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The Assessment of Oxidative Stress Biomarkers in Different Serum and Plasma Specimens

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ABSTRACT

Objective: Appropriate selection of the type of blood specimen tube employed is of great importance to the accurate and reliable measurement of oxidative stress biomarkers. This study examined the effect of specimen variety on measurement results using serum and plasma samples obtained from blood tubes with six different contents for measuring ischemia-modified albumin (IMA), total oxidant status (TOS), and total antioxidant status (TAS).

Materials and Methods: Ischemia-modified albumin, TOS, and TAS levels were assayed in serum and plasma specimens obtained from blood collected from 16 volunteers and placed into serum collection tubes, plain tubes, and anticoagulant tubes containing dipotassium EDTA, trisodium citrate, lithium heparin, and sodium fluoride/disodium EDTA (NaF/Na_2EDTA). IMA was measured using the Bar-Or method, and TOS and TAS were measured using TOS and TAS assay kits, respectively. Total albumin assay was performed using a Beckman Coulter AU5800 autoanalyzer.

Results: In all tubes, IMA, IMA/albumin ratio, TOS, TAS, and oxidative stress index values were compared, and all these parameters were found to be statistically significant between six different tubes (p=0.0001). Ischemia-modified albumin and TOS measurements in plasma specimens obtained from tubes containing dipotassium EDTA and NaF/Na_2EDTA differed significantly from the results of the other specimens.

Conclusion: We conclude that IMA and TOS cannot be measured from blood specimens with dipotassium EDTA and NaF/Na_2EDTA. However, TAS can be determined in all specimen types. The selection of the specimen type to be used in the measurement of IMA, TOS, and TAS is of great importance and now requires standardization.

Keywords: Anticoagulants, ischemia, oxidative stress, plasma, serum

INTRODUCTION

Oxidative stress, representing an alteration in the pro-oxidant/antioxidant balance in favor of pro-oxidants with increased reactive oxygen species, is known to be involved in the pathophysiology of chronic/degenerative diseases such as atherosclerosis, neurodegenerative disease, and cancer (1). Considerable use of oxidative stress biomarkers is today made both in scientific research and in the diagnosis, treatment, and follow-up of diseases in clinical practice. Although numerous biomarkers are associated with oxidative stress, ischemia-modified albumin (IMA) is frequently used in the literature in order to determine acute ischemic conditions, total oxidant status (TOS) parameters are used to determine total oxidant capacity, and total antioxidant status (TAS) parameters are used to determine total antioxidant capacity (2–4). The Bar-Or method has usually been employed to determine IMA levels in previous research, while TOS and TAS assay kits have been employed to determine TOS and TAS levels, respectively. Blood specimens have generally been employed for the measurement of these biomarkers. In addition, despite the use of the same method for measuring a specific parameter, assays have also been performed using different types of blood specimen. Serum specimens (from serum collection tubes) and plasma specimens (from dipotassium EDTA, lithium heparin, and sodium fluoride/disodium EDTA (NaF/Na_2EDTA)) have been used for IMA measurement. Additionally, serum specimens (from serum collection tubes) and plasma specimens (from lithium heparin and dipotassium EDTA) have all been used for TOS and TAS assays (5–7). Therefore, there is a significant possibility of discrepancies arising between measurements depending on the use of different specimen tubes. This may, in turn, reduce reliability in the results and make it difficult to compare study findings with previously reported results (8).

The purpose of this study, planned in the light of these concerns, was to examine the effects of specimen types on IMA, TOS, and TAS level results obtained in serum and plasma specimens elicited from six different blood tubes (serum collection tubes, plain tubes, and anticoagulant tubes containing dipotassium EDTA, trisodium citrate, lithium heparin, and NaF/Na_2EDTA). We think that the study data will make a significant contribution to the accurate selection of the most appropriate type of specimen to be used in the measurement of oxidative stress biomarkers and to the most reliable results being obtained.

