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*Review Article*

# Diagnostic and Therapeutic Application of Proteomics in Infectious Disease

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The study of an organism's genome, often known as "genomics," has advanced quickly, producing a wealth of publicly accessible genetic data. Despite how valuable the genome is; proteins essentially control most aspects of cell function. Proteomics, or the comprehensive study of proteins, has emerged as an important technology for disease characterization, diagnosis, prognosis, drug development, and therapy. Proteomics technologies are now used to support the diagnosis and treatment of both infectious and noninfectious diseases. Nevertheless, it is more difficult to describe a proteomic profile since a single gene product may result in a number of unique proteins, and proteins have a wider range of chemical configurations. The proteome profiles of a particular organism, tissue, or cell are impacted by a variety of environmental factors, including those triggered by infectious agents. This review intends to highlight the applications of proteomics in the study of disease diagnosis and treatment. In this review, the different technologies used in proteomics studies, like two-dimensional gel electrophoresis, mass spectrometry, and protein microarray as well as biomarker discovery and drug target identification using proteomics, have also been focused on.

## 1. Introduction

Several microbes are found in our surroundings, though most of these microbes are not appreciated through the naked eyes. Microorganisms like viruses, bacteria, fungi, and protozoa that exist in the environment are the cause of infectious disease [1]. A relatively small number of these infectious microorganisms cause the death of humans and animals. Death of humans and animals due to infectious microorganism accounts the highest proportion, especially in developing countries [2–4]. Despite their simple cellular structure and less-culturing conditions, microorganisms have been subjected to extensive studies [5]. Compared to larger species such as plants and animals, microbes possess relatively small genomes, which are more feasible for molecular sequencing [6]. In addition to sequencing, more sophisticated technologies are needed to help understand these disease-causing microorganisms and enhance novel methods for diagnosis and treatment of the disease [7, 8]. Proteomics is one such methodology that is increasingly being used as a tool to study a variety of disease states [2, 5].

Protein which is the polymer of amino acids is a highly complex substance that is present in all living organisms [9, 10]. Each specific amino acid encoded by the messenger ribonucleic acid (mRNA) determines the primary structure of proteins which again the proper folding will lead to protein secondary structure. The alpha helix and beta pleated sheet are protein secondary structures. Turns and coils interact chemically with each other to form the unique three-dimensional shape of the final protein [11]. Many proteins, however, have several different polypeptide subunits that make the final active protein. For these proteins, the interactions between the different subunits form the quaternary structure. These all can be studied in the field of proteomics

[12]. Proteins have several functions in the living organism. Among the most promising results of studying proteins are the identification of drug sites and the discovery of biomarkers for the treatment and diagnosis of disease. This relies on proteomics to identify proteins associated with a disease [9, 13]. For the normal functioning of proteins, posttranslational modification (PTM) is very essential. The modification is important for protein stability, biochemical activity, and protein targeting and signaling. PTM most often occurs in the endoplasmic reticulum and the Golgi apparatus of the cell. Among the many forms of modification, protein phosphorylation is the common proteoform involved in the regulation of the physiological process [14].

Proteomics is crucial for early disease diagnosis and prognosis and for monitoring disease development. Technologies like mass spectrometry (MS), two-dimensional gel electrophoresis (2-DE), and protein microarrays are the techniques that can be used to study proteomics [14, 15]. In particular, MS and proteomics-based technologies have increasingly been used to characterize the molecular details of pathogen-host relationships and provide insights into the biological basis of infectious diseases. MS-based proteomics is in a high level of supporting the development of diagnostics and therapies and the emerging role of multiomics strategies in providing a systems biology view of pathogen-host relationships [1]. Protein microarray is a high throughput method, which is rapid, automated, economical, and highly sensitive consuming only small quantities of samples and reagents for tracking the interactions and activities of proteins [16]. Therefore, the objective of this review is to provide a comprehensive understanding of some of the techniques that have been used in proteomics and the role of proteomics on infectious disease diagnosis and treatment.

## 2. Techniques Used in Proteomics

Analytical and bioinformatics tools are used in proteomics to characterize and analysis of protein structure and functions. Analytical techniques like 2D gel electrophoresis and matrix-assisted laser desorption ionization-time of flight-(MALDI-TOF-) MS are used as part of the proteomic study. In the case of bioinformatics, numbers of software tools are used [9, 17].

*2.1. Two-Dimensional Gel Electrophoresis.* Two-dimensional gel electrophoresis is a powerful method for the analysis of complex proteins [2, 8]. 2-DE technique is mainly used to compare two similar samples to find specific protein differences [9, 16]. This technique can separate over a thousand of protein spots per gel. Then, individual proteins are excised from the gel and purified for identification, usually by MS analysis. The process starts with the extraction of proteins from the biological sample followed by loading on a pH gradient. The first dimension also called isoelectric focusing separates different molecules by their isoelectric point. The second dimension, sodium dodecyl sulphate (SDS), separates protein based on their molecular size [8, 18].

