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Review Article

Use of Malondialdehyde as a Biomarker for Assessing Oxidative Stress in Different Disease Pathologies: a Review

***Zorawar SINGH** [1], **Indrakaran P. KARTHIGESU** [2], **Pramjit SINGH** [3], **Rupinder KAUR** [1]*

1. *Post Graduate Department of Zoology, Khalsa College, Amritsar, India*
2. *Dept. of Environmental and Occupational Health, Faculty of Medicine and Health Sciences, University Putra Malaysia, Kuala Lumpur, Malaysia*
3. *Dept. of Environmental and Health Science, Faculty of Biomedicine and Health Sciences, Asia Metropolitan University, Selangor, Malaysia*

***Corresponding Author:** E. mail: zorawarsinghs@rediffmail.com

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Abstract

Malondialdehyde (MDA) is widely used as a biomarker for assessing oxidative stress in biomedical fields. Lipid peroxidation is a chain phenomenon resulting in the formation of various active compounds that result in cellular damage. Biomonitoring of MDA has been used in both in-vivo and in-vitro studies as a key biomarker for various disease patterns including hypertension, diabetes, atherosclerosis, heart failure and cancer. Higher levels of MDA are reported in patients of various categories including lung cancer patients, complex regional pain syndrome patients and glaucoma patients. The findings suggest the validity of the MDA assay as a reliable tool in finding out the oxidative stress in different disease pathologies. The present review emphasizes on the reliability and efficacy of MDA estimation in various health disorders.

Keywords: Malondialdehyde, Lipid peroxidation, Free radicals, Oxidative stress

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Introduction

Oxidative stress is the state of imbalance between the reactive oxygen species (ROS) and the ability of a biological system to detoxify readily the reactive intermediates. Development of oxidative stress because of free oxygen radical generation has been implicated in the pathogenesis of many diseases including Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction and even cancer. FRs are unstable moieties that tend to interact with cellular structures. ROS and FR are continuously formed in the body of animals by a partial reduction of oxygen. The superoxide radical ($\cdot O_2^-$), one of the ROS, is known to be generated in brain. It is involved in the reduction of certain iron complexes including cytochrome C and ferricethylenediaminetetraacetic acid (Fe^{3+}-EDTA) (1). Similarly, nitric oxide (NO·) and peroxyl ($RO_2\cdot$) radicals are highly unstable moieties formed in the human body that disrupt proteins and promote DNA damage. ROS are also engaged in disruption of the integrity of polyunsaturated fatty acids. Hypochlorous acid (HOCl), peroxynitrite ($ONOO^-$), hydrogen peroxide (H_2O_2) and ozone (O_3) are the non-radical forms of ROS that can easily enter free radical reactions.

The antioxidant system of the body defends the ROS produced in the body. Superoxide dismutases (SOD) help the body to remove the superoxide radicals by converting it to hydrogen peroxide (H_2O_2). Catalases further catalyze the conversion of hydrogen peroxide to water and

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oxygen. Glutathione peroxidases are also involved in the removal of H_2O_2.If the production of FRs increases beyond a certain level in the body of the organism, the defensive enzyme systems fail and the condition leads to oxidative stress. Oxidative stress inturn may lead to increase in the free calcium ions and iron within the cells in mammals and this rise in intracellular free Ca^{2+} may result in DNA damage by endonuclease activation (2). Severe oxidative stress can result in cell damage and even cell death (1).

Lipid peroxidation

Lipid peroxidation is a chain reaction occurring during oxidative stress leading to the formation of various active compounds including propanedial and 4-hyrdoxynonenal (HNE) resulting in the cellular damage. It provides a continuous supply of FRs that lead to further peroxidation. In a toxicity study of the oxidative products, peroxide (LOOH) was found to be more toxic than 4-hyrdoxynonenal (HNE) and much more than malondialdehyde (MDA) (3). Lipid peroxidation can be initiated by any chemical species that can extract a hydrogen atom from side chain of a polyunsaturated fatty acid (PUFA) which is generally present in the cell membranes. Arachidonic acid is a polyunsaturated omega-6 fatty acid present in the cell membranes, brain, muscles and liver that contains many uninterrupted methylene double bonds that serve as a source of hydrogen atoms for the FRs. Arachidonic acid induces the platelets to produce large amounts of MDA. Arachidonate conversion to MDA catalyzed by human platelet microsomes is inhibited by vinblastine (4). MDA formation by platelets is also prevented by aspirin or indomethacin (5).

