

Research Article

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High dose ascorbic acid treatment in COVID-19 patients raised some problems in clinical chemistry testing

[COVID-19 Hastalarında Kullanılan Yüksek Doz Askorbik Asit Tedavisi Klinik Kimya Testlerinde Bazı Sorunlara Neden Olmaktadır]

<https://doi.org/10.1515/tjb-2020-0237>

Received May 15, 2020; accepted July 9, 2020; published online September 7, 2020

Abstract

Objective: COVID-19 pandemia still continues to threaten the whole world. High dose ascorbic acid (AA) infusion is a choice of treatment and its efficiency is still being investigated. AA interferes with many clinical chemistry tests. However, data about the interference of high concentrations of AA is not sufficient. In this study, we aimed to investigate the interference of AA at high concentrations on commonly used chemistry assays.

Materials and Methods: Serum samples at AA concentrations of 200, 150, 100, 75, 50, 25, 10, 5, 2 and 0 mg/dL were prepared by using the stock solution of 15000 mg/dL AA. Each sample was analyzed by using the most common 30 chemistry tests (Abbott Architect C8000, Illinois, USA) and a POCT glucometer (STANDARD GlucoNavii, Gyeonggi-do, Republic of Korea).

Results: Creatinine, sodium and glucose (POCT) tests were found to be positively interfered by increasing AA concentrations; while direct bilirubin, lipase, UIBC, triglyceride, total cholesterol, HDL/LDL cholesterol tests were

negatively interfered. Absolute interference (%) increased as the AA concentration increased.

Conclusion: This is the largest and first study to investigate the interference of high dose AA, which is used in severe COVID-19 patients nowadays. Manufacturers and clinicians should be aware of the possibility of aberrant results due to high dose AA infusion. Clinicians should not forget to consult a laboratory specialist, since he is the only person to monitor the reactions in all assays, and know the technical subjects like interferences, assay method specifications. This issue is very important for correct decision-making and interpretation of the data-mining studies accurately and efficiently.

Keywords: ascorbic acid; bias; cholesterol; COVID-19; glucometer; interference.

Öz

Amaç: COVID-19 pandemisi hala tüm dünyayı tehdit etmeye devam etmektedir. Yüksek doz askorbik asit (AA) infüzyonu, tedavide bir seçenektir ve etkinliği hala araştırılmaktadır. AA birçok klinik biyokimya testini interfere eder. Fakat yüksek konsantrasyonlardaki AA interferansı hakkında veriler yetersizdir. Bu çalışmada, yüksek doz AA'nın yaygın kullanılan biyokimya testleri üzerindeki interferansını araştırmayı amaçladık.

Gereç ve Yöntem: 15000 mg/dL AA stok solüsyonu kullanılarak 200, 150, 100, 75, 50, 25, 10, 5, 2 and 0 mg/dL AA konsantrasyonlarına sahip serum örnekleri hazırlandı. En yaygın kullanılan 30 biyokimya testi (Abbott Architect C8000, Illinois, ABD) ve bir hasta başı test cihazı (HBTC) glukometre (STANDARD GlucoNavii, Gyeonggi-do, Kore

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Cumhuriyeti) kullanılarak, hazırlanan her serum örneğinin analizi yapıldı.

Bulgular: Artan AA konsantrasyonlarıyla; kreatinin, sodyum ve glukoz (HBTC) testlerinde pozitif interferans olduğu; direkt bilirubin, lipaz, UIBC, trigliserit, total kolesterol, HDL/LDL kolesterol testlerinde ise negatif interferans olduğu bulundu. AA konsantrasyonu arttıkça interferans (%) büyüklüğü de artmaktaydı.

Sonuç: Bu çalışma, son zamanlarda ciddi seyirli COVID-19 hastalarında kullanılan çok yüksek dozda AA'nın biyokimya testleri üzerinde neden olduğu interferansı araştıran ilk ve en geniş çalışmadır. Üreticiler ve klinisyenler, yüksek doz AA infüzyonuna bağlı ortaya çıkabilecek anormal test sonuçlarının farkında olmalıdır. Klinisyenler böyle durumlarda bir laboratuvar uzmanına danışmayı unutmamalıdır. Çünkü laboratuvar uzmanı; tüm testlerdeki reaksiyonları takip edebilen, test metot özellikleri ve interferanslar gibi teknik konuları bilen tek kişidir. Doğru karar verme ve veri madenciliği çalışmalarının doğru ve verimli olarak yorumlanması açısından bu konu çok önemlidir.

