



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

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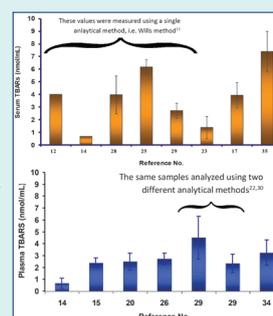
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Abstract

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. Thiobarbitoric 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 al¹⁹ 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 olanzapine 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 Machado-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

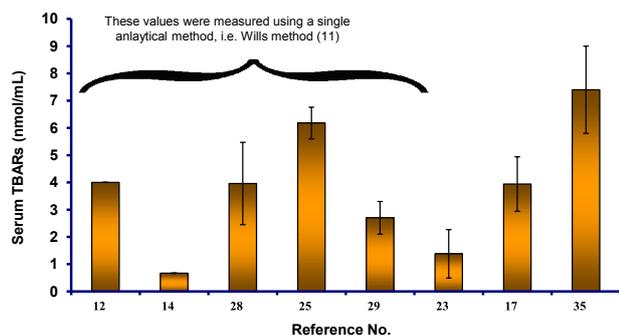


Fig. 1. Serum TBARs (\pm SD) of healthy controls reported by various research groups (one datum [~ 60 nmol/mL³³] was excluded from figure).

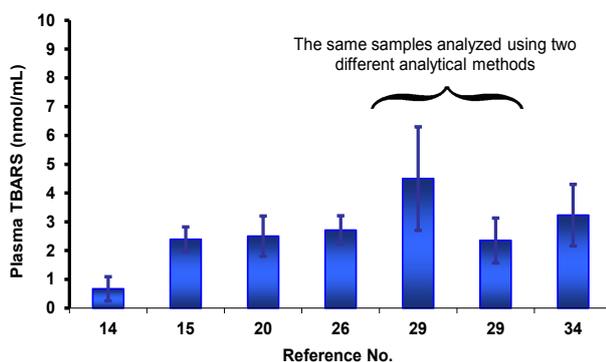


Fig. 2. Plasma TBARs (\pm SD) of healthy controls reported by various research groups (one datum [~ 62.7 nmol/mL¹⁹] was excluded from figure).

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/mL²⁴ 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

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.

solutions.^{2,39} Some authors claimed preventive effects of addition of butylated hydroxyl toluene⁴⁰ 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.

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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.

