Application of emerging biomarkers as a novel approach for diagnosis of diseases

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ABSTRACT

Over past decade, exponential progress has been made in the area of biomarker research. Biomarkers are indicators whose presence, absence, or abnormal levels is indicative of a normal state, disease, or exposure to xenobiotics. Several novel biomarkers have been discovered and many more are being identified each year. In addition, significant improvement has occurred in techniques which are utilized for detection and quantification of these biomarkers apart from development of novel techniques. Biomarkers have been used for variety of purposes such as diagnosis of diseases, their prognosis, risk assessment and even in their prediction. The focus of this paper is on biomarkers which are utilized in diagnosis of diseases. The paper emphasizes on various perspectives of diagnostic biomarkers including ideal diagnostic biomarkers, their discovery, their applications in diagnosis of various diseases and technologies that have been utilized in their identification and quantification. The paper sheds light on potential of biomarkers in diagnosis, challenges posed in their application in clinical setting and future potentials.

Keywords: - *Biomarkers, diagnosis, genetic biomarkers, epigenetic biomarkers, cancer biomarkers, neurodegenerative diseases biomarkers, cardiovascular diseases biomarkers.*

1. INTRODUCTION

Biomarkers are indicators whose presence, absence, or abnormal levels (i.e., either higher than normal or lower than normal level) is indicative of a normal state, medical condition, or exposure to xenobiotics (allergens, pollutants, drugs). For example, abnormal levels of glucose, either higher or lower than normal, is indication of hyperglycemia and hypoglycemia, respectively and on measuring its levels using glucometer, can serve as marker for diabetes and normal glucose levels indicates absence of diabetes and normal sugar metabolism. The term is often used synonymously for molecular markers, but they are just one among the several types of biomarkers, thus biomarker is inclusive of molecular as well as other indicators of biological nature including cellular imaging and other indicators. By identifying, detecting, and quantifying the biomarkers accurately using one or other technique, the subject can be diagnosed for the presence or absence of medical condition(s), for the state of health, and for exposure to xenobiotic. As there is great diversity in biomarkers , detection of each type requires diverse technologies ranging from simple biosensors to sophisticated equipment. For one disease several biomarkers maybe available and many novel markers are discovered each year, yet all may not reach the clinical setting for diagnosis. Only few of the indicators are able to get validated and selected for application in clinical diagnostics. It includes those indicators which fulfill most of the characteristics of ideal biomarkers of which specificity for the disease , quick response time on detection and minimally invasive nature of isolation are of prime importance.

There are number of types of biomarkers based upon their characteristics and involvement in pathophysiology of the disease like for example if mutation in gene is giving rise to disease, then it will fall under genetic biomarkers

though it will also fall under molecular marker as genes are biomolecules, hence a single biomarker may fall into more than one category. Some of these categories have been discussed in this paper. Further the paper lays emphasis on applicability of biomarkers in diagnosis of various diseases. Although majority of diseases can be diagnosed with biomarkers , some of the diseases which have other technological interventions for diagnosis and have potential for replacement with biomarker-based diagnosis have been highlighted in this paper such as various types of cancer, cardiovascular diseases , neurodegenerative diseases , and sleep disorders. Lastly, the paper discusses about challenges and shortcomings that create hinderance in implementation of biomarkers in clinical diagnostics which can be addressed through large scale research and technological advancement in biomarker identification, detection, and quantification methods.

2. REVIEW OF LITERATURE

2.1 Ideal Diagnostic Biomarkers

A biomarker may be called as an ideal one if it possesses most of the characteristics as discussed subsequently. It should be non-invasive or minimally invasive in nature which offers benefits such as minimal damage or influence on body, greater acceptability, and ease of isolation. It should be affordable requiring minimal, inexpensive, and simple equipment for detection. Biomarker must give reproducible and quick results [1]. The level must be greatly variable than normal even in early stage of diseases and hence allow for early diagnosis of disease [1]. This will in turn allow for early treatment. It should be highly specific for a condition and should not be affected by comorbid conditions and environmental factors. A biomarker whose level is elevated or reduced in more than one medical condition is not ideal for diagnosis as it is not specific for one condition and cannot be used for differential diagnosis. This occurs especially when two or more conditions are related to each other and have similar effect on levels of markers. For example, inflammatory biomarker such as NF-κB is associated with several conditions including diabetes, sleep apnea, asthma, CVDs, Alzheimer's disease, and cancer hence it is not an ideal biomarker for differential diagnosis as its level is affected as consequence of inflammation which is a manifestation of multiple diseases [2]. Another factor required for a biomarker to be ideal is that it should allow for isolation from acceptable sources such as body fluids like blood or its components, urine, and saliva. Once isolated from such sources, it should be easy to identify and quantify the marker. It is usually not possible for a marker to have all the characteristics as discussed but it should offer at least most of these characteristics if not all.

