Clin. Lab. 2021;67:XXX-XXX

ORIGINAL ARTICLE

Effects of a Serum Heating Procedure for Inactivating COVID-19 on Common Biochemical Tests

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SUMMARY

Background: **COVID-19 has recently been declared an epidemic by the WHO, and there is an urgent need for affected countries and laboratories to assess and treat people at risk of COVID-19. A heat procedure has been suggested for specimen inactivation. This study was designed to evaluate the effect of serum heating on biochemical indexes, and providing a basis for accurate detection results of the COVID-19 patients.**
Methods: **We collected 29 normal cases of two tubes of 5 mL whole blood. One tube was analyzed directly, and the other was analyzed after heating at 56°C 30 minutes.**
Results: **A total of 34 serum biochemical index quantitative results were obtained, 28/34 indexes were not significantly affected by the heat inactivation and remained clinically interpretable. As the thermal inactivation for these indexes showed good correlation, ALB ($p = 0.04$, Pearson R = 0.91, 2.6% mean increase), CysC ($p = 0.03$, Pearson R = 0.98, 9.9% mean increase), CO2CP ($p < 0.001$, Pearson R = 0.96, 13% mean decrease), they were still interpretable. Four biochemical indexes ALP, CK, CK-MB, and insulin were inactivated and showed significant statistical differences ($p < 0.001$).**
Conclusions: **Our study showed CK, CK-MB, ALP, and insulin were sensitive to heat and will be inhibited or degrade after heating, indicating that the rapid decrease of this indexes in the COVID-19 patients may be caused by sample heat inactivation. For safety and diagnostic accuracy, we recommend the use of a point-of-care device for blood gases, electrolytes, troponin, and liver and renal function tests within a ISL 2 or above biosafety cabinet with level 3 or above biosafety laboratory practice.**
(Clin. Lab. 2021;67:xx-xx. DOI: 10.7754/Clin.Lab.2020.200743)

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Manuscript accepted August 6, 2020

KEY WORDS

COVID-19, biochemical test, heat inactivation, creatine kinase, CK-MB, ALP

INTRODUCTION

An outbreak of 2019 novel coronavirus disease (COVID-19) has rapidly spread throughout the world [1]. 2019-nCoV belongs to the β-genus coronavirus, with a linear positive single-stranded RNA genome. It is the seventh coronavirus known to infect humans [2]. Common symptoms include fever, dry cough, and my-

algia [3]. For physicians to make important diagnostic and treatment decisions, timely reporting of laboratory results is critical. On March 3, the Handbook of Prevention and Treatment of the Pneumonia Caused by the Novel Coronavirus (2019-nCoV (version 7) included that blood routine, urine routine, CRP, biochemical indexes (liver enzyme, myocardial enzyme, kidney function, etc.) should be monitored according to the condition [4].

Samples from these patients should only be handled by experienced laboratory personnel in laboratories equipped with P-3 safety facilities. Unfortunately, only a few such laboratories exist and they may be limited in the type of clinical analyses. There is another concern that the COVID-19 is infectious in laboratory generated aerosol. Hence, stringent guidelines for laboratory personnel with respect to handling of laboratory specimens containing COVID-19 have been published in China. According to the new type of coronavirus laboratory biosafety guidelines (version 2), the infectious materials must be inactivated using a reliable method before serological detection, biochemical analysis, and virus antigen detection in a level BSL-II laboratory [5].

