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Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders

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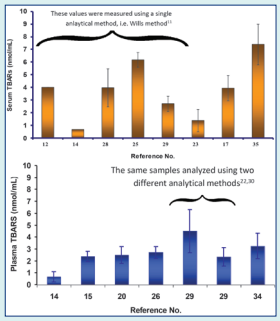
Abstract

Despite very wide variations of malondialdehyde (MDA) concentrations in biological samples, it is still used as a biomarker of oxidative stress in clinical investigations. In the current perspective study, we aimed to summarize a number of critical analytical points for determination of MDA. Technical problems and controversial findings in healthy people and some psychiatric disorders reveal that the reliability of MDA as a biomarker of oxidative stress needs to be re-evaluated by experts.



Malondialdehyde (MDA) is the most frequently used biomarker of oxidative stress in many health problems such as cancer, psychiatry, chronic obstructive pulmonary disease, asthma, or cardiovascular diseases. This perspective study aimed to collect some evidence for low reliability of the MDA as a biomarker of oxidative stress. Our main hypothesis is that MDA assay is not able to provide valid analytical data for biological samples due to its high reactivity and possibility of various cross-reactions with co-existing biochemicals. Thiobarbituric acid (TBA) assay is the most commonly used method for determination of the MDA in biological fluids.[1] The assay is based on a condensation reaction of two molecules of TBA with one molecule of MDA, in which the reaction rate depends on temperature, pH and concentration of TBA. The reaction is carried out in acidic solution and temperature of ~ 100˚C within one hour time course and most of MDA is produced during reaction process from decomposition of products of lipid peroxidation.[2] The rapidity, ease of use and cost of TBA assay made it the most common method in spite of some consideration and limitations of the method. These mainly are:

- Non-specificity of TBA reactivity on MDA[3,4] and production of MDA from reactions other than lipid peroxidation.[5] Concerning these characteristics of TBA assay and cross-reaction of other aldehydes produced from lipid peroxidation, most of researchers used total values of TBA reactive substances (TBARs) as a biomarker of oxidative stress instead of MDA values,
- Effects of procedural modifications on MDA-TBA adduct development,[3]
- Low stability of MDA in biological samples due to its high tendency for reacting with proteins, amino acids etc.[6] and rapid enzymatic degradation,[7]
- Poor reproducibility of analytical results,[4,8,9]
- Low recovery test results.[10]

Variations of TBARs in biological samples of a number of psychiatric patients were investigated and compared with the values of healthy controls (for details see Table 1).[9,11-36] In a more recent article, de Sousa et al[19] compared plasma

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Table 1. A summary of TBARs variations in some psychiatric disorders

