# Determination of malondialdehyde levels in serum and plasma samples of children affected with sickle cell anemia

Mr. Sunmeet Matkar[1], Dr. Ajit K Gangawane[2]
PhD Scholar, LifeWave Diagnostic and Research Centre, Mumbai, Maharashtra, India[1]
Dean, Faculty of Vocational Education & Deputy Registrar, Parul University, Limda, Waghodia, Vadodara, Gujarat-391769[2]

**Abstract**

Sickle cell anemia (SCA) is an inherited disorder of hemoglobin synthesis categorized by sickle-shaped erythrocytes. Hemoglobin S present in deformed erythrocytes demonstrated an elevated rate of auto-oxidation as compared to normal hemoglobin A. Increased auto-oxidation produces additional reactive oxygen species (ROS) that ultimately promotes oxidatively stressed environment. Reactive oxygen species is capable of degrading the membranes of sickle-shaped erythrocytes composed of polyunsaturated lipids and form malondialdehyde (MDA) as a by-product. Objective of this study is to determine and compare the levels of MDA in serum and plasma samples of children affected with sickle cell anemia. A total of 100 children aged 10–15 years were divided into two groups: Group A (n = 50) consisting of children affected with sickle cell anemia and Group B (n = 50) consisting of healthy children. The study samples included serum and plasma, which were extracted from blood that was collected aseptically from both the study groups. Both study samples were subjected to thiobarbituric acid (TBA) assay for determination of the levels of MDA. Absorbance was estimated by spectrophotometer at 531 nm, and the values of concentration of MDA were derived. Our study reported that the mean levels of MDA in serum and plasma samples as 8.9825 ± 1.04 and 0.5152 ± 0.28, respectively, in Group A and they were found to be higher than mean MDA levels of serum (5.87 ± 0.92) and plasma (0.2929 ± 0.06) of Group B. The difference of their mean was found to be statistically significant. Our study recorded significant correlation between the levels of MDA in the plasma and serum samples of the children affected with SCA. Our study suggests that plasma can be effectively employed as an alternative for assessing the oxidative stress in children with SCA.

**Keywords**:-Malondialdehyde, plasma, serum, sickle cell anemia, reactive oxygen species.

## I. INTRODUCTION

Sickle cell anemia (SCA) is the most common and most severe form of sickle cell disease (SCD), a group of inherited blood disorders caused by a genetic mutation.[1] SCA affects the body's production of hemoglobin, the oxygen-carrying protein in red blood cells. It is associated with a significant morbidity and mortality due to episodic vaso-occlusive events, pain crises, and multi-organ damage.[2] SCA has been traced to a single point mutation that substitutes valine for glutamic acid in the β-globin subunit. Normal erythrocytes incorporate hemoglobin A (HbA) and are biconcave, in contrast to this, the erythrocytes of SCA patients incorporate hemoglobin S and are sickle shaped. HbS exhibits an enhanced rate of

auto-oxidation in comparison to HbA in the presence of reactive oxygen species (ROS), namely, superoxide, peroxide, and hydroxyl radical.[3] Under normal physiological conditions, antioxidant enzymes and oxygen radical scavengers inhibit basal fluxes of ROS. However, when the production of ROS overwhelms the endogenous antioxidant defense mechanism, it results in an oxidatively stressed environment. Children affected with SCA show an impaired antioxidant status due to reduced antioxidant defenses. Oxidative stress has been related to the etiopathogenesis of several chronic diseases.[4] It can damage specific molecular targets such as lipids, proteins, and carbohydrates resulting in cell dysfunction and/or cell death. However, lipids are the most commonly affected class of biomolecules and its oxidation gives rise to a number of secondary products.[5] Membranes of sickle-shaped erythrocytes are high in polyunsaturated fatty acids which are more susceptible to endogenous free radical-mediated oxidative damage. Thus, it affects the hemostatic environment. ROS degrade polyunsaturated lipids, forming malondialdehyde (MDA) as a by-product which is said to be the biomarker of increased oxidative stress.

MDA is the by-product of the radical-initiated oxidative decomposition of polyunsaturated fatty acids, and hence, it is a frequently measured biomarker of oxidative stress.[6,7] Estimation of oxidative stress is an essential part of routine blood investigations that are employed for monitoring health and disease. There are very few literature studies which estimate oxidative stress using plasma MDA, especially in individuals suffering from sickle cell anemia. Therefore, the present study was undertaken to evaluate and correlate the levels of MDA in serum and plasma in children suffering from sickle cell anemia.