2.2 Types of Biomarkers

Biomarkers can be classified on basis of primary purpose for which they are detected and quantified into diagnostic, predictive, prognostic, drug response and risk assessment markers. On basis on of their nature, they may be categorized into molecular, cellular, and imaging markers. Markers may also be classified on basis of their prime function and characteristics as inflammatory, immunological, hematological, genetic, epigenetic, and other categories. Diagnostic biomarkers may also be categorized on basis of the type of disease for which they are detected into cancer biomarkers, metabolic disease markers, autoimmune disease markers, infectious disease markers and so on. Although there are vast array of biomarkers that have been investigated so far and many novel markers are still being explored for their potential in diagnosis of diseases, there is no specific or standard classification for them nor there is distinct well-defined boundary between various sub-categories discussed here and hence overlap may exist.

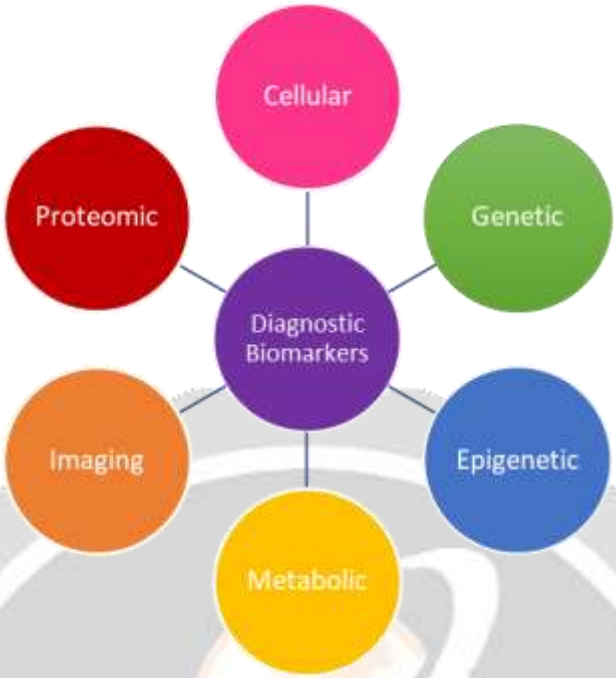

Fig -1: Types of diagnostic biomarkers

2.3 Genetic biomarkers

Genetic biomarkers comprise of genes which are either overexpressed or are downregulated as a consequence of presence of disease in subject. It also includes mutated genes associated with pathophysiology of a genetic disorder or due to exposure to xenobiotics such as carcinogens. Such mutated genes can serve a biomarker by assisting in diagnosis of genetic disorders, neoplastic diseases occurring due to mutations and genetic predisposition to particular disease associated with mutated gene. Foreign genes of invading bacteria, viruses or other pathogens also fall under genetic markers as they allow for diagnosis of infectious diseases occurring due to pathogenic invasion. Recent example of such foreign genes that have been applied in diagnosis are E gene, N gene, RdRp gene and ORF8 gene of SARS CoV-2 which have been used as diagnostic markers of COVID-19 by utilizing qPCR technology for detection although other technologies like CRISPR Cas 12 are also being explored for detection of such genes [3][4][5]. Foreign proteins associated with pathogens that are encoded by their genes also serve as proteomic biomarker for infectious diseases.

2.4 Epigenetic biomarkers

Epigenetic markers include stable and reproductible epigenetic indicators and modified epigenetic processes that can assist in diagnosis of ill-health as well as normal state of body. Although such markers can be derived from sources such as tissue and cellular biopsy samples and from biological fluids such as blood, plasma, serum, and urine, plasma is preferred for isolating markers like miRNA and cell free DNA as isolation of plasma it is least invasive in comparison with other sources. Epigenetic biomarkers such as histones, vast array of micro RNAs, methylated and cell free DNA can be easily derived from biological fluids including blood plasma, serum, urine, and semen [6]. Methylated DNA can also be derived from stool sample. These markers can be detected and subsequently quantified using various molecular methods as described by Giménez et al., 2019 [6].

2.5 Biomarkers in diagnosis of diseases

2.5.1 Cancer biomarkers

Early diagnosis of Cancer, which is amongst the leading cause of death using biomarkers can help in reduction of the mortality rate and hence the global disease burden. Owning to the number of advantages offered by biomarkers such as minimal invasiveness, ease of isolation from body fluids, high specificity, and early diagnosis, they act as