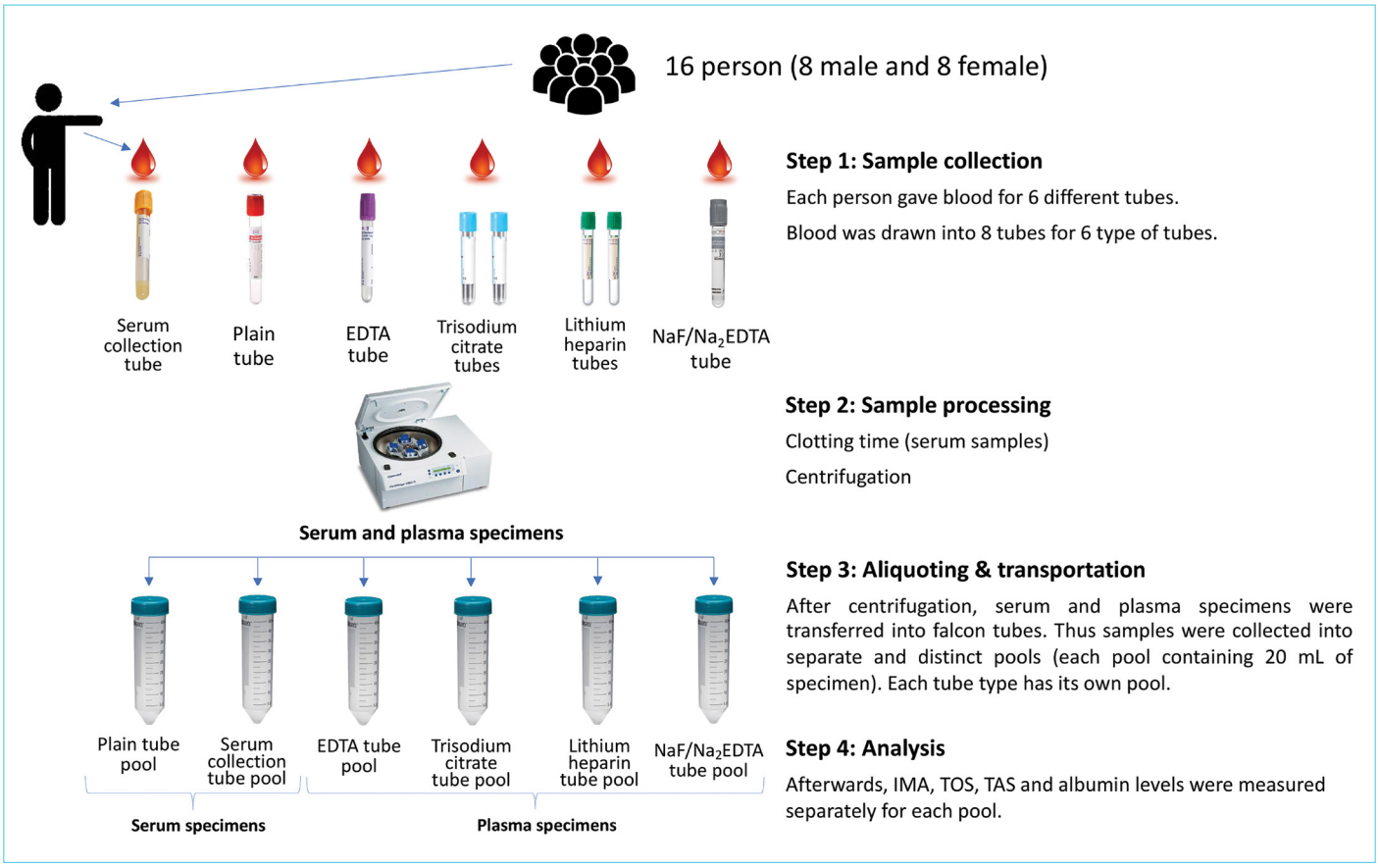

Figure 1. Schematic of the study design and experimental workflow
IMA: Ischemia-modified albumin; TOS: Total oxidant status; TAS: Total antioxidant status

MATERIALS and METHODS

Design and Setting

The study was performed at the Karadeniz Technical University Faculty of Medicine Department of Medical Biochemistry, Turkey. Blood specimens were collected from 16 healthy volunteers (eight males and eight females, aged 26–35 years).