Serum biochemical tests are the most basic and common tests for patients, especially those requiring intensive care and critically ill patients. At present, most general serum biochemical tests are performed by automated analyzers, but for highly infectious specimens, adequate disinfection and virus inactivation cannot be carried out, causing occupational health hazards to laboratory staff. 2019-nCoV was sensitive to ultraviolet and heat, it can be inactivated effectively by 56°C 30 minutes, ethyl ether, 75% ethanol, and chlorine-containing disinfectant [6]. Heating has long been recognized as an effective method of virus inactivation [7], and 56°C 30 minutes can inactivate the 2019-nCoV completely for routine laboratory examination. However, it has not been reported whether thermal inactivation has an impact on serum biochemical indexes or not. This study was designed to compare the biochemical indexes in heated or unheated blood samples. Evaluating whether the biochemical indexes after heat inactivation had significant differences and providing a statistical delineation for heat inactivation procedure effects on COVID-19 patients' serum biochemical tests.

MATERIALS AND METHODS

Whole blood samples were collected in two EDTA-containing tubes (5 mL) and kept at 4 ± °C to be analyzed within 24 hours. One tube was isolated serum directly from whole blood centrifuged at 3,000 r/minute for 15 minutes and analyzed immediately. The other tube was heat inactivated at 56°C for 30 minutes. Then serum was isolated by centrifugation and analyzed. The heat inactivation procedure was performed according to the new type of coronavirus laboratory biosafety guidelines (version 2).

A total of 34 serum biochemical indexes were analyzed, including insulin, C-Peptide, 25-OH VitD, Hs-CRP, UA, CO2CP, Glu, HCY, liver function indexes (GLB, A/G, GGT, ALP, ALT, TBA, PA, ALB, TP), kidney function indexes (CysC, Urea, Cr, β_2-MG), myocardial enzyme indexes (α-HBDH, LDH, CK-MB, CK, AST), and serum lipid level (Apo A1, Apo B, Apo A1/Apo B, LP(a), TC, TG, HDL-C, LDL-C). All the serum biochemistry detection was performed using the automatic biochemical analyzer (C501, Roche, Germany).

Data were statistically analyzed using SPSS 19.0 software. Measurement data were expressed as mean ± standard deviation ($\bar{x}$ ± SD). *t*-test was used to evaluate the characteristics between heated or unheated group. We considered a p-value less than 0.05 as statistically significant. We also calculated the correlation coefficient for the accuracy of each index and considered Pearson R > 0.90 to show good correlation. The effect of heat inactivation on an analyte was considered insignificant if the linearity was preserved and correlation coefficient/rank correlation was ≥ 0.9, and the constant bias was evaluated as to its statistical significance by paired *t*-test. If an analyte did not fulfil the above two criteria, the heat inactivated measurement result was considered to have lost the diagnostic values.

RESULTS

We collected 29 normal cases of whole blood. There were 9 males and 20 females. Age range from 21 to 48 years old, mean 31.69 ± 6.88. The participants were confirmed to have no history of COVID-19 infection and no symptoms.

A total of 34 serum biochemical index quantitative results were obtained. These indexes reflected the risk of liver function, kidney function, myocardial injury, lipid profile, endocrine dyscrasia, and independent risk factors (Table 1). Combined with a *t*-test p > 0.05 and Pearson R > 0.90, 28/34 indexes were not significantly affected by the heat inactivation and remained clinically interpretable (Figure 1).

Despite the proportional differences, analytical results for ALB (p = 0.04, Pearson R = 0.91, 2.6% mean increase), CysC (p = 0.03, Pearson R = 0.98, 9.9% mean increase), and CO2CP (p < 0.001, Pearson R = 0.96, 13% mean decrease) were still interpretable, as the thermal inactivation for these indexes showed good correlation. The effect of heat inactivation on an analyte was considered insignificant if the slope of the regression line was between 0.95 and 1.05, the linearity was preserved, and the correlation coefficient/rank correlation was ≥ 0.9 (Figure 2).

Four biochemical indexes ALP, CK, CK-MB, and insulin were inactivated and showed significant statistical differences (p < 0.001). They all showed poor correlation (R = 0.82, R = 0.50, R = 0.64, R = 0.42). After the samples were heated, they were all obviously decreased, CK from 77.46 ± 26.36 to 16.97 ± 5.01, CK-MB from

Table 1. Laboratory results of 34 biochemistry indexes between heated and unheated groups.