Anahtar kelimeler: COVID-19; askorbik asit; interferans; bias; kolesterol; glukometre.

Introduction

Coronavirus disease (COVID-19) is still spreading across the globe; since it has firstly emerged in Wuhan, Hubei, China in December 2019. The disease has been caused by a new virus, named as 2019 novel coronavirus (2019-nCoV) by World Health Organization (WHO) tentatively, before it was named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by International Committee on Taxonomy of Viruses [1].

As this pathogen was an unknown virus, scientists started to investigate different treatment options to alleviate its dangerous complications on patients, such as sepsis and acute respiratory distress syndrome (ARDS). Ascorbic acid (vitamin C) is one of these treatment options. Ascorbic acid (AA), known with its antioxidant characteristics, was found to have a protective effect on alveolar capillaries in patients with sepsis [2]. Thus, some scientists have begun new clinical trials to investigate the effects of high dose AA infusion on severe COVID-19 patients [3–5].

AA is a water-soluble vitamin found in plasma at a reference concentration of 0.4–1.5 mg/dL [6]. Intravenous (i.v.) AA administration is known to be a choice of treatment in complementary and alternative medicine, and is widely used in diseases such as advanced cancer and ARDS [7]. In these cases, used AA dose is too high to compare with the physiological plasma levels. In a phase one clinical

trial, up to 110 g/m² dose of AA was found to be tolerable, which corresponded to 187 g approximately. When this huge amount of AA is administered intravenously, plasma AA peak level rises to the values above 700 mg/dL [8]. This is about 500 folds of its physiological level. Aforementioned clinical trial results show that if 30 g/m² (about 50 g) of AA is given to a patient, peak plasma level is 400 ± 150 mg/dL and elimination half-life ($t_{1/2}$) is 2.1 ± 0.9 h [8]. In another study, a 72-year-old male patient was given 60 g of AA, and his peak level was about 480 mg/dL; while his peak level was about 180 mg/dL for 30 g AA dose [9].

Our hospital was assigned as a pandemic hospital by Turkish Ministry of Health, during COVID-19 pandemic. Doctors of intensive care unit (ICU) in our hospital use high dose AA treatment for severe COVID-19 patients. Daily 30 g of AA is administered i.v. to these patients. This dose may be as high as 50 g or more for some COVID-19 patients. In order to monitor treatment and evaluate the prognosis, clinicians request clinical chemistry testing. And at this point, some problems have come up. Because AA interferes with many chemistry tests in serum and urine dipstick analysis [10, 11]. Manufacturers usually do not investigate interference of such high levels of AA. Thus, there is no sufficient data for such high concentrations of AA in related inserts and other previous studies.

The aim of this study was to investigate the interference of AA at high concentrations on 30 commonly used chemistry tests and one point-of-care test (POCT) by glucometer.

Materials and Methods

Interference study was performed according to CLSI guideline EP07--Interference Testing in Clinical Chemistry. First of all, we created a nonhemolyzed and non-icteric serum pool, using the surplus samples. Serum pool was kept in room temperature in a closed tube in dark for 12 h to make sure that preexisting AA level fallen to zero or undetectable [12]. AA preparation used in ICU (15000 mg/dL AA) and serum pool were mixed in different ratios to create different concentrations of AA. CLSI EP07 recommendation for test concentration for AA is 342 µmol/L (6.02 mg/dL) [13]. However, this concentration was not sufficient for this study. So; serum samples at AA concentrations of 200, 150, 100, 75, 50, 25, 10, 5, two and 0 (serum pool itself) mg/dL were prepared by using the stock solution of 15000 mg/dL. Glucose [hexokinase], blood urea nitrogen (BUN) [urease], aspartate aminotransferase (AST) [NADH-UV], alanine aminotransferase (ALT) [NADH-UV], potassium [ion selective electrode, ISE], chloride [ISE], creatine kinase (CK) [NADPH-UV], alkaline phosphatase (ALP) [para nitrophenyl phosphate], lactate dehydrogenase (LDH) [lactate to pyruvate], gamma-glutamyl transferase (GGT) [gamma-glutamyl-3-carboxy-4-nitroanilide], amylase [2-Chloro-4-Nitrophenyl-α-Maltotrioxide], total bilirubin [diazonium salt], total protein [biuret], albumin [bromocresol green], iron [ferene], uric acid [uricase + Trinder reaction], magnesium [enzymatic-NADPH], calcium

