Original
ISSN 2413-0516

Serum Levels of Homocysteine, Troponin-I, and High Sensitive C-Reactive Protein in Iraqi COVID-19 Patients

Shahad Sabah Khalid*, Zhahraa Mohamed Ali, Mohanad Faris Raheem

Department of Laboratory Sciences, College of Pharmacy, University of Baghdad, Baghdad, Iraq.
*Correspondence to: Shahad Sabah Khalid (E-mail: shahad_almukhtar@ymail.com)
(Submitted: 04 March 2022 – Revised version received: 16 March 2022 – Accepted: 25 April 2022 – Published online: 26 June 2022)

Abstract

Objective: This study aimed to investigate if the Homocysteine has recently been proposed as a serious predictive biomarker for Covid-19 infection severity.

Methods: In this case-control study, which involved 90 participants, 5 ml of venous blood specimen was reserved for each participant, to measure homocysteine, troponin-I, and high sensitive C-reactive protein in their blood, to find if there was an association between these markers levels and COVID-19 infection by using STATA version 23.

Results: The current study found a significant increase in measured values of homocysteine in patients' serum than controls *P*-value = 0.004 which is < alpha (0.05) with an area under the curve of 0.678, also found a significant increase in measured values of cardiac troponin-I; and hs-CRP in COVID-19 patients than controls, *P*-values were 0.02 and 0.00 respectively which are < alpha (0.05) with an area under the curve of 0.686 and 0.739 respectively.

Conclusion: Homocysteine has been noted as a strong predictive biomarker for COVID-19 infection severity in many articles, the current study showed that homocysteine had a moderate predictive biomarker for COVID-19 infection. Cardiac troponin-I showed a moderate predictive biomarker for COVID-19 infection, while hs-CRP was noted as a good predictive biomarker. Many studies reported an association between high levels of hs-CRP and mortality rate in COVID-19 patients.

Keywords: COVID-19, homocysteine, troponin I, C-reactive protein

Introduction

A novel form of coronavirus, "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) was described in Wuhan in 2019, is an RNA airborne virus, that causes varied comorbidities and mortalities.[1] COVID-19 is occurring in two phases; first is viral replication then inflammatory response. The flare-up of coronaviruses begins to spread quickly through the world, which induces the World Health Organization (WHO) to state the disease "a worldwide health threat". The exponential elevation in infected cases worldwide may be because of the lack of pre-virus immunity.[2]

Homocysteine (Hcy) is a non-essential α-amino acid, that does not take part in the protein synthesis.[3] Homocysteine has recently been suggested as a serious predictive biomarker for Covid-19 infection severity, Different forms of Hcy are existing in the blood circulation these include: bound to plasma proteins (70–80%), joined with other Hcy molecules to form a disulfide (20–30%), while free thiol represents the smallest part (~1%).[4]

SARS-CoV-2 passes on methyl group for viral RNA supply from the host cell S-adenosylmethionine (SAM), which will be transformed into S-adenosylhomocysteine (SAH). Homocysteine is formed as an intermediate product when SAH hydrolase (SAHH) removes adenosine from SAH, homocysteine reprocessed by the remethylation and trans-sulphuration pathway in the human body.[5] SARS-CoV2 entry into cells is through its spike proteins that attach to the ACE2 cellular receptors to form a tunnel through which the virus enters the cell.[6] Homocysteine attaches also to the ACE2 enzyme, in addition to the attachment to various ion-channel cellular receptors, and then enters cells.[7,8]

Vasculitic damage is not only pertinent in the lung, where it leads to edema and acute respiratory distress syndrome, but also has a considerable turn in the cardiovascular diseases (ischemia, deep venous thrombosis, pulmonary thromboembolism) and cerebral injuries (ischemia, hemorrhage); its severity is regrettably not easily expected through currently used laboratory biomarkers such as D-Dimer or prothrombin time/activated partial thromboplastin time (PT/aPTT).[9,10]

