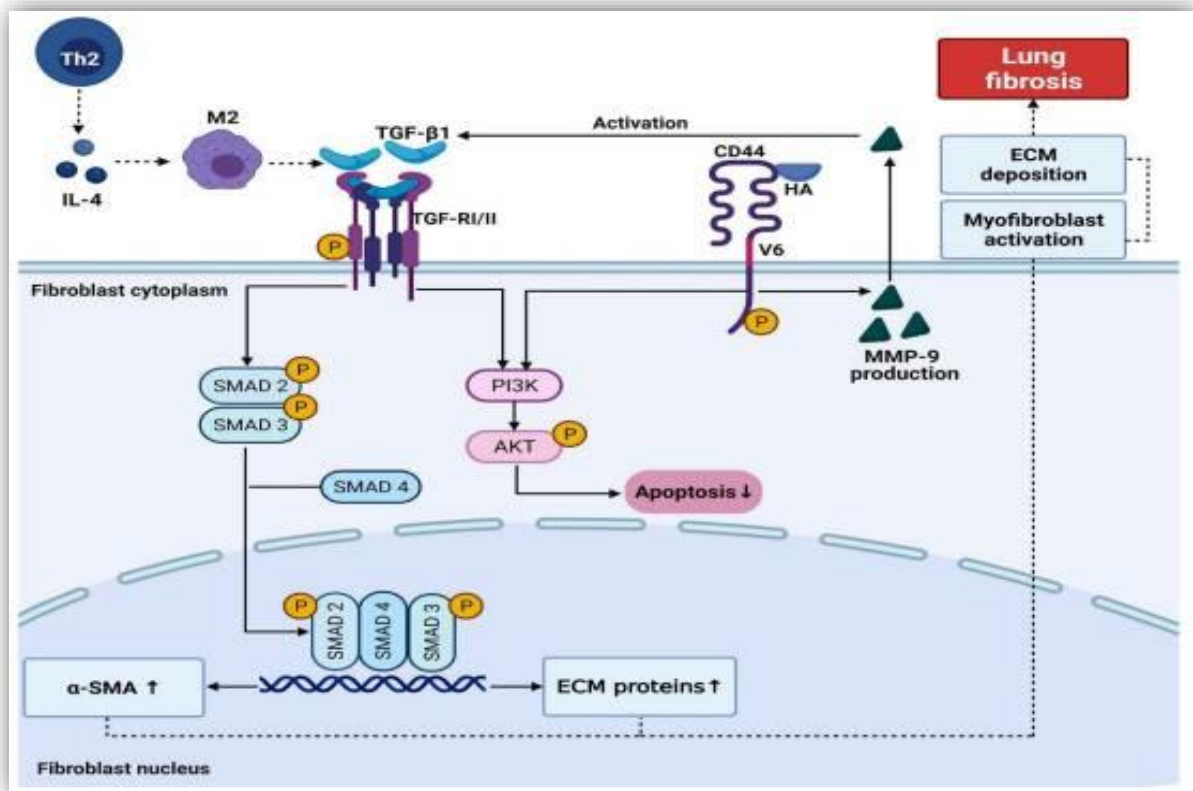


Figure 1.1 :- TGF-B 1 some signaling cascades intracellular (5)

A plasmin cleavage item d-dimer the item of -fibrin corruption), and plays the unthinking part in thrombo-inflammation in COVID-19. A few thinks about have related hoisted D-dimer with expanded seriousness and antagonistic results of COVID-19 (13). Other than considers have detailed raised standard D-dimer levels in patients hospitalized for direct to extreme coronavirus malady 2019 (COVID-19). These lifted standard D-dimer levels have been related with illness seriousness and mortality in review cohorts (14).

An intracellular chemical called Lactate dehydrogenase, does catalyze alter the pyruvate to lactate and NADH , NAD⁺. It present in every tissue, When these tissues are harmed, they discharge LDH into the circulatory system or other body

liquids. On the off chance that your LDH blood or fluid levels are high, It can expose certain tissues in your body to severe damage by disease or harm [15]. The level of the enzyme is expected to rise as long as it is present in the lung tissue.

Also, in thrombotic microangiopathy connected with renal disappointment and cardiac harm, LDH grade are high (16,,17). The ordinary present of CRP in blood, is below than 10 mg/L; in any case, it rises quickly inside 6 - 8 hours and give the most noteworthy top in (48 hours) from the illness onset. 7 Clinical thinks about illustrated that changed levels of a few blood markers could be connected with the degree of severity and mortality of patients with COVID-19. CRP could be a sort of protein delivered by the liver that serves as an early marker of disease and aggravation. (18,19) The point of current consideration is to assess the coherence of TGF-B1 and viral load with levels of several biomarkers such as (CRP, LDH, D-dimer) and their role in the severity of COVID-19 disease in Iraq.