[arsenazo] and phosphorus [phosphomolybdate] analysis for each sample were performed by Abbott Architect C8000 (Illinois, USA) autoanalyzer, by using original manufacturer reagents [Data in square brackets indicates the test method]. Ferritin and high sensitive (hs) Troponin I [chemiluminescent microparticle immunoassay (CMIA)] were analyzed by Abbott Architect i1000 (Illinois, USA) autoanalyzer. This was the first group, on which AA showed no interference. Data about the interfered tests (second group) [creatinine, sodium, direct bilirubin, lipase, triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol, unsaturated iron binding capacity (UIBC), glucose (POCT)] were presented in detail in Table 1.

Number of replication for each test was determined according to the following formula. [13]:

$$R = 2 \times (3.29 \times s / d_{\max})^2$$

R : number of replicates (at 95 % confidence level and 95 % power)

s : precision

$d_{\max}$: maximum allowable interference

To determine the $d_{\max}$ bias value according to the biological variation data (desirable specifications) was used for all analytes [14] except for three of them. Within-subject and between-subject biological variation of sodium is very small, so that CLIA criteria for sodium (CLIA requirement: 4 mmol/L) was employed [15]. As there is no biological variation data for UIBC, we have used American Association of Bioanalysts (AAB) criteria which is 25% [16]. Similarly, glucose (POCT) test assessment has different criteria of 12.5%, according to CLSI document POCT12-A3 Point-of-Care Blood Glucose Testing in Acute and Chronic Care Facilities [17]. We estimated the bias limit for these tests, according to these data and state of the art [18]. Eventually, $d_{\max}$ values were determined as 2.5 mmol/L for sodium, 10% for UIBC and glucose (POCT).

Each sample was analyzed, considering the replication number of each test. Scatter plots were prepared between the AA concentrations and the test results, and regression line was drawn with 95% confidence interval (CI). Then, "serum pool $\pm d_{\max}$ " concentration limits were marked on graph. Using the regression equations with 95% CI, corresponding AA levels were calculated for lower and upper concentration limits for each test. The smaller of these results was the AA concentration where significant interference started.

Interference (%) was calculated for each test at each AA level by the following formula:

$$\text{Interference (\%)} = 100 \times (\text{Sample} - \text{Pool}) / \text{Pool}$$

Interference (%) and AA concentrations were used to draw interferograms, and correlation analysis was performed between these parameters for each test.

Statistical analysis

To sum up briefly; test results were plotted against AA concentrations and a regression equation with 95% CI was obtained for each test. To determine the interfering AA concentration from each equation, we used "pool $\pm d_{\max}$ " limits. If the test results were within these limits, this indicated that there was no interference at these AA concentrations. Pearson correlation analysis was performed between interference (%) and AA concentrations. When the correlation coefficient (r) was greater than 0.7 and $p < 0.05$, the correlation was considered as strong and significant. Thus, corresponding AA concentrations, where

the interference started, were assigned; and dose dependency of this interference was proved.

Results

Creatinine, sodium and glucose (POCT) tests were found to be positively interfered by increasing AA concentrations; while direct bilirubin, lipase, UIBC, triglyceride, total cholesterol, HDL cholesterol and LDL cholesterol tests were negatively interfered (Figure 1). The other 21 chemistry tests (glucose, BUN, AST, ALT, potassium, chloride, CK, ALP, LDH, GGT, amylase, total bilirubin, total protein, albumin, iron, uric acid, magnesium, calcium, phosphorus, ferritin and hs-Troponin I) were not significantly affected by AA. Interference study was performed at 283 ng/mL and 17.3 ng/L for ferritin and hs-Troponin I, respectively. AA concentration, where interference began for each test, was presented in Table 2.

