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CTGF A Prognostic Bioindicator For COVID-19 Secondary Pulmonary Fibrosis And Hint For Survival

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Abstract

COVID-19 infection results in acute lung injury and has derailed the global social and economy. CTGF shows dramatic up-regulation in the areas of severe injury and inflammatory diseases. Its Quadra modular structure, enables to interact with multiple cell surface receptors, growth factors and matrix components which have been implicated in several cellular events that are crucial in wound healing and spectrum of development and progression of fibrosis. This study examined the usefulness of plasma CTGF levels in predicting disease severity in COVID-19 patients. Sixty-four patients with confirmed COVID-19 between January 2021 to October 2021 were enrolled and twenty healthy individuals also included in the study as control. Further patients were classified into subgroups based on their clinical severity. The CTGF concentrations were measured using an enzyme-linked immunosorbent assay. Significantly higher mean plasma CTGF levels were observed in severe and control cohorts compared to the control group. . Herein, we propose that CTGF is a promising biomarker in distinguishing COVID-19 patients to indicate disease severity, hint for pulmonary involvement and and survivability.

Keywords: CTGF, TGF-β, COVID-19, ARDS, HRCT

Introduction

Globally, the COVID-19 crisis has resulted in unprecedented damage to public health and economy. This outbreak has affected almost all the countries and each exhibited significant variation in morbidity and mortality(1). The clinical spectrum of infection appears to be mostly asymptomatic or with mild symptoms, whereas a small portion of infected cases develop severe illness, and some die tragically(2). Even after testing negative for COVID-19, a considerable proportion of discharged survivors have reported symptoms ranging from simple lethargy and body aches to most fatal lung fibrosis as sequelae. It has been reported that around 7%–8% of recovered patients from COVID-19, especially (who were critically ill) underwent critical illness during treatment, developed post-ARDS lung fibrosis(3). Moreover, the combined prevalence in admitted and non-hospitalized patients may be even more. Therefore, greater attention is being devoted now and necessitates the development of potential biomarkers for early prediction of patients who will develop this lethal sequelae.

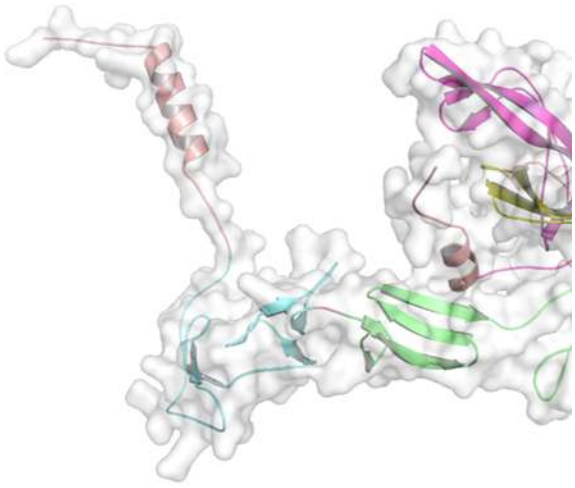
Although impressive progress has been made over the past few years in diagnostic technology, no biomarkers are currently available in clinical practice for pulmonary fibrosis(4). In this context, many studies suggest that plasma CTGF could be a

potential biomarker for diagnosis and long-term prognosis as its levels are markedly increased in various organ fibrosis, including lungs(5)(6). However, there are no solid ( ) studies examining whether it is useful in prediction of post ARDS pulmonary fibrosis and survival rate.

The aim of this study is to evaluate the prognostic significance of plasma CTGF levels in early discrimination (identification) of pulmonary fibrosis suspects among patients hospitalized for COVID-19 pneumonia. Also investigating its role as surrogate marker for survival in critically ill COVID-19 patients who are prone to fibrosis.

Background:

CTGF is a 38kDa heparin binding, cysteine-rich, multifunctional regulatory glycoprotein(7)(8). It has been reported as an autocrine downstream profibrotic mediator of transforming growth factor-β (TGF- β) for fibrogenesis in various organs(9). It consists of a distinct functional quadra module architecture that facilitates multiple biological actions through the interaction of cell surface receptors, cytokines, integrins (αIIβ3 in platelets and αMβ2 in monocytes), other extracellular matrix proteins, and growth factors(7). Interaction with such molecules has been shown to be dependent on the different domains of CTGF. These interactions are widely regarded as the key cell signalling events in tissue remodelling and fibrogenesis(10).