The most widely used biomarker in the diagnosis of acute myocardial injury (AMI) is troponin.[11] Troponin is constitutive of cardiac and skeletal muscles. The troponin complex is formed of three subunits; troponin T (TnT) which is considered as tropomyosin-binding subunit, and troponin I (TnI) which is considered an inhibitory subunit, and troponin C (TnC) which is the calcium-binding part. Troponin monitors the interaction that is mediated between actin and myosin by the effect of calcium which results in the contraction and relaxation of striated muscles.[12,13]

Troponin I (TnI) is the inhibitory subunit responsible for inhibiting the ATPase activity of actinomyosin, It is a polar protein with a surplus of positively charged remnants. The expression of TnI is based on the level of ontogenesis; both cardiac and slow skeletal types are available in the heart of the human fetus. After birth, the number of the slow skeletal type is diminished and the number of cTnI is increased. It has been recorded that baby in age of nine months has cTnI exclusively with no slow skeletal type.[14]

Troponin is present in the blood circulation 4 to 6 hours after AMI,[15] reaches a peak after nearly 18 to 24 hours, and may stay increased for up to 14 days estimation of cTn-I by automated assay is recently one of the most sensitive and specific methods for diagnosing AMI.[16,17]

The presently most favorable laboratory marker for cardiovascular risk is the estimation of CRP at low levels [high-sensitivity CRP (hs-CRP)], CRP comprises five identical symmetrically organized 23-kDa promoters, which are folded into

two antiparallel -sheets, similar to the structure of lectins, and is generally raised in human plasma after bacterial infections, cancer, or after surgical procedures.[18]

This study aimed to measure homocysteine level, troponin-I, and high sensitive C-reactive protein in serum of COVID-19 infected patients participating in this study.

Methods

In a case-control study, which involved 90 participants, 45 were hospitalized patients diagnosed clinically with COVD-19, and with a positive result of nucleic acid amplification testing by real-time reverse transcription-polymerase chain reaction (RT-PCR) of respiratory samples which were nasal/oropharyngeal swabs for COVID-19 infection and 45 of them were healthy as control.

The study was carried out in two centers for COVID-19 in Baghdad, Iraq, these centers were: Al-Ataa, and Dhare Alphaeadh hospitals, during the period from October/2021 to February/2022.

All COVID-19 patients were treated with the same protocol, all blood samples were taken from the participants in the same conditions, all patients were on Continuous positive airway pressure therapy (CPAP), participants were interviewed by the researcher, and sociodemographic data including; name, age, gender, and history of any past disease and medication taken from them.

Inclusion criteria; Adult (30–60) years old, patients with COVID-19 infection with positive PCR, have no chronic disease. Exclusion criteria; patients with cardiovascular disease, diabetes, and hypertension, autoimmune disease, pregnant and lactating women, patients with a history of asthma, smoking, malignancy, patients on steroid or immunosuppressive drugs as long term therapy previous to COVID-19 infection. 5 ml of venous blood specimen was reserved for each participant and collected in a gel tube and left for an appropriate time at room temperature to allow them to clot then centrifuged for 10–15 minutes at 4400 rounds per minute (rpm) to get the serum that was used in the analysis for studied homocysteine, troponin-I, and hs-CRP levels.

Statistical analysis: Statistical analysis was conducted using the statistical package for social sciences (SPSS) version 23 software for Windows.

Descriptive statistics are presented as median, Interquartile range, and mean rank T-test- Mann Whitney was used to measure the difference between the means of non-normally distributed variables. The receiver operating characteristic curve (ROC) for measuring the area under the curve was used also.

Results

In the present study, there was a significant increase in serum levels of homocysteine in COVID-19 patients' serum than controls (*P*-value = 0.004) which is < alpha (0.05), where the median (IQR) for COVID-19 patients was 11.0 (30.735) while for control was 6.800 (4.295) a shown in Table 1 and Figure 1.

Also there was a significant increase in measured values of cardiac troponin-I, with *P*-value = 0.02 < 0.05, where COVID-19 patients' median (IQR) was 0.00646 (0.0056) while median (IQR) for control was 0.004135 (0.0041). The *P*-value for hs-CRP was 0.00 < 0.05 which refers to a significant increase in hs-CRP level in COVID-19 patients than in control, where the median (IQR) for COVID-19 patients was 52.955 (36.81) while for controls was 1.9810 (51.32) as shown in Table 1, Figures 2 and 3.