## II. Experiment and Result

A total of 100 children in the age group of 10–15 years participated in the study which consisted of two groups. Group A (n = 50) included children who were randomly selected from the patients attending primary health centre at Nagpur Municipal Corporation Hospital, Nagpur, Maharashtra, India whereas Group B (n = 50) consisted of healthy controls. Children with any other systemic diseases (Hypertension, Diabetes, Cardiovascular disease, Thalassemia and HIV), immune-compromised status, or having any history of vaso-occlusive crisis in the past 3 months, who had a blood transfusion or any serious illness, were excluded from the study. The ethical guideline of 1975 declaration of Helsinki was followed and a written informed consent was obtained from the parents/guardians of all the children. The study protocol was approved by the Institutional Ethical Committee, Nagpur Municipal Corporation Hospital, Nagpur, India. The biochemical analysis for the study was conducted in the Department of Biochemistry, LifeWave Diagnostics and Research Centre, Mumbai, India.

In order to minimize diurnal variations, all the study samples were collected in the morning hours. The study participants were instructed not to eat or drink anything except water for at least 2 h before the sample collection. Two types of blood samples (plasma and serum) were involved in this study. To obtain serum and plasma samples, 2 ml of blood was drawn by a 24-gauge needle from the cubital vein of the left arm from all the study subjects. Intravenous blood was collected and centrifuged at 3000 rpm for 5 min in order to separate serum and plasma. Both serum and plasma were then stored at −20°C and later used to estimate the biochemical parameters. Estimation of MDA levels of plasma and serum was done using thiobarbituric acid (TBA) assay method as given by Satoh.[8] The TBA reacts with MDA giving rise to a high absorptivity adduct which can be easily assessed with a

spectrophotometer at 531 nm. A standard graph was plotted, and concentration of MDA was expressed as nmol/ml.

**Results:** The values of MDA detected in plasma and serum within both the study groups are shown in Table 1.

**Table 1:** Comparative evaluation of levels of malondialdehyde (MDA) in serum of group A and group B

| Groups | n | Mean±SD | SEM | Mean difference | t | P |
|---|---|---|---|---|---|---|
| Group A | 50 | 0.5152±0.28195 | 0.03256 | 0.22227 | 6.669 | 0.001* |
| Group B | 50 | 0.2929±0.06166 | 0.00712 | | | |

*P<0.05, significant; SD=Standard deviation; SEM=Standard error of mean

The levels of MDA in serum were compared between children with SCA and healthy controls using the student's unpaired t-test, and the result was statistically significant (P < 0.05).

Additionally, when the levels of MDA in plasma were compared between the two groups using the student's unpaired t-test, the results was statistically significant (P < 0.05). Table 2.

**Table 2:** Comparative evaluation of malondialdehyde levels in plasma of Group A and Group B

| Groups | n | Mean±SD | SEM | Mean difference | t | P |
|---|---|---|---|---|---|---|
| Group A | 50 | 8.9825±1.04329 | 0.12047 | 3.10587 | 19.288 | 0.001* |
| Group B | 50 | 5.8767±0.92536 | 0.10685 | | | |

*P<0.05, significant; SD=Standard deviation; SEM=Standard error of mean

The correlation of MDA levels in serum and plasma sample of study subjects with SCA is represented in Table 3 and the results. Analysis was done using Pearson's correlation coefficient.

**Table 3:** Correlation between malondialdehyde levels of serum and plasma in sickle cell anemic (case) children

| Groups | n | Mean±SD | Correlation (r) | P |
|---|---|---|---|---|
| Serum level | 50 | 8.9825±1.04329 | −0.002 | 0.98 |
| Plasma level | 50 | 0.5152±0.28195 | | |

Correspondingly, the correlation of the levels of MDA between serum and plasma sample of healthy study subjects is shown in Table 4. Analysis was done using Pearson's correlation coefficient.

**Table 4:** Correlation between malondialdehyde levels of serum and plasma in healthy (control) children

| Groups | n | Mean±SD | Correlation (r) | P |
|---|---|---|---|---|
| Serum level | 50 | 5.8767±0.92536 | 0.323 | 0.005* |
| Plasma level | 50 | 0.2929±0.06166 | | |

Upon comparison, the levels of MDA between plasma and serum samples using Pearson's correlation coefficient showed a highly significant result ($P < 0.05$). When the levels of MDA in serum and plasma were correlated with age, the statistical analysis revealed a non-significant result in study subjects with SCA while significant result in healthy study subject controls ($P < 0.05$) (Tables 5 and 6).