Matrix	Conditions	TBARs (nmol/mL)	Reference of analytical method	Reference of TBARs data
Serum	Euthymic	~ 6.2	11	12
Serum	Depressed	~ 5.0	11	12
Serum	Manic	~ 7.8	11	12
Plasma	Schizophrenia	1.15± 0.35	13	14
Plasma	Schizophrenia - medicated	0.93 ± 0.65	13	14
Plasma***	Social phobia*	2.32 ± 0.38	9	15
Plasma***	Social phobia**	4.52 ± 0.42	9	15
Serum	Bipolar disorder before lithium therapy	6.63 ± 1.51	16	17
Serum	Bipolar disorder after lithium therapy	6.33 ± 1.16	16	17
Plasma	Bipolar disorder before lithium therapy	60.77 ± 45.14	18	19
Plasma	Bipolar disorder after lithium therapy	37.88 ± 35.85	18	19
Plasma	Schizophrenia*	3.8 ± 0.8	11	20
Plasma	Schizophrenia* after haloperidol therapy	3.4 ± 0.7	11	20
Plasma	Schizophrenia* after clozapine therapy	4.4 ± 0.7	11	20
Serum	Schizophrenia - paranoid	5.1 ± 1.7	11	21
Serum	Schizophrenia - disorganized	5.0 ± 1.3	11	21
Serum	Schizophrenia - undifferentiated	5.4 ± 1.4	11	21
Serum	Schizophrenia – partial remission	4.9 ± 1.6	11	21
Serum	Schizophrenia – marked symptoms	5.9 ± 2.0	11	21
Serum	Schizophrenia - deteriorated	4.7 ± 1.3	11	21
Serum	Schizophrenia	1.34 ± 0.97	22	23
Serum	Euthymic	0.62 ± 0.02	11	24
Serum	Depression	0.89 ± 0.02	11	24
Serum	Manic	1.73 ± 0.16	11	24
Plasma	Schizophrenia	4.76 ± 0.79	9	26
Plasma	Bipolar disorder	4.26 ± 0.46	9	26
Serum	Schizophrenia	4.95 ± 1.56	11	27
Serum	Bipolar disorder - euthymic	6.36 ± 1.46	11	27
Serum	Bipolar disorder - manic	7.54 1.74	11	27
Serum	Bipolar disorder - depressed	5.28 ± 1.54	11	27
Serum	Mania before lithium therapy	5.1 ± 1.1	11	28
Serum	Mania after lithium therapy	3.6 ± 0.3	11	28
Plasma	Schizophrenia - chronic	8.01 ± 5.5	22	29
Plasma	Schizophrenia - chronic	5.16 ± 1.85	30	29
Plasma	Adult attention-deficit hyperactivity disorder	2.44 ± 0.84	31	32
Serum	Schizophrenia** after haloperidol therapy	~ 78		33
Serum	Schizophrenia** after quetiapine therapy	~ 75		33
Serum	Schizophrenia** after olanzepine therapy	~ 73		33
Serum	Schizophrenia** after risperidone therapy	~ 80		33
Plasma***	Schizophrenia	4.06 ± 1.79	22	34
Serum	Mania	9.8 ± 5.0	18	35
Serum	Schizophrenia – acute phase	3.5 ± 1.2	18	36
Serum	Schizophrenia – after antipsychotic treatment	3.6 ± 1.5	18	36

* Non-smoker.
** Smoker.
*** EDTA treated.

TBARs and some other markers in healthy individuals and patients with bipolar disorder (BD) before and after lithium therapy. Plasma samples were collected during two years, stored at -80°C and TBARs were measured using a spectrophotometric analysis. The reported TBARs for healthy controls was 62.74 ± 37.58 nmol/mL, those of BD patients before and after lithium therapy were 60.77 ± 45.14 and 37.88 ± 35.85 nmol/mL, respectively, and a significant decrease was observed from baseline to endpoint TBARs (p=0.023) in patients with lithium therapy.[19] Decreased TBAR levels after lithium therapy (3.6 ± 0.3 vs. 5.1 ± 1.1 nmol/mL) has been confirmed by Machedo-Vieira et al,[28] whereas no significant difference (6.33 ± 1.16 vs. 6.63 ± 1.15 nmol/mL) was observed in another investigation.[17] Further, no change in TBAR levels in healthy people receiving lithium was observed by the same group of de Sousa.[25] There are also other controversies on TBARs of healthy people (e.g., for plasma ranging from 0.67[14] to 62.74[10] nmol/mL) among various reports (Figs. 1 and 2). In both Figs. 1 and 2, one datum with very high deviation was excluded, however, significant variations were observed for healthy

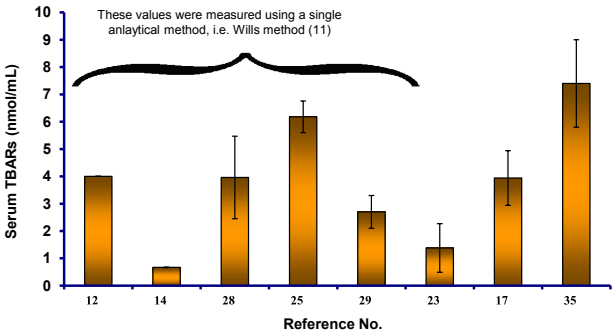

Fig. 1. Serum TBARs (±SD) of healthy controls reported by various research groups (one datum [~ 60 nmol/mL[33]] was excluded from figure).