Domain-1 (Cyan) at N- terminal which has shown to be homologous to IGF-1 (Insulin-like growth factor) Binding Protein followed by domain-2 VWF-C/CR (pale green), von Willebrand factor type C/chordin-like cysteine-rich domain binds to TGFβ and helps in augmenting profibrotic activity. The hinge region presents between the modules II, and III are susceptible cleavage site for proteinases. Domain-3 TSP1, (yellow), which is homologous to thrombospondin 1 repeat can bind to low-density lipoprotein receptor-related protein-1 (LRP-1). CT, carboxy terminal domain (magenta) which contains a cysteine knot motif and has a binding affinity towards heparan binding sulphate proteoglycans and has an important role in proliferative activity through Ras/MEK/ERK signalling.

It was reported that CTGF was noted to have diverse functions depending on cell types. However, precise molecular mechanism is still unclear.

Materials and methods:

Study population:

Sixty-four patients who had been diagnosed with COVID-19 and all hospitalised at COVID unit Apollo Hospitals jubilee hills, Hyderabad for treatment were enrolled. All patients underwent HRCT examination of the lungs after admission. We grouped patients according to the degree of clinical severity (Mild pneumonia-20, moderate pneumonia-10, severe pneumonia-13 and critical-20) and this evaluation relies majorly on the chest HRCT features. Of these, patients with critical and severe pneumonia required admission into intensive care unit out of which 20 needs Non-Invasive ventilation and eight required mechanical ventilation. During follow up fifteen patients (Nine, Four, and two from critical illness, severe and moderate COVID-19 cohort respectively) developed post ARDS pulmonary fibrosis. Eleven patients were demised succumbed during follow up and all belongs to severe and critical cohorts and all developed pulmonary fibrosis during follow up.

All
these patients had varying degrees of pulmonary involvement observed by HRCT examination. Clinical characteristics of post-COVID-19 pulmonary fibrosis assessed between the survived and deceased patients. An additional cohort of 20 healthy individuals of equal sex ratio (Age-25±5 years) with no history of COVID-19 symptoms were included as control group. This group members were RTPCR negative upon enrolment in the study.

Volume 7, Issue 2; March-April 2024; Page No 191-198

Whole blood samples were collected from all patients before the commencement of any drug therapy and healthy cohort into a EDTA coated vacutainers on the day of admission. Plasma was separated by centrifugation at 3000 RPM for 30 min at 4°C and Plasma aliquots were stored frozen at -80°C until assay. CTGF values were ascertained by using solid phase sandwich ELISA method with human CTGF ELISA kit (R&D systems, Human CTGF: cat DY9190-05) in accordance with the instructions provided by the manufacturer. the assay range for CTGF was 31.3 to 2000pg/ml. Assay was validated for accuracy and precision and performed in duplicates and the mean value was reported.

Patients who had a history of chronic obstructive pulmonary disease (COPD), chronic kidney disease, pulmonary fibrosis interstitial lung disease were excluded. All patients written informed consent was obtained to the study and the study protocol was approved by our local ethics committee (AHJ-ACD-033/01-21).

Chest CT scan

The chest CT images of the patients were undertaken in the supine position without intravenous contrast using 128 slice multidetector CT scanner (Philips Medical Solutions Netherland). The transverse images were reconstructed to 0.5 to 0.75mm. The CT scans were performed at the time of admission and further scans are carried out according to the treating physician which is mostly based on the patient condition after examined by the physician. The CT severity score (CT-SS) was calculated to differentiate clinical forms of COVID-19. The CT-SS was defined by dividing lung into 5 lobes. The score with the range of 0-5 assigned to each lobe based on parenchymal opacification. The total CT-SS was determined by summing up individual lobar scores and its theoretic range from 0-25.

Results:

After the analysis and examination of the data obtained from the patients at our hospital, the plasma concentrations of CTGF were found to be predominantly high in severe patients in the intensive care unit (ICU) supported with intubation and mechanical ventilation. Inversely, the CTGF concentrations were low in patients with mild-moderate patients with less severe respiratory impairments.