**Table 5:** Correlation of age with levels of malondialdehyde in serum and plasma of sickle cell anemic study subjects

| Groups | n | Mean±SD | Correlation (r) | P |
|---|---|---|---|---|
| Age | 50 | 8.2±3.25 | | |
| Serum level | 50 | 8.9825±1.04329 | −0.018 | 0.877 |
| Plasma level | 50 | 0.5152±0.28195 | 0.080 | 0.494 |

**Table 6:** Correlation of age with levels of malondialdehyde in serum and plasma of healthy (control) subjects

| Groups | n | Mean±SD | Correlation (r) | P |
|---|---|---|---|---|
| Age | 50 | 8.17±3.05 | | |
| Serum level | 50 | 5.8767±0.92536 | 0.619 | 0.001* |
| Plasma level | 50 | 0.2929±0.06166 | 0.313 | 0.006* |

Oxidative stress has been implicated in many chronic diseases.[9] Oxidative stress is induced by reactive oxygen species (ROS) who have been reported to play a very important role in cell signalling and metabolic processes and also have been thought to be implicated in the pathogenesis of a variety of inflammatory disorders.[10] The most commonly involved biological targets of oxidative stress are polyunsaturated fatty acids thereby providing malondialdehyde (MDA) as a by-product on peroxidation.[11] MDA is able to damage several physiological mechanisms of the human body through its ability to react with molecules such as DNA and proteins. It is, therefore, useful to consider this molecule as something more than a lipid peroxidation by-product. In the past 20 years, MDA has been recognized as a relevant lipid peroxidation marker, and as such, the measurement of MDA levels in biological samples from participants affected by several diseases has been widely utilized. Recent research has revealed potential applications of antioxidant/free radical manipulations in prevention or control of diseases.[12]

Basis on these introductory observations, our study put forwards that the differences in levels of MDA exist between SCA patients and healthy controls and that increased levels of MDA may be a feature of both local and peripheral extracellular fluids in patients with SCA. Consequently, the current study studied both the plasma and serum levels of MDA in the study participants with SCA and healthy controls. Numerous methods have been reported for measuring the levels of MDA in biological fluids.[13,14] However, no single assay can be

considered as ideal even though levels of MDA measurement can be carried out in an aqueous as well as in a lipophilic environment.[15] In the present study, the thiobarbituric acid (*TBA*) *method* , a quantitative assay was used for the quantification of the levels of MDA. Therefore, in the current study, quantification of levels of MDA was carried out using un-stimulated plasma. The blood sample has been the gold standard for conducting several medical tests.[16-19]

In the present study, the levels of MDA in serum were found to be elevated in study subjects with SCA compared to control study subjects. These elevated levels of MDA could be attributed to the enhanced ROS formation in SCA which forms a very stable structure by extracting electrons from other sources including enzymatic and non-enzymatic antioxidants.[20] Several studies have reported the increased levels of MDA in serum within various systemic conditions such as diabetes mellitus, malignancies of the stomach, breast, cervix, and premalignant lesions, and conditions such as leukoplakia and oral submucous fibrosis[20-21] including SCA[21]. The results of the current study are in accordance with the results of above studies. Findings of this study further emphasizes the role of oxidative stress in the pathophysiology of SCA and any intervention aimed at increasing the antioxidant capacity of these patients shall be beneficial.

The levels of MDA in plasma obtained from group B were found to be elevated while the levels were within the normal range in group A. The presence of MDA levels higher than normal in SCA patients may be due to oxidatively stressed environment which also suggests enhanced utilization of antioxidants and leading to a reduction of total antioxidant capacity in plasma.

## III. CONCLUSION

The level of MDA concentration was found to be elevated in children affected with SCA, and a significant correlation between the levels of MDA in serum and plasma indicates that changes in serum may be reflected equally in plasma. Therefore, assessment of levels of MDA in the plasma sample of SCA patients could serve as an alternative to that in serum. In our study, elevated oxidative stress may account for raised MDA level which serves as a biomarker.

