Biomarkers of Oxidative Stress

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Abstract

Free radicals (FRs) and/or reactive oxygen species (ROS) are bioactive substances generated inevitably during the metabolic process of organisms. To combat excessive free radical and/or reactive oxygen production, living organisms have evolved many sophisticated peroxide-antioxidant defense systems. These systems are located in a dynamic equilibrium state under normal physiological conditions, while the body antioxidant system could be unbalanced and lead to oxidative stress in pathological states. Oxidative stress is closely related to the occurrence and development of various diseases, including cancer. Therefore, FRs and/or ROS involved in pathological reactions can be used as markers of oxidative stress. Although most oxidation-antioxidant markers are not difficult to be measured by modern medical detection technology separately, the detection of each oxidation-antioxidant substance is not only time- and energy-consuming, but also inaccurate. One of the reasons for inaccuracy is the incomplete understanding and detection of oxidation-antioxidant substances in the organism. The other is the superposition effect produced by various oxidation-antioxidant substances which have a synergistic effect in the same system. In view of this, only combined total oxidant status (TOS) with total antioxidant status (TAS) and oxidant stress index (OSI) can accurately assess the oxidant stress status of subjects. In this chapter, the species of single oxidation or antioxidant, TOS, TAs and OSI, and the determination method of end products of lipid hydroxides (malondialdehyde and 4-hydroxynonene) are introduced.

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Free radicals (FR) and reactive oxygen species (ROS) are naturally produced in all aerobic organisms [1]. On the one hand, FR and ROS perform many normal physiological functions in the body. On the other hand, excessive generation of FR and ROS may attack biological macromolecules, resulting in oxidative damage to the body. It has been confirmed that intracellular oxidative damage is mainly caused by FR and ROS [2]. FR can inhibit the function of many components in normal cells, react with unsaturated bonds in membrane lipids, denature proteins, and damage nucleic acids, etc. The metabolic form of ROS and the scavenging rate of the body's antioxidants constitute the peroxidation-antioxidant system of the body. Under physiological conditions, the system is in a state of dynamic equilibrium. But in the case of diseases (such as inflammation, trauma, infection, and cancer), the former's generation is faster than the latter's clearance, disordering the dynamic balance [3]. The subsequent body response leads to oxidative stress (OxS) state, resulting in oxidative damage of biological macromolecules such as proteins, lipids, and nucleic acids, interfering with normal life activities. Therefore, OxS refers to when the body encounters various harmful stimuli, highly active molecules in the body, such as ROS and reactive nitrogen species (RNS) excessively produced, and at the same time, the degree of oxidation exceeds the removal of oxides, causing an imbalance of oxidation-antioxidant system, which leads to oxidative damage of biological macromolecules such as proteins, lipids, and/or nucleic acids, and interferes with normal life activities, resulting in a serious state of antioxidant stress.

32.1 Single Oxidation or Antioxidant

Aerobic organisms have evolved an antioxidant defense system that removes FR and ROS. The system can be divided into three levels [4]: ① primary antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), glutathione S-transferase (GSH), paraoxonase 1 (PON1) enzyme, whose function is to prevent the production of new FR and/or ROS; ② secondary antioxidants, such as vitamins (Vit) A, Vit C, Vit E, uric acid, glutathione, α-lipoic acid, carotene, trace elements copper, zinc, manganese, and selenium, whose function is to clear FR and/or ROS before FR and/or ROS trigger lipid peroxidation chain reaction; ③ tertiary antioxidants, such as DNA repair enzymes and methionine oxysulfide reductase, whose function is to repair nucleic acid chains damaged by FR and/or ROS oxidation and maintain the normal physiological function of cells. However, in some pathological conditions, the body cannot defense against the increase of oxidations or decrease of antioxidants, and the balance between oxidation and

antioxidation is transformed to the oxidative state, which will inevitably lead to OxS reaction.