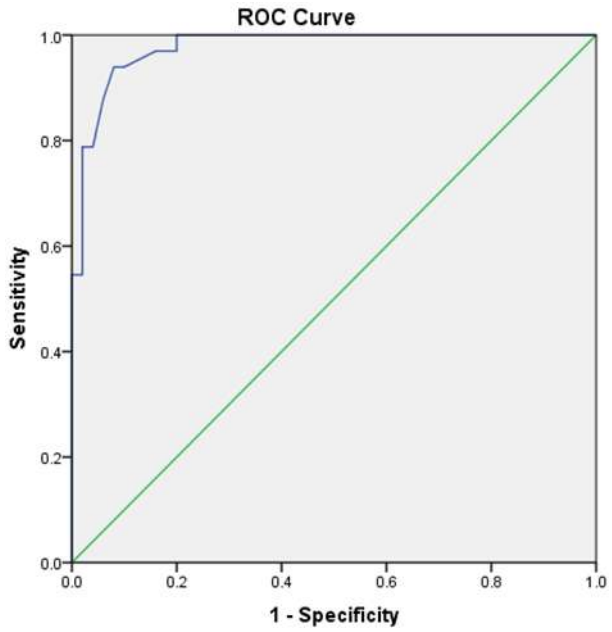
CTGF	Mean	SD	P value
Mild	75.31	2.32	
Moderate	84.88	2.14	
Severe	90.04	11.72	
Critical	126.60	49.13	
Control	75.31	2.32	<0.001

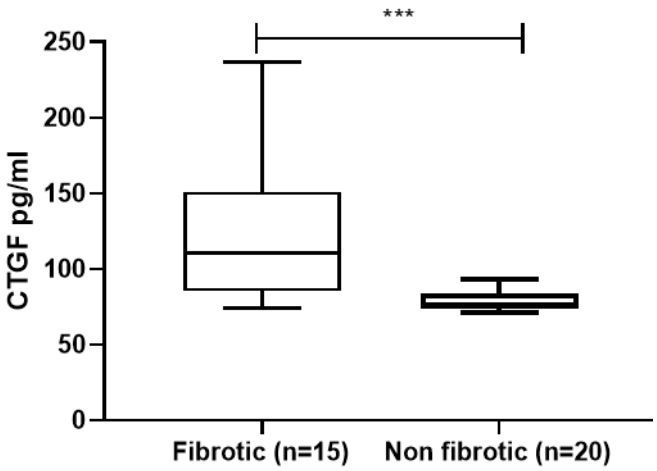
The current proposed study holds the potential in predicting increased mortality among COVID-19 patients through the CTGF as a biomarker.

The CTGF values were significantly high in critically ill COVID-19 patients with compared to every other cohort in this study.

ROC analysis showed that basal CTGF values of control group had good accuracy in discriminating patients based on HRCT features (AUC=0.976, P value=<0.001, Best cut off= 84.54, sensitivity=93.9, Specificity=92), Patients with high Plasma CTGF levels developed typical fibrotic features like traction bronchiactesis, interlobular septal thickenings, honey combing and distortion of lung architecture during follow up. Patients with lower CTGF levels shown no considerable pulmonary fibrotic features. Thus, CTGF levels indicating significant correlation with the progression of post ARDS pulmonary fibrosis. They may be a useful guide for clinicians in evaluating proportion of pulmonary involvement among patients.

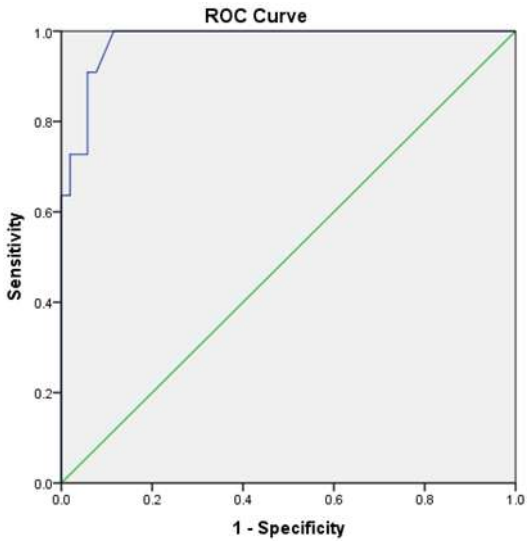
Volume 7, Issue 2; March-April 2024; Page No 191-198





Biomarkers which predict patient's survival can play a vital role in medical diagnosis and guide treatment decisions. In this study CTGF serves as predictor for disease severity, hint for pulmonary sequalae and patient survivability. Compare to other laboratory markers CTGF values among alive and dead patients strongly correlating with the criticality of the patient condition and helps in determining the patients with chances of less survival. ROC analysis showed that CTGF values of dead patients is markedly high compared to the patients survived from COVID-19 (AUC=0.979, p value=<0.001, best cut off=92.04, sensitivity=100, specificity=88.5).