Collection and Preparation of Samples

Blood specimens were collected by a single specialist phlebotomist, between 08.00 and 09.00 a.m. after overnight fasting in order to avoid diurnal variations, particularly in case of oxidative stress parameters. All volunteers assumed a seated position for 15 min prior to phlebotomy in order to eliminate potential posture-related interference in blood distribution. At the end of this period, approximately 24 mL of blood was collected from a single donor via venipuncture using a 21-gauge needle (Vacusera, Disera, Cat. No. 250021K, Izmir, Turkey) and was directly placed into eight tubes (six different types), in the same order for each donor: one 4.0 mL plain tube (Vacutube, Cat. No. VP40031S, Selangor, Malaysia), one 5.0 mL serum collection tube (BD Vacutainer, Cat. No. 367955, Plymouth, UK), two 1.8 mL trisodium citrate tubes (BD Vacutainer, Cat. No. 363047, Plymouth, UK), two 2.0 mL lithium heparin tubes (BD Vacutainer, Cat. No. 368494, Plymouth, UK), one 3.0 mL dipotassium EDTA tube (BD Vacutainer, Cat. No. 368856, Plymouth, UK), and one 4.0 mL NaF/Na_2EDTA tube (BD Vacutainer, Cat. No. 368520, Plymouth, UK) (9). Trisodium citrate (1.8 mL) and lithium heparin (2.0 mL) tubes have limited volume. Therefore, blood was taken into these tubes twice in order to provide sufficient sample. Blood was drawn into eight tubes for six types of tubes. This was done in order to obtain a sufficient sample volume for repeated measures without requiring a new pool. Blood collection was performed using the vacutainer system. The same amount of blood was placed into the same tubes. Plasma tubes were centrifuged immediately, whereas the serum tubes were stored upright for 30–45 min at room temperature for clotting and were centrifuged at the end of that period. All samples were centrifuged at 1800 g for 10 min at room temperature. No hemolysis or lipemia was observed in any specimens following separation of serum and plasma. All serum and plasma specimens were transferred into separate sterile 50 mL polypropylene tubes (Falcon, Cat. No. 352070, New York, USA) using an automatic pipette within 30 min. Samples obtained from plain, serum collection, dipotassium EDTA, lithium heparin, trisodium citrate, and NaF/Na_2EDTA tubes were thus collected into separate and distinct pools (each pool contained 20 mL of specimen). At the end of this process, total albumin, IMA, TOS, and TAS values in the specimens were measured immediately (without freezing). Figure 1 shows the schematic of the study design and experimental workflow.

Measurements were repeated 20 times for each test (20 technical replicates per sample), and the repeatability of tests was observed by calculating intraassay repeatability (CV%) values.

Measurement of IMA

Ischemia-modified albumin levels were analyzed using the rapid and colorimetric method developed by Bar-Or et al. (10). Briefly, 200 µL of serum and plasma was placed into plastic vials, to which 0.1% of 50 µL cobalt chloride (Sigma-Aldrich, Cat. No. C8661, Saint Louis, MO, USA) was then added and gently shaken. The mixture was allowed to stand for 10 min to ensure sufficient albumin binding to cobalt. Next, 50 µL of 1.5 mg/mL dithiothreitol (DTT) (Sigma-Aldrich, Cat. No. D5545, Saint Louis, MO, USA) was added as a coloring agent. After being allowed to stand for 2 min, 1 mL of 0.9% NaCl was added to stop the reaction. Blind sampling was performed for each specimen. Each serum and plasma cobalt blind sample without DTT was prepared by adding 50 µL of distilled water instead of 50 µL of 1.5 mg/mL DTT during the DTT addition stage. Specimen absorbances were measured at a wavelength of 470 nm on a spectrophotometer (Shimadzu UV-1601 PC, Auburn, Australia) and recorded. Color formation in specimens with DTT was compared with that in the blind tubes, and the results were expressed as absorbance units (ABSU). CV% values for this method were 5.2% serum from serum collection tubes, 8.9% serum from plain tubes, 8.3% trisodium citrate plasma, and 6.4% lithium heparin plasma, while dipotassium EDTA plasma and NaF/Na_2EDTA plasma were below limits of detection.