All biochemical antioxidants involved in the body's antioxidant defense system are biomarkers of OxS. In order to evaluate the antioxidant status in vivo, it is necessary to detect the antioxidants in the body. These antioxidants such as SOD, GSH-Px, CAT, GSH, PON1, Vit A, Vit C, Vit E, and uric acid in the oxidation defense system all can be detected separately. In the past, many researchers have often used these indicators to reflect the body's antioxidant status. However, due to the tremendous number of different antioxidants in plasma, serum, urine, or other biological samples, it is difficult to implement a single determination of various antioxidants.

In the body's oxidation-antioxidation system, the opposite of antioxidants is oxidations, which are mainly FR and oxidants. The FR that can be generated in organisms by enzymatic and/or nonenzymatic reactions include ① ROS, such as $\cdot O_2$-, $\cdot OH_2$; ② RNS, such as ·NO, $\cdot NO_2$, and ·ONOO-. Commonly mentioned oxidants are $^1O_2$, H_2O_2, etc. The FR and oxidants often mentioned in the study are shown in Table 32.1. Strictly speaking, $^1O_2$ and H_2O_2 are not oxygen free radicals, but active oxygen.

The following oxidizing substances are all OxS biomarkers (Table 32.2), but oxidizing substances are not easier to detect than anti-oxidizing substances, and are more difficult to detect individually.

Tables 32.3 and 32.4 list the biomarkers of oxidative stress commonly used in clinical or scientific research or reported in the literature (except for the nitro-oxidative stress class). Among them, many markers have been commonly recognized as OxS biomarkers.

32.2 Total Oxidant Status and Total Antioxidant Status

There are two types of antioxidant systems in the human body. One is the enzyme antioxidant system, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), and the other is the nonenzyme antioxidant

Table 32.1 Oxyradical abbreviation and terminology

Abbreviation	Terminology
$O_2^{\cdot-}$	Superoxide anion radical
$HO_2^{\cdot}$	Hydrogen peroxide radical
H_2O_2	Hydrogen peroxide
HO·	Hydroxyl radical
RO·	Oxygen organic free radical
ROO·	Organic peroxide radical
$^1O_2$	Singlet oxygen
ROS	Reactive oxygen species
L·	Lipid free radical
LOO·	Lipid peroxide radical

Table 32.2 Reactive oxygen species with the significance of oxidative stress

Species	Terminology	Characteristics
$O_2^{\cdot-}$	Superoxide anion	The single electron reduction state; formed by many oxygen reactions (such as flavin protein, redox cycle)
$HO_2^{\cdot}$	Hydrogen peroxy	Formed by the protonation of $O_2^{\cdot-}$; enhanced fat solubility
H_2O_2	Hydrogen peroxide	Two-electron reduced state; formed by disproportionation of $O_2^{\cdot-}\left(HO_2^{\cdot}\right)$, or directly formed by O_2
HO·	Hydroxyl radical	Three-electron reduction state; formed by Fenton reaction and metal-catalyzed Haber–Weiss reaction; is highly active
RO·	R-oxygen radical, alkoxy radical	Oxygen organic free radicals (such as lipids)
ROO·	R-Peroxy radical, alkyl peroxy radical	Formed from organic hydroperoxide (ROOH), such as lipid, by hydrogen extraction (or homolysis)
ROOH	R-hydroperoxide	Organic hydroperoxides (such as fatty acids and thymine hydroperoxides)
O_2^* or $^1O_2$	Singlet oxygen	First excitation; higher than ground state oxygen (O); red (bimolecular) or infrared (monomolecular) light emission
^{3}R'R"CO ($R'R"CO^*$)	Triplet carbonyl	Excited carbonyl compounds, blue-green light emission (i.e. via dioxane intermediates)