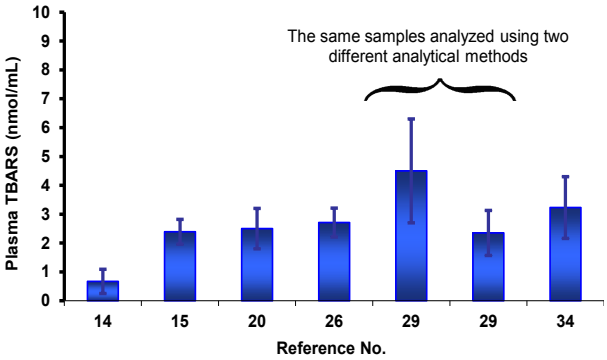

Fig. 2. Plasma TBARs (±SD) of healthy controls reported by various research groups (one datum [~ 62.7 nmol/mL[19]] was excluded from figure).

people, which is obviously questionable finding. One might consider analytical methods as a source of these discrepancies. When we considered a given analytical technique, namely, Wills method,[11] discrepancies were still present varying from 0.67 ± 0.01 nmol/mL[24] to 6.18 ± 0.58 nmol/mL.[25] We would like to point out some considerations on TBARs measurements and to discuss their validity from a bioanalytical point of view. A number of reasons could be considered as possible causes of these observations including variations in sample preparation, storage, pre-treatment, and analysis which are briefly reviewed in this perspective. Full details could be found in a recent review article.[37]

There are some concerns in serum preparation, since TBARs are increased during coagulation process.[38] In plasma preparation, one should consider the effects of EDTA on TBAR values. A significant increase in TBARs for plasma samples (1.39 ± 0.26 nmol/mL) treated with EDTA was reported when compared with the corresponding serum (1.07 ± 0.27 nmol/mL) and heparinized plasma (1.11 ± 0.18 nmol/mL) samples.[31]

Storage of serum/plasma samples at -20°C without addition of antioxidants increased TBAR levels by a factor of two after 3-7 days and the addition of EDTA + glutathione increased the stability of TBARs up to 35 days.[31]

Most of TBARs are produced during the heating of acidic solutions.[2,39] Some authors claimed preventive effects of addition of butylated hydroxyl toluene[40] and some others denied such effect.[41] It has been shown that different acids and their concentrations could affect the results of TBAR assay.[41]

Measurement of TBARs using spectroscopic methods is simple, low cost, convenient, and widely used in clinical studies. Modifications were made on analytical conditions, therefore various values could be produced which make the comparison of reported values very difficult and even impossible. As mentioned above, poor selectivity of spectroscopic method is another disadvantage and the methods based on separation of analytes may overcome this limitation. However, some of these methods resulted in poor recovery, reproducibility, and repeatability values.[42] As clearly noted by Wade and van Rij,[43] most of problems associated with TBA assay were ignored by many researchers. Despite of these points, MDA is still used as an oxidative stress biomarker.[44,45]

Concerning above mentioned points on TBAR levels in healthy individuals and patients, controversial findings on TBARs in psychological disorders, official definitions of a biomarker,[46] poor reproducibility, low repeatability, non-specificity, low stability of the standard solutions of TBA assay, and lack of full validation data of TBARs measurements in biological fluids, the reliability of TBARs as a biomarker of oxidative stress in the psychological disorders is questionable. As a conclusion, a number of bioanalytical points are summarized and the discussions on other viewpoints are open. We believe that MDA as an oxidative stress biomarker needs to be re-evaluated by an expert panel.

Perspective Highlights

What is current knowledge?

√ MDA is used as a biomarker of oxidative stress in various diseases despite its wide variations even in healthy people.

What is new here?

√ According to the collected evidence, most of the technical problems on MDA measurement are unresolved and need further investigation, and the biomarker role of MDA should be re-evaluated by experts.

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Ethical issues

There is none to be declared.

Competing interests

We have no conflicting interests with regard to the present submission.

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