

Review Article

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Serum Malondialdehyde (MDA) Levels as A Biomarker of Covid-19- A Diagnosis, Surveillance and Preventive Measure

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Abstract

The last two to three years were quite frightening for the medical health sector. In this time interval, the whole human civilization was facing serious problems pandemic outbreaks in all over the globe. Due to covid-19 no medicine was working in this pandemic. In view of the potential threat of pandemic, scientists around the world have been running to understand SARS-CoV-2 and discover the screening methods of this disease to find out potential screening, surveillance and prevention techniques invented. In this review article we discuss new approach to screening and monitoring the covid-19 infection. Advances in medical biosciences technology provide new methods for novel virus detection, quantify and severity. Analytical tools like spectroscopic /HPLC techniques for MDA molecular markers estimation offer a valuable alternative to covid-19 diagnosis and evaluation studies, management. The different approach to standardization of MDA estimation system which have been developed need to be field tested and the field data is collected so that the complete technology packages could be ready for commercialization and transfer to the user agencies.

Keywords: severe acute respiratory syndrome (SARS); Covid-19; MDA; lipid per-oxidation; ROS; oxidative stress; communicable diseases; screening

Introduction: Overview of COVID-19

COVID-19 is a worldwide, zoonotic disease caused by the novel coronavirus, a member of the Coronaviridae family. Coronaviruses are enveloped viruses with non-segmented, single-stranded, positive-sense RNA genomes [1]. There are six known human coronaviruses, with severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) being highly pathogenic. Coronaviruses are classified under the order *Nidovirales*, family *Coronaviridae*, and subfamily *Coronavirinae*. *Coronavirinae* is divided into four genera: α -coronavirus, β -coronavirus, Gama-

coronavirus, and Delta-coronavirus [2]. Beta-coronavirus has four distinct lineages (A, B, C, D), and SARS-CoV and MERS-CoV belong to lineage B. Coronaviruses have pleomorphic or spherical structures with an average diameter of 80–120nm [3]. The virion surface is decorated with club-like projections made up of the trimeric spike (S) glycoprotein. Some beta coronaviruses (e.g., HCoV-OC43 and HCoV-HKU1) have dimeric projections made up of the hemagglutinin-esterase (HE) protein. Other structural proteins include the membrane (M) glycoprotein, envelope (E) protein, and nucleocapsid (N) protein (Figure-1) [4].

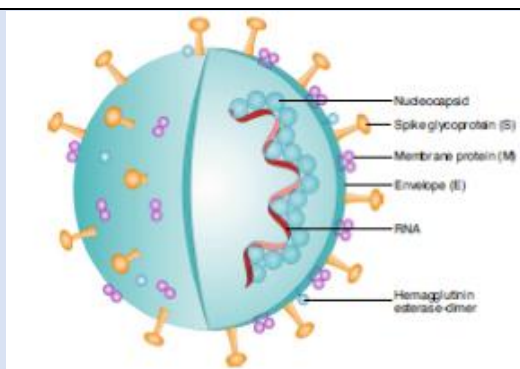


Figure 1: Schematic representation of SARS-CoV-2 structure. This is an enveloped, positive-sense RNA virus with four main structural proteins, including spike (S) and membrane (M) glycoproteins, as well as envelope (E) and nucleocapsid (N) proteins

Diagnostic methods include parasitological, radiological, and histo-pathological studies, as well as serological tests. Screening and therapeutic approaches for COVID-19 face challenges due to the novelty of the disease, and effective drugs and vaccines are still unavailable [5]. Collection of proper respiratory tract specimens at the right time and from the correct anatomical site is crucial for accurate molecular diagnosis. Real-time RT-PCR assays are the preferred molecular tests for diagnosing SARS-CoV-2 infections. Antigen-antibody-based techniques are being introduced as supplemental tools [6]. Current screening/diagnosis techniques have limitations, and there is a need for alternative tools that are easy to handle, cost-effective, and highly accurate [7]. Random access, integrated devices available at the point of care with scalable capacities are proposed to facilitate rapid and accurate screening and monitoring of SARS-CoV-2 infections [8]. This covers recent issues and challenges for the laboratory screening/diagnosis of infections caused by SARS-CoV-2. In the pre-analytical stage, collecting the proper respiratory tract specimen at the right time from the right anatomic site is essential for a prompt and accurate molecular diagnosis of COVID-19. Appropriate safety and efficacy measures are compliance to keep laboratory staff safe while producing rational test results. In the analytic stage, real-time RT-PCR assays remain the molecular test of choice for the etiologic diagnosis of SARS-CoV-2 infection while antigen-antibody based techniques are being introduced as supplemental tools. But all present screening/diagnosis techniques have a limitations and risk for negative, false positive result accuracy. In based upon above limitation we looking for other screening tools which have easy to handling, cost-effective and high accuracy potentials [9]. Therefore, currently proposed techniques, random access, integrated devices available at the point of care with scalable capacities will facilitate the rapid and accurate screening and monitoring of newly SARS-CoV-2 infections and greatly assist in the control of this outbreak.