## References

1. Ademola Samson Adewoyin, “Management of Sickle Cell Disease: A Review for Physician Education in Nigeria (Sub-Saharan Africa),” Anemia, vol. 2015, Article ID 791498, 21 pages, 2015. https://doi.org/10.1155/2015/791498.
2. McGann, P. T., Nero, A. C., & Ware, R. E. (2013). Current management of sickle cell anemia. Cold Spring Harbor perspectives in medicine, 3(8), a011817. doi:10.1101/cshperspect.a011817
3. Erfan Nur,1,2* Bart J. Biemond,2 Hans-Martin Otten,1 Dees P. Brandjes,1 and John-John B. Schnog, 2011, Oxidative stress in sickle cell disease; pathophysiology and potential implications for disease management, American Journal of Haematology, 86:484–489, 2011.
4. Damanhouri GA, Jarullah J, Marouf S, Hindawi SI, Mushtaq G, Kamal MA. Clinical biomarkers in sickle cell disease. Saudi J Biol Sci 2015;22:24-31.
5. Jenny Schröter and Jürgen Schiller, “Chlorinated Phospholipids and Fatty Acids: (Patho) physiological Relevance, Potential Toxicity, and Analysis of Lipid

Chlorohydrins," Oxidative Medicine and Cellular Longevity, vol. 2016, Article ID 8386362, 26 pages, 2016. https://doi.org/10.1155/2016/8386362.
6. Metkari S, Tupkari JV, Barpande SR. An estimation of serum malondialdehyde, superoxide dismutase and Vitamin A in oral submucous fibrosis and its clinicopathologic correlation. J Oral Maxillofac Pathol 2007;11:23-7.
7. Paulose S, Rangdhol V, Ramesh R, Jeelani SA, Brooklyin S. Estimation of serum malondialdehyde and assessment of DNA damage using comet assay in patients with oral submucous fibrosis. J Investig Clin Dent 2016;7:286-293.
8. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 1978;90:37-43.
9. Biswas S. K. (2016). Does the Interdependence between Oxidative Stress and Inflammation Explain the Antioxidant Paradox?. Oxidative medicine and cellular longevity, 2016, 5698931.
10. Schieber & Chandel (2014) Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Current Biology. 2014;24(10):R453–R462. doi: 10.1016/j.cub.2014.03.034.
11. D'souza D, Subhas BG, Shetty SR, Balan P. Estimation of serum malondialdehyde in potentially malignant disorders and post-antioxidant treated patients: A biochemical study. Contemp Clin Dent 2012;3:448-51.
12. Baskol G, Korkmaz S, Erdem F, Caniklioglu A, Kocyigit M, Aksu M. Assessment of nitric oxide, advanced oxidation protein products, malondialdehyde, and thiol levels in patients with restless legs syndrome. Sleep Med 2012;13:414-418.
13. Irfan Rahman & Saibal K. Biswas (2004) Non-invasive biomarkers of oxidative stress: reproducibility and methodological issues, Redox Report, 9:3, 125-143, DOI: 10.1179/135100004225005219
14. Sousa B. C., Pitt A. R., Spickett C. M. Chemistry and analysis of HNE and other prominent carbonyl-containing lipid oxidation compounds. Free Radical Biology & Medicine. 2017 doi: 10.1016/j.freeradbiomed.2017.02.003.
15. Elias DB, Freitas RM, Gonçalves RP, Magalhães HY, Sousa JH, Magalhães SM. Evaluation of the concentration of malondialdehyde and nitrite in patients with sickle cell anemia treated or not with hydroxyurea. Einstein (Sao Paulo) 2010;8:414-418.
16. Trivedi S, Lal N, Mahdi AA, Mittal M, Singh B, Pandey S. Evaluation of antioxidant enzymes activity and malondialdehyde levels in patients with chronic periodontitis and diabetes mellitus. J Periodontol 2014;85:713-720.
17. Tonguç MÖ, Öztürk O, Sütçü R, Ceyhan BM, Kilinç G, Sönmez Y, et al. The impact of smoking status on antioxidant enzyme activity and malondialdehyde levels in chronic periodontitis. J Periodontol 2011;82:1320-1328.
18. Turki A, Naji M. Measurement of serum malondialdehyde (MDA) levels as a marker of lipid peroxidation in neonatal sepsis. Med J 2011;5:9-17.
19. Beg M, Ahmad S, Gandhi S, Akhtar N, Ahmad Z. A study of serum malondialdehyde levels in patients of cerebrovascular accident. Indian Acad Clin Med 2005;6:229-31.
20. Ghallab NA, Hamdy E, Shaker OG. Malondialdehyde, superoxide dismutase and melatonin levels in GCF of aggressive and chronic periodontitis patients. Aust Dent J 2016;61:53-61.
21. Weiss SL, Deutschman CS. Elevated malondialdehyde levels in sepsis – Something to 'stress' about? Crit Care 2014;18:125.