*2.2. Mass Spectrometric Analysis.* Mass spectrometry is one of the analytical techniques requiring only a few nanomoles of a sample to obtain characteristic information pertaining to the structure and molecular weight of the analyte [19]. It is a versatile tool in large-scale proteomics in which the spectra are used to classify the molecules, atoms, or isotope stereotype signature of a sample, such as peptides [9, 17]. The technique identifies a molecule by sorting it according to the mass-to-charge ratio of the substance [8, 20]. MALDI-TOF is the most useful technique for protein identification [8, 9].

The protein can be breakdown into pieces with trypsin enzyme, which breaks it down at particular amino acid sequences and form peptide fragments [19]. The resulting peptide fragments will be energized by different ionization techniques, most often by a laser (MALDI); the sample for analysis will be coated with energy absorbent and sometimes by electrospray ionization (ESI)/desorption ionization method, a soft ionization technique with good sensitivity and reliability of determining the molecular weight of proteins and peptides without fragmenting the macromolecules into smaller charged fragments. In the first step of the analysis, the protein must be transformed into the gas phase [14, 21]. The charged peptide fragments are then resolved based on their mass-to-charge ratio on the spectrometer. The fragments that have been separated tamper with a detector, which gauges the strength of each fragment's signal and produces a mass spectrum [8, 22].

The peptide fragment is represented by the graph produced due to signal intensity against the mass-to-charge ratio. The height peak represents the most common form/abundance of that fragment. Most often, one peptide fragment differs from its neighbor by a single amino acid. As a result, the peptide sequence can easily be determined. This process is referred to as peptide mass fingerprinting. The peptide sequence determined is then correlated with protein databases to identify the actual protein [8].

*2.3. Protein Microarrays.* In the field of proteomics, protein microarrays are the vital and emerging techniques which enable for the high throughput detection of proteins from small number of samples. They can be analytical, functional, and reverse-phase microarrays [14, 23].

Analytical protein microarray also called capture microarray in which a library of antibodies and aptamers are arrayed on the support surface. Hence, antibody microarray is a class of this microarray. The captured antibodies enabled for the detection of labeled proteins. The technique is very important to measure the expression level and binding affinities of proteins [24, 25]. Microarray immunoassays have been used for the detection arrange of microorganisms like *Staphylococcal enterotoxin B*, cholera toxin, *Bacillus globigii*, and B ricin [26].

Functional protein microarray is the second type of analysis focused on the different interactions including protein to DNA, protein to RNA, protein to protein, protein to drug, protein to lipid, and enzyme-substrate relationship [23]. Functional protein microarray was first applied for the analysis of the specificity of the substrate in yeast protein kinases

[27]. The roles of proteins are described by functional protein microarrays [14, 28].

The other type called reverse-phase protein microarray is a sensitive and high throughput immunoassay for protein analysis from various samples usually tissue samples, cells, and body fluids. The technique involves, on the nitrocellulose slide, cell lysates/antigens of various states, which are immobilized as a capture molecule and analyzed with antibodies against target proteins. Then, antibodies are detected by using fluorescent, chemiluminescent, and colorimetric assays [17]. Protein quantification can be done by using reference peptides printed on the slide. Certain disorder determination through the abnormal/altered protein identification is achieved by these microarrays [23]. The reverse-phase protein microarray analysis on hematopoietic stem cell and primary leukemia samples shows that it is highly reproducible and accurate for large-scale phosphorylation status and protein expression analysis in human stem cells and acute myelogenous leukemia cells [29]. For quantitative analysis of phosphoproteins and other cancer-related proteins in non-small cell lung cancer (NSCLC) cell lines, the reverse phase protein microarray approach was evaluated by monitoring apoptosis, DNA harm, cell cycle control, and signaling pathways [14, 30].

## 3. Diagnostic and Therapeutic Application of Proteomics

Proteomic studies consider the metabolic states of an organism on a large scale level unlike the conventional biochemical experiments that are focusing on particular and/or specific protein complexes of an organism. Under proteomic studies, genome-wide recognition and quantitative measurement of microorganism proteins are included [2, 31]. The wide range of protein recognition provides a solid basis for proteomic studies, such as protein quantitation, cellular localization, and the interaction between proteins to study its area of application [2, 32]. In general, proteomic applications are mentioned below.