Recently diagnosis challenges

Recommends using Nasopharyngeal (NP) swabs for early diagnosis or screening due to higher diagnostic yields, better patient tolerance, and increased operator safety. Suggests combining NP and Oropharyngeal (OP) swabs for increased sensitivity but acknowledges the need for twice the number of swabs [10]. Proposes alternatives like self-collected

saliva or nasal washes for epidemiological screening, especially for asymptomatic individuals ("worried well"). Advocates reserving NP swabs for hospitalized patients, with the possibility of using deep sputum or Broncho-alveolar Lavage (BAL) samples for those who initially test negative [11]. Highlights the importance of understanding the need for repeated testing or the use of bronchoscopy in severe cases if the initial screening test is negative. Calls attention to the insufficiently studied role of rectal swabs in advanced infections or as a test of infectivity/cure, stressing the urgency of further research in this area [12]. Stresses the need for broad screening/testing, involving both molecular and serological testing, to accurately estimate the true mortality rate and other epidemiological markers. Quick Development of Point-of-Care Molecular Devices: Emphasizes the critical importance of developing integrated, random access, point-of-care molecular devices for the accurate screening of COVID-19 infections. Describes these rapid STAT tests as significant for real-time patient management and infection control decisions, especially in situations where resources for respiratory isolation are limited. In summary, the commentary underscores the importance of optimizing screening methods, resource allocation, and the development of rapid diagnostic tools for effective and efficient management of the COVID-19 pandemic [13]. The emphasis on understanding the dynamics of testing, exploring alternative specimens, and broadening testing approaches contributes to a comprehensive strategy in addressing the challenges posed by the virus [14].

Overview of MDA

The role of super oxygen radicals (SOR) or Reactive Oxygen Species (ROS) in biological systems, particularly in the context of parasitic infections [15]. It also introduces the concept of oxidative stress and its potential physiological and pathological impacts. Additionally, the study mentioned focuses on measuring the serum concentration of malondialdehyde (MDA), an end-product of lipid peroxidation, in humans with COVID-19 to understand its relevance in the pathological mechanism of the disease [16]. ROS, including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^\bullet$), are produced by aerobic organisms and can have both physiological and harmful effects [17]. ROS are highly reactive and can be toxic, mutagenic, or carcinogenic. Despite their potential harm, ROS at sub-toxic concentrations play

crucial physiological roles in biological systems [18]. The passage mentions an active area of research exploring the role of highly reactive oxygen free radicals in the pathogenesis of parasitic infections. Polymorphonuclear neutrophils (PMNL) and monocyte/macrophage cells, part of the non-specific immune system, generate large amounts of toxic substances, including ROS and reactive nitrogen species (RNS) [19-20]. ROS and RNS, such as nitric oxide (NO), can have cytotoxic effects on bacteria, parasites, and tumor cells[21]. ROS and RNS have the capacity to degrade biomolecules, leading to oxidative stress [22,23,24]. Lipid peroxidation, involving the

degradation of membrane lipids, can compromise cell integrity and function. The study mentioned in the passage measures the serum concentration of malondialdehyde (MDA), an indicator of lipid peroxidation, in humans with COVID-19 [25,26]. Increased levels of MDA are associated with lipid peroxidation and have been correlated with various chronic diseases, including parasitic infections [27,28,29]. The study aims to understand the potential involvement of oxidative stress, ROS, and lipid peroxidation in the pathological mechanisms of COVID-19 by assessing MDA levels in infected individuals.

Table 1: Main viruses associated with reactive oxygen species (ROS) production.