Lipid peroxides, which are derived from polyunsaturated fatty acids, are unstable. They readily decompose to form a complex series of compounds, which include malondialdehyde (MDA). MDA is the major metabolite of arachidonic acid and serves as a reliable biomarker for oxidative stress. MDA is a mutagenic, tumorigenic and highly reactive three-carbondialdehyde produced during polyunsaturated fatty acid peroxidation and arachidonic acid metabolism. MDA is also generated during the breakdown of prostaglandin endoperoxide (PGH_2) into 12-hydroxyheptadecatrienoate (HHT). It has the molecular formula $C_3H_4O_2$ with molar mass of 72.02 g mol^{-1} and boiling point of 108^0C. The melting point ranges between 72-74 ^{0}C (6). The general appearance of MDA is solid and needle like (7). It has the pKa value of 4.46 and exists as its conjugate base (–O–CH=CH–CHO) (6). MDA takes part in different biological reactions inside the cells including covalent binding to proteins, RNA and DNA (8).

Malondialdehyde as a bio-indicator

The monitoring of MDA levels in different biological systems can be used as an important indicator of lipid peroxidation both in-vitro and in-vivo for various health disorders. The endogenous formation of MDA during intracellular oxidative stress and its reaction with DNA forms MDA-DNA adducts which makes it an important biomarker of endogenous DNA damage (9). Determination of MDA in blood plasma or tissue homogenates is one of the useful methods to predict the oxidative stress levels. MDA falls in the category of Thiobarbituric Acid Reactive Substances (TBARS) and the later are an index of lipid peroxidation. Various techniques are used to measure the levels of MDA in different samples including serum, plasma or tissues. Thiobarbituric acid (TBA) assay is the commonly used method for determination of MDA. However, TBA assay is a non-specific test for MDA as TBA also reacts with other aldehydes that may be present in biological samples. Those entities also form coloured species that interfere with the MDA assay. New analytical methods for the isolation and quantification of the MDA-TBA adduct have been developed including HPLC and gas chromatography mass spectrometry (GC-MS) to improve the investigation.

Hydrogen peroxide (H_2O_2) and tert-butyl hydroperoxide (t-BOOH) were found to be responsible for increased MDA levels in hepatoma cell (HepG2) culture (10). MDA was measured by HPLC as its 2, 4-dinitrophenylhydrazone derivative in human hepatoma HepG2 cells in culture and higher MDA levels were found in conditions

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of oxidative stress. Derivatisation of MDA with 2, 4-dinitrophenylhydrazine (DNPH) has also been described for determination of MDA in human urine (11). Detection limit of the method was 56 nM of MDA and the levels of MDA were found to be 0.019 ± 0.012 mM/mmol creatinine. Similarly, HPLC was used for determination of plasma MDA levels in normal male and female volunteers and the average was found to be 1.076 nmol/ml with a coefficient of variation of about 58% (12).
Another simple and sensitive method for determining MDA concentration is gas chromatography mass spectrometry (GC-MS). This method has been described by various researchers including Shin (13) for the determination of MDA in blood. They used headspace-solid phase micro-extraction gas chromatography mass spectrometry (HS-SPME-GC-MS) with Acetone-d (6) as internal standard. The mean concentration of MDA in normal human blood (n=20) was found to be 2.61 μmol/L. The psychostimulent drug methamphetamine (MA) can harm brain dopamine neurons, possibly by causing oxidative damage (14). Significantly, higher levels of 4-hydroxynonenal (67%) and MDA (75%) in the dopamine rich caudate nucleus and frontal cortex (48 and 36% respectively) were found using GC-MS. MDA was found to form Schiff-base adducts with lysine residues and cross-link proteins in-vitro. Reduced forms of Lysine-MDA [3-(N epsilon-lysino) propan-1-ol (LM)] and Lysine-MDA-Lysine iminopropene cross- link [1,3-di(N epsilon-lysino) propane (LML)] were prepared and GC/MS assay was used to quantify the reduced compounds in protein (15). The levels of LM and LML in freshly isolated low-density lipoprotein (LDL) of healthy subjects were found to be 0.002-0.12 mmol/mol of lysine. LM and LML increased with the formation of conjugated dienes during the copper-catalyzed oxidation of LDL.