Homocysteine showed an area under the curve (AUC) of 0.678 which was moderate strength a predictive value for COVID-19 severity. The optimal cut-off value for Hcy as a predictive value of COVID-19 infection severity was estimated to be 8.10 (nmol/ml); sensitivity and specificity were 66.7% and 68.9%, respectively (as shown in Table 2 and Figure 4).

Cardiac troponin-I had an AUC of 0.686, the optimal cut-off value was 0.004635 (ng/ml) with sensitivity and specificity of 75.5% and 60% respectively, while the AUC for hs-CRP was 0.739 making it a good predictive marker of COVID-19

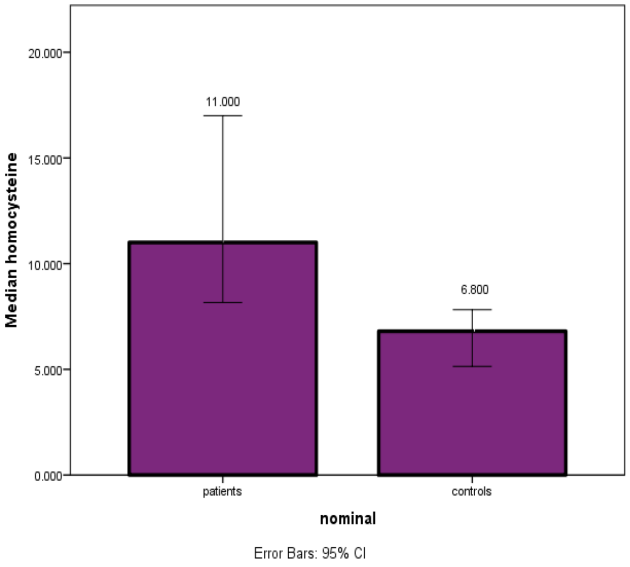

Fig. 1 **Homocysteine levels in studied groups.**

Table 1. **Assessment of Homocysteine, Troponin-I, And hs-CRP Levels in Studied Groups**

Parameter	Group	Median	Interquartile range	Mean rank	*P*-value
Homocysteine nmol/ml	Patients	11.00	30.735	53.52	*0.004
	Controls	6.800	4.295	37.48	
Cardiac troponin-I ng/ml	Patients	0.00646	0.0056	53.89	*0.002
	Controls	0.004135	0.0041	37.11	
Hs-CRP µg/ml	Patients	52.955	36.81	56.23	*0.00
	Controls	1.9810	51.32	34.77	

**P* < alpha (0.05) significant; *r*, spearman correlation coefficient.

severity, the optimal cut-off value was 8.7220 (µg/ml) with sensitivity and specificity of 77.8% and 68.9% respectively Figures 5 and 6.

Discussion

Homocysteine has been suggested as a crucial biomarker for cardiovascular complications in patients admitted to hospital with COVID-19 infection.[19]

Analysis by Receiver Operating Characteristic (ROC) for estimating the sensitivity and specificity of the cut-off at which