Virus
Human immunodeficiency virus (HIV)
Hepatitis B virus (HBV) and hepatitis C virus (HCV)
Epstein-Barr virus (EBV)
Herpes simplex type 1 (HSV-1) and vesicular stomatitis virus (VSV)
Respiratory syncytial virus (RSV)
Influenza viruses
Human T cell leukaemia virus type 1 (HTLV-1)

Biochemistry and generation of MDA In-vivo and In vitro

In-depth explanation of the generation and effects of reactive oxygen species (ROS) and focuses on the role of malondialdehyde (MDA) as a reactive terminal degradation product [30,31]. Cellular respiration in aerobic organisms produces energy, but it also generates ROS as by-products. ROS are produced endogenously in mitochondria, peroxisomes, the endoplasmic reticulum, and the plasma membrane. Exogenous sources include UV light, heat, bacterial agents, tobacco smoke, and ionizing radiation [32]. ROS can react with membrane lipids, particularly polyunsaturated fatty acids (PUFAs), and leading to lipid peroxidation. Lipid peroxidation, both enzymatic and non-enzymatic, can result in the perturbed integrity of cellular structures, potentially leading to cellular death. Enzymatic mechanisms involve the activation of lipoxygenases, myeloperoxidases, cyclooxygenases, and cytochrome P450. Peroxydienyl radicals are generated, leading to the production of lipid hydroperoxide molecules (LOOH) [33,34,35]. Non-enzymatic mechanisms involve free radicals generated by NADPH oxidases and nitric oxide synthases [36]. Free radicals remove hydrogen from PUFAs, resulting in the generation of lipid-peroxides. Cellular antioxidant mechanisms involve enzymes such as glutathione peroxidases,

superoxide dismutases, and catalases [37]. Antioxidants like Vitamin C and Vitamin E act non-enzymatically to counteract the effects of ROS. Imbalance between oxidative stress and antioxidant mechanisms can lead to pathological oxidation [38]. End products of lipid peroxidation, including malondialdehyde (MDA), are used as indicators of oxidative stress in higher eukaryotic organisms [39]. MDA is an aldehyde with the chemical formula C₃H₄O₂ that mainly exists in its enol form at physiological pH7.6 (figure-2) [40,41]. Newly generated MDA can modify free amino acids, proteins, nucleotides, and phospholipids, creating stable covalent epitopes. Major carriers of MDA epitopes include apoptotic/necrotic cells, micro-vesicles (MV), and oxidized lipoprotein particles [42, 43]. Arachidonic acid (AA, 20:4) and docosahexaenoic acid (DHA, 22:6) in membrane phospholipids are substrates for MDA generation [44, 45]. Proposed mechanisms involve the formation of intermediates and breakdown through thermal or acid-catalyzed reactions. MDA can also be generated as a side product of thromboxane A₂ synthesis and through the oxidation of spermine, UV-irradiation of skin surface lipid squalene, or gamma irradiation of carbohydrates. The detailed explanation provides insights into the intricate processes of ROS

generation, lipid peroxidation, and the significance of MDA in cellular responses to oxidative stress [46].

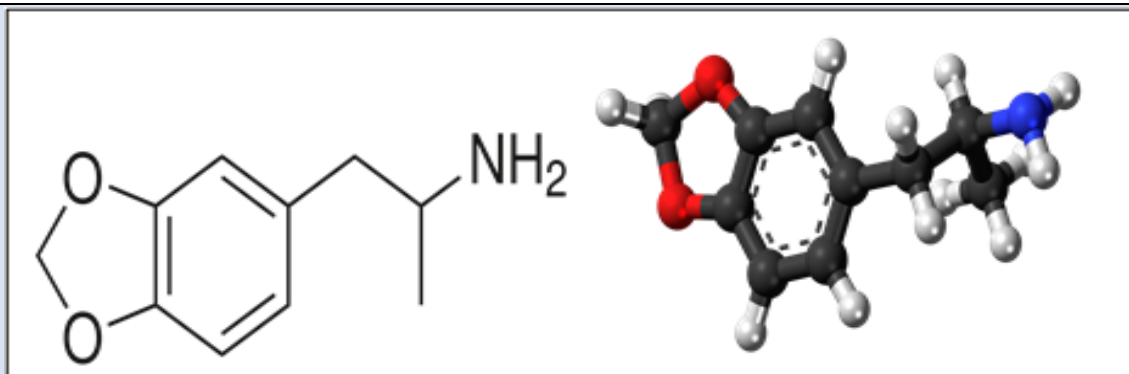


Figure 2: Chemical structure of MDA(2D,3D)

Generation of MDA epitopes

The characteristics and reactivity of malondialdehyde (MDA), particularly in the context of its pH-dependent behavior and its potential to form immunogenic epitopes, such as malondialdehyde-acetaldehyde (MAA) [47]. Liberated MDA exists as a bi-functional electrophile, and its reactivity is influenced by pH levels. At physiological pH (7.6), MDA primarily exists in the enolate-ion form. Lowering the pH (acidic conditions) can increase MDA reactivity, favoring the formation of beta-hydroxyacrolein and enhancing its affinity towards nucleophilic molecules [48].