Total albumin levels in specimens were assayed before every measurement. Total albumin assays were performed using the bromocresol green method (colorimetric) in the biochemistry laboratory on an automated system (Beckman Coulter AU5800, Shizuoka, Japan). CV% values for this method were 0.7% serum from serum collection tubes, 0.4% serum from plain tubes, 0.2% dipotassium EDTA plasma, 1.2% trisodium citrate plasma, 0.4% lithium heparin plasma, and 1.1% NaF/Na_2EDTA plasma. In order to prevent changes in the contents of total albumin affecting IMA levels, IMA/albumin ratio (IMAR) was calculated using the formula IMAR=IMA/total albumin, and the IMA results were standardized.

Determination of TOS

Total oxidant status levels in blood specimens were assayed on the basis of colorimetric measurement TOS assay kits (Rel Assay, Cat. No. RL0024, Gaziantep, Turkey). The measurement principle of these kits relies on the conversion of Fe^{2+} to Fe^{3+} with different oxygen species in an acidic environment and the measurement at 530 nm of the color changed caused by Fe^{3+} with xylenol orange. The measurements were performed on a microplate spectrophotometer (Molecular Devices VersaMax, CA, USA). The standard concentration is 10 µmol H_2O_2 equivalent/L (11). The results were expressed as µmol H_2O_2 equivalent/L. CV% values for this assay were 5.7% serum from serum collection tubes, 7.8% serum from plain tubes, 14% trisodium citrate plasma, and 14% lithium heparin plasma, while dipotassium EDTA plasma and NaF/Na_2EDTA plasma were below limits of detection.

Determination of TAS

Total antioxidant status assay kits (Rel Assay, Cat. No. RL0017, Gaziantep, Turkey) based on colorimetric measurement were used for TAS assay. The measurement principle of these kits relies on the photometric determination of the conversion by antioxidants in the specimen of the dark blue–green ABTS (2,2′-azino-di-[3-ethylbenzthiazoline sulphonate]) radical into the colorless ABTS form. The total antioxidant capacity in a specimen is inversely proportional to the color intensity measured at 660 nm on a microplate spectrophotometer (Molecular Devices VersaMax, CA, USA). The standard solution in this method was prepared with trolox. The standard concentration is 1.0 mmol trolox equivalent/L, and the results were expressed as mmol/L trolox equivalent (12). CV% values for this assay were 5.5% serum from serum collection tubes, 6.1% serum from plain tubes, 6.9% dipotassium EDTA plasma, 8.9% trisodium citrate plasma, 8.7% lithium heparin plasma, and 6.9% NaF/Na_2EDTA plasma.

OSI Calculation

Total oxidant status and TAS were used in the calculation of OSI% values. TAS value units were converted from mmol trolox equivalent/L into µmol trolox equivalent/L and were then calculated using the following formula (13):

OSI%=[(TOS, µmol H_2O_2 equivalent/L)/(TAS, µmol trolox equivalent/L)] × 100

Statistical Analysis

Values for all groups were expressed as mean±standard deviation. Statistical analysis was performed on Statistical Package for the Social Sciences (version 23.0, NY, USA) statistical software. Compatibility with normal distribution was assessed using the Kolmogorov–Smirnov test for all groups. Since the results were normally distributed, parametric tests were then applied. Group differences were determined using a one-way repeated-measures analysis of variance (ANOVA) for repeatedly measured continuous variables. If the main-effects F-ratio was significant, differences among groups were subsequently identified using a Bonferroni posthoc analysis. Values of $p<0.05$ were regarded as statistically significant.

RESULTS

Oxidative Stress Biomarker Levels in Serum and Plasma Specimens

Ischemia-modified albumin, IMAR, TOS, TAS, and OSI values determined in serum (from serum collection and plain tubes) and plasma (from dipotassium EDTA, trisodium citrate, lithium heparin, and NaF/Na_2EDTA tubes) are shown in Table 1. In all tubes, IMA, IMAR, TOS, TAS, and OSI values were compared, and all these parameters were found to be statistically significant between six different tubes ($p=0.0001$).