Interference (%) for the designated AA concentration for each analyte was shown in Figure 2. AA concentration and interference (%) data of the interfered tests were strongly and significantly correlated (Table 2), indicating that the interference (%) increased as the AA concentration increased.

Tests, in which oxidase/peroxidase system employed, were more affected from increasing AA concentrations. For example, interference in lipase analysis was about -50% when AA level was 200 mg/dL. Triglyceride, total cholesterol, HDL and LDL cholesterol tests were so much affected that their results fell to zero if the AA level was too high. When AA level was 24.69 (23.70–25.73 CI 95%) mg/dL, triglyceride result was 0 mg/dL; which meant the interference was -100%. Similarly AA levels of 74.39 (74.37–74.41 CI 95%), 24.69 (23.70–25.72 CI 95%) and 144.2 (140.7–147.9 CI 95%) mg/dL caused the results of total cholesterol, HDL and LDL cholesterol fall to 0 mg/dL, respectively.

Interference on glucose (POCT) results incredibly reached to 375%, at peak AA concentrations of 200 mg/dL. It was interesting that ISE analysis for sodium was interfered with AA, while potassium and chloride was not affected at all. Creatinine-AA interferogram showed a different course, as well. AA interference on creatinine increased till 25 mg/dL of AA concentrations, reached to an interference of about 8% and went steady till 150 mg/dL of AA, and after that concentration the interference increased dose dependently again (Figure 2). That was why the correlation between interference (%) on creatinine and AA concentrations was ($r=0.739$, $p=0.015$) not as strong as the other interfered tests (Table 2).

Table 1: Data about the interfered tests and study design. [R: number of replicates (at 95% confidence level and 95% power), s: precision, d_{max}: maximum allowable interference].

Analyte	Method	Manufacturer	Instrument	Pool analyte concentration	Bias (%)	d _{max}	s	R
<i>Creatinine</i>	Alkaline picrate	Abbott Laboratories (Illinois, USA)	Architect C8000	1.32 mg/dL	3.96	0.052 mg/dL	0.016 mg/dL	2
<i>Sodium</i>	Ion selective electrode	Abbott Laboratories (Illinois, USA)	Architect C8000	136 mmol/L	0.23	2.5 mmol/L	0.91 mmol/L	3
<i>Direct Bilirubin</i>	Diazo reaction	Abbott Laboratories (Illinois, USA)	Architect C8000	0.30 mg/dL	14.19	0.042 mg/dL	0.013 mg/dL	2
<i>Lipase</i>	Coupled enzyme + Trinder reaction	Abbott Laboratories (Illinois, USA)	Architect C8000	32 U/L	11.31	3.5 U/L	1.02 U/L	2
<i>Triglyceride</i>	Glycerol phosphate oxidase + Trinder reaction	Abbott Laboratories (Illinois, USA)	Architect C16000	117 mg/dL	9.57	11.2 mg/dL	2.19 mg/dL	2
<i>Total Cholesterol</i>	Cholesterol oxidase + Trinder reaction	Abbott Laboratories (Illinois, USA)	Architect C16000	171 mg/dL	4.10	7.03 mg/dL	2.6 mg/dL	3
<i>HDL Cholesterol</i>	Specific detergents + Cholesterol oxidase + Trinder reaction	Archem Diagnostics (Istanbul, Turkey)	Architect C16000	46.2 mg/dL	5.60	2.59 mg/dL	0.78 mg/dL	2
<i>LDL Cholesterol</i>	Specific detergents + Cholesterol oxidase + Trinder reaction	Archem Diagnostics (Istanbul, Turkey)	Architect C16000	111.5 mg/dL	5.46	6.09 mg/dL	1.81 mg/dL	2
<i>UIBC</i>	Ferrozine	Archem Diagnostics (Istanbul, Turkey)	Architect C16000	245 µg/dL	10	24.5 µg/dL	6.88 µg/dL	2
<i>Glucose (POCT)</i>	Glucose dehydrogenase - FAD biosensor	SD Biosensor (Gyeonggi-do, Republic of Korea)	STANDARD GlucoNavii	101 mg/dL	10	10.1 mg/dL	3.7 mg/dL	3