*3.1. Proteomics in Biomarker Discovery.* The most dominant diagnostic tools currently in use are immunological techniques, particularly those focused on selected antibodies to detect the plethora of blood proteins known as biomarkers [33, 34]. In general, a biomarker refers to disease-related proteins or a biochemical predictor that can be used in a clinic to diagnose or control disease activity, prognosis, and disease development, as well as to guide molecular target treatment or therapeutic response evaluation [35]. A biomarker in aspects of medicine can be used as a traceable substance that could be inserted into an organism as a means for evaluating the status of health. Prostate-specific antigen is one example of a commonly used biomarker in medicine [7].

When protein expression changes in biological pathways during disease conditions, monitoring of these altered proteins in tissue, blood, urine, or other biological samples can provide indicators of the disease [36]. Disease-specific biomarker calcification by using global protein profiling can be done by proteomics technology [37]. Proteomics expression provides biomarker identification by comparing the profile of protein expression between normal samples and those affected by the disease. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), in which protein profiles are matched between normal and disease samples, is the simplest method used in biomarker discovery [38].

Disease-specific biomarkers can be categorized into diagnostic for early detection; prognostic to predict disease recurrence; and treatment-predictive biomarkers. Predictive biomarkers classify patients into categories of responders and nonresponders [38]. This classification also is important in drug design applications. So these biomarkers, in general, could reflect how patients feel and survive [39]. Biomarkers must be processed using bioinformatics analysis after their detection by a mass spectrometry-based method and must also be replicated in various populations. It does not mean the technique is full of success; rather, a few established biomarkers using proteomics technology have been approved by the Food and Drug Administration for clinical use [40].

Proteomics technology is a promising method for disease-related biomarker identification in biological fluids, including urine, plasma, and serum. Body fluid samplings are less invasive and have low-cost advantages for proteomics research. In diseases like cancer, cardiovascular diseases, AIDS, renal diseases, and diabetes, the proteomic biomarker discovery is advanced [41]. Despite many technical advances, the body fluids are composed of a range of proteins that has to be solved for the effectiveness of the biomarker discovery [42]. Blood is a suitable fluid for biomarker discovery to be used in human illness, as it has several merits over other samples. On the other hand, it is difficult to identify biomarkers using plasma proteomics due to the high dynamic range of plasma protein, low abundance of invaluable plasma biomarkers, and patient heterogeneity [43]. The most popular approaches used to classify biomarkers for various diseases are 2D-PAGE/MALDI-TOF and surface-enhanced laser desorption/ionization (SELDI)/protein chip methods. In a number of diseases, SELDI-MS is used for biomarker detection due to its ease of operation and high-throughput application [7, 44].

*3.2. Proteomics in the Diagnosis of Infectious Diseases.* Nowadays, individual proteins as disease markers are very important for disease identification. But it is not always reliable because the high level of antigen may be detected in benign cancer like the benign form of prostate cancer. The advancement of proteomics technology favors for the analysis of low molecular weight proteins, which may divulge the patterns of disease and are useful for early detection and determining the prognosis of the disease progress. Both infectious and noninfectious diseases like cancer, acquired immune deficiency syndrome (AIDS), tuberculosis, malaria, measles, hepatitis, and severe acute respiratory syndrome (SARS) have been investigated using proteomics [5].

Proteomic methods can also be applied for the diagnosis of tuberculosis in recent years. Tuberculosis is a global concern that can affect a number of individuals worldwide, and drug-resistant strains of *Mycobacterium tuberculosis* are a growing concern. Tuberculosis can be diagnosed via direct

methods like culture, biochemical assay, antigen detection by monoclonal sera, analysis of lipid composition by chromatography, and detection of the DNA/RNA of the bacteria. Tuberculosis can also be diagnosed by indirect methods by detecting IgG or IgM antibody against *Mycobacteria* and cellular immunity via skin test. Serological tests are capable of detecting preclinical infection that allows for early intervention and potentially reduces the rate of transmission. Perhaps proteomic methods enable for the establishment of proteins secreted by the clinical isolates in vitro. Among these, rRv0566c, rRv3874, and rRV3369 have shown potential as serodiagnostic antigens with a sensitivity of 43%, 74%, and 60%, and a specificity of 84%, 97%, and 96%, respectively [45]. Therefore, kit-based serum screening test can be performed by using such groups of proteins [8].

Proteomics plays a significant role in the diagnosis of sera proteins during periods of SARS-CoV-2 outbreak. Severe acute respiratory syndrome is a viral infectious disease which was the cause of a number of deaths. For the treatment and control of this disease, a precise diagnostic approach is important. MS-based proteomic techniques have been used to detect SARS-CoV-2 viral proteins in both man and animal. Sera proteomic study of SARS patients revealed possible protein markers of truncated forms of $\alpha$1-antitrypsin (TF-$\alpha$1-AT) that were consistently detected with higher concentrations in SARS patients than healthy controls [46]. These markers are proven useful as therapeutic targets, vaccine targets, and diagnostic tool for SARS patients [8].