system, including Vit C, Vit E, glutathione, melatonin, alpha-lipoic acid, carotenoids, trace elements copper, zinc, selenium, etc. With existing biochemical and/or molecular biology technologies, most of the antioxidants in the antioxidant system can be independently detected. However, detection of each antioxidant separately is time-consuming, labor-intensive, expensive, complicated, and inaccurate. The reason for this inaccuracy is that the antioxidants have a synergistic effect in the same system and will produce a superimposed effect. Moreover, both the oxidizing substance and the anti-oxidizing substance have what we know and we have not yet known. Therefore, the determination of one and/or several oxidative or antioxidant substances or their metabolites alone cannot correctly evaluate the oxidative or antioxidant status of the body. In addition, oxidative/antioxidant substances can be divided into known and unknown. Using existing inspection methods, the known oxidation/antioxidants can be detected, but it is time-consuming and laborious, and the unknown ones still cannot be detected. Otherwise, the effects of different oxidation/antioxidants can be superimposed, and the detection of only a few oxidative/anti-oxidant substances does not represent a change in overall levels, because the changes of other oxidative/anti-oxidant substances are not clear.

In view of this, the concepts of total antioxidant status (TAS) and total oxidant status (TOS) came into being. TAS represents the overall level of enzymes and nonenzyme antioxidants in the organism. It is also called total antioxidant capacity (TAC), total antioxidant activity (TAA), total antioxidant power (TAOP), total antioxidant response (TAR), total reactive antioxidant potential (TRAP), etc. TAS

Table 32.3 OxS biomarkers commonly used in clinical practice or research (antioxidants)

Abbreviation	Full name	Abbreviation	Full name
AOPP	Advanced oxidation protein products	Mel	Melatonin
ALA	α-Lipoic acid	Myase	Myeloperoxidase
apoA-I	Apolipoprotein A-I	OMP	Oxidatively modified protein
ADMA	Asymmetric dimethyl-L-arginine	DHN	1,4-dihydroxynonene
CbP	Carbonylproteine	Anti-oxLDL	Ox-LDL antibody
ACR	Carotene	PHPA	Para-hydroxyphenylacetic acid
CAT	Catalase	PON1	Paraoxonase 1
CoQ10	Coenzyme Q10	PMN-Elae	Polymorphonuclear leukocyte elastase
Cu	Copper	GSH	Reduced glutathione
CRP	C-reactive protein	Se	Selenium
COX	Cyclooxygenase	SOD	Superoxide dismutase
8-OHdG	8-hydroxy-20-deoxyguanosine	SDMA	Symmetrisches Dimethylarginin
F_2-IsoP	F_2-isoprostane	Try	Tryptophan
FB	Free biotin	DNP	2,4-Dinitrophenylhydrazine
GSSG	Glutathione disulfide	Ubi	Ubiquinone
GSHPx	Glutathione peroxidase	UA	Uric acid
GSH	Glutathione S-transferase	VitA	Vitamin A
Hp	Haptoglobin	VitB	Vitamin B6
IDO	Indolamin-2,3-Dioxygenase	VitB12	Vitamin B12
Kyn	Kynurenin	VitAC	Vitamin C
Lyso	Lysozym	Vit/E	Vitamin E
Mn	Manganese	Zn	Zinc

is synonymous with the body's total antioxidant level. It not only represents the sum of enzymes and nonenzyme antioxidants in the body but also reflects the relationship of mutual connection and synergism between the antioxidants. There is a close relationship between the strength of the body's antioxidant defense system and its health and disease status. When it decreases, it will inevitably cause inflammation, tumors, and immune system diseases. Therefore, the TAS level reflects the comprehensive information of the body's antioxidant capacity in different states.

Compared to TAS, total oxidant status (TOS) represents the overall level of all oxidants in the oxidation-antioxidant system that maintains the body's antioxidant defense capabilities. It is also named total peroxide (TP), serum oxidation activity (SOA), reactive oxygen metabolites (ROM), oxygen radical absorbance capacity (ORAC), or some other synonyms. TOS is synonymous with the total oxidation level of the body. Like the TAS, it not only represents the sum of oxidations in the body but also reflects the relationship of mutual connection and synergism between oxidations. Oxidation is an essential component of the human body's antioxidant

Table 32.4 OxS biomarkers commonly used in clinical practice or research (oxidants)

