

## Original Research Paper

# Estimation of Time Passed Since Death by New Biochemical Parameters: MDA (Malondialdehyde) and Total Thiol

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### Abstract

Determination of time passed since death is extremely important medico-legal issue. It requires meticulous examination, documentation and subsequent necessary evaluation by various pathological and biochemical examination. Two biochemical parameters, MDA breakdown product of lipid per oxidation and Total Thiol which is responsible for the protection of cells against oxidative stress are measured to estimate Time Passed since Death. Pearson correlation in various causes of death shows MDA level is significantly correlated with time passed since death, whereas Total Thiol level is inversely proportional. This study establishes role of MDA and Total Thiol as biochemical parameters to estimate time passed since death.

**Key Words:** Time passed since death, Biochemical parameters, MDA, Total Thiol. Causes of death

### Introduction:

Determination of time passed since death is extremely important for any investigation into the cause of death in the cases where death is sudden, suspicious or unnatural. [7] The post mortem changes give us clues about the time elapsed since death and this single vital information when calculated accurately has the potential to reveal many unfolded medico-legal mysteries. [8]

Several studies were conducted to see the role of biochemical parameters in determination of time since death. Lactic acid, amino acid nitrogen and non protein nitrogen (NPN), different enzymes like acid and alkaline phosphatases and transaminases (ALT) had shown an increasing trend. [1] LDH levels in serum also showed a linear rise. Lipid peroxidation is defined as the oxidative deterioration of polyunsaturated fatty acid.

The basic structures of all cell and organelle membranes are lipid bilayer, which mainly contain polyunsaturated fatty acid (PUFA) side chains. [6] Free radicals Hydroxyl, peroxy, hydroperoxy, alkoxy etc are capable of oxidizing PUFA.

Free MDA which arises largely from per oxidation of PUFA, measuring is the commonest assay of lipid per oxidation in vitro. [2] Low molecular weight Thiol, an organo-sulfur compound, and their associated enzymatic recycling systems are responsible for the protection of cells against oxidative stress.

Thiol containing compound is a Glutathione peroxidase. The Glutathione peroxidase super family present in plasma, in the cell lining of gastro intestinal tract, in the cytosol of red blood cells and also attached to the inside of the erythrocytic membrane.

During cell death the cell membrane as well as the enzymatic system all destroyed. So there will be certainly a change in MDA and Thiol levels in blood after death.

### Materials and Methods:

This cross-sectional study was done in FSM Department and Biochemistry Department of NRS Medical College, Kolkata. Total 90 cases were selected according to cause of death.

Blood sample was collected from the great vessels, the heart of the deceased by the help of disposable syringe in plain vial with known passage of time. The serum was separated from the clotted blood by centrifugation. Estimation of MDA was done by

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method of Ohkawa et al and of Thiol done by M.L.Hu method.

**1. MDA (Malondialdehyde) Estimation:**

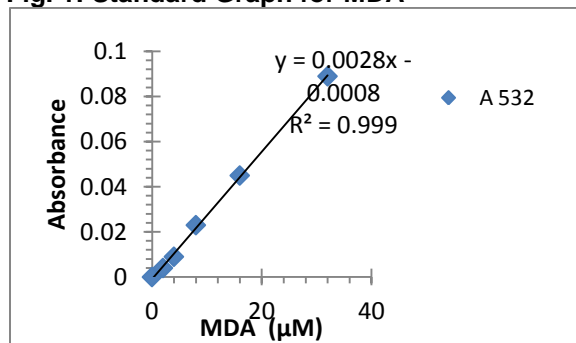
**Reagents Used:** TBA (Thiobarbituric acid), SDS (Sodium Dodecyl Sulphate), 20% Acetic Acid (pH 3.5), Mixture of n-butanol and pyridine (15:1), MDA as standard

**Protocol:** Here 1ml of serum was added to 1.5 ml of 0.8% aqueous solution of TBA, 0.4ml of 8.1% SDS and 1.5ml Acetic Acid (pH 3.5).The mixture was finally made up to 5ml by adding distilled water and heated at 95°C in a water bath for 1 hour. After cooling 1ml of distilled water and 5ml of n-butanol, pyridine (15:1) was added. The mixture was shaken vigorously after that centrifuged at 4000 round per minute for 10minutes. The absorbance of the organic layer was measured at 532 nm (A) against water blank (B).

The calculation of MDA is = (A – B)

The standard graph for MDA is plotted and the test values were extrapolated from the standard graph.

**Fig. 1: Standard Graph for MDA**



**2. Total Thiol Estimation:**

**Reagents used:** Tris-EDTA buffer, DTNB, Absolute Methanol.

**Protocol:** 200 µl serum was mixed with 600 µl Tris-EDTA (0.25M), EDTA (20mM) buffer, at pH 8.2 followed by addition of 40 µl, 10 mM DTNB and 3.16 ml absolute methanol.

It is kept for 15 minutes at room temperature. The absorbance of supernatant is measured at 412 nm (A) spectrophotometrically, and subtracted from a DTNB blank (B) and a blank containing the sample without DTNB (0.03). Total S-H groups were calculated using an absorptivity of 13600 cm<sup>-1</sup>M<sup>-1</sup>(extinction coefficient). The calculation of measurement of total Thiol was (A-B-0.03) X 4/0.2÷13.6 mM= (A-B-0.03) X 1.47 m

**Results:**

**Table 1: Pearson correlation of Time passed since with MDA and total Thiol in death from different causes**

Cause Of Death	Time Passed Since Death	
	MDA	TOTAL THIOL
Burn	0.682	-0.530
Asphyxia	0.382	0.131
Poisoning	0.640	-0.325
Fall from height	0.555	-0.305
Road traffic accident	0.479	-0.275

**Discussion:**

Lipid oxidation gives rise to a number of secondary products. Malonaldehyde is (MDA) one of the most frequently used indicators of lipid peroxidation. [4] The degree of lipid peroxidation is determined by measuring MDA concentration in postmortem blood. [5] This aldehyde is a highly toxic molecule and should be considered as more than just a marker. [3]

MDA Pearson correlation of Time passed since death with MDA and Total Thiol in the cases of various cause of death shows that MDA level is significantly correlated with time passed since death, i.e. as time elapsed since death increases the level of MDA in post mortem blood also increases as cells are disrupted after death. On the other hand Total Thiol level is inversely proportional with time passed since death, means negatively correlated. it indicate that as time passed since death increases the level of Total Thiol in post-mortem blood decreases and protection of cells from oxidative stress lost.

**Conclusion:**

This study shows that MDA and Total Thiol can be helpful and important biochemical parameters to estimate time passed since death.

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