2. Methodology and Material

2.1 Protocols and Study cohort

Study was conducted from February 2021 to May 2021 . All subjects were with a duration of diagnosed covid 19 for at least 5 day. The age range of all patients were from 21 year to 81 year. All enrolled patients were attending the AL-Kindy teaching hospital/Baghdad and Ibn Al-Qiph Patient selected for this study 100 patients infected with Covid 19 participated and were with covid 19 and categorized into two bunches the first group who were cured and discharged recovered.As for the second group, it included those who died as a result of the severity of the disease, as well as patients who were entered to the ICU .

All persons examined to confirm that they were infected with SARS-COV2 by investigate the swab of nasopharyngeal using RT-PCR system .The history of comorbidity was operationally based on rescored of specialist . All dimorphic data as age ,sex, smoking ,comorbidites were extricated from patient's drum record and specialist and employing information collection frame, The information was collected using questioner prepared by us, All members marked the moral assent established the Al-Nahrain University Institutional Review Board (IRB) granted consent for this study

2.3 blood sampling

About 3 ml of blood was drawn from every patient in a sterile plane tube. Blood test was centrifuged at “4,000” rpm for 15 min after (15 minute) within the course of clotting .The resulting serum was kept at (-20 °C) until being dealt with.

2.4 Estimation of immunological and biochemical serum level

Levels of TGF- B1 in serum tests were estimated utilizing Sandwich -ELISA strategy utilizing Horseradish Peroxidase (HRP)-conjugated counter acting agent particular for TGF-B1 ELISA was performed in Micro ELISA strip plate in line with

the informative of producer. The serum level of D-Dimer in tests assessed utilizing the procedure of (Antigen-Antibody reaction)as reviewed in (Poudel A et al., 2021). CRP and LDH, were measured in completely robotized analyzer called (Beckman Coulter Analyzers).

2.6 Statistically analysis

SPSS computer program 25.0 (SPSS, Chicago), statistical investigations were performed Persistent data subjected to ordinariness test (Shapiro Wilk test). Data with customarily scattering were shown as cruel and standard deviation, and analyzed with Understudy t-test. Information with(non-normal distribution) displayed as median and rang analyzed with the Mann-Whitney-U test. Categorical factors communicated as number and rate analyzed with chi-square test. Pearson's relationship test was utilized to investigate the conceivable relationship of distinctive markers with duration seriousness of the COVID-19 and viral load was assessed through the calculation of proportion (OR) and their comparing 95% certainty interims (CI) utilizing twofold calculated relapse. A p-value less than (0.05) was considered to show significant deference.

3. Results

3.1 Patients Outcome

After 30-day follow up, 77 patients (77%) discharged home well, 23 patients (23%) required ICU admission/died (Figure 3-1).