Classification	Abbreviation	Full name
Oxidant	ALE	Advanced lipoxidation end product
	8-iso-$PGF_2\alpha$	8-iso-prostaglandin $F_2\alpha$
	Fe^{2+}	Ferrous ion
	4-HNE	4-hydroxy-2-nonenal
	HNA	4-hydroxynonenoic acid
	HydrP	Hydroperoxide
	DDG	7,8-dihydro-8-oxo-20-Deoxyguanosine
	LO	Lipid alkoxyl radical
	LOOH	Lipid hydroperoxide
	LOO	Lipid peroxyl radical
	MDA	Malondialdehyd
	Nrf2	Nuclear factor-like 2
	OMP	Oxidatively modified proteins
	oxLDL	Oxidierte LDL
	di-Tyr	O,o'-dityrosine
	ProC	Protein carbonyl
	SNST	S-nitrosothiols
	NHPA	3-nitro-4-hydroxyphenylacetic acid
	Cl-Tyr	3-chlorotyrosine
Integral	OSI	oxidant stress index
	TAS	Total antioxidant status
	TOS	Total oxidant status

defense system. There is a close relationship between the strength of the body's antioxidant defense system and health or disease states. When oxidation is elevated, it will inevitably cause inflammation, tumor, and immune system diseases. Therefore, the TOS level reflects comprehensive information about oxidizing ability in different states.

It can be seen that TAS and TOS are necessary detection indicators to fully reflect the body's antioxidant effect. At present, both TAS and TOS can realize automatic detection, with high precision and good reproducibility, and can be used for the detection of any biological sample, which is easy to popularize.

Taking Hitachi automatic biochemical analyzer as an example, the instrument setting parameters for automatic detection of TAS (Table 32.3) and TOS (Table 32.4) are given here. Other brands and models of automatic biochemical analyzers can refer to this parameter and instrument performance, which can be easily modified settings.

32.2.1 Measurement of Total Antioxidant Status

The following is a brief introduction of the TAS full-automatic detection method.

Table 32.5 TAS test parameter setting of Hitachi LAbOSPECT 008AS automatic biochemical analyzer

Test parameters	Instrument setting
Method	2-Point End
Sample volume	4 μL
R1	160 μL
R2	16 μL
Reaction time	10 min
Temperature	37 °C
Read points	21, 38
Primary wavelength	405 nm
Secondary wavelength	546 nm
Calibration type	Linear
Unit	mmol/L

32.2.1.1 Detection Principle

The TAS assay relies on the ability of antioxidants in the sample to inhibit the oxidation of the peroxidase methemoglobin from ABTS (2,2'-azino-di-3-ethylbenzthiazoline sulfonate) to $ABTS^+$. The amount of $ABTS^+$ produced can be monitored at 600 nm using an automatic biochemical analyzer. Under the reaction conditions used, the antioxidants in the sample suppress the absorbance at 600 nm to an extent proportional to their concentration.

32.2.1.2 Reagent Composition

Reagent 1 (R1): 0.1 mol/L citric acid/trisodium citrate dihydrate buffer (pH = 5.8).

Reagent 2 (R2): 0.1 mol/L citric acid/trisodium citrate dihydrate buffer (pH = 3.6), 8.0 mmol/L ABTS [2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] and 2.0 mmol/L H_2O_2. After the reagent is prepared, it will be kept away from light and at room temperature for at least 1 h, and it can be used only after ABTS is fully oxidized.

pH Correction: Use 0.5 mol/L citric acid or 0.1 mol/L NaOH to accurately adjust the pH = 3.6 of R2.

The reagent can be stored for 2 weeks at room temperature (20–25 °C) and at least 6 months at 4 °C.

Standard solution: According to different biological samples, a kind of water-soluble vitamin E analogue 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (AR) was used to prepare different concentrations as standard.