	Normal range	Non heated group	Heated group	*t*-test
		(Means ± SD.)	(Means ± SD.)	p-value
A/G	1.5 - 2.5	1.63 ± 0.21	1.71 ± 0.23	0.16
ALB (g/L)	35 - 52	46.45 ± 2.25	47.67 ± 2.07	0.04
ALP (U/L)	40 - 130	72.24 ± 22.44	30.31 ± 13.16	< 0.001
ALT (U/L)	7 - 40	21.46 ± 21.07	16.18 ± 14.97	0.28
ApoA1 (g/L)	1.08 - 2.25	1.39 ± 0.16	1.4 ± 0.15	0.87
ApoA1/ApoB	0.8 - 2.2	2.04 ± 0.54	2 ± 0.53	0.79
ApoB (g/L)	0.6 - 1.17	0.72 ± 0.17	0.75 ± 0.16	0.58
AST (U/L)	15 - 40	22.14 ± 11.62	22.13 ± 11.53	1.00
CK (U/L)	20 - 200	77.46 ± 26.36	16.97 ± 5.01	< 0.001
CK-MB (U/L)	0 - 25	11.96 ± 3.51	5.62 ± 1.72	< 0.001
CO2CP (mmol/L)	21 - 31	23.9 ± 2.05	20.74 ± 2	< 0.001
Cr (μmol/L)	45 - 84	55.79 ± 12.58	54.97 ± 13.12	0.81
GGT (U/L)	7 - 45	24.1 ± 30.62	23.19 ± 30.66	0.91
GLB (g/L)	20 - 40	28.93 ± 3.56	28.28 ± 3.66	0.49
Glu (mmol/L)	3.9 - 6.1	5.41 ± 0.75	5.43 ± 0.76	0.91
HCY (μmol/L)	0 - 15	12.37 ± 6.52	12.4 ± 6.36	0.98
HDL-C (mmol/L)	1 - 1.55	1.32 ± 0.28	1.3 ± 0.3	0.83
Hs-CRP (mg/dL)	0 - 0.5	0.11 ± 0.18	0.11 ± 0.18	0.95
LDH (U/L)	135 - 214	175.31 ± 31.35	175.1 ± 31.82	0.98
LDL-C (mmol/L)	1.9 - 3.1	2.58 ± 0.64	2.53 ± 0.62	0.75
PA (g/L)	0 - 10	0.26 ± 0.05	0.26 ± 0.05	0.96
TBA (μmol/L)	0 - 10	3.73 ± 2.61	4.01 ± 2.88	0.71
TC (mmol/L)	0 - 5.2	4.33 ± 0.84	4.39 ± 0.84	0.77
TG (mmol/L)	0 - 2.26	1.41 ± 1.22	1.45 ± 1.22	0.90
TP (g/L)	65 - 85	75.38 ± 4.32	75.94 ± 4.33	0.62
UA (μmol/L)	119 - 416	304.07 ± 81.09	306.03 ± 81.48	0.93
Urea (μmol/L)	2.78 - 8.07	3.87 ± 1.11	3.94 ± 1.18	0.80
α-HBDH (U/L)	72 - 182	121.48 ± 23.96	123.31 ± 25.85	0.78
β2-MG (mg/L)	0.8 - 2.2	1.62 ± 0.3	1.59 ± 0.24	0.64
CysC (mg/L)	0.59 - 1.03	0.81 ± 0.13	0.89 ± 0.13	0.03
Lp(a) (mg/dL)	0 - 30	30.99 ± 36.76	30.10 ± 35.86	0.93
Insulin (μU/mL)	3 - 25	7.12 ± 3.23	3.55 ± 1.78	< 0.001
C-Peptide (μU/mL)	0.80 - 4.20	2.16 ± 0.90	2.14 ± 0.90	0.95
25-OH VitD (ng/mL)	30 - 100	21.19 ± 5.81	23.21 ± 6.74	0.32

11.96 ± 3.51 to 5.62 ± 1.72, ALP from 72.24 ± 22.44 to 30.31 ± 13.16, insulin from 7.12 ± 3.23 to 3.55 ± 1.78. ALP and CK were not acceptable with a significant decrease, even below the reference range after heat inactivation (Figure 3).