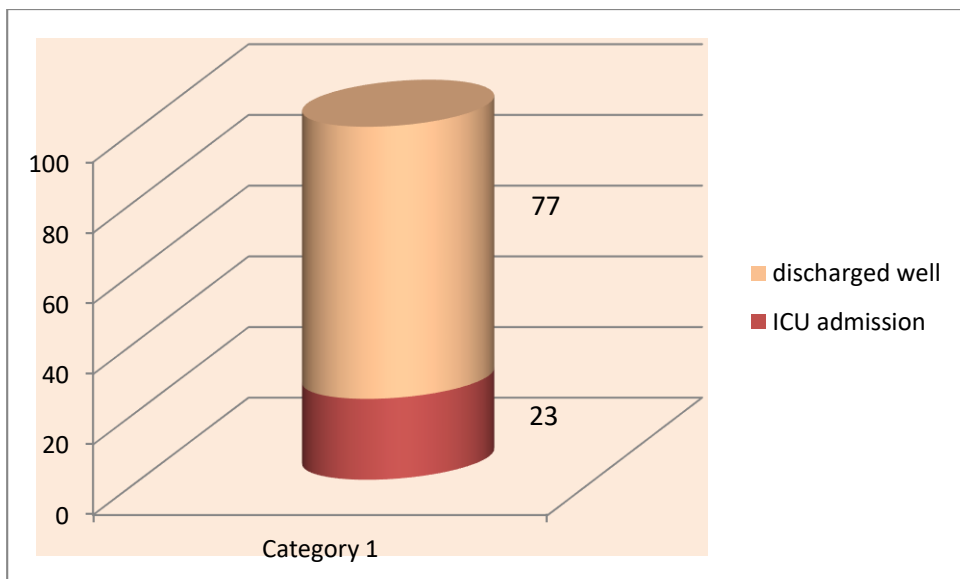


Figure 3-1: The result of the patients with the SARS-COV2 disease

3.2 Relations of the Statistic Characteristics with the infection Result.

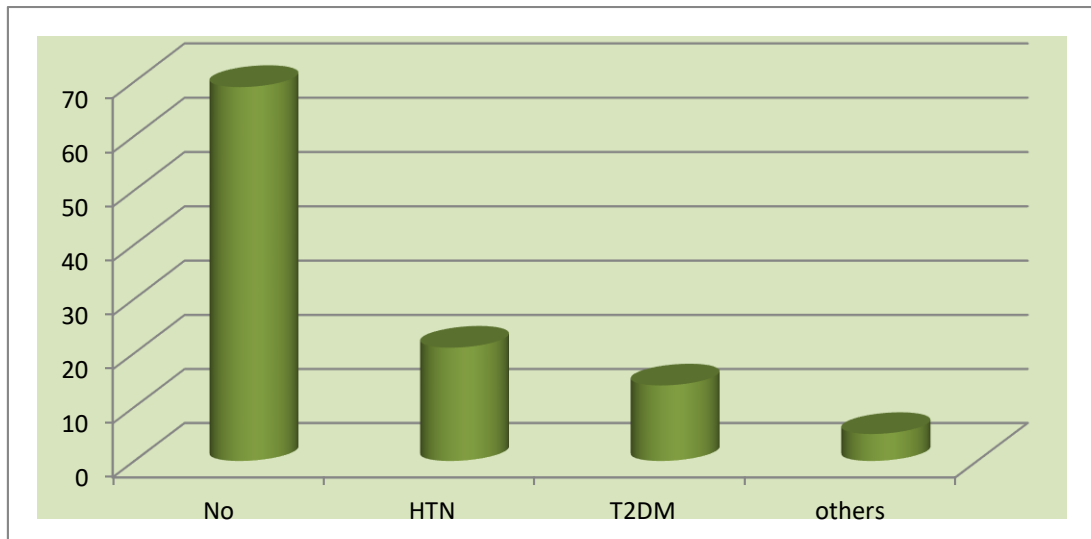
The mean age of the recovering patients was 38.83 ± 11.74 years which was lesser than that of patients who need ICU/died (59.0 ± 12.67 years) with a highly

significant difference. More than one-quarter (26.07%) of patients required ICU/died were ex/current smokers compared with only 9.09% of discharged well patients who had such habit with a significant difference. The frequency of HTN and diabetes in patients required ICU/died was 47.83% and 39.13%, respectively compared with 13% and 6.49% in discharged well patients with highly-significant differences (Table: 3.1 and Figure: 3.2).

Table (3.1) the association of the Statistic Characteristics with infection Result and demographic data

Variables	Need ICU admission/died (n=23)	Discharged well (n=77)	p-value
Comorbidities			
No	6(26.09%)	63(81.82%)	<0.001*
HTN	11(47.83%)	10(13%)	0.001
T2DM	9(39.13%)	5(6.49%)	<0.001
Others	2(8.7%)	3(3.9%)	0.324
Smoking			
Never	17(73.91%)	70(90.91%)	0.033*
Ex/current	6(26.07%)	7(9.09%)	
Gender			
Male	17(73.91%)	46(59.74%)	0.217*
Female	6(26.09%)	31(40.26%)	
Age, years			
Mean±SD	59.0±12.67	38.83±11.74	<0.001†

Diabetes mellitus (DM), hypertension(HTN) *Chi square test / The exact Fisher test, † Student t – test



DM: diabetes mellitus, HTN: hypertension

Figure: 3.2 most common disease statistic in covid-19 patient.

3.3 Patients Clinical Characteristics

Disease period ranged from 3-18 day with a mean of (8.22±3.06) days. More than two-third (68%) of the patients no required O₂ ventilation throughout hospitalization. Viral load ranged from 0-43×10⁶/ml, usually, below 50% in all cases with a mean of 28.66±7.87. (Table :-3-2).

