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Is Malondialdehyde an Acceptable Biomarker of Oxidative Stress In Children with Down Syndrome?

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The concentrations of malodialdehyde (MDA) in biological samples were measured using simple spectrophotometric methods and considered as a biomarker of oxidative stress in many related diseases including down syndrome (DS) (1-7). The aim of this communication is not to discuss the effects of oxidative stress on DS, cell aging or neurologic disorders as one may find lots of evidence to support the effects of oxidative stress in many pathological conditions. Despite numerous reports in the medical literature dealing with the variations of MDA in health and disease conditions, our main concern is on the reliability and repeatability of MDA as a biomarker of oxidative stress in clinical investigations. Although MDA has been used for this purpose, there are major concerns on its reliability criteria. MDA could be produced from a number of biological sources including lipid peroxidation (8). MDA is routinely measured by spectrophotometric,/spectrofluorimetric./ liquid chromatographic, /capillary electrophoretic and some other analytical techniques after derivatization with thiobarbituric acid in an acidic solution at high temperature. These derivatization conditions resulted in wider variations of MDA levels, since MDA could be produced/released from biological samples in acidic solution and high temperature. In addition there are some concerns on reproducibility, repeatability, stability and some other analytical validation criteria of the used method for quantification of MDA. Most of these items were reviewed in a recent work and readers of this journal could refer to the paper (8). Despite these critical points, it has been used in many research projects even in more recent papers. As an example, He et al. (1) reported the serum MDA of 6.075 nmol/mL for DS against 5.625 nmol/mL for control group in which a significant ($P < 0.013$) increase was found for DS versus control group. As shown in figure 1 of the original paper (1), very wide variations were observed for MDA levels in DS group. We believe that a part of this wide variation is due to the non-validity of the used analytical methods. MDA has been used in some other papers as a biomarker of oxidative stress and details of available MDA data on DS cases are summarized in Table 1. Significant increase in MDA levels was also supported by findings from earlier investigations (2, 6). In other reports, significant and non-significant increases in MDA levels of DS group in different age subgroups have been reported (3, 5). A controversial finding was also reported by Gromadzinska et al. (4) in which MDA in DS group was significantly less than in control group in both age subgroups. Slight decreases were observed in control (4.14 vs 4.04) and (3.06 vs 2.49) groups in two investigated age subgroups which is in agreement with the other findings (5).

Mochova et al. (2) reported a significant increase in serum MDA levels of DS vs control group. When they compared the differences in four, i.e. 1 - 6, 6 - 13, 13 - 20 and > 20 years age subgroups, no significant differences were observed (2). In contrast for whole group of Mehar Sulthana et al. (5) significant increase for DS group vs control group was observed whereas non-significant difference was obtained in an age subgroup. Non-significant increases were reported for plasma samples for 1 - 8 years (3), and 8 - 14 years (5) which are not in agreement with other reports.

Casado et al. (6) reported increased erythrocyte MDA levels with increased age in both DS and healthy control groups. Age-matched groups showed significantly increased levels in DS group (6). There are ~ 50 folds difference between erythrocyte MDA levels of Casado et al. (6) and Machova et al. (2) and it has been claimed that this may be due to different analytical methods used for determination of MDA (6). The widest MDA level variations ranging from 1.6 up to 263 nmol/mg Hb was observed for DS group in various studies listed in Table 1. More variations on erythrocyte MDA levels were reported from the same research group where the maximum mean value for MDA levels for > 80 years old group was 620.60 ± 244.76 nmol/mg Hb (9). Controversial findings were also reported for male and fe-

Table 1. Available Data on MDA in Down Syndrome and the Control Groups[a]

Matrix/Unit	MDA for Cases	MDA for Controls	P Value	Ref
Serum, nmol/mL	6.075 (36)	5.625 (40)	0.013	1
Serum, μmol/L	8.39 ± 0.34 (31)	7.34 ± 0.27 (30)	0.021	2
Plasma, μmol/L, 1 - 8 y	0.20 ± 0.13	0.16 ± 0.10	NS	3
Plasma, μmol/L, 6 - 16 y	3.06 ± 0.78 (6)	4.14 ± 1.05 (68)	0.02	4
Plasma, μmol/L, 17 - 30 y	2.49 ± 0.89 (8)	4.04 ± 0.89 (9)	0.005	4
Plasma μmol/L, 0.25 - 4 y	6.0 ± 2.8	2.8 ± 1.3	0.0002	5
Plasma, μmol/L, 4 - 8 y	6.7 ± 2.5	3.3 ± 0.8	0.0102	5
Plasma, μmol/L, 8-14 y	5.8 ± 1.4	4.0 ± 0.5	NS	5
Plasma, μmol/L, Whole group	6.1 ± 2.5	3.1 ± 1.2	< 0.001	5
Erythrocyte, μmol/g Hb	2.20 ± 0.09 (37)	2.09 ± 0.08 (33)	NS	2
Erythrocyte, nmol/mg Hb, < 1 y	112.37 ± 5.14 (16)	86.92 ± 5.14 (20)	< 0.05	6
Erythrocyte, nmol/mg Hb, 2 - 4 y	136.25 ± 5.06 (17)	114.36 ± 5.32 (20)	< 0.05	6
Erythrocyte, nmol/mg Hb, 5 - 9 y	161.39 ± 5.34 (18)	132.68 ± 3.09 (18)	< 0.05	6
Erythrocyte, nmol/mg Hb, 10 - 14 y	184.75 ± 4.16 (14)	156.11 ± 5.02 (15)	< 0.05	6
Erythrocyte, nmol/mg Hb, 15 - 19 y	228.19 ± 5.28 (14)	189.36 ± 4.71 (15)	< 0.05	6
Erythrocyte, nmol/mg Hb, 20 - 29 y	263.36 ± 6.15 (11)	217.22 ± 5.30 (12)	< 0.05	6
Erythrocyte, μmol/g Hb, 23.2 y	1.582 ± 0.020 (42)	1.504 ± 0.024 (24)	0.019	7

Abbreviation: NS, non-significant.
[a]Values are expressed as mean ± SD (n).

male subgroups in different age ranges where higher erythrocyte MDA levels for female healthy group with 36 - 64 years (9). This is in agreement with a previous report of Inal et al. (10) in which similar findings were reported for plasma MDA and the increase was justified concerning menopause (11). Lower eryhthrocyte MDA values were observed for female healthy subjects in 65 - 79 and > 80 years subgroups which are not in agreement with above findings. The other important points are that apparently Gil et al. (9) did not exclude smoker subjects in these age subgroups and the standard deviation for > 80 years was very high.

In conclusion and concerning ideal characteristics of a biomarker of oxidative stress, technical and practical problems associated with analysis of MDA in biological samples, very wide variations of MDA in healthy controls (and also in case) groups and controversial findings from the literature (8), using MDA as a biomarker of oxidative stress is questionable and the above mentioned topics should be further investigated and re-evaluated by an expert panel. Most of published articles on MDA even in recent years have employed non-valid analytical methods for determination of MDA in biological samples. We would like to recommend researchers to use the analytical methods for MDA measurements after partial or full validation of the methods. More details on validation procedures for bioanalytical methods could be found in Food and Drug Administration guidelines.

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