References

- Janero DR, Burghardt B. Analysis of cardiac membrane phospholipids peroxidation kinetics as malondialdehyde: unspecificity of thiobarbituric acid-reactivity. *Lipids* **1988**; 23: 452–58. doi: 10.1007/BF02535519
- Gutteridge JM, Toeg D. Iron-dependent free radical damage to DNA and deoxyribose. Separation of TBA-reactive intermediates. *Int J Biochem* **1982**; 14: 891–3. doi: 10.1016/0020-711X(82)90071-4
- Halliwell B, Gutteridge JM. Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. *FEBS Lett* **1981**; 128: 343–6. doi: 10.1016/0014-5793(81)80114-7
- Kikugawa K, Kojima T, Yamaki S, Kosugi H. Interpretation of the thiobarbituric acid reactivity of rat liver and brain homogenates in the presence of ferric ion and ethylenediaminetetraacetic acid. *Anal Biochem* **1992**; 202: 249–55. doi: 10.1016/0003-2697(92)90102-D
- Liu J, Yeo HC, Doniger SJ, Ames BN. Assay of aldehydes from lipid peroxidation: Gas chromatography-mass spectrometry compared to thiobarbituric acid. *Anal Biochem* **1997**; 245: 161–5. doi: 10.1006/abio.1996.9990
- Shin BC, Huggins JH, Carrawa KL. Effects of pH, concentration and aging on the malondialdehyde reaction with proteins. *Lipids* **1972**; 7: 229–33.
- Siu GM, Draper H H. Metabolism of malondialdehyde in vivo and in vitro. *Lipids* **1982**; 17: 349–55. doi: 10.1007/BF02535193
- Schoenmakers AW, Tarladgis BG. Reliability of the thiobarbituric acid test in the presence of inorganic ions. *Nature* **1966**; 1153. doi: 10.1038/2101153a0
- Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta* **1978**; 90: 37–43. doi: 10.1016/0009-8981(78)90081-5
- Mendes R, Cardoso C, Pestana C. Measurement of malondialdehyde in fish: A comparison study between HPLC methods and the traditional spectrophotometric test. *Food Chem* **2009**; 112: 1038–45. doi: 10.1016/j.foodchem.2008.06.052
- Wills ED. Mechanisms of lipid peroxide formation in animal tissues. *Biochem J* **1966**; 99: 667–79.
- Andreazza AC, Cassini C, Rosa AR, Leite MC, de Almeida LMV, et al. Serum S100B and antioxidant enzymes in bipolar patients. *J Psychiat Res* **2007**; 41: 523–9. doi: 10.1016/j.jpsychires.2006.07.013
- Konings WT, Drijver EB. Radiation effects on membranes I: Vitamin E deficiency and lipid peroxidation. *Rad Res* **1979**; 80: 494–501. doi: 10.2307/3574991
- Arvindakshan M, Sitasawad S, Debsikdar V, Ghate M, Evans D, Horrobin DF, et al. Essential polyunsaturated fatty acid and lipid peroxide levels in never-medicated and medicated schizophrenia patients. *Biol Psychiat* **2003**; 53: 56–64. doi: 10.1016/S0920-9964(02)00284-0
- Atmaca M, Kuloglu M, Tezcan E, Ustundag B. Antioxidant enzyme and malondialdehyde levels in patients with social phobia. *Psychiat Res* **2008**; 199: 95–100. doi: 10.1016/j.psychres.2002.12.004
- Dahle LK, Hill EG, Holman RT. The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. *Arch Biochem Biophys* **1962**; 98: 253–61. doi: 10.1016/0003-9861(62)90181-9
- Banerjee U, Dasgupta A, Rout JK, Singh OP. Effects of lithium therapy on Na⁺-K⁺-ATPase activity and lipid peroxidation. *Prog Neuro-Psychopharmacol Biol Psychiat* **2012**; 37: 56–61. doi: 10.1016/j.pnpbp.2011.12.006
- Cayman commercial kit, Cayman Chemical Company, Ann Arbor, MI, USA.
- de Sousa RT, Zarate CA Jr, Zanetti MV, Costa AC, Talib LL, Gattaz WF et al. Oxidative stress in early stage bipolar disorder and the association with response to lithium. *J Psychiat Res* **2014**; 50: 36–41. doi: 10.1016/j.jpsychires.2013.11.011
- Gama CS, Salvador M, Andreazza AC, Kapczinski F, Silva Belmonte-de-Abreu P. Elevated serum superoxide dismutase and thiobarbituric acid reactive substances in schizophrenia: A study of patients treated with haloperidol or clozapine. *Prog Neuro-Psychopharmacol Biol Psychiat* **2006**; 30: 512–5. doi: 10.1016/j.pnpbp.2005.11.009
- Gama CS, Salvador M, Andreazza AC, Lobato MI, Berk M, Kapczinski F, et al. Elevated serum thiobarbituric acid reactive substances in clinically symptomatic schizophrenic males. *Neurosci Lett* **2008**; 433: 270–3. doi: 10.1016/j.neulet.2008.01.018
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* **1979**; 95: 351–8. doi: 10.1016/0003-2697(79)90738-3
- Huang TL, Liou CW, Lin TK. Serum thiobarbituric acid-reactive substances and free thiol levels in schizophrenia patients: Effects of antipsychotic drugs. *Psychiat Res* **2010**; 177: 18–21. doi: 10.1016/j.psychres.2009.01.017
- Kapczinski F, Dal-Pizzol F, Teixeira AL, Magalhaes PVS, Anna MKS, Klamt F, et al. Peripheral biomarkers and illness activity in bipolar disorder. *J Psychiat Res* **2011**; 45: 156–61. doi: 10.1016/j.jpsychires.2010.05.015
- Khairova R, Pawar R, Salvatore G, Juruena MF, de Sousa RT, Soeiro-de-Souza MG, Salvador M, Zarate CA, Gattaz WF, Machado-Vieira R. Effects of lithium on oxidative stress parameters in healthy subjects. *Mol Med Reports* **2012**; 5: 680–2. doi: 10.3892/mmr.2011.732
- Kuloglu M, Ustundag B, Atmaca M, Cantan H, Tezcan AE, Cinkilinc N. Lipid peroxidation and antioxidant enzyme levels in patients with schizophrenia and bipolar disorder. *Cell Biochem Funct* **2002**; 20: 171–5. doi: 10.1002/cbf.940
- Kunz M, Gama CS, Andreazza AC, Salvador M, Cereser KM, Gomes FA, et al. Elevated serum superoxide dismutase and thiobarbituric acid reactive substances in different phases of bipolar disorder and in schizophrenia. *Prog Neuro-Psychopharmacol Biol Psychiat* **2008**; 32: 1677–81. doi: 10.1016/j.pnpbp.2008.07.001
- Machado-Vieira R, Andreazza AC, Viale CI, Zanatto V, Cereser V Jr, Vargas RS, et al. Oxidative stress parameters in unmedicated and treated bipolar subjects during initial manic episode: A possible role for lithium antioxidant effects. *Neurosci Lett* **2007**; 421: 33–6. doi: 10.1016/j.neulet.2007.05.016
- Mahadik SP, Mukherjee S, Scheffer R, Correnti EE, Mahadik JS. Elevated plasma lipid peroxides at the onset of nonaffective psychosis. *Biol Psychiat* **1998**; 43: 674–9. doi: 10.1016/S0006-3223(97)00282-5
- Young IS, Trimble ER. Measurement of malondialdehyde in plasma by high performance liquid chromatography with fluorometric detection. *Ann Clin Biochem* **1991**; 28: 504–8. doi: 10.1177/000456329102800514
- Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol* **1990**; 186: 421–31. doi: 10.1016/0076-6879(90)86135-I
- Bulut M, Selek S, Gergeriloglu S, Savas HA, Yilmaz HR, Yuce M, Ekici G. Malondialdehyde levels in adult attention-deficit hyperactivity disorder. *J Psychiatry Neurosci* **2007**; 32: 435–8.
- Padurariu M, Ciobica A, Dobrin I, Stefanescu C. Evaluation of antioxidant enzymes activities and lipid peroxidation in schizophrenic patients treated with typical and atypical antipsychotics. *Neurosci Lett* **2010**; 479: 317–20. doi: 10.1016/j.neulet.2010.05.088
- Ranjekar P, Hinge A, Hegde MV, Ghate M, Kale A, Sitasawad S, et al. Depressed antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients. *Psychiat Res* **2003**; 121: 109–22. doi: 10.1016/S0165-1781(03)00220-8
- Tsai MC, Huang TL. Thiobarbituric acid reactive substances (TBARS) is a state biomarker of oxidative stress in bipolar patients in a manic phase. *J Affective Disorders* **2015**; 173: 22–6. doi: 10.1016/j.jad
- Tsai MC, Liou CW, Lin TK, Lin IM, Huang TL. Changes in oxidative stress markers in patients with schizophrenia: The effect of antipsychotic drugs. *Psychiat Res* **2013**; 209: 284–90. doi: 10.1016/j.psychres.2013.01.023
- Khoubnasabjafari M, Ansarin K, Jouyban A. Critical review of malondialdehyde analysis in biological samples. *Cur Pharm Anal*