Table (3.2) Clinical Characteristics of the Patients : n=100

Clinical variables	Patients (number=100)
Disease duration, days	
Mean±SD	8.22±3.06
Median	7.0
Range	3-18
O₂ Requirement	
No	68(68%)
Yes	32(32%)
Viral load, × 10⁶ / ml	
Mean±SD	28.66±7.87
Range	0.0-43

3.4 Association of Clinical Characteristics with disease Outcome

The median disease duration in well- discharged patients was 7.0 days (3-18 days) compared with 9 days (6-14 days) in patients required ICU/died with a significant difference. Furthermore, the vast majority of patients required ICU/died (95.65%) required O₂ versus Only 13% of well- discharged patients with a highly significant difference (Table 3.3)

Table (3.3) Number of Patients' clinical characteristics (n=100).

Variables	Discharged well (n=77)	Need ICU admission/died (n=23)	p-value
Disease duration, days			
Median	7.0	9.0	0.003
Range	3.0-18.0	6.0-14.0	
O₂ Requirement			<0.001
No	67(87%)	1(4.35%)	
Yes	10(13%)	22(95.65%)	
Viral load, ×10⁶/ml			0.065
Mean±SD	29.5±8.05	25.92±6.69	

3.5 Assotiation between Duration and Viral level with some Markers

Relationship called Spearman's test was utilized to investigate the conceivable relationship of age viral level with diverse markers. Viral level illustrated noteworthy relationship with CRP (r= -0.300; p= 0.003). TGF beta 1 appeared

noteworthy association with viral level ($r = -0.262$; $p = 0.045$). At long last, time had a positive noteworthy relationship with each of CRP ($r = 0.349$; $p < 0.001$) -D-dimer ($r = 0.319$; $p = 0.002$), -LDH ($r = 0.265$; $p = 0.025$) Table (3.4).

Table:- (3. 4) Some markers show correlation with viral level and duration.

Variable	Durationtime		Viral level	
	r	p-value	r	p-value
TGF- β 1	- 0.054	0.622	- 0.262	0.045
L.D.H	0.265	0.025	- 0.255	0.031
C.R.P	0.349	< 0.001	- 0.300	0.003
D-dimer	0.319	0.002	0.165	0.104