MDA in different disease patterns

Higher levels of MDA concentration has been reported in different studies including lung cancer patients (16). Table 1 shows various studies based on the use of MDA as a biomarker. High levels of MDA were also found to play a role in atherogenesis in rabbit aortae (17). A positive correlation between artheromatous lesions and MDA concentration was found. Incubation of cultured endothelial cells of human umbilical vein with MDA (200μM) for 24 hours led to an increase in the cell stiffness (18). A marked increase in MDA levels was observed following the course of circulatory shock in human muscular tissue (19). Plasma extracellular superoxide dismutase (SOD3) activity, MDA and CD4 counts were studied were recorded in HIV positive subjects in Kano state, Nigeria and a negative correlations between the serum MDA concentration and CD4 cell count (Pearson $r = -0.68$, $P< 0.01$) and SOD3 (Pearson $r = -0.71$, $P< 0.01$) were found (20). MDA formation is also associated with exposure to insecticides. Exposure of human erythrocyte solutions to Trichlorfon was found to increase the levels of MDA in a dose dependent manner. A decrease in glutathione peroxidase (GSH-Px) and reduced glutathione (GSH) levels was also seen following Trichlorfon exposure (21).

MDA and ocular pathologies

Lipofuscin (LF) accumulation is associated with various retinal diseases and LF accumulation in the retinal pigment epithelium (RPE) may interfere with the normal retinal function. Proteins in the LF granules are oxidatively modified by MDA (31). These proteinaceous LF compounds in RRE were found to be involved in lipofuscinogenesis and may contribute to cytotoxic effects of LF in retinal diseases. The distribution of phospholipid-MDA adducts was measured in human lenses (32). Lipid extracts from sections of the central core and from the equatorial region of eye lenses were measured over an age range of 13-65 years. The MDA adduct concentration was found to be highest in the anterior nuclear section of the central core and lowest in the equatorial section.

MDA in ischemic conditions

Human growth hormone was found to increase lipid peroxidation in rats which inturn increases MDA level of hypoxic-ischemic newborn rat brain tissue (33).

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Table 1: Some studies using MDA concentration as a parameter

STUDY (Reference no.)	YEAR	SUBJECTS	MDA levels	
			Exposed	Controls
22	2003	Early breast cancer patients	2.7 ± 0.2 μM/mL	2.2 ± 0.2 μM/Ml
23	2009	Breast cancer patients	1.41 ± 0.11	0.34 ± 0.03
8	2010	Oral cancer patients	14.34 ± 1.43 etamol/ml	5.10 ± 2.32 etamol/ml
24	2013	Cancer bearing dogs	4.68 ± 1.32 μM/L	2.95 ± 0.61 μM/L
25	2013	Complex Regional Pain Syndrome patients	5.2 ± 0.9 μM/L	5.4 ± 0.5 μM/L
26	2013	Alcohol dependent patients	1.28 ± 0.58 μM	0.9 ± 0.21 μM
27	2013	Atherosclerosis patients with familial Mediterranean fever	1.08 ± 0.66 nmol/L	1.08 ± 0.33 nmol/L
28	2013	Patients with ovarian and endometrial carcinomas	10.1 ± 1.1 μM	7.5 ± 2.7 μM
29	2013	Glaucoma patients	0.97 ± 0.37 μM/L	0.45 ± 0.39 μM/L
30	2014	Patients with chronic obstructive pulmonary disease	220 ± 17.5 nmol/L	85.9 ± 11.3 nmol/L