At high concentrations of MDA in an acidic milieu (pH range 4-7), long oligomers of MDA can form. Hydrolytic cleavage of these MDA oligomers generates MDA and acetaldehyde (AcA) [48]. Monitoring the formation of MDA oligomers is crucial, as AcA and MDA can react to create malondialdehyde-acetaldehyde (MAA) immunogenic epitopes. MAA epitopes are more complex forms with potential immunogenic properties. Free AcA and MDA possess the ability to create epitopes on major cellular macromolecules in different tissues [49]. They serve as mediators of oxidative stress due to their chemical nature. The formation of stable epitopes with biomolecules, suggested to increase the half-life of MDA in vivo, is highlighted. MDA modifications of lipids, nucleotides, free amino acids, and proteins may lead to functional loss, structural integrity compromise, and altered cellular responses [50]. The passage indicates that modifications of each type of macromolecule (lipids, nucleotides, free amino acids, and proteins) will be discussed separately, potentially providing a detailed exploration of the consequences of MDA-induced modifications [51]. This segment

underscores the dynamic reactivity of MDA, its dependence on pH, and the potential implications of its interactions with acetaldehyde in forming immunogenic epitopes[52]. The consequences of MDA modifications on different macromolecules are highlighted as essential aspects of understanding its role in oxidative stress and cellular responses.

MDA epitopes in diseases

Diseases associated with increased levels of malondialdehyde (MDA) and highlights the role of MDA in various chronic and acute conditions related to oxidative stress [53]. Additionally, it mentions the involvement of highly reactive oxygen free radicals (ROS) in the pathogenesis of parasitic infections and viral diseases [54]. Cardiovascular diseases Neurodegenerative diseases Metabolic diseases Communicable diseases MDA levels in the plasma of healthy individuals show great variability, influenced in part by the method of blood drawing and sample preparation. *Leishmania Plasmodium falciparum* (malaria)*Ascaris lumbricoides* *Toxoplasma gondii* *Trypanosoma cruzi* [55]. Viral infections can trigger ROS production, either through cytokines secreted in response to the pathogen or by viral components [56]. Lipid peroxidation, a physiological process, is implicated in the pathogenesis (intensity of virulence) of various parasitic diseases [57]. ROS-induced lipid peroxidation disrupts cell membranes, leading to necrotic death. Infection with various parasites is associated with a marked elevation in lipid peroxidation [58]. The commonly used TBARS assay for MDA estimation is known to be non-specific. More reliable methods, such as MDA-specific antibodies or mass spectrometry-based assays, exist but their reliability as biomarkers for all diseases remains unclear [59]. The passage emphasizes the

broad spectrum of diseases linked to increased MDA levels and highlights challenges in accurately estimating MDA, especially in healthy individuals and in the context of specific diseases [60]. It underlines the need for more specific and reliable biomarkers for assessing oxidative stress in various pathological conditions.

Estimation methods of MDA methods

Spectrophotometric estimation

Commonly used method for measuring lipid peroxidation as an indicator of reactive oxygen species (ROS)-mediated damage to cell membranes [61]. The method involves using a spectrophotometer at specific wavelengths or a spectrometer for detection. Malondialdehyde (MDA) is highlighted as one of the well-studied end-products of lipid peroxidation and is frequently used to estimate oxidative stress conditions in clinical samples [62]. Lipid peroxidation is commonly used as an indicator of ROS-mediated damage to cell membranes. Measurement is done using a spectrophotometer at an absorbance of 520-535nm or by a spectrometer at specific excitation and emission wavelengths (λ excitation = 515nm, λ emission = 555nm) [63,64]. Malondialdehyde (MDA) is a well-studied end-product of peroxidation of polyunsaturated fatty acids [65]. The amount of MDA is frequently estimated using the thiobarbituric acid reactive substances (TBARS) technique. Various methods for MDA estimation include those described by Donnan [66], Yagi [67], Mihara and Uchiyama [68], Buege and Aust [69], Ohkawa et al. [70], Yoshioka et al [71]. In these methods, MDA reacts with TBARS in an acidic medium at 100°C to produce a pink/red-colored product [72]. MDA may also react with TBARS, producing derivatives that absorb light in the same wavelength range. A kit for MDA estimation is mentioned as being available, providing a convenient option for researchers and laboratories [73]. This passage outlines the practical aspects of measuring lipid peroxidation, particularly focusing on MDA estimation using the TBARS technique [74]. The described methods offer a fast, sensitive, and easy approach for assessing oxidative stress conditions in clinical samples.