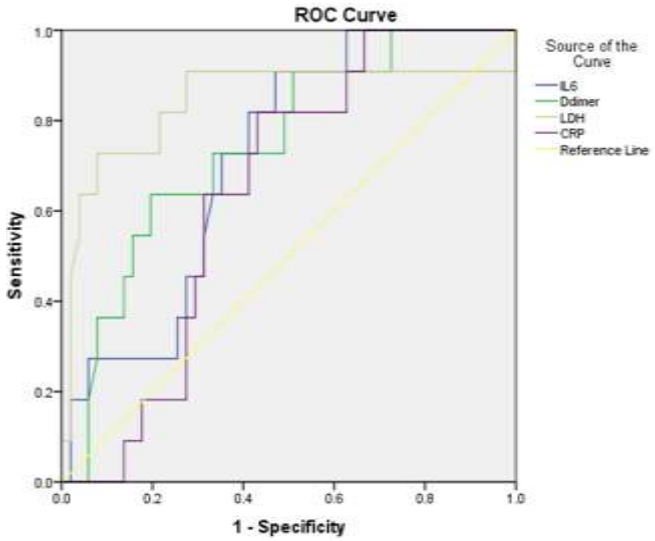
Volume 7, Issue 2; March-April 2024; Page No 191-198

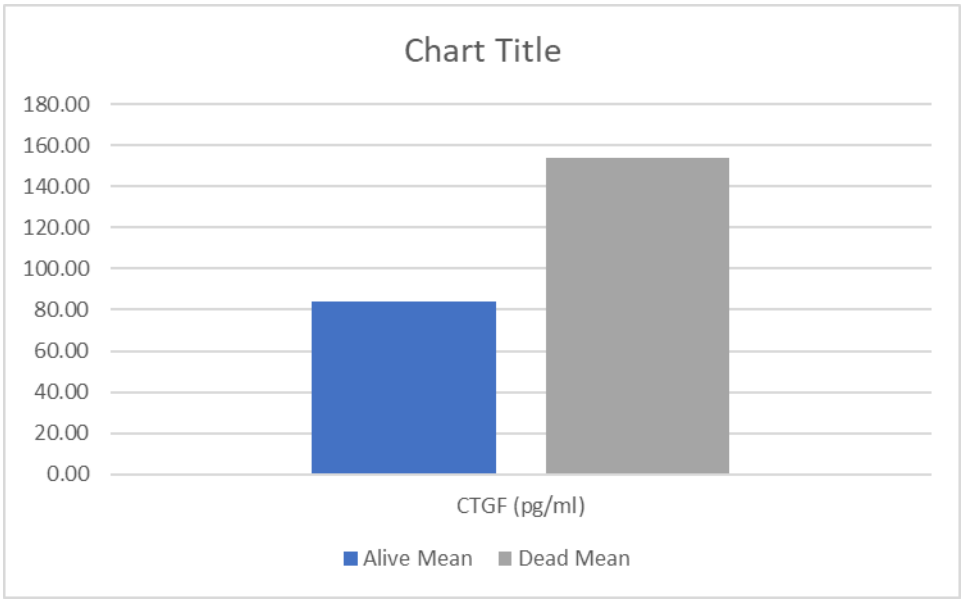


Parameters	Alive		Dead		t value	p value
	Mean	SD	Mean	SD		
CTGF	83.94	10.14	153.88	51.95	9.168	<0.001
IL6	45.23	42.21	72.63	44.37	1.938	0.057
D dimer	609.16	948.68	966.36	717.49	1.177	0.244
LDH	407.48	169.14	787.36	461.54	4.719	0.022
CRP	64.79	23.41	62.78	86.14	0.077	0.939

Parameters	**AUC**	**p value**	**Best cut off**	**sensitivity**	**specificity**
IL6	0.716	0.026	30.55	90.9	52.9
D-Dimer	0.744	0.012	711	63.6	80.4
LDH	0.844	<0.001	618.9	72.7	92.2
CRP	0.643	0.138	46.8	81.8	56.9

Volume 7, Issue 2; March-April 2024; Page No 191-198





Statistical analysis:

All results are expressed as mean ± standard error of mean (SEM) and analysed with the GraphPad Prism 5.0 software. All data were analysed by one-way or two-way ANOVA with multiple comparisons or Student t-test where appropriate. In all cases, $P < 0.05$ was considered statistically significant. $P < 0.05$(*), $P < 0.01$(**), $P < 0.001$(***).