DISCUSSION

2019-nCoV infection mainly involves the respiratory tract, and it also causes liver dysfunction, kidney injury, and myocardial injury [8]. Therefore, it is of great sig-

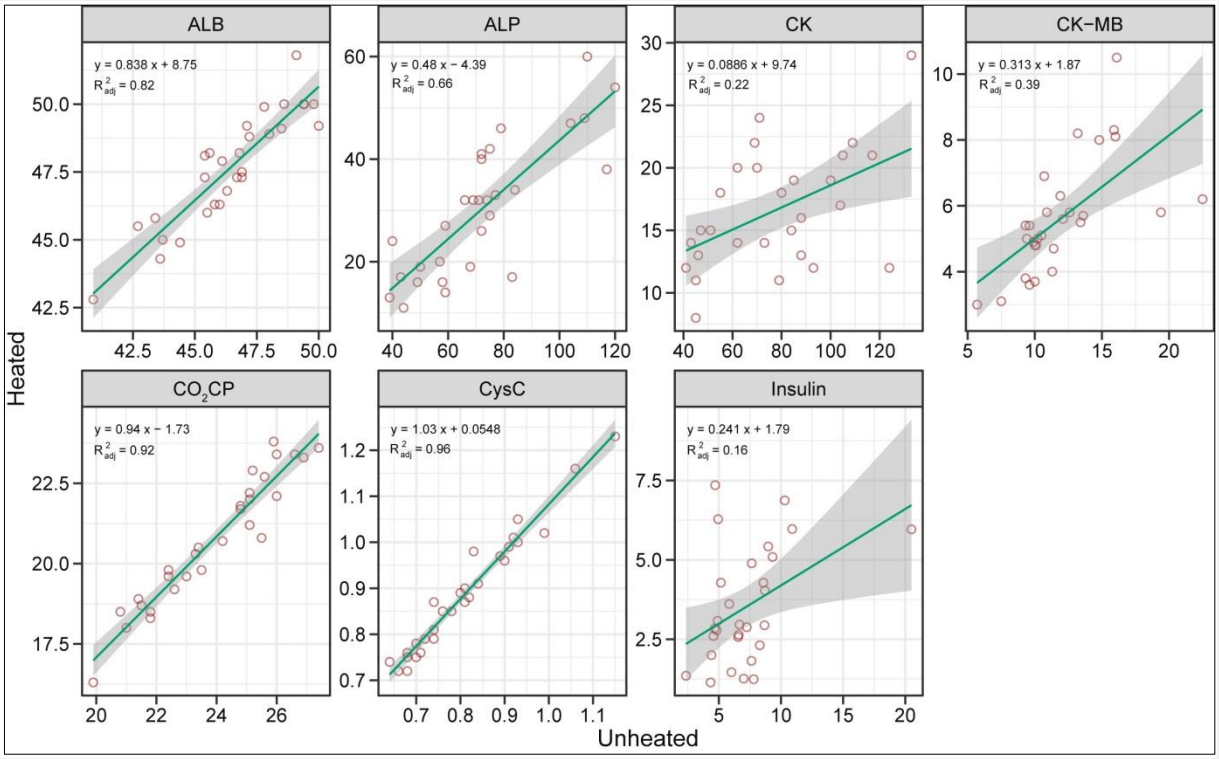

Figure 1. Correlation coefficient of 34 biochemical indexes between heated and unheated samples.