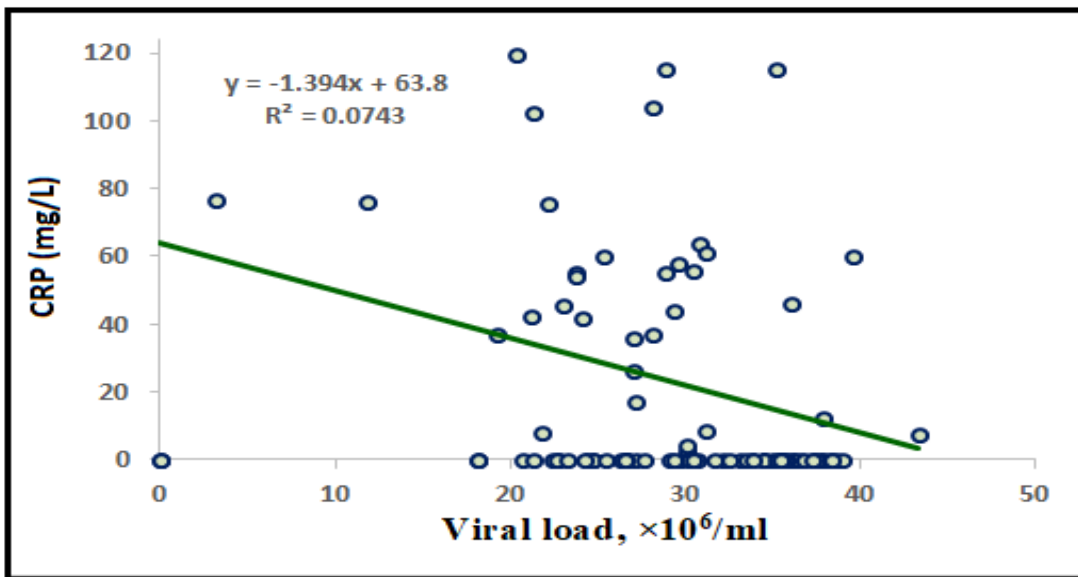


Figure (3.3): Scramble plot and regression line among viral load with CRP

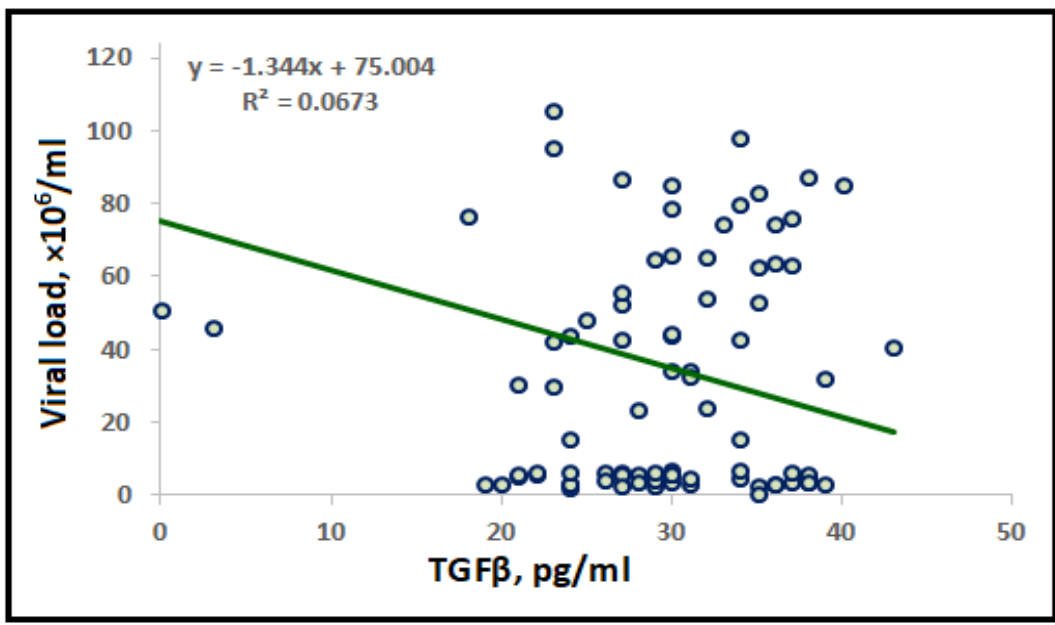


Figure (3.4): Scramble plot and regression line among TGF-β1 with the viral load

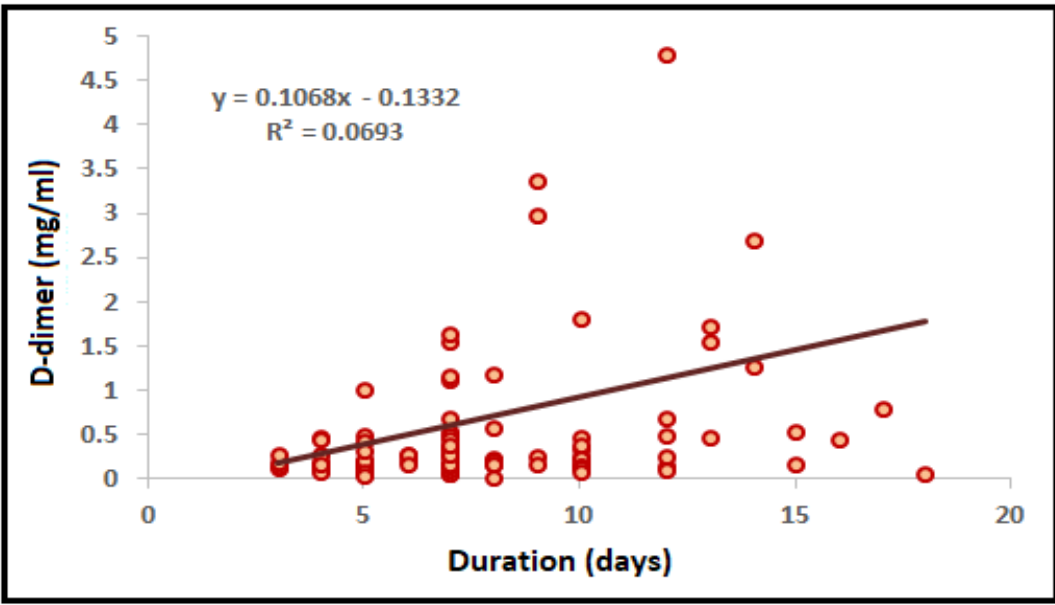


Figure (3-5): Scramble plot and regression line among duration and D-dimer

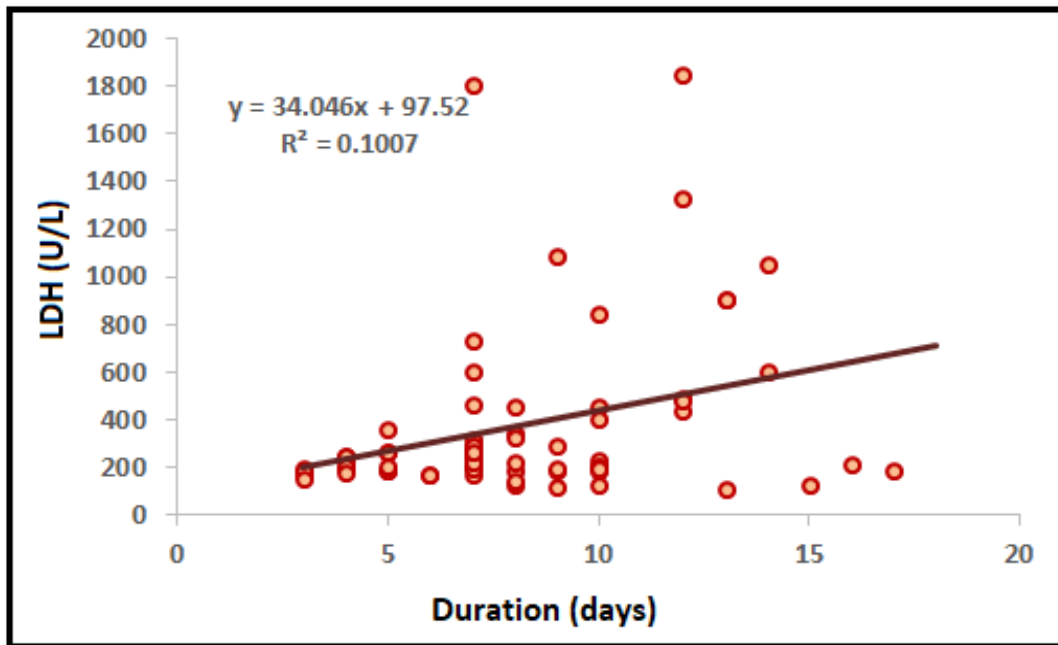


Figure (3-6): Scramble plot and regression line among duration with LDH

4. Discussion

In show disdain toward of The larger part of patients were male , our data revealed no basic influence ($P > 0.05$) of sex with respect to the result of COVID-19 infection. The writing of audit appeared conflicting discoveries with respect to the impact of sex on COVID-19 result. For illustration, previous study in china gathered a death rate 1.7% and 2.8% between the women and male group patients, separately [21]. similar study previously conducted on Chinese revealed low death rate of ladies (0.4%) compare with men rate 7.2% [22]. Precedent analysis study conceded on 5057 SARS-COV2 infected patients, exposed the increase death range between the hospitalized male bunch (23). Study of Biswas et al, on 64,676 crown virus infection -cases , showed male patients were altogether connected to lifted chance of death compare with women with significant deference (P-value < 0.00001) (24). Reliable information , Al-Bari and co-worker, deduced non significant deference in mortality between Bangladeshi patients men and woman who infected with SARS-COV2 (25).