Comparison of IMA, IMAR, TOS, TAS, and OSI Values of Serum Samples Obtained from Serum Collection Tubes with Other Serum and Plasma Specimens

Ischemia-modified albumin, IMAR, and OSI values differed significantly from those measured in serum (from plain tubes) ($p=0.014$, $p=0.003$, and $p=0.002$, respectively).

Ischemia-modified albumin, IMAR, and OSI values differed significantly from those measured in plasma specimens containing dipotassium EDTA, lithium heparin, and NaF/Na_2EDTA ($p=0.0001$).

Ischemia-modified albumin, IMAR, and OSI values differed significantly from those measured in plasma specimens containing trisodium citrate ($p=0.0001$, $p=0.0001$, and $p=0.007$, respectively).

Table 1. Oxidative stress biomarker values in serum and plasma specimens from six different types of blood tube

Parameter	Blood tubes						
	Serum collection Mean±SD	Plain Mean±SD	Dipotassium EDTA Mean±SD	Trisodium citrate Mean±SD	Lithium heparin Mean±SD	NaF/Na_2EDTA Mean±SD	p
IMA	0.396±0.020	0.361±0.030	0.005±0.003	0.256±0.021	0.517±0.033	0.005±0.002	0.0001
IMAR	0.084±0.004	0.075±0.006	0.001±0.0006	0.063±0.004	0.110±0.007	0.001±0.0005	0.0001
TOS	1.398±0.080	1.534±0.120	0.099±0.060	1.547±0.218	1.937±0.270	0.062±0.042	0.0001
TAS	1.887±0.103	1.840±0.112	2.04±0.142	1.725±0.151	1.729±0.151	1.892±0.131	0.0001
OSI	0.074±0.006	0.083±0.008	0.004±0.002	0.090±0.016	0.113±0.020	0.003±0.002	0.0001

SD: Standard deviation; EDTA: Ethylenediaminetetraacetic acid; IMA: Ischemia-modified albumin; IMAR: IMA/albumin ratio; TOS: Total oxidant status; TAS: Total antioxidant status; OSI: Oxidative stress index. P shows differences between the tubes according to repeated-measures ANOVA. Values of $p<0.05$ were regarded as statistically significant

Table 2. Comparison of IMA, IMAR, TOS, TAS, and OSI p values of serum samples obtained from serum collection tubes with other serum and plasma specimens

Parameter	Blood tubes				
	Serum collection				
	p, Plain	p, Dipotassium EDTA	p, Trisodium citrate	p, Lithium heparin	p, NaF/Na_2EDTA
IMA	0.014	0.0001	0.0001	0.0001	0.0001
IMAR	0.003	0.0001	0.0001	0.0001	0.0001
TOS	0.004	0.0001	0.171	0.0001	0.0001
TAS	1.000	0.027	0.001	0.047	1.000
OSI	0.002	0.0001	0.007	0.0001	0.0001

EDTA: Ethylenediaminetetraacetic acid; IMA: Ischemia-modified albumin; IMAR: IMA/albumin ratio; TOS: Total oxidant status; TAS: Total antioxidant status; OSI: Oxidative stress index. P shows differences between the tubes according to repeated-measures ANOVA followed by Bonferroni's posthoc test

Total antioxidant status values differed significantly from those measured in plasma specimens containing dipotassium EDTA, trisodium citrate, and lithium heparin (p=0.027, p=0.001, and p=0.047, respectively).

Total oxidant status values were also significantly different from those measured in serum (from plain tubes) and plasma specimens containing dipotassium EDTA, lithium heparin, and NaF/Na_2EDTA (p=0.004, p=0.0001, p=0.0001, and p=0.0001, respectively). All results are presented in Table 2.

Comparison of IMA, IMAR, TOS, TAS, and OSI Values of Serum Samples Obtained from Plain Tubes with Plasma Specimens

Ischemia-modified albumin and IMAR values were significantly different from those measured in plasma specimens containing dipotassium EDTA, trisodium citrate, lithium heparin, and NaF/Na_2EDTA (p=0.0001).