Discussion

High dose AA infusion has become a choice of treatment for severe COVID-19 patients in many hospitals. This medication causes some trouble in clinical chemistry testing, as high dose AA has been found to deteriorate the analytical process of some assays. An altered test result may cause misdiagnosis and inappropriate treatment [10].

In our study, we found that AA had a positive interference on creatinine and sodium analysis, similar to previous studies [19, 20]. The highest AA concentrations used in these studies were similar to our study. At peak AA concentration, about 25% increase was detected in those studies; and we found about 11% increase in creatinine concentrations. Meng et al. reported significant interference of AA on all ISE tests (sodium, potassium, chloride) and calcium [19]. However, we determined AA interference only on sodium analysis among all ISE tests, and there was no interference on calcium (arsenazo method) analysis according to our results. This was due to the differences in test methodologies and autoanalyzers, as the manufacturers were not the same. In a case report with aberrant electrolyte results, the reason for increase in ISE results

was explained with the diffusion of AA from membrane to the surface of electrode and getting oxidized there [21].

AA is one of the most commonly known antioxidant organic compounds. Thus, it interferes with especially Trinder based reactions [22]. Since the lipase assay in our laboratory was based on Trinder reaction, a significant negative interference was found. This is the first study to report AA interference on lipase assay. Negative interference of AA on triglyceride, total cholesterol, HDL and LDL cholesterol results of other studies were similar to our study [10, 19, 20]. Nevertheless, the decrease in our results was much extreme with increasing AA concentrations.

Meng et al. indicated that AA had negative interference on total bilirubin and no interference on UIBC [19]. However, we proved that AA had negative interference on direct bilirubin similar to another study [20] and negative interference on UIBC. Different manufacturers and assay methodologies might be the reason. Additionally, aforementioned previous studies were not designed according to CLSI EP07 guideline. They analysed only the statistical differences by ANOVA, paired t-test, etc. Strength of our study is the design according to CLSI EP07 guideline and wide spectrum of the chemistry tests (30 tests). 15