Considering the age of COVID-19 disease, the current show that the hazardous gather tainted with COVID-19 a high passing rate included patients with of 59.0 ± 12.67 . While, the foremost influenced bunch incorporate patients maturing 38 a long time. previous study in Iraq appeared halfway comparative comes about to the show finding where the age categories of 30-39 and 60-69 a long time were the foremost influenced and the most noteworthy passing rate bunches, separately (26). Moreover, Saeed et al., refer to that the foremost influenced bunch of COVID-19 patients experienced from (30 to 39) a long time (27). In USA, the cure rate among the bunch patients of gently malady and youthful ages is less

unmistakable. Besides, the seriousness of COVID-19 was incredibly diminished among patients who less than 17 a long time ago especially out hospitalized patients (28). (Bis was et.al.) study, illustrated that COVID-19-patients at age less than or equal to 50 a long time ago had a noteworthy raised chance of death when compared with cases with age (< 50) years (29). Similarly Italian study, show most notable death rate among COVID-19 patients was watched between the great age individuals from eighty one to ninety a long time ago 42.7% taken after by individuals who was 71-80 a long time ago (35.6%) (30). This consider show that most of the infected persons are from the age of 38 a long time ago and they are the foremost defenseless gather to contamination by ideals of being the dynamic individual in a society whereas working in different areas. On the other hand, the foremost likely to kick the bucket was those matured 59 and over, which is the starting of incredible age with which a person's resistance starts to decrease and he is uncovered to comorbidities.