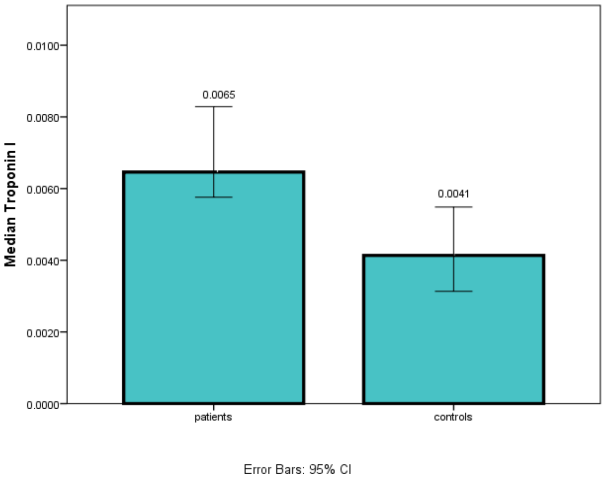

Fig. 2 **Troponin-I levels in studied groups.**

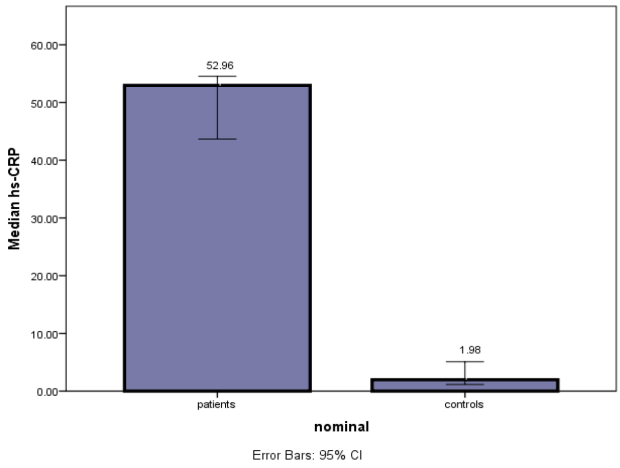

Fig. 3 **Hs-CRP levels in studied groups.**

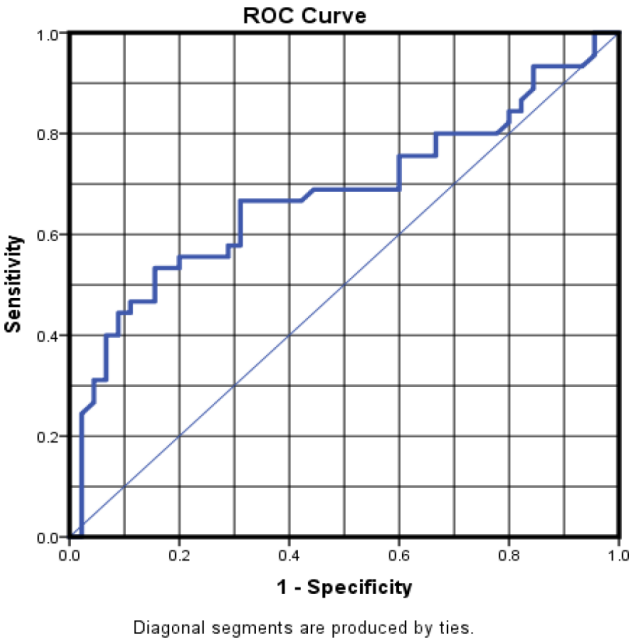

Fig. 4 **Receiver operating characteristic curve for measuring the area under the curve of homocysteine in patients with COVID-19 infection.**

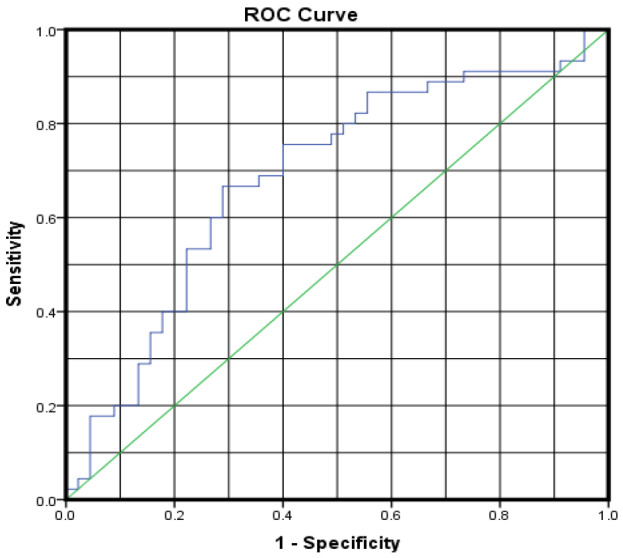

Fig. 5 **Receiver operating characteristic curve for measuring the area under the curve of cardiac troponin-I in patients with COVID-19 infection.**

Table 2. **Receiver operating characteristic curve for measuring the area under the curve Of homocysteine, cardiac troponin-I, and Hs-CRP levels in patients with COVID-19 infection**

Variable	AUC	95% CI of AUC	*P*-Value	Optimal cut-off	SN	SP
Homocysteine nmol/ ml	0.678	0.565–0.792	*0.004	8.100	0.667	0.689
Cardiac Troponin I ng/ml	0.686	0.575–0.798	*0.002	0.004635	0.756	0.600
Hs-CRP µg/ml	0.739	0.634–0.843	*0.000	8.7220	0.778	0.689

Where: *p< alpha (0.05) significant; AUC, the area under the curve; CI, is confidence interval; SN, sensitivity; SP, specificity (from the curve *X*-axis is 1-specificity).