Total antioxidant status values differed significantly from those measured in plasma specimens containing dipotassium EDTA and trisodium citrate (p=0.001 and p=0.025, respectively).

Total oxidant status and OSI values were significantly different from those measured in plasma specimens containing dipotassium EDTA, lithium heparin, and NaF/Na_2EDTA (p=0.0001). All results are presented in Table 3.

Comparison of IMA, IMAR, TOS, TAS, and OSI Values of Plasma Samples Obtained from Four Different Anticoagulant Blood Tubes

Ischemia-modified albumin, IMAR, TOS, TAS, and OSI values measured in plasma containing dipotassium EDTA were significantly different from those measured in plasma containing trisodium citrate and lithium heparin (p=0.0001).

Ischemia-modified albumin, IMAR, and TOS values measured in plasma containing trisodium citrate were significantly different from those measured in plasma containing lithium heparin (p=0.0001, p=0.0001, and p=0.004, respectively).

Ischemia-modified albumin, IMAR, TOS, and OSI values measured in plasma containing trisodium citrate were significantly different from those measured in plasma containing NaF/Na_2EDTA (p=0.0001).

Ischemia-modified albumin, IMAR, TOS, TAS, and OSI values measured in plasma containing lithium heparin were significantly

Table 3. Comparison of IMA, IMAR, TOS, TAS, and OSI p values of serum samples obtained from plain tubes with plasma specimens

	Blood tubes			
	Plain			
Parameter	**p, Dipotassium EDTA**	**p, Trisodium citrate**	**p, Lithium heparin**	**p, NaF/Na_2EDTA**
IMA	0.0001	0.0001	0.0001	0.0001
IMAR	0.0001	0.0001	0.0001	0.0001
TOS	0.0001	1.000	0.0001	0.0001
TAS	0.001	0.025	0.405	1.000
OSI	0.0001	1.000	0.0001	0.0001

EDTA: Ethylenediaminetetraacetic acid; IMA: Ischemia-modified albumin; IMAR: IMA/albumin ratio; TOS: Total oxidant status; TAS: Total antioxidant status; OSI: Oxidative stress index. P shows differences between the tubes according to repeated measures ANOVA followed by Bonferroni's post hoc test. P<0.05 were regarded as statistically significant

Table 4. Comparison of IMA, IMAR, TOS, TAS, and OSI p values of different plasma specimens

	Blood tubes					
	Dipotassium EDTA			Trisodium citrate		Lithium heparin
Parameter	**p, Trisodium citrate**	**p, Lithium heparin**	**p, NaF/Na_2EDTA**	**p, Lithium heparin**	**p, NaF/Na_2EDTA**	**p, NaF/Na_2EDTA**
IMA	0.0001	0.0001	1.000	0.0001	0.0001	0.0001
IMAR	0.0001	0.0001	1.000	0.0001	0.0001	0.0001
TOS	0.0001	0.0001	0.601	0.004	0.0001	0.0001
TAS	0.0001	0.0001	0.170	1.000	0.010	0.008
OSI	0.0001	0.0001	1.000	0.065	0.0001	0.0001

EDTA: Ethylenediaminetetraacetic acid; IMA: Ischemia-modified albumin; IMAR: IMA/albumin ratio; TOS: Total oxidant status; TAS: Total antioxidant status; OSI: Oxidative stress index. P shows differences between the tubes according to repeated measures ANOVA followed by Bonferroni's post hoc test. P<0.05 were regarded as statistically significant

different from those measured in plasma containing NaF/Na_2EDTA (p=0.0001, p=0.0001, p=0.0001, p=0.008, and p=0.0001, respectively). All results are presented in Table 4.

DISCUSSION

Oxidative stress is currently the subject of considerable interest and research since it has been linked to numerous diseases (1). Blood specimens are commonly employed in studies of oxidative stress. However, our review of the literature revealed numerous studies using different types of blood specimen for the same marker assayed using the same method. Divergent and inconsistent results have therefore been reported (14–17). The procedure of blood collection for analysis is a very important factor and is responsible for a significant proportion of preanalytic errors. A blood specimen being placed into an incorrect tube can give rise to a number of undesirable outcomes such as additional blood collection, loss of time, extra workload, increased cost, and reduced confidence in the results (18). This makes it impossible to compare the results of studies of oxidative stress biomarkers. These difficulties indicate the need for standardization of the blood specimens employed in the measurement of oxidative stress biomarkers. This study therefore investigated the effects on oxidative stress biomarker assay results of different blood specimen types (in blood specimens with six different contents).