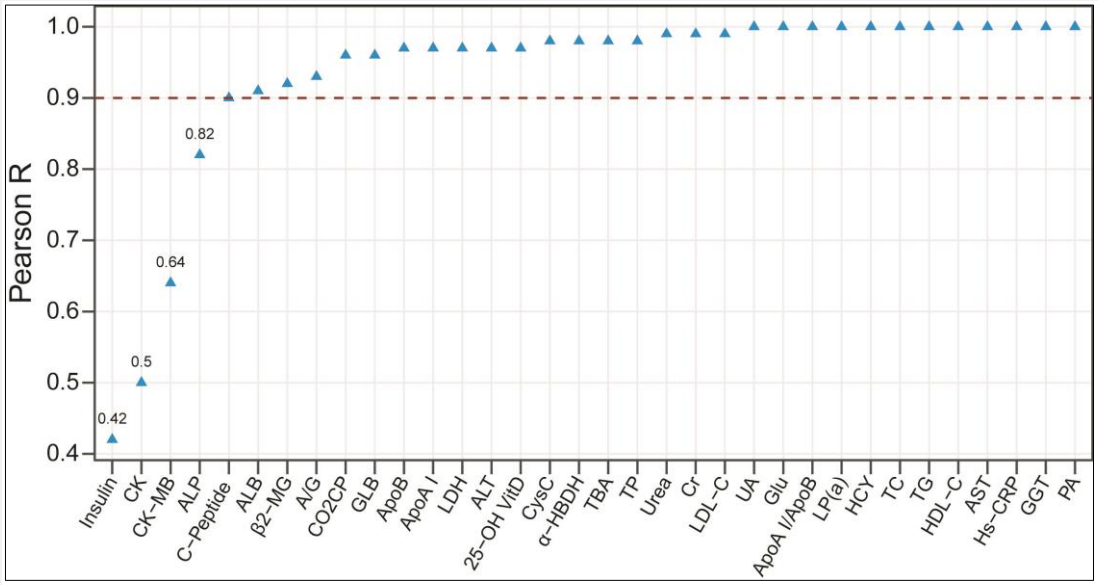

Figure 2. Linear regression expression of seven biochemical indexes with $p \leq 0.05$ by *t*-test.