Plasma MDA was determined as a function of ischemia and reperfusion in rats and humans using ion-pairing high performance liquid chromatography and the study revealed that oxygen deprivation induces the production of a low, but detectable amount of MDA in both heart and brain tissues of rats, whereas reperfusion causes a marked increase of MDA in both the tissues (34). In humans, plasma MDA was affected only in patients suffering from acute myocardial infarction with successful thrombolysis, thus indicating the occurrence of oxygen radical-mediated tissue injury. Oxidative stress was measured in human and rat hearts which were temporarily made ischemic by measuring oxidized glutathione (GSSG) and MDA levels. It was found that after ischemia, human and rat heart show signs of oxidative stress by releasing GSSG whereas human heart was found to have no MDA under normoxic and ischemic conditions. Cardioplegia induced a 41% (P = 0.08) decrease in rat heart MDA content (35).

MDA in reproductive pathologies

MDA in seminal plasma of normal and abnormal men was measured by thiobarbituric acid test and it was found that abnormal semen samples had significantly lower number of viable spermatozoa (59.06 ± 9.63 Vs. 68.33 ± 5.46%, P<0.005) and higher levels of MDA (2.53 ± 0.66 Vs. 1.66 ± 0.37 nmol/ml, P<0.05) as compared to normal semen samples (36). Similarly, Gomez et al. (37) used spectrophotometric assay for the measurement of MDA and 4 hydroxyalkenals (MA+ 4HA) for the detection of sperm pathologies involving oxidative stress and found highly significant correlations (P<0.001) between the loss of motility of spermatozoa and oxidative stress created either with xanthine oxidase or by prolonged aerobic incubation.

MDA and hypertension

Different studies have been conducted with the aim to measure the levels of MDA during hypertensive conditions. Higher serum MDA levels were found in hypertensive patients as compared to normotensive control individuals (38). Higher levels of serum-MDA and decreased catalase activity were found in hypertensive pregnant women as compared to controls (39). El-Banaet et al. (40) studied the maternal and cord plasma concentration of MDA in pre-clamptic and healthy pregnant women. The concentration of MDA in pre-clamptics was found to be significantly lower in cord plasma as compared to maternal plasma (P<0.001). This hypothesized that antioxidant capacity of the cord blood was sufficient to shield

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the foetus from oxidative injury due to increased oxidative stress of a pre-clamptic mother.

MDA, prostaglandins and lipid hydroperoxides

MDA and 15(S)-8-iso-prostaglandin (2aplha) belong to the group of post frequently analysed biomarkers of oxidative stress in basic and applied clinical research. The effects of haemolysis on free MDA and total (free + esterified) 15(S)-8-iso-Prostaglandin (2aplha) concentration in human plasma were examined and was found that in both in-vivo (r = 0.74) and in-vitro (r = 0.87) conditions, there was a positive significant correlation between haemolysis degree (0 - 0.2%) and plasma-MDA concentration (50 - 250 nmol/L). It was hypothesized that free haemoglobin catalyzes the formation of MDA and 15(S)-8-iso-PGF (2aplha) from free and esterified arachidonic acid (41). Tesoriere et al. (42) examined the exposure of RBC to MDA in glucose containing phosphate saline buffer and found a 16% hemolysis within 6 hours. From the result, it was suggested that exposure to MDA caused rapid intracellular oxidative stress and it resulted in oxidative cascades on RBCs resulting in their lysis and dysfunction. MDA caused marked variations in RBC's membrane by formation of conjugated diene (CD) lipid hydro-peroxides. The protective effect of melatonin against cytotoxic actions of MDA on human RBC's was examined. Melatonin was found to prevent the formation of CD lipid hydro-peroxides and protect the RBCs from MDA induced time-dependent haemolysis (43).