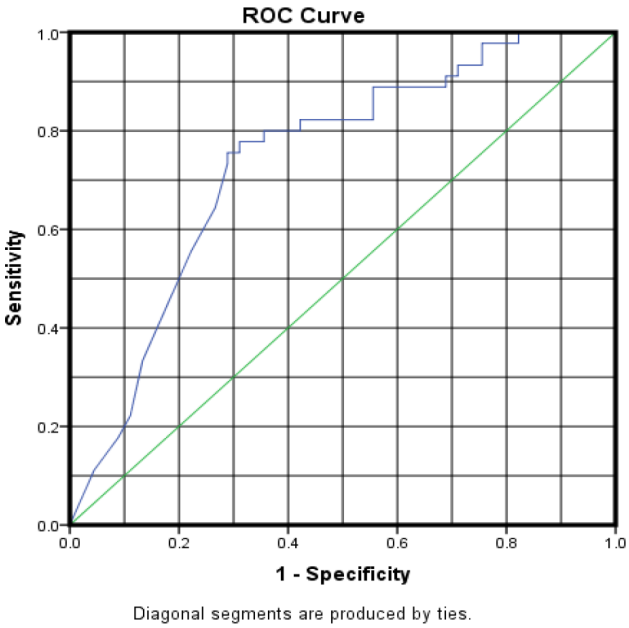

Fig. 6 **Receiver operating characteristic curve for measuring the area under the curve of hs-CRP in patients with COVID-19 infection.**

patients are considered infected with severe COVID-19 disease is equal to or higher than the specified cut-off value, so the current study showed that homocysteine had an optimal cut-off value as a predictive value of COVID-19 severe infection estimated to be (8.10) mol/ml sensitivity and specificity were 66.7% and 68.9%, respectively, and as a moderate predictive biomarker for COVID-19 infection (AUC of 0.678).

Ponti G et al. in a multicenter, retrospective analysis, Ponti G et al. including patients hospitalized for COVID-19 from April to September 2020, the study suggest that homocysteine level equal to or more than 16 µmol/L was the optimal cut-off value for Hcy as predictive of in-hospital mortality with sensitivity and specificity of 41 and 83%, respectively; with AUC is 0.55.[20]

Many articles suggested Hcy as a strong predictive biomarker for COVID-19 infection severity.[21] In a group of 273 Chinese patients that were hospitalized with COVID-19 disease and had mild symptoms, over 40 parameters were measured at admission. Disease progression was recorded for 72 patients, Hcy serum levels and monocyte-to-lymphocyte ratio (MLR) were the solely significant predictors in hyperhomocysteinemic patients (>15.4 µmol/L), evaluated to correspond with a three-fold elevated risk of disease evolution at radiological images. The most important thing is that Hcy is the solely predictive marker specified which can be easily modifiable.[22]

C-reactive protein (CRP), is an acute-phase protein[23] synthesized in the liver as a response to interleukin-6 (IL-6) and is a broadly available biomarker of inflammation.[24] Several recent studies have reported an association between higher CRP concentrations and greater disease severity in COVID-19 disease.[25]

In the current study, hs-CRP was recognized as a good predictive biomarker with an AUC of 0.739. Many studies reported an association between high levels of hs-CRP and mortality rate in COVID-19 patients. A study done among 375 patients with confirmed SARS-CoV-2 infection detected that increased hs-CRP levels were notably correlated with high mortality risk.[26] Juan Li in a study aimed to explore the influencing factors on critical COVID-19 disease estimated the hs-CRP level median (IQR) for survival patients to be 18.4 (59.90) (mg/L) while for non-survivor was 113.30 (93.20) (mg/L) with *P*-value < 0.001 and AUC (95% CI) was 0.879 (0.815–0.944).[27]