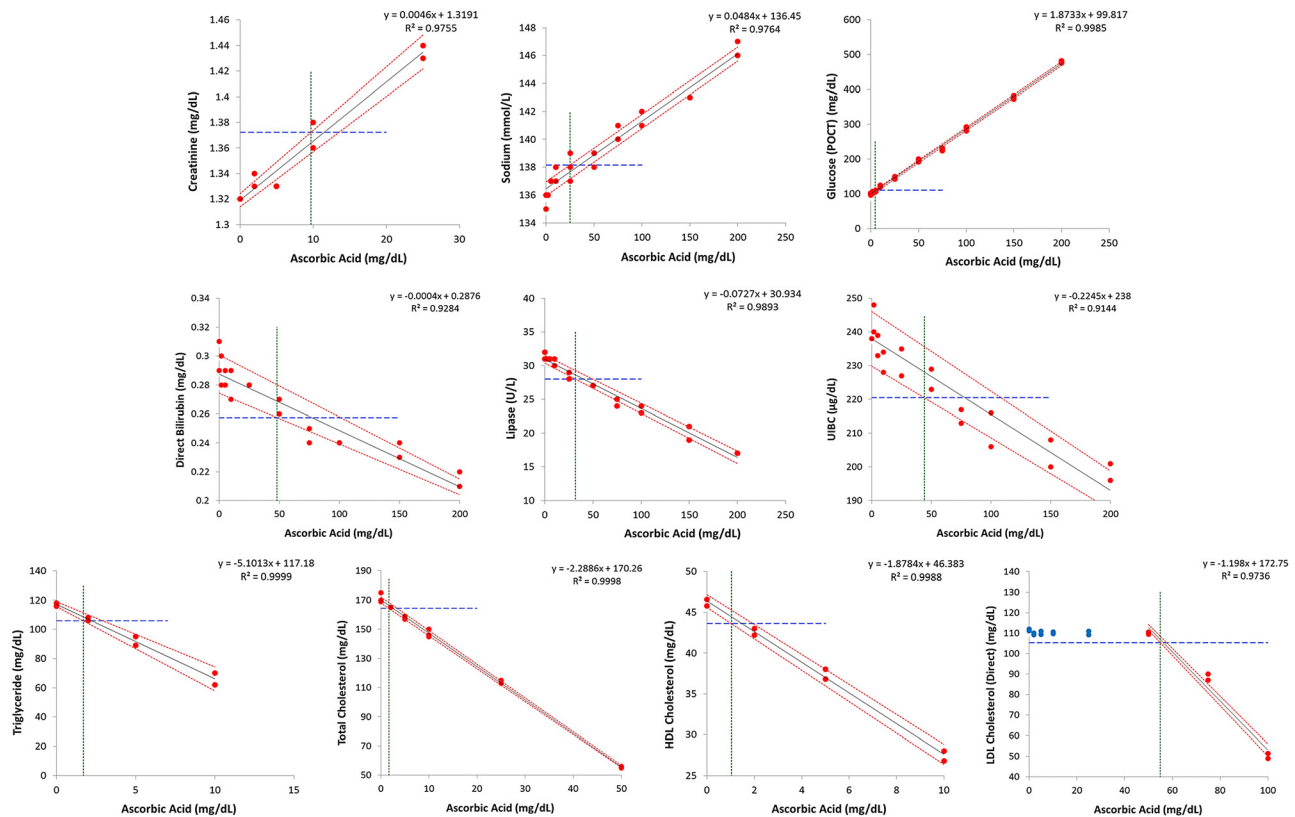


Figure 1: Scatter plots of the interfered tests against ascorbic acid (AA) concentration. [Red dashed lines indicate the confidence interval (CI) of the regression line. Blue horizontal line is the acceptable concentration of each test calculated from pool concentration and maximum allowable interference. The point where blue horizontal line intersects the regression line first (including the CI), shows the minimum interferent AA concentration, and is marked with a green vertical dashed line.] [Regression line of LDL cholesterol was started where the results began to decrease from pool concentration. Thus, blue dots in scatter plot of LDL cholesterol were not included to regression to obtain a better trend line.] [If a certain concentration of AA caused the test result to fall to zero, further concentrations of AA were not included in regression to obtain a better trend line.].

Table 2: Results of the ascorbic acid (AA) interference study.

Analyte	Interferent AA concentration (mg/dL)	Correlation of interference (%) and AA concentration	
		R	P
Creatinine	≥9.71	0.739	0.015
Sodium	≥25.08	0.988	<0.001
Direct Bilirubin	≥48.24	-0.964	<0.001
Lipase	≥31.76	-0.995	<0.001
Triglyceride	≥1.69	-0.999	<0.001
Total Cholesterol	≥1.72	-0.999	<0.001
HDL Cholesterol	≥1.03	-1.000	<0.001
LDL Cholesterol	≥54.93	-0.934	<0.001
UIBC	≥44.3	-0.956	<0.001
Glucose (POCT)	≥4.28	0.999	<0.001

commonly used chemistry tests [glucose (hexokinase), BUN, AST, ALT, CK, ALP, LDH, GGT, amylase, total protein, albumin, iron, magnesium, calcium, phosphorus,] were not found to be interfered by AA similar to the previous studies [10, 19, 20] except for calcium [19], glucose [20], ALP and phosphorus [20]. Ferritin and hs-Troponin I were not affected by AA, as well. To our knowledge, this is the first study to investigate the effect of such high concentrations of AA on hs-Troponin I and ferritin.