The data will be analysed using SPSS version 24 software. the descriptive variables will be expressed as frequency and percentage. The continuous variables will be expressed as means and standard deviation.

Assess the degree of association in the qualitative variables chi square test will be used.

To compare the quantitative variables student t test will be used. Receiver operator curve analysis was done to estimate the best cut off, sensitivity and specificity

The differences have been considered as statistically significant when the p-value was < 0.05.

Discussion:

In recent years biomarkers have played a central role in disease detection, risk stratification and differential status of disease progression. In COVID-19 era fibrosis is a major aftermath of concern and chest CT

Volume 7, Issue 2; March-April 2024; Page No 191-198

findings had potential contribution in disease diagnosis and to assess its severity. However, it also associated with major drawbacks of exposing patients to radiation and disease transmission at radiology units by surface aerosol contamination. Since fibrosis is a highly dynamic and progressive disease. It is asymptomatic at early stages. Thus, accurate and validated biomarkers are highly desired to monitor clinical course of the disease and to advance clinical management timely.

In accordance with the pathobiology of fibrosis, we have chosen CTGF as it is reported as one of the main downstream mediators in the pathobiological process of fibrotic disease of various organ systems. We determined Plasma CTGF levels to evaluate its diagnostic value in differentiating patients with fibrotic lung damage.

In severe patients admitted to intensive care units who required intubation and mechanical ventilation, plasma concentrations of CTGF were observed considerably higher, in comparison with mild-moderate patients who had less severe respiratory impairment.

However, the strength of the current study lies in the fact that we have investigated prognostic role of CTGF first time in patients with COVID-19 of varying degree which were not mutually investigated before. Plasma concentrations of CTGF seemed to reflect lung involvement in COVID-19 patients as reflected by HRCT features

The clinical value of CTGF as bio-indicator should be explored further to assess the risk of ARDS pulmonary fibrosis and aid in monitoring the clinical course of post COVID-19 pulmonary fibrosis. On the other hand, our study identified some associations that deserve further consideration and may lead to improvements in the risk stratification, monitoring, and management of COVID-19 patients.

Limitations: There are few limitations in our current study should be reported. Namely, due to the small sample size, single centre study and CTGF concentration was determined at only one time point. Larger studies at multiple centres at more time points are required in a longer follow-up to validate our results.

Conclusion:

In the increased cases of post covid complications, the current study illustrates a relative to non-severe COVID-19, severe or critical COVID-19 characterization by increased CTGF expression. The data represents timely way to diagnose the susceptible fibrotic condition which might help in initiating early anti-fibrotic therapy in a considerate way for such patients. However, these data will be further evaluated in a longer follow-up to be confirmed. It needs to be validated demographically in diverse populations with fibrotic disease.

We suggest a close follow-up of recovered patients to find this sequela and an individualized treatment plan according to the degree of fibrosis. Early diagnosis and interventions may help in improving clinical outcomes for patients with pulmonary fibrosis. Increased plasma CTGF in hospitalized COVID-19 patients may help to in early discriminate identification of severe patients and predict those developing fibrotic lung sequelae.

Ethical statement:

The current study was reviewed and approved by the Institutional Review Boards of the local ethics committee of The Apollo hospitals Hyderabad jubilee hills approved this retrospective study and the need for patient's informed consent was waived. This study was conducted in compliance with the Declaration of Institutional Review board of Apollo hospitals.

Informed consent

All subjects gave their written informed consent to the study. All members of both groups were given detailed information concerning the research, and were enrolled following the receipt of written consent forms.

Conflict of interest:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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Volume 7, Issue 2; March-April 2024; Page No 191-198

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Volume 7, Issue 2; March-April 2024; Page No 191-198