Cardiac troponin-I shows a moderate predictive biomarker for COVID-19 infection with an AUC of 0.686, Shi and colleagues explained in a huge sequential patient cohort with COVID-19 that myocardial injury, identified at admission, was correlated with a higher risk of in-hospital mortality. For 416 hospitalized patients with COVID-19 in China, 82 had an initial cTn-I higher than the upper reference range, proposing approximately 20% of the cohort had evidence of myocardial injury. Those with elevated cTn-I, compared to those without, progress with a more severe disease on multiple measures. Those with elevated cTn-I had many more cardiac comorbidities and showed a higher risk of death, both during the time from symptom onset (hazard ratio, 4.26 [95% CI, 1.92–9.49]) and admission to the endpoint (hazard ratio, 3.41 [95% CI, 1.62–7.16]).[28] Juan Li (2021) in a study aimed to explore the influencing factors of critical coronavirus disease 2019 (COVID-19) estimated the cTn-I level median (IQR) for survival patients to be 2.55 (4.73) (mg/L) while for non-survivor was 40.75 (652.83) (mg/L) with *P*-value < 0.001.[27]

Conclusion

Homocysteine may be a beneficial biomarker that can assess clinicians to recognize patients who are at higher risk for severe COVID-19 infection. Hcy levels in plasma can be determined easily by a simple and affordable laboratory test. Good and integral food and supplements of vitamin B especially B12, and Folic acid could have protective clinical effects for patients with infectious disease, and also clinical management of COVID-19 infection can be improved by early determination of many biomarkers to control therapeutic intervention efficacy and/or foretell the clinical course of the COVID-19 disease not only for those diagnosed with COVID-19 disease but also for subjects suspected and waiting for confirmation.

Limitation

The current study is limited by the inclusion of hospitalized patients with COVID-19 infection who had no previous chronic disease. The results cannot, therefore, be generalized to all COVID-19 patients; also the number of cases is limited and so gave limited results.

Several exclusion criteria were used in the study to decrease the confusing effect; the baseline markers levels before being infected with COVID-19 disease were unavailable, which made the accurate development of changes in the studied markers uncertain.

Abbreviations

SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; hs-CRP, high sensitive C-reactive protein; WHO, World Health Organization; ACE2, angiotensin-converting enzyme 2; Hcy, homocysteine; AUC, area under the curve.

Conflicts of Interest

None.