- 2015, in press. doi: 10.2174/1573412911666150505185343
38. Poubelle P, Chainti-Euil J, Besadoux J, Boitman F, Simon L, Crastes de Paulet A. Plasma lipoperoxides and aging critical assessment of the thiobarbituric acid method for the measurement of lipoperoxides and malondialdehyde. *Biomedicine* **1982**; 36: 164-6.
39. Gutteridge JMC. Free-radical damage to lipids, amino acids, carbohydrates and nucleic acids determined by thiobarbituric acid reactivity. *Int J Biochem* **1982**; 14: 649-53. doi: 10.1016/0020-711X(82)90050-7
40. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol* **1978**; 12: 302-10. doi: 10.1016/S0076-6879(78)52032-6
41. Lepage P, Heckel C, Humbert S, Stahl S, Rautmann G. Preparative steps necessary for the accurate measurement of malondialdehyde by high-performance liquid chromatography. *Anal Biochem* **1991**; 197: 277-83. doi: 10.1016/0003-2697(91)90392-7
42. Hackett C, Linley-Adams M, Lloyd B, Walker V. Plasma malondialdehyde: A poor measure of in vivo lipid peroxidation. *Clin Chem* **1988**; 34: 208.
43. Wade CR, van Rij AM. Plasma malondialdehyde, lipid peroxides, and the thiobarbituric acid reaction. *Clin Chem* **1989**; 35: 336.
44. Nur Aksoy S, Saygili EI, Bulbul F, Bahar A, Savas H, Virit O, Altindag A, Bayram A, Taracioglu M. Myeloperoxidase enzyme levels and oxidative stress in bipolar disorder. *Afr J Biotechnol* **2010**; 9: 3318-23. doi: 10.5897/AJB10.067
45. Bulut M, Selek S, Bez Y, Kaya MC, Gunes M, Karababa F, Celik H, Savas HA. Lipid peroxidation markers in adult attention deficit hyperactivity disorder: New findings for oxidative stress. *Psychiatry Res* **2013**; 209: 638-42. doi: 10.1016/j.psychres.2013.02.025
46. Ilyasova D, Scarbrough P, Spasojevic I. Urinary biomarkers of oxidative status. *Clin Chim Acta* **2012**; 413: 1446-53. doi: 10.1016/j.cca.2012.06.012