Other alternative approach

The alternative methods for estimating blood plasma malondialdehyde (MDA) levels, along with other lipid peroxidation biomarkers. These methods offer different approaches with varying levels of reproducibility, reliability, and practicality [75].

HPLC using a C-18 reversed-phase column is mentioned for estimating blood plasma MDA levels. The method is described by Victorino et al. and provides reproducibility and reliability [76]. MDA estimation can also be done by GC-MS on a capillary column following trans-methylation with sodium methoxide. While more reproducible and reliable, this method is considered time-consuming, labor-intensive, and impractical for individual sample processing [77]. Various biomarkers, such as 8-isoprostaglandinF2 α (8-iso-PGF2 α), 4-hydroxy-2-nonenal (4-HNE), conjugated dienes (CD), and lipid hydroperoxides (LOOH), are mentioned. 8-Iso-PGF2 α , a result of non-enzymatic peroxidation of arachidonic acid, can be measured using rapid ultra-high-performance liquid chromatography-tandem mass spectrometry [78]. The method is noted for being labor-intensive and requiring specialized and expensive instrumentation. Unsaturated hydroxyalkenal 4-HNE can be evaluated in tissues, preferably using immunohistochemistry (IHC) or HPLC. CDs, resulting from free radical-induced autoxidation of polyunsaturated fatty acids (PUFAs), can be investigated based on the maximal absorption of UV light at 233nm. LOOHs, primary oxidation products of PUFAs, can be estimated by the ferrous oxidation xylenol-orange (FOX) assay. This assay is based on the ability of LOOH to oxidize ferrous iron in the presence of xylenol orange, forming a colored ferric-xylenol orange complex with absorbance at 560nm [79]. These alternative methods and biomarkers provide diverse options for researchers to assess lipid peroxidation and oxidative damage to cell membranes. Each method has its advantages and limitations, and the choice depends on the specific requirements of the study.

Application of Serum MDA Estimation for covid-19 pandemic

1. Very easy error free sampling
2. Human error is very less almost negligible at sample collection
3. It will be mostly using randomized/surveillance testing in general population
4. Easy operative and result interpretation techniques.
5. Very helpful to knowing virus load in infected patient
6. Easy to knowing severity of infection
7. It will also enable also asymptomatic covid-19 infected individuals

8. it will be very economical so easily used as whole population
9. It will be helpful for therapeutic management of covid-19 patient
10. It will also enable to measure immunity index of general population
11. Reduce the risk of lab personals which handling the sample
12. Testing cost approximately 25/test manual method, 55/kit method, 200/ hplc method.
13. Testing time 1hour spectroscopic method and 3-hour hplc methods
14. Less safety measure required for sampling transportation
15. Not specific, extra training required to lab technicians
16. It will be helpful to research also (for therapeutic research)
17. Setup of surveillance lab easily as well as establish in very remote area
18. Less lab facility required
19. This testing techniques also helpful to assessing health index of population
20. It will be applicable assessing healthy person immunity level

Limitations

1. Standardization required
2. Cross examination is measure issue
3. Set the infection range value
4. Increase the accuracy

Practical relevance

The challenges and cost matter of covid-19 infection diagnosis has made necessary the development of new user-friendly methods. Advances in medical biosciences technology provide new methods for novel virus detection, quantify and severity. Analytical tools like spectroscopic /HPLC techniques for MDA molecular markers estimation offer a valuable alternative to covid-19 diagnosis and evaluation studies, management. This research may summarize the alternate cost-effective techniques in plant COVID-19 pandemic investigation and surveillance with special accentuation on the screening and monitoring efforts of the MDA quantification.

Executive Summary

Novel corona virus pandemic is also central to innovative areas of current research scenario, including diagnosis and therapeutic management. The prime importance of covid-19 pandemic of surveillance/screening, entire population. Due to cost

and sampling and facility challenges would be to required cost effective and simple handling utility techniques for covid-19 Diagnosis and evaluation. The significance of an efficient MDA estimation protocol would be too easy and cost-efficient techniques to screening maximum number of populations in minimum period of time with proper safety and accuracy along with therapeutic index against covid-19 pandemic. The different approach to standardization of MDA estimation system which have been developed need to be field tested and the field data is collected so that the complete technology packages could be ready for commercialization and transfer to the user agencies

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