32.2.1.3 Instrument Setting

Take Hitachi LAbOSPECT 008AS automatic biochemical analyzer as an example, the instrument parameters of TAS automatic detection are listed in Table 32.5. It is easy to adjust the test parameters according to the principle of the experiment, reagent composition, and instrument performance. The overall antioxidant levels in the samples are calculated using a known concentration of antioxidant. TAS values are expressed as mmol Trolox equivalent/L (mmol Trolox equiv./L).

32.2.2 Measurement of Total Oxidant Status

The following is a brief introduction of TOS full-automatic detection method.

32.2.2.1 Detection Principle

The TAS assay relies on the oxidation of ferrous ions to ferric ions in an acidic medium in the presence of various oxidative species. Ferric ion concentration was measured by xylenol orange. The assay was calibrated with a hydrogen peroxide standard (unit: μmol/L). Results were expressed in μmol H_2O_2 equivalent/L (μmol H_2O_2 equiv./L).

32.2.2.2 Reagent Composition

Reagent 1 (R1): 150.0 μmol/L xylenol orange, 140.0 mmol/L NaCl, 1.35 mol/L glycerol, and 16.0 mmol/L HCl.

Reagent 2 (R2): 5.0 mmol/L ferrous ammonium sulfate, 10.0 mmol/L o-dianisidine dihydrochloride, and 17.8 mmol/L HCl.

pH Correction: Use 0.1 mol/L HCl or 0.1 mol/L NaOH to accurately adjust the R1 and R2 reagents to pH = 1.75.

The reagent can be stored for 2 weeks at room temperature (20–25 °C) and at least 6 months at 4 °C.

Standard solution: 30% hydrogen peroxide (GR), according to the different biological samples, different standard concentrations will be prepared.

32.2.2.3 Instrument Setting

Take Hitachi LAbOSPECT 008AS automatic biochemical analyzer as an example; the instrument parameters of TAS automatic detection are listed in Table 32.6. It is easy to adjust the test parameters according to the principle of the experiment, reagent composition, and instrument performance. Likewise, the overall oxidant levels in the samples are calculated using a known concentration

Table 32.6 TOS test parameter setting of Hitachi LAbOSPECT 008AS automatic biochemical analyzer

Test parameters	Instrument setting
Method	2-Point end
Sample volume	21 μL
R1	162 μL
R2	12 μL
Reaction time	10 min
Temperature	37 °C
Read points	19, 38
Primary wavelength	570 nm
Secondary wavelength	800 nm
Calibration type	Linear
Unit	μmol/L

of hydrogen peroxide. TOS values are expressed as μmol H_2O_2 equivalent/L (μmol H_2O_2 equiv./L).

32.3 Calculation of Oxidant Stress Index

The oxidant stress index (OSI) is an index that reflects the state of redox balance in the human body. It can be calculated by the following formula:

$$\mathrm{OSI} = \mathrm{TOS}/\mathrm{TAS}.$$

When the TOS unit is μmoL H_2O_2equiv/L and the TAS unit is μmoL Troloxequiv/L, the above formula can be converted into:

$$\mathrm{OSI}(\text{arbitrary unit}) = [(\mathrm{TOS}, \mu\mathrm{moL}\, H_2O_2\mathrm{equiv}/\mathrm{L})/(\mathrm{TAS}, \mu\mathrm{moL}\, \mathrm{Troloxequiv}/\mathrm{L}] \times 100$$

Whether in a physiological state or pathological conditions, the results of the individual detection of one or several oxidized and/or antioxidant substances alone may rise or decrease compared to the levels of healthy individuals. The most puzzling thing is that TAS or TOS may also change (rise or fall) to varying degrees. However, when TAS and TOS simultaneously increase or decrease proportionally, the body will not produce OxS at this time if the ratio OSI does not change significantly (that is, there is a small fluctuation within the allowable range). At this moment, if only one or several oxidants and/or antioxidants, even TAS or TOS levels, changed significantly compared with those in healthy individuals, the observer is likely to judge that OxS occurred. However, OxS does not occur-if OSI remains relatively stable.

It can be seen that OSI is the key indicator for judging whether the oxidation-antioxidation balance of the body is disordered, which leads to the occurrence of OxS.