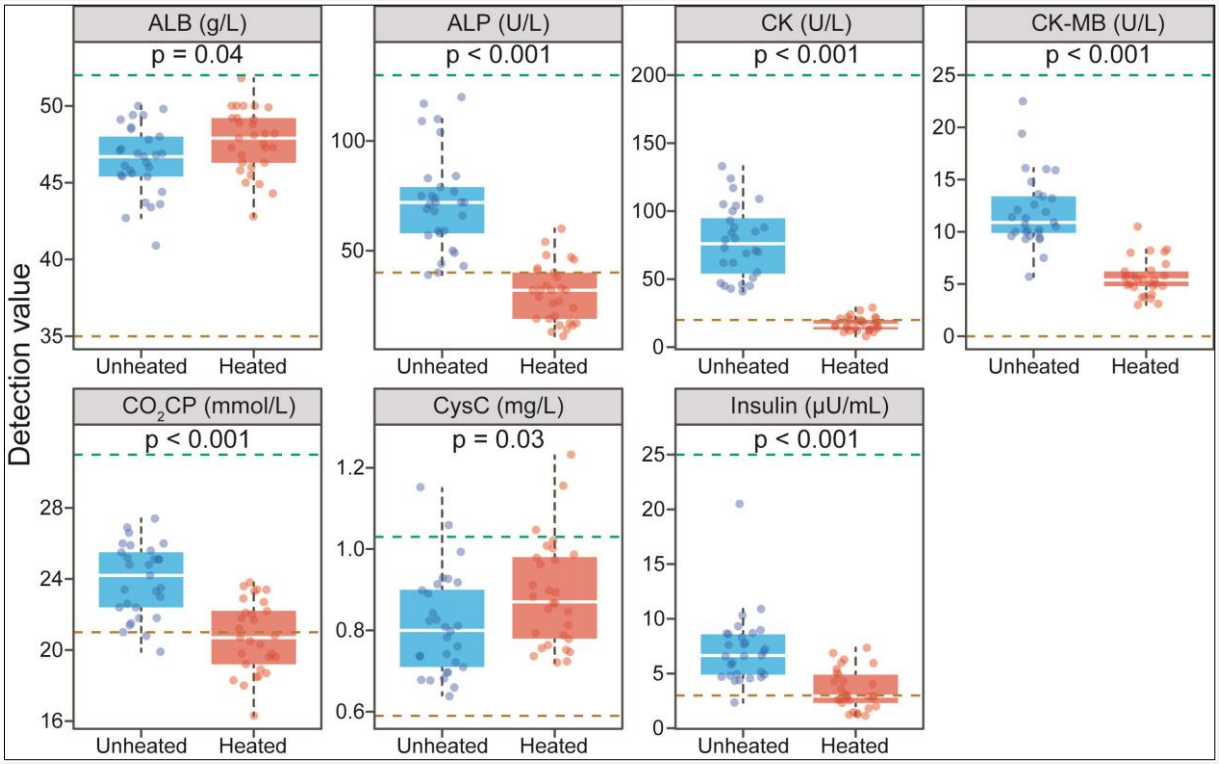

Figure 3. Seven biochemical indexes' statistical differences between heated and unheated samples.

Red dashed line: lower limit, Blue dashed line: upper limit.

nificance to detect the blood biochemical indexes of patients, which can continuously reflect the course of disease and the prognosis for recovery patients. According to the Guideline for determination of biochemical indexes in serum on Roche cobas c501, the cap of the EDTA-tube is removed during sample testing, which may increase aerosol contamination and increase the risk of infection for the environment and operators. Therefore, virus inactivation of samples is needed before detection.

To the best of our knowledge, this is the first time that a comparison of the 34 serum biochemical indexes between the heated and unheated samples was made. Our results showed that 30/34 indexes were well correlated, among which 28/30 has no significant difference. No abnormal renal function indexes were noticed through the whole process. There was a $p < 0.05$ of ALB by *t*-test; however, the data had good correlation between two groups and there was no abnormality beyond the reference range. So, we believe this index is within acceptable limits, which may be related to the small number of samples. CO2CP can reflect the ability of respiration to regulate the acid-base balance [9]. As CO2 molecules have a strong dispersion ability, it is possible that heating will accelerate the CO2 loss. Our assay of AST, LDH, ALT showed no significant difference despite more adverse effects reported by Bhagat et al. [10] and Mak et al. [11]. This may be caused by differences in analytical assays. Therefore, every clinical laboratory should evaluate their own assays.

Biochemical analysis of CK, CK-MB, LDH, and α-HBDH are biomarkers of myocardial damage and are used as a potential adjunct test in clinical and forensic medicine [12]. As recently reported, 2019-nCoV infection can also injur the cardiovascular system, especially causing severe myocardial damage. Laboratory examination was accompanied by an increase in cTnt, CK, and LDH [13]. Our data showed a significant difference ($p < 0.001$) in statistics after heat inactivation in CK and CK-MB levels, indicating that CK and CK-MB were sensitive to heat and will be inhibited or degraded after heating. Serum CK is relatively unstable, and the active center contains easily oxidized cells. So, CK should be determined as soon as possible after specimen collection [14]. In 1982, Fusae Kanemitsu reported the residual CK activity of the atypical CK after heating at 56°C

for 60 seconds was 77% [15]. The indexes of LDH and α-HBDH were stable and showed no significant difference after heating. In the evaluation of myocardial injury, more clinical diagnosis should be combined to make a judgment. This is the first time the endocrine indexes insulin, C-peptide, and 25-OH VitD were evaluated. Our data showed insulin was inactivated and showed a significant decrease and poor correlation, which suggests that for the detection of diabetic patients, attention should be paid to the impact of heat inactivation, which has lost its clinical significance.