Ischemia-modified albumin assay in serum and plasma specimens obtained from blood tubes with six different contents was performed using the method described by Bar-Or et al. (10), and IMAR was calculated from these. IMA values measured in sera (from serum collection tubes) and in heparin-containing plasma specimens obtained from healthy individuals were roughly similar to those measured in sera (from serum collection tubes) and in heparin-containing plasma specimens in control data from studies in the literature (Table 5) (14, 16, 19). IMA measurements in serum specimens obtained from plain tubes were significantly lower than serum results obtained from serum collection tubes (Table 5). This difference between IMA levels in serum specimens from tubes with different contents was detected for the first time in the present study, and our review of the literature revealed no similar findings. This is an interesting phenomenon and requires further investigation. Serum collection tubes contain a certain quantity of clot activator particles in addition to the separator (8). The difference in IMA results in serum specimens from the different tube types may derive from the separator and clot activator contained.

In our studies performed using the Bar-Or method in blood specimens with different contents, IMA levels in plasma specimens obtained from blood placed into tubes containing dipotassium EDTA and NaF/Na_2EDTA were significantly lower, and even at undetect-

Table 5. Comparison of our findings with ischemia-modified albumin control data in the literature

IMA									
Serum collection			Lithium Heparin		Dipotassium EDTA			NaF/Na_2EDTA	
Our findings	Doğan et al. (control)(15)	Atik et al. (control)(20)	Our findings	Hazini et al. (control)(17)	Our findings	Dahiya et al. (control)(16)	Işık et al. (control)(21)	Our findings	D'souza et al. (control)(18)
0.396±0.020	0.360±0.040	0.447±0.010	0.517±0.030	0.347±0.050	0.005±0.003	0.325±0.010	0.790±0.090	0.005±0.002	0.425±0.120

Values are expressed as mean±standard deviation (SD). IMA: Ischemia-modified albumin; EDTA: Ethylenediaminetetraacetic acid

Table 6. Comparison of our findings with total oxidant status and total antioxidant status control data in the literature

	Serum collection		Lithium heparin		Dipotassium EDTA	
	Our findings	Güven et al. (control)(5)	Our findings	Dilek et al. (control)(6)	Our findings	Bukhari et al. (control)(7)
TOS	1.398±0.080	0.745±0.281	1.937±0.270	6.300±3.500	0.099±0.060	5.760±0.140
TAS	1.887±0.103	1.698±0.731	1.729±0.151	2.700±0.600	2.040±0.142	1.480±0.030

Values are expressed as mean±SD. TOS: Total oxidant status; TAS: Total antioxidant status; TOS: μmol H_2O_2 equivalent/L; TAS: mmol trolox equivalent/L

able levels, compared to control levels in dipotassium EDTA- and NaF/Na_2EDTA-containing plasma specimens in the literature (Table 5) (15, 17, 20). Although we used the same method as that employed in previous studies and repeated each measurement at least 20 times, our IMA values in plasma specimens containing both dipotassium EDTA and NaF/Na_2EDTA were extremely low. We particularly attribute this to the potential effect of the anticoagulant content of both tube types. Both dipotassium EDTA- and NaF/Na_2EDTA-containing tubes contain specific quantities of EDTA. EDTA is one of the most powerful known chelators, and it binds Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, and Fe^{2+} ions (21, 22). When IMA is measured using the Bar-Or method, the Co^{2+} ions that are further added during the experiment probably form a complex with EDTA and are removed from the environment. Photometric IMA measurement is therefore impossible since insufficient free Co^{2+} ions remain to produce color by interaction with DTT. Exceedingly low IMA levels in plasma specimens containing dipotassium EDTA and NaF/Na_2EDTA may therefore be expected, and we are doubtful of the results reported from other studies. These findings once again show the importance of the selection and standardization of blood specimens to be used in the measurement of oxidative stress biomarkers.