32.4 End Products of Lipid Hydroperoxide

The main target of reactive oxygen species is polyunsaturated fatty acids on the cell membrane, which triggers lipid peroxidation and leads to cell structure and function damage. In addition, the decomposition of lipid hydroperoxide produces many end products, such as compounds containing aldehyde groups (malondialdehyde, MDA), keto groups and hydroxyl (4-hydroxynonene, 4-HNE), organic hydrocarbons alkane, alkene and new oxygen free radicals, etc., which can accelerate biological oxidation in cells.

Lipid peroxidation is a free radical chain reaction. The formation of lipid peroxides has the following two types:

1. Enzymatic reactions: Some lipoxygenases can promote the reaction of oxygen with polyunsaturated fatty acids to form lipid peroxides. For example, 5-lipoxygenase and 12-lipoxygenase can promote the carbon atoms in the fifth and twelfth sites of arachidonic acid to be oxygenated to form 5-hydrogen peroxy-arachidonic acid and 12-hydrogen peroxy-arachidonic acid.
2. Nonenzymatic reaction: Polyunsaturated fatty acids have multiple double bonds, and more active hydrogen atoms are located on the methylene group between the two double bonds. For example, the C-H bond located in the methylene but not affected by the double bond has a dissociation energy of 393.56 kJ/mol, while the C-H bond located in the methylene and affected by two double bonds has a dissociation energy of only 355.85 kJ/mol. Therefore, when subjected to the action of light, radiation, and free radicals, etc., polyunsaturated fatty acids can easily remove hydrogen atoms from the methylene group located between two double bonds to form lipid radicals, and then double bonds and unpaired electron sites transfer to form relatively stable conjugated double bonds and then react with oxygen to form products such as lipid peroxy radicals and lipid peroxides.

Under the action of light, radiation, or free radicals, lipid molecules (LH) remove 1 hydrogen atom to form lipid free radicals (L·). Lipid free radicals react with oxygen to form lipid peroxyl radicals (LOO·); then LOO· radicals attack other lipid molecules and seize their hydrogen atoms to generate lipid radicals (L·) and lipid hydrogen peroxide (LOOH). This reaction is repeated in this manner, resulting in continuous consumption of lipids and large production of lipid peroxides.

RO, RO_2·, and ROOH are lipid peroxidation products; however, the content of these lipid peroxidation products in the human body is extremely low in normal physiological conditions, and their products will be converted to harmless substances, even if there is a chance of lipid peroxidation. Lipid peroxide can be decomposed into aldehydes, ketones, alcohols, ethers, carboxylic acids, and alkanes, among which malondialdehyde is the most representative lipid peroxidation product. Consequently, many researchers have tested malondialdehyde to determine whether a lipid peroxidation reaction has occurred in a system. But in terms of the human body, it is extremely one-sided to determine whether OxS occurs in the human body with this method [5].

The products of lipid peroxidation are commonly used as biomarkers of OxS or oxidative stress/damage. Lipid peroxidation generates a variety of relatively stable end products for decomposition, which can then be measured as an indirect biomarker of OxS in biological samples.

It is possible to estimate antioxidant activity in vivo by detecting the changes of lipid, protein, and/or DNA oxidative damage markers in biological samples. However, most of these markers are nonspecific, and their detection may also be interfered with by compounds of non-peroxidative origin [6]. There are many methods available for detecting oxidative damage to human lipid, protein, and DNA. A series of peroxidative products involved in the methods have been applied, including thiobarbital acid reactive substances (TBARS), converged dienes,

hydrocarbons, lipid peroxides, F_2-isostandards, protein carbonyls, 8-hydro-deoxyguanosine, etc.

Among them, the method of detecting MDA based on the TBARS reaction principle has been widely used with its simpleness in technology. However, it is interfered with by compounds of non-peroxidative origin in human biological samples. It is also affected by Fe content in buffers and reagents. There are significant differences in the values of healthy subjects between different laboratories. High-performance liquid chromatography has improved specificity, but it is not easy to be popularized because of the limitation of instrument price and technical difficulty. In view of these factors, only the spectrophotometry of MDA is introduced here.