With respect comorbidities with other infections among COVID-19 patients, the show finding deduces the high frequency of comorbidity between ICU admitted/died patients when compared to released well patients. Previous study conducted in the state Iraq, illustrated that diabetes, asthma, and serious weight were the foremost predominant comorbidities in association to COVID-19 attack rate (31). In a past study conducted in Bahrain from May 2, 2020 to July 31, 2020, it was concluded that the foremost common comorbidities were relegated hypertension (29.61%), DM (27.9 1%), and G6PD lack (11.64%) (32). Decreased immunological work may be connected to comorbidity. As diabetes patients' common resistant work is altogether lessened, which may restrain the body's capacity to make fitting antibodies against any ailment [33]. Study conducted in China in 2020 appeared that the comorbidities among COVID-19 patients were as take after: diabetes (20%), hypertension 15%, and cardiovascular maladies (15%) (34). Comorbidities of other restorative conditions with COVID-19 were taken note as take after the chronic lung illness (34.6%), diabetes mellitus (28.3%), and cardiovascular diseases 27.8% (35).

Our finding appeared infinite, lion's share of patients required ICU/died (95.65%) required O₂ versus Only 13% of well-discharged patients with exceedingly noteworthy contrast. Numerous COVID-19 individuals have low rate of oxygen carried on blood, even when they feel healthy. low blood oxygen may be an early sign requires health care. Oxygen level known briefly as SpO₂. A sound person with typical lungs, will have a blood vessel oxygen immersion is more often than not 95% or higher. A few individuals with inveterate lung illness or breathing stop while resting may have typical range about 90%. The "SpO₂" perusing on a beat oximeter appears the level of O₂ in person's blood. (36,37) Another uncertain angle, which report influence SARS2 defilement on prosperity and insight of RBCs of these persons. Early on modeling considers by (38), expected the spike S1 protein can bind with Hb to decrease both oxygen fondness and add up to Hb substance, but the work was along these lines tended to due to a require of exploratory back (39).

The fundamental realization of cellular oxygen accessibility beneath COVID-19 conditions isn't completely caught on and is or maybe questionable. In order to oxygen get to cells and eventually to mitochondria for the oxidative digestion

system its require ordinary oxygen transport capacity as decided through Hb rate , and its capacity to discharge oxygen in to tissue (40) . By as it were a number of examinations so distant, both oxygen transport ability as well read blood cell astuteness, are influenced beneath corona virus-19 Hence, the exhibit establish no critical affect of viral level on oxygen prerequisites between two COVID-19 patients group . Liu et al., prove patients showing serious side effects illustrated viral stack that was 60 crease higher than viral level of patients showing gentle side effects (41). At most, the impact of COVID-19 viral level on infection seriousness is broadly challenged. the think conducted in Bahrain by Abdulrahman et al found the nonattendance of noteworthy affiliation ($P>0.05$) between malady seriousness as demonstrate by oxygen necessities, and viral stack, as prove by cycle threshold (Ct)which don by real-time PCR (42).Conversely, systematically review eight from fourteen studies established the presence of significant relation between the level of the virus and severity of the disease in terms of oxygen requirements (43). Additionally, another past study verified the nonappearance of the subordinate relation between the passing rate (mortality) and the rise viral level (44). When seen within the setting of infection screening and time since indication begin, the truth that people who displayed more truly on affirmation had lower cycle threshold levels than those who displayed less extremely may make more sense (42).Hence, viral shedding may take place over a long time, imposing higher cycle threshold values in patients upon hospital admission when compared to outpatients who had only recently infected (42). The study of Singanayagam et al., had detailed the relationship between time since indications starting and most elevated cycle threshold levels (45). Separately, a think about of 205 people found reverse relationship between sickness seriousness and the viral load, which they guessed might be due to the length of time since contamination started (46). As a result, whereas assessing the relationship between viral level and malady severity on confirmation, the period since side effect begin may be a key confounder. In any case, such a conclusion cannot be drawn with certainty without stratifying the two bunches and bookkeeping for the predisposition and mistake in test collecting. While the time since sign began may jumble the affiliation among the level of the virus and ailment severity, additionally conceivable that there is no essential association (42).

With respect to the length time of the problem with clinical introductions, the current uncovered that there was a noteworthy affiliation ($P<0.05$) with the out come . In past study, it was concluded that in decently debilitated COVID-19 outpatients, genuine time -PCR tests held on positive along 49 days(47). Additionally, the test inspiration and term of the indication term related essentially with the introductory viral stack (47). The height of viral stack is exceptionally conceivably to be the vital figure forcing overburden to body's resistant reaction that would be go with movement into extreme sickness (48). Within the line of the aforementioned information, it is apparent that the relationship between the viral level of COVID-19 and the ailment seriousness is still not completely caught on. This would require crucial got to carry out advance thinks to support investigation and divulging the atomic instruments behind this confounder.