Our study reminded that it is critical to pay attention to the myocardial enzyme CK and CK-MB level. The rapid decrease in the COVID-19 patients may be caused by sample heat inactivation. The detection indexes showed a large degree of decline, which would affect the clinical judgment, thus missing the abnormal indicators of myocardial injury.

Ethics Approval and Consent to Participate:

This study was approved by the Ethics Committee of Shenzhen Samii Medical Center and all healthy participants were written informed consent.

Financial Support:

This work was supported by Research Foundation of Shenzhen Samii Medical Center (grand number: SSMC-2020-A8).

Declaration of Interest:

The authors declare no competing financial interest.

References:

1. World Health Organization Novel coronavirus - China. Jan 12, 2020. https://www.who.int/csr/don/12-january-2020-novel-coronavirus-china/en/

2. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. Lancet 2020;395(10224):565-74 (PMID: 32007145).

3. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan. China Lancet 2020;395(10223):497-506 (PMID: 31986264).

4. Handbook of Prevention and Treatment of the Pneumonia Caused by the Novel Coronavirus (2019-nCoV). http://www.chinadaily.com.cn/a/202002/03/WS5e380559a31012821727483d.html

5. Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19). http://www.nhc.gov.cn/xcs/zhengcwj/202001/0909555408d842a58828611dde2e6a26.shtml

6. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019;17(3):181-92 (PMID: 30531947).

7. Mitchell SW, McCormick JB. Physicochemical Inactivation of Lassa, Ebola, and Marburg Viruses and Effect on Clinical Laboratory Analyses. J Clin Microbiol 1984 Sep;20(3):486-9 (PMID: 6490832).

8. Conti P, Ronconi G, Caraffa A, et al. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. J Biol Regul Homeost Agents 2020 Mar 14;34(2):327-31 (PMID: 32171193).

9. Li S, Zhu L, Cheng X, Wang Q, Feng J, Zhou J. The significance of CO2 combining power in predicting prognosis of patients with stage II and III colorectal cancer. Biomark Med 2019 Sep;13(13):1071-80 (PMID: 31497992).

10. Bhagat CI, Lewer M, Prins A, Beilby J. Effects of heating plasma at 56 degrees C for 30 minutes and at 60 degrees C for 60 minutes on routine biochemistry analytes. Ann Clin Biochem 2000; 37:802-4 (PMID: 11085629).

11. Chong YK, Ng WY, Chen SP, Mak CM. Effects of a plasma heating procedure for inactivating Ebola virus on common chemical pathology tests. Hong Kong Med J 2015 Jun;21(3):201-7 (PMID: 25904476).

12. Kwong TC, Arvan DA. How many creatine kinase 'isoenzymes' are there and what is their clinical significance? Chn Chim Acta 1981;115:3-8 (PMID: 7261404).

13. Chen C, Chen C, Yan JT, Zhou N, Zhao JP, Wang DW. [Analysis of myocardial injury in patients with COVID-19 and association between concomitant cardiovascular diseases and severity of COVID-19]. Zhonghua Xin Xue Guan Bing Za Zhi 2020 Mar 6; 48(0):E008. (PMID: 32141280).

14. Chengyang Xu, Tianyi Zhang, Baoli Zhu, Zhipeng Cao. Diagnostic role of postmortem CK-MB in cardiac death: a systematic review and meta-analysis. orensic Sci Med Pathol 2020 Jun;16(2): 287-294 (PMID: 32193705).

15. Kanemitsu F, Kawanishi I, Mizushima J. The origin of a cathode-migrating creatine kinase found in serum from a cancer patient. Clin Chim Acta 1982 Jul 15;122(3):377-83 (PMID: 7105420).