32.5 Measurement of Malondialdehyde

In this book, a modified spectrophotometric method of the thiobarbituric acid (TBA) test is introduced for the determination of MDA.

32.5.1 Detection Principle

MDA in LPO degradation products can combine with thiobarbituric acid to form a red complex TBARS, which has a maximum peak at 532 nm. The concentration of MDA in the sample can be calculated by comparing it with the standard of equivalent tests.

32.5.2 Reagent Composition

Reagent 1 (R1): 10% trichloroacetic acid.
Reagent 2 (R2): 0.67% thiobarbituric acid.

Standard solution: 6.0 μmol/L 1,1,3,3-tetramethoxypropane. It can be prepared into a standard storage concentrated solution of 1.0 mmol/L and can be stored in a refrigerator at 4 °C for at least 3 months.

32.5.3 Manipulation Steps

Step 1: Deproteination.
Take a clean 5 mL test tube, first add 200 μL of the sample, then add 400 μL of reagent R1, and shake vigorously or use a micro shaker to make it thoroughly mixed. Then centrifuge at 5000 rpm for 10 min.
Step 2: Color reaction.

Take 300 μL of supernatant and add 300 μL of reagent R2. Then boil the sample in an open water bath for 10 min, remove, and cool to room temperature.

Step 3: Colorimetric determination.

Read the absorbance using a spectrophotometer at the wavelength of 532 nm. The concentration of MDA in the sample can be obtained by calculating the standard tube operated at the same time.

Although the method is simple and easy to be popularized. it cannot be automatically detected because of the need for centrifugation and boiling, which can be seen from the **Manipulation** steps.

32.6 Conclusions

Oxidative stress refers to the imbalance between the oxidation and antioxidant system in the body, which causes a pathological process of oxidative damage of cells and/or tissues. When OxS occurs, the oxidation-antioxidant system tends to be unbalanced in the direction of oxidation, resulting in inflammatory infiltration of neutrophils, increased secretion of proteases, and the production of a large amount of oxidation intermediates [7]. Therefore, OxS is a negative effect from the free radicals in the body. In physiological conditions, it can not only promote the aging of the body but also promote the occurrence and development of diseases in pathological conditions. More than 95% of the free radicals in the body are oxygen free radicals, with the characteristics as follows: (1) the human body can not only produce free radicals but also scavenge them to keep the dynamic balance, so that the body can protect cells, tissues, and organs from oxidative damage; (2) oxygen free radicals can not only cause damage to the body but also promote certain physiological functions of the body; (3) the production and removal of oxygen free radicals are in a dynamic balance. If this dynamic balance is broken, it will cause damages to cells, tissues, and/or organs leading to the occurrence and development of disease.

Antioxidants are the substances that the body fights against oxygen free radicals (oxidants). At present, there are many kinds of related biomarkers (i.e., OxS biomarkers) used to reflect the oxidation/oxidation status of the body. But so far, no matter what kind of disease, there is still no widely accepted and highly specific biomarkers of OxS as an evaluation indicator of diagnosis, risk prediction, and prognosis for clinical application. Many oxidative damages may be a cascade reaction, which not only has complicated disease course but also involves a special tissue structure. Thus, the use of a single biomarker of oxidative stress is very limited, because it can only reflect a certain stage or aspect of damage to cells or tissues. TAS, TOS, and OSI could reflect the state of OxS in a system (cell, tissue, organ, or whole body), and the combined detection of them is the best choice to evaluate the OxS system. However, these three indexes have no tissue specificity, and they can only reflect the overall level of the body. With the continuous development of science and technology, a more understanding of proteomics, metabonomics, and bioinformatics will promote the development of OxS

biomarkers with tissue organ specificity, high accuracy, and sensitivity to provide reliable clinical evidence for disease prevention and treatment.

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