GlucoNavii (SD Biosensor, Republic of Korea) POCT device [using glucose dehydrogenase-flavin adenine dinucleotide (GDH-FAD) method] showed a poor performance with increasing AA concentrations. Positive interference started at 4.28 mg/dL of AA concentration. Glucose result was 101 mg/dL at AA concentration of 0 mg/dL, while it was 480 mg/dL for the same sample containing 200 mg/dL AA. Such results may cause an erroneous

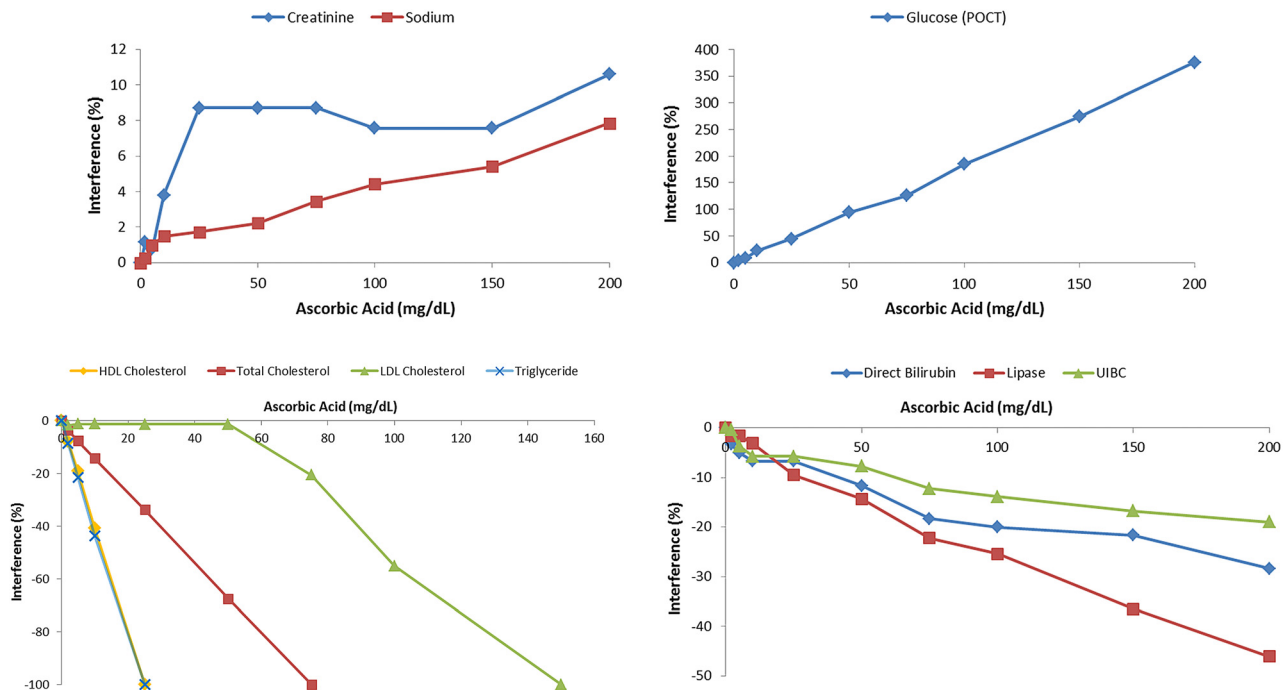


Figure 2: Interferograms of all the affected assays. [When interferences reached to -100%, further concentrations of AA were not marked on the graphs to prevent the superposition's.] [Positive and negative interferences were shown separately not to be superposed and to be seen clearly.].

insulin administration and may be life-threatening. Other studies about AA interference on glucometers also introduced similar results with us, and the reason for this situation was indicated as the increase of current on biosensor due to AA [23]. In these cases, clinicians should not make a decision based on only this result, and they should send a sample to the core laboratory. However, some manufacturers use glucose oxidase method in their reagents and auto analyzers which also is affected by AA [22]. So, the clinician or hospital information system should inform the laboratory about the given medication to the patient to achieve an accurate and reliable result. Hexokinase method is the method of choice in these cases.

There are two common methods to overcome AA interference. First one is a practical method, in which autooxidation property of AA is utilized. After keeping the sample for a certain time, AA level falls spontaneously in the sample because of autooxidation. Keeping the sample for 24 h and then reanalyzing is a choice especially for Trinder based tests [24]. For triglyceride analysis on samples of high content AA, the National Cholesterol Education Program recommends to keep sample for a certain time and then analyze, to get rid of AA interference [25]. Second method is the addition of an enzyme called AA oxidase into the reagents of interfered assays [26–28], especially the Trinder based assays.