As for the immune regulator TGF- β , the present exposed no significant variation in level of TGF- β between two encompassed groups in this current . Recent study carry out by Ferreira-G has been suggested that in acute COVID-19 cases, SARS-CoV-2 would activate a chronic immune response; which instructed by TGF- β , and is unfocused from itself (49). Besides transforming growth factor - β , documented as an exceptional immune controller (50), it does support fibrosis (51), and comorbidity acute cases (52). So, curative target the TGF- β could be a key to improve acute disease . particularly, if putting into concern the ability to causes fibroses of TGF- β (53). Some evidence found that a number of cytokines, including interleukins IL-8, IL-1, IL-6, TNF,with (IFN) showed clear changes in COVID-19 patients, however, the hypercytokinemia was not clearly recognized because it is a complex network. few researcher have centered on the modify and portion of changing advancement factor- β (TGF- β) in the midst of COVID-19 infection

Shen et al., created a commonsense demonstrate to recognize the characteristics of hypercytokinemia a common sign in febrile and SARS-COV2 infection (54). It is mainly suitable model recognize the febrile and irresistible infections as COVID-19. (55) Agreeing to this show, characteristics of hypercytokinemia and pathogenesis of COVID-19 have been hypothesized to be a result of the disequilibrium within the cytokine organize that happen as a result of the lifted organic action of TGF- β . TGF- β can source fever because its endogenous pyrogen, but relatively powerless pyrogeny and commonly produce low fever only (55) That would absolutely enforce distinct clinical introduction within the shape of dry cough, fever, pneumonia, fatigue, and missing of olfactory in some cases. Investigate and clarification of the COVID-19 pathogenicity will contribute to exactness treatment. Diverse anti-TGF- β treatments could be found as possibilities within the treatment of COVID-19 with diminished mortality rate (54).

LDH is another serum demonstrative biomarker for disease with COVID-19. Lactate dehydrogenase (LDH) does catalyze the ultimate arrange of oxygen consuming glycolysis (56).The display revealed a critical increment within the level of LDH within the ICU conceded /kicked the bucket bunch in comparison to the well- discharged gather. LDH rise in COVID19 patients evidences wounds in lung and tissue (57). A pooled examination appeared that the hoisted LDH levels were related with a ~6-fold rise in chances of movement of intense ailment and a ~16-fold rise in odds of passing inCOVID-19 patients (58). A few ponders illustrating that LDH isn't connected with destitute forecast (57). Be that as it may, efficient survey plus meta-analysis consider including (10399) from 21 ponder awarded the association of LDH with destitute determination in COVID-19 persons (59). The current consider appeared a positive noteworthy relationship among the height of LDH and age. In any case, JW Martha plus co-worker uncovered no affiliation among the rise of- LDH with the age.

With regard to tobacco use, the present finding reflects a significances distinction (P- Value below 0.05) between the two enrolled groups of COVID-19 patients: well-discharged group and ICU-admitted / died group (table 3.1). amazingly though a few have guessed that high rates of smoker in China clarified a few of the morbidity in those patients, Caroline ,et al found smokers to be related to

expanded hazard of clinic affirmation or of the fundamental ailment; in reality, it indeed appeared defensive for healing center confirmation. In any case, this may be arti factual: patients with obscure smoking category had altogether higher danger of affirmation and of fundamental sickness. It is conceivable that information is excessively lost to current or previous smokers who might not care to reply this address; on the off chance that so, would constrict clear advantage of smoking. Few patients had a recorded history of smoking; separated examinations might not be conducted for this accumulate (5).

5. Conclusion

However, we found significant association between viral load and CRP ,LDH susceptibility to covid-19 in the current study sample population. The TGF B1 on the other hand, is close significant etiological factor in link to viral load ($P = 0.045$).but confirm no significances among enrolled group . However, TGF show no role in the severity . Gender did not force any critical result to sickness. The three serum biomarkers LDH, D-dimer and CRP, they displayed critical rise rate among required ICU-admitted/died patients in comparison to their rate within well-discharged patients. Our finding appeared endless-lion's share of patients required ICU/died (95.65%) required O2 versus Only 13% of well-released patients with exceedingly noteworthy contrast. To further reveal the molecular mechanisms behind the role of the abovementioned biomarkers in the severity of COVID-19 disease, it is recommended to conduct a large-scale study together with the study of the gene for TGF beta and quantitative study for viral load To find out the real role of all above in the severity of the disease .

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