The mean IMA level in plasma specimens separated from tubes containing trisodium citrate was 0.256±0.021 ABSU. This differed significantly from the values in serum and other plasma specimens. We also assayed total albumin levels in serum and plasma specimens. Similarly to IMA, we observed significantly lower total albumin levels in plasma specimens separated from tubes with trisodium citrate compared to serum specimens. We think that this suppression of total albumin levels in specimens with trisodium citrate may also have affected IMA levels. We therefore conclude that in order to reduce the effect of total albumin levels on IMA results to a minimum, the results need to be standardized by means of IMAR calculation. However, since there are no data available for IMA and/or IMAR values in trisodium citrate-containing plasma in the literature, we were unable to perform a detailed analysis and comparison.

Total oxidant status and TAS assays were also performed in this study, and OSI was calculated therefrom, using TOS and TAS assay kits in serum and plasma specimens obtained from blood tubes with six different contents. Our TOS and TAS values measured in both serum (from serum collection tubes) and plasma specimens containing lithium heparin were roughly similar to control data measured in serum (from serum collection tubes) and lithium heparin-containing plasma specimens in the existing literature (5, 6). However, at TOS and TAS assays using the relevant assay kits in plasma specimens containing both dipotassium EDTA and NaF/Na_2EDTA, only TOS values were very much lower than results obtained from other tubes (7). TAS measurement was found to be possible in all specimens (Table 6). Fe^{2+} ions used in the TOS assay kit and added to the assay environment will be removed from that environment by forming a complex with EDTA in plasma specimens, and measurement may therefore be expected to occur at low levels. This in all likelihood explains our exceedingly low TOS levels in specimens containing dipotassium EDTA and NaF/Na_2EDTA. We regard the much higher TOS results reported in other studies using the same method and sample type as in the present study with caution owing to the interfering effect of EDTA, and we again think that these findings require confirmation.

Total oxidant status and TAS were also measured in plasma specimens separated from tubes containing trisodium citrate, with mean values of 1.547±0.218 and 1.725±0.151 being determined, respectively. These values differed from those measured in serum specimens, but since there are no available data for TOS and TAS values in plasma samples containing trisodium citrate, no detailed analysis was possible.

Oxidative stress index is a marker of oxidative stress encountered by living organisms and is calculated as the ratio of TOS to TAS. OSI values calculated in our study were compatible with TOS values and were therefore exceedingly low in plasma specimens with dipotassium EDTA and NaF/Na_2EDTA.

Oxidative stress biomarkers are frequently employed in research and also in the clinical setting, and blood samples are widely employed for measurement. However, the most suitable blood specimen for the particular oxidative stress marker to be measured is unclear, and there is once again no standardization on the subject. Our review of the literature revealed no studies examining the effects of serum and plasma specimens obtained from different blood tubes on oxidative stress parameter measurement results. The present study was planned accordingly, and levels of oxidative stress parameters (IMA, TOS, and TAS) measured in serum and plasma specimens from different blood tubes differed significantly from one another. We conclude that IMA measurement using the Bar-Or method and TOS measurement using TOS assay kits in plasma samples containing dipotassium EDTA and NaF/Na_2EDTA are not appropriate for use. In conclusion, the selection of the type of blood specimen to be used in the measurement of the biomarkers IMA, TOS, and TAS is extremely important, and we think that this now needs to be standardized if accurate and reliable test results are to be obtained.

Ethics Committee Approval: The Karadeniz Technical University Faculty of Medicine Ethical Committee granted approval for this study (date: 24.03.2017, number: 24237859-190).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – ME, SCK, AM; Design – ME, SCK, AM, SÖY, SD; Supervision – SCK; Resource – SCK, AM; Materials – SCK, AM, SD; Data Collection and/or Processing – ME, SCK; Analysis and/or Interpretation – ME, SÖY; Literature Search – ME, SÖY, SD; Writing – ME; Critical Reviews – SCK.

Conflict of Interest: The authors have no conflict of interest to declare.

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