The influence of Leukotriene C4 (LTC4) on aggregation and MDA formation in human blood platelets was studied by Ponicke and Forster (44) and it was found that MDA formation was enhanced by LTC4. The presence of certain protozoans including *Leishmania* is associated with higher levels of MDA. *Leishmania* sp. are obligate intercellular protozoans that infect and replicate within mammalian macrophages. Macrophages, neutrophils and other phagocyctic cells are capable of generating large amount of reactive oxygen species (ROS) and RNS (Reactive nitrogen species).The overproduction of ROS and RNS results in oxidative stress and the acceleration of lipid peroxidation in cutaneous leishmaniasis patients, resulting from altered enzymatic antioxidant activities as compared to controls (45).

MDA-lipid associations

The MDA association with plasma lipoproteins alters the lipid structures via apoprotein or apoprotein/lipid associations (46). When plasma lipoproteins react with MDA, a complex change occurs in the resonance Raman banding of beta-carotene in the 1500-1600 cm^{-1} region. MDA also modifies the acoustical region (70-200) cm^{-1} of low-density lipoproteins (LDL). Malondialdehyde-modified low-density lipoproteins (MDA-LDL) were also detected in sera of 40 healthy individuals by an immunosorbent assay technique in which a monoclonal antibody was used against MDA-LDL complex (47). Adducts of MDA with apolipoprotein (apo-B) were also found in plasma of nine patients with severe atherosclerosis (48). Malondialdehyde modified high density lipoproteins (MDA-HDL) were studied and MDA-HDL was found to be less effective in decreasing cellular cholesterol content (49). It also contributed to the progress of atherogenesis by decreasing cholesterol efflux from peripheral tissues. The relationship between MDA and Atherogenic Index (AI) was studied and the levels of lipid profile and antioxidant status in the serum of 15 hyperlipidemic patients and 30 matched normolipidemic control subjects were measured. The normolipidemic subjects were divided into lower and higher lipid group. An increase in the levels of MDA triglycerides, total cholesterol and LDL were observed in higher lipid group and a significant increase was seen in hyperlipidemic patients. A positive correlation was found between MDA and AI (r = 0.61, $P< 0.05$) (50).

MDA-DNA interactions

It has been reported that MDA reacts with DNA bases to form a series of adducts of deoxyadenosine (M1A), deoxycytidine (M1C) and deoxyguanosine (M1dG) (51). 3–(2-deoxy-beta-d-erythro-pentofuranosyl pyrimido) [1, 2- alpha] purin-10 (3H) one, M1dG is the major adduct derived from the reaction of DNA with MDA and the

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DNA peroxidation product base propenal. Cyclooxygenase-2 (COX-2) activity in human colon cells also results in formation of MDA and generation of M1dG adducts (52). Maddukuri et al. (53) conducted an experiment to evaluate bypass of M1dG by human Y-family DNA polymerases kappa, iota and Rev 1. The result indicated that DNA hPol kappa or the combined action of hPol iota or Rev 1 and hPol kappa bypass M1dG residues in DNA and generate products that are consistent with some of mutations induced by M1dG in mammalian cells.

Sun et al. (54) used an immuno-enriched ^{32}P-postlabeling HPLC method for detection of M1dG in human breast and liver tissue. The detection limit in biological samples was approximately 200 amol M1dG from 10 μg of DNA, corresponding to 6-adducts /10^9 nucleotides. The lipid peroxidation products can accumulate at high levels in the breast tissues of women with breast cancer. Wang et al. (55) used a modified nuclease P1-enhanced version of ^{32}P-postlabeling assay for analysis of MDA induced DNA adducts in breast tissues of 51 breast cancer patients. It was found that breast tissues from cancer patients exhibited significantly higher levels of the putative MDA adducts than those found in non-cancerous controls (Median : 42.5 Vs. 15.67 ; P = 0.0001, Mann-Whitney U test). DNA was isolated from the blood of 10 healthy human donors and the levels of the malondialdehyde-deoxyguanosine adduct were measured by Rouzer et al. (56). They observed significant differences between the levels in females (5.1 ± 0.4 adducts /10^8bases) and males (6.7 ± 1.1 adducts / 10^8 bases). Mutagenic potential of MDA was also determined by Niedernhofer et al. (57) by reacting MDA with pSP 189 shuttle vector DNA, which was transfected into human fibroblasts for replication. The MDA increased the mutation frequency (up to 15-fold) in supF reporter gene as compared to untreated DNA.