According to the inserts provided by manufacturers; triglyceride (Abbott, Illinois, USA), HDL cholesterol (Archem, Istanbul, Turkey) and lipase (Abbott, Illinois, USA) reagents used in this study did not contain AA oxidase; while total cholesterol (Abbott, Illinois, USA) and LDL cholesterol (Archem, Istanbul, Turkey) reagents contained this enzyme. LDL cholesterol reagent had the highest amount of AA oxidase. That was why LDL cholesterol test could tolerate the highest AA concentration which was 54.93 mg/dL. AA concentrations above 145 mg/dL made LDL cholesterol result fall to zero. On the other hand, HDL cholesterol test was prone to interference by AA even at very low levels. Even physiological AA seemed to cause interference on HDL cholesterol assay. AA concentrations above 25 mg/dL made HDL cholesterol result fall to zero. With this aspect, triglyceride and HDL cholesterol reagents were similar, as triglyceride reagent did not contain AA oxidase either. Total cholesterol reagent had the lowest amount of AA oxidase. As the AA interference on total cholesterol started at 1.72 mg/dL of AA concentration, the enzyme amount seemed insufficient. This interference was detected when serum total cholesterol level was 171 mg/dL. Increasing the amount of AA oxidase in total cholesterol reagent might be considered.

Another interesting finding of this study was that there was no interference of AA, even at peak levels, on uric acid

test; although this assay employed a Trinder based method. Previous studies reported negative interference of AA on uric acid [10, 19, 20]. Since uric acid reagent (Abbott, Illinois, USA) used in our study contained AA oxidase, there was no interference. Uric acid assays which contain this enzyme or utilize UV method is not interfered by AA. With this aspect, AA oxidase may be added to the lipase reagents used in this study, which utilize Trinder reaction, as well.

In our hospital, we sometimes come across this kind of interference, as many COVID-19 patients have been hospitalized and high dose AA has been used for the severe ones. To prevent the possible interferences due to AA, there are three choices: i. collects the blood sample after minimum 12 h from i.v. 30 g AA administration, considering its elimination half-life [8] ii. if the time of blood collection is not accurate, keep the sample at least 24 h to allow AA to autooxidize iii. analyze the sample with an appropriate method which is not interfered by AA (e.g. AA oxidase containing reagents or assays with different methods). Nevertheless, be aware of the possibility of aberrant results and do not forget to consult a laboratory specialist since he is the only person to monitor the reactions in all assays.

There are some limitations of this study. The most important tests in COVID-19 evaluation include some other tests such as D-Dimer. Since we had difficulties in getting the other related reagents, we could not add them to our study. As we had not an opportunity to design this study with different methods, manufacturers, and the reference methods of the stated assays; one should consider that the interference results of this study included only the assay methods and manufacturers already available in our laboratory (Table 1). Other assay manufacturers may have different results due to the reagent and method differences.

The purpose of this study was to attract attention to this subject and prevent the erroneous decision-making. There are some data-mining studies investigating the relation between COVID-19 and its prognosis with some chemistry tests. Many studies indicated a relation between poor prognosis of COVID-19 with low HDL/LDL cholesterol and high glucose results [29–34]. These studies should be carefully and cautiously assessed, considering the possible AA medication in subjects included to study. The number of these retrospective data-mining studies is getting more and more. These studies should definitely be designed with the inclusion of a laboratory specialist, as the clinicians do not know the technical issues like interferences, assay method specifications, etc. This is very important for correct decision-making, and interpretation of the present and retrospective data accurately and efficiently.

Acknowledgments: We would like to thank Dr. Pınar Eker and Biol. Yasemin Tunakan from Public Hospital Services Directorate-2 Core Laboratory, for their technical support. FY and FKI researched literature and conceived the study. FY and FKI designed the study and analyzed data. FY wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Ethical statements: Not applicable.

Informed consent: Not applicable.

Competing interests: Authors state no conflict of interest.

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