References

1. Bchetnia M, Girard C, Duchaine C, Laprise C. The outbreak of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): A review of the current global status. Journal of Infection and Public Health. 2020 Nov 1;13(11):1601–10.
2. Sisó-Almirall A, Kostov B, Mas-Heredia M, Vilanova-Rotllan S, Sequeira-Aymar E, Sans-Corrales M, et al. Prognostic factors in Spanish COVID-19 patients: A case series from Barcelona. PLoS One. 2020;15(8):e0237960.
3. McCully KS. Homocysteine and the pathogenesis of atherosclerosis. Expert Rev Clin Pharmacol. 2015 Mar;8(2):211–9.
4. Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. Lancet (London, England). 1999 Jul;354(9176):407–13.
5. Romagnoli S, Peris A, De Gaudio AR, Geppetti P. SARS-CoV-2 and COVID-19: From the Bench to the Bedside. Physiol Rev [Internet]. 2020 Jun 4;100(4):1455–66. Available from: https://doi.org/10.1152/physrev.00020.2020.
6. Hammons A, Summers C, Woodside J, Mcnulty H, Strain JJ, Young I, et al. Folate/homocysteine phenotypes and MTHFR 677C>T genotypes are associated with serum levels of monocyte chemoattractant protein-1. Clin Immunol. 2009 Aug 1;133:132–7.
7. Chen C-H, Beard RS, Bearden SE. Homocysteine impairs endothelial wound healing by activating metabotropic glutamate receptor 5. Microcirculation [Internet]. 2012 May;19(4):285–95. Available from: https://pubmed.ncbi.nlm.nih.gov/22221504.
8. Naser NH, Alibeg AAA. Exacerbation of COVID 19 in Hypertensive Patients? A review? Iraqi J Pharm Sci (P-ISSN 1683-3597, E-ISSN 2521-3512). 2021;30(2):23–30.
9. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet [Internet]. 2020 Feb 15;395(10223):497–506. Available from: https://doi.org/10.1016/S0140-6736(20)30183-5.
10. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet [Internet]. 2020 Feb 15;395(10223):507–13. Available from: https://doi.org/10.1016/S0140-6736(20)30211-7.
11. Alqubbanchi FB, Al-Hamadani FY. A Pharmacoeconomics Study for Anticoagulants used for Hospitalized COVID-19 Patients in Al-Najaf Al-Ashraf city–Iraq (Conference Paper). Iraqi J Pharm Sci (P-ISSN 1683-3597, E-ISSN 2521-3512). 2021;30(Suppl.):48–59.
12. Ahmad M. Biomarkers in Acute Myocardial Infarction. J Clin Exp Cardiol. 2012 Nov 10;3.
13. Ruseva A. Laboratory diagnosis of acute myocardial infarction. Trakia J Sci. 2005;3(1):8–14.
14. Farah CS, Reinach FC. The troponin complex and regulation of muscle contraction. FASEB J [Internet]. 1995 Jun 1;9(9):755–67. Available from: https://doi.org/10.1096/fasebj.9.9.7601340.
15. Sasse S, Brand NJ, Kyprianou P, Dhoot GK, Wade R, Arai M, et al. Troponin I gene expression during human cardiac development and in end-stage heart failure. Circ Res. 1993 May;72(5):932–8.
16. Christensen H, Johannesen HH, Christensen AF, Bendtzen K, Boysen G. Serum cardiac troponin I in acute stroke is related to serum cortisol and TNF-α. Cerebrovasc Dis. 2004;18(3):194–9.
17. Ishii J, Cui W, Kitagawa F, Kuno T, Nakamura Y, Naruse H, et al. Prognostic value of the combination of cardiac troponin T and B-type natriuretic peptide after initiation of treatment in patients with chronic heart failure. Clin Chem. 2003;49(12):2020–6.
18. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. Structure. 1999 Feb;7(2):169–77.
19. Ponti G, Manfredini M, Oliva G, Ozben T, Fontana C, Tomasi A. Predicting COVID-19 Hospitalized Patients' Outcome with Homocysteine. J Clin Cardiol. 2021;2(1).
20. Ponti G, Roli L, Oliva G, Manfredini M, Trenti T, Kaleci S, et al. Homocysteine (Hcy) assessment to predict outcomes of hospitalized Covid-19 patients: a multicenter study on 313 Covid-19 patients. Clin Chem Lab Med. 2021;59(9): E354–7.
21. Ibrahimagić O, Smajlović D, Dostović Z, Vidović M, Tupković E, Kunić S. COMMENT ON AN ARTICLE: "Homocysteine as a potential predictor of cardiovascular risk in patients with COVID-19". Med Hypotheses. 2020;143.
22. Yang Z, Shi J, He Z, Lü Y, Xu Q, Ye C, et al. Predictors for imaging progression on chest CT from coronavirus disease 2019 (COVID-19) patients. Aging (Albany NY). 2020;12(7):6037.
23. Tillett WS, Francis Jr T. Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. J Exp Med. 1930;52(4):561.
24. Morley JJ, Kushner I. Serum C-reactive protein levels in disease. Ann N Y Acad Sci. 1982;389(1):406–18.
25. Potempa LA, Rajab IM, Hart PC, Bordon J, Fernandez-Botran R. Insights into the use of C-reactive protein as a diagnostic index of disease severity in COVID-19 infections. Am J Trop Med Hyg. 2020;103(2):561.
26. Yan L, Zhang H, Goncalves J. An interpretable mortality prediction model for COVID-19 patients. Nat. Mach. Intell. 2, 283–288 (2020).
27. Li J, Wang L, Liu C, Wang Z, Lin Y, Dong X, et al. Exploration of prognostic factors for critical COVID-19 patients using a nomogram model. Sci Rep. 2021;11(1):1–6.
28. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. hospitalized patients with COVID-19 in Wuhan, China. JAMA Cardiol 2020;5:e200950. JAMA Cardiol. 2020;5(7):802–10.