One of the prominent factors for increased lipid peroxidation can be smoking. The increase in the level of MDA in plasma can be due to the presence of free radicals in cigarette smoke. Plasma MDA (*P*-MDA) was measured in EDTA- treated plasma of 213individuals after derivatization by thiobarbituric acid (TBA) and separation on HPLC. It was found that daily smokers had a significantly higher concentration of *P*-MDA than non-smokers did (P = 0.05) (58). An immunohistochemical method was used for detection of adducts in human oral mucosa cells with respect to smoking habit. The level of DNA damage was found to be higher among 25 smokers (mean relative staining intensity, 97 ± 41) as compared to 25 non-smokers (74 ± 17, P<0.02) (9).

In men, positive associations of M1dG adduct level with height and age, and an inverse association with body mass index was found. Legumes, fruits, salads and whole meal bread were inversely associated with M1dG adducts, whereas consumption of offal, white meat, beer and alcohol were positively associated with elevated levels. In women, there was an inverse association of the adducts with the ratio of polyunsaturated: saturated fatty acids (P=0.019) and a weak positive correlation with saturated fat (P< 0.061) (59).

Rat and human urine have also been examined for the presence of deoxyguanosine-MDA adducts (60). dG-MDA was identified in both rat and human urine samples by HPLC and fluorescence detection. The rate of excretion by five-week-old rats (28.54 ± 2.28 nmol/kg/24h) was higher than nine-week-old rats (6.29 ± 1.02) and much higher than humans (0.40 ± 0.05). This indicated that dG-MDA excretion is related to metabolic rate, which declines during growth. Similar experiment was also performed by Hadley and Draper (61). They isolated a 1:1 guanine-malondialdehyde adduct from rat and human urine and suggested its endogenous origin as indicated by its presence in rat urine fed on MDA free diet.

MDA-DNA adducts and cancer

Xing et al. (62) detected M1dG by ^{32}P-postlabeing method. They found a significant increase in the levels of M1dG adducts in the cancerous human esophageal tissue (median 14.1, range 1.4/10^8 nucleotides, P<0.0001) as compared to normal esophageal tissue (median 3-4,

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range $1.7/10^8$ nucleotides). This study suggested that M1dG adducts may be involved in the initiation and progression of cancer with its mutagenic and carcinogenic effects. Colorectal biopsies from normal mucosa of participants were analyzed for the presence of M1dG. The mean adduct levels were found to be 4.3 ± 3.0 and 4.6 ± 2.9 per 10^7 total bases in men and women respectively (63). In adenoma patients, the adduct levels were compared with controls and a trend for higher values was found in individuals with adenomas (P<0.005). Leuretti et al. (59) developed an immuno slot-blot (ISB) assay for the measurement of M1dG in one µg of DNA. A series of human samples was analyzed and levels of 0.3-6.43 M1dG per 10^7 normal bases were detected in 42 gastric biopsy samples and 0.7-16.65 M1dG per 10^7 normal bases in 28 samples of leukocyte DNA.

Conclusion

Malondialdehyde (MDA) is a useful biomarker for lipid peroxidation and oxidative stress. Different researchers have used MDA assay as a parameter for different sample types. Increased levels of oxidative stress have been associated with various disease patterns. MDA and MDA-DNA adduct determination has been found to be a valuable tool in finding out the associations of oxidative stress levels and occurrence of various pathologies including cancer. Conclusively, MDA estimations can be used as a reliable tool to assess oxidative stress levels and finding its relationships with different disease patterns.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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The authors declare that there is no conflict of interest.

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