Cancer-related deaths are in the alarming rate in today's world. Hepatocellular carcinoma (HCC) is among the deadly cancers which are mainly caused by hepatitis B virus (HBV). A number of deaths have been recorded in patients infected with HBV [47, 48]. Chronic hepatitis C virus (HCV) infection can also play a role to HCC, although it is not comparable with HBV cancer cases per year [5]. A low percentage of HCC cases which are caused by a third form of hepatitis called hepatitis G virus have been reported. HCC can be diagnosed by using various diagnostic approaches like imaging and serum tumor marker measurement. Alpha-fetoprotein (AFP) is a commonly used serum tumor markers of HCC. AFP lectin fraction L3 (AFP-L3) and des-gamma-carboxy prothrombin (DCP) also played a significant role clinically [48].

*3.3. Proteomics in Drug Discovery and Treatment of Disease.* Proteomics-based research has extended its role in almost all scientific areas of research over the past few years. Proteomic technology plays a significant role in the area of therapeutics in drug target identification and evaluation of drug efficacy and toxicity level of the drug. As the discovery of drugs is an inherently complex process and high-cost values, modern emerging technologies like proteomics can facilitate the processes of discovery [49].

There are several stages of drug development, and it is also a multidisciplinary area using genomics, proteomics, metabolomics, bioinformatics, and system biology. Proteomics experiments are also useful in investigating drug action, toxicity, tolerance, and efficacy. Although genomics is applied for drug discovery process, proteomics-based methods are preferred because gene expression level is not an absolute indicative of protein expression [50], and genomics presents cellular events only to the transcriptome stage [51]. Therefore, emphases on proteomics studies that assess large-scale proteins and their posttranslational modifications (PTMs) shall be concentrated. Proteomics enables to compare changes in protein levels in normal and diseased tissue which are important in drug target identification. The first step in the drug discovery process is target recognition, and the recognition and early validation of disease-modifying targets are important steps in the drug discovery pipeline. In reality, proteomics technology plays a significant role in the identification of drug target proteins, as proteins are important drug targets in disease conditions [52].

A wide-ranging understanding of disease-associated pathways often plays an important role in designing an agent to inhibit or increase a certain chemical pathway or cycle in the drug development procedure. Drugs are designed to target specific proteins in the cellular network. The use of proteomics-based technologies is, therefore, appropriate in order to understand this network of protein interactions, to locate the drug candidates on their protein targets, and to expose cellular mechanisms resulting in the observed phenotype [7, 53].

In the treatment of many infectious diseases, drug resistance is also a significant clinical issue, and, in many cases, the cause is unclear. The sequenced genetic and protein data of several microorganisms enables for the identification and classification of novel drug-resistant genes [3, 54, 55]. For example, ergosterol biosynthesis protein Erg10p, which is expressed in *Candida albicans*, has been linked to azole resistance [56]. This is the principal drug target for the *Candida species* [8]. Chloroquine, the known malarial remedy, has become out of the market in most part of the world due to the increased emergence of resistance. Proteomics plays a significant role to resolve this challenge by identifying the possible drug targets in *Plasmodium* organisms as well as evaluating the host-pathogen interaction and interaction of protein drugs [10]. To date, developments include the identification of variations between *Plasmodium* species, immune target identification for vaccination and immune defense, and a detailed understanding of chloroquine cell targets and mechanisms of chloroquine resistance. A number of disease resistance in microorganisms can be resolved by using the proteomics technology via new drug target identification or understanding the host-pathogen interaction [8].

In the near future, proteomics is predicted to have a significant effect on drug development. Potential drug targets are proteins that are expressed differently between healthy and diseased ones. These can be used to classify lead compounds, which are prospective novel therapeutics with in vitro action against the target, using commercially accessible chemical compound libraries. Exploiting this advanced new era of knowledge to develop better treatments can be the next challenge [57].

## 4. Conclusion

Proteomics, which is the comprehensive study of the organism's whole protein, is a very important tool in the diagnosis

of disease and for the discovery of drug targets at conditions where drug resistance is a growing problem. The protein-protein interaction, protein modification, the three-dimensional structure of proteins, and protein function are studied and monitored in this area of "omics." The introduction of 2-DE, MS analysis, and protein microarray plays a valuable role for the study of proteins. The discovery of biomarkers for the diagnosis of the disease is a major area of proteomic studies beyond a number of industrial applications, though it is not widely used all over the world. Proteomics is not usually practiced in the developing world even though it has indispensable role in the different areas of research. Generally, this review mainly focuses on the role of proteomics in disease diagnosis and therapeutics in association with biomarker discovery and drug target identification.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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