suitable diagnostic alternatives or complement to conventional diagnostic methods such as biopsies which are more invasive in nature. Due to number of abnormal alterations at multiple levels in cancer cells which distinguishes them from normal cells and normal metabolic process, several biomarkers have been explored and established at various levels for cancer diagnosis. These levels include cellular, metabolic, and molecular alterations. Molecular level biomarkers include genetic, epigenetic, genomic, and proteomic diagnostic markers. Apart from this, imaging biomarker are also used in diagnosis of cancer e.g., imaging of breast morphology by mammography for breast cancer diagnosis. For certain cancer types, such as breast cancer, biomarkers are not frequently applied in diagnosis. Epigenetic markers have also been investigated for diagnostics and they can be easily derived from sputum, stool and fluids like blood and urine thereby assisting in overcoming challenges posed by tissue biopsies such as possibility of triggering infection at site of isolation and also difficulty in isolation if site of isolation is out of or difficult to reach [7]. Of the sources of epigenetic indicators, blood serves as source for marker for many cancer types, urine serves as source of sample isolation for prostate and bladder cancer, sputum for lung cancer and lastly excreta for colorectal cancer [7][8]. For example, hypermethylated SEPT9 can be easily derived for diagnostics of colorectal cancer in blood and diagnostic tests have been developed for its detection [7]. Similarly, owning to elevation in its levels in serum in individuals with colorectal cancer, miRNA 21 can serve as marker in diagnostics of the same [9] . Although over past 20 years, as many as thousands of biomarkers have been investigated, only a handful of them are able to clear the subsequent validations and applied in clinical diagnostics.

Table -1: Biomarkers utilized in diagnosis of various cancer types [7][8]

S. No.	Cancer Type	Marker
1.	Breast, Ovarian	Carbohydrate antigen 125
2.	Colorectal	Hypermethylated SEPT9
		miRNA 21
3.	Testicular	Human Chorionic Gonadotropin β
4.	Thyroid carcinoma	Calcitonin
5.	Hepatocellular carcinoma	Alpha fetoprotein

2.5.2 Neurodegenerative diseases biomarkers

As the name suggests, neurodegenerative disease is a collective term for group of incurable neurological diseases involving reduction or loss in functionality or structure of neurons, which progresses and worsens with increasing age. It includes diseases such as Huntington's (HD), Parkinson's (PD), Alzheimer's (AD), and other diseases. Conventionally, behavioral manifestations have been utilized in diagnosis of these diseases, however they have number of disadvantages including late onset of these manifestations when disease has progressed significantly and false negative in mild presentations of these symptoms even with established behavioral test such as ADAS-Cog which is a diagnostic behavioral test for AD [10]. Although currently there is no cure available to treat neurogenerative diseases and clinical presentations may occur as late as ten to twenty years post onset of disease, early diagnosis of these diseases using biomarkers may allow for early treatment and prevention of their progression. These biomarkers can be detected using Immunoassays, imaging technologies such as NMR, MRI, and PET and other methods [10][11]. Some of the biomarkers that have been investigated for diagnosis of neurodegenerative diseases have been listed in the subsequent table [12][13][14].

Table -2: Biomarkers for diagnosis of neurodegenerative diseases [12][13][14]

S. No.	Disease	Biomarker	Level	Detection Technology	Source
1.	Alzheimer's	Neurogranin	Elevated	Immunoassay	CSF

	Disease	T-tau	Elevated	ELISA	CSF
		P-tau	Elevated	ELISA	CSF
		Aβ42	Elevated	ELISA, PET, mass spectrometry	CSF
		Aβ42/Aβ40	Decreased	ELISA	CSF
2.	Parkinson's Disease	Orexin A	Decreased	RIA	CSF
		GFAP	Elevated	RIA	CSF
		8-Hydroxy-2′-Deoxyguanosine	Elevated	–	Serum, CSF
3.	Huntington's Disease	Vasopressin	Elevated	Blood test	Serum
		Glutathione Peroxidase	Decreased	Blood test	Erythrocytes
		Cytokines	Elevated	Blood test	Plasma

2.5.3 Cardiovascular Diseases (CVD) biomarkers

Having highest global mortality burden, CVDs are group of diseases affecting circulatory system. Multiple novel markers are being investigated for holding potential for diagnosis of various CVDs at early stage. Genetic markers and markers affecting their expression are being investigated for diagnosis and risk prediction of CVDs owning to factor that they present themselves at very early stages since birth over imaging biomarkers and biomarkers that are present in circulation. For analyzing the expression of genes technologies such as microarray have been adopted in order to search and subsequently validate biomarkers associated with CVDs [15]. Apart from genetic investigation for novel CVD markers, proteomic search is done by utilizing technologies such as 2D gel electrophoresis, dot blot, ELISA, immunoprecipitation, and Western blotting [15]. Another approach utilized for proteomic marker search is Mass Spectrometry which offers high throughput and proves advantageous over other methods as it does not require additional step of separating the proteins [15]. Some of the biomarkers that have been investigated for their association with CVDs and allowing for their diagnosis have been listed in following table.

Table -3: Biomarkers for diagnosis of cardiovascular diseases [15]

S. No.	Condition	Marker
1.	Atherosclerosis	MMP 3, 9, 14
		Angiotensin I converting enzyme 1
		IL 6, 9, 18
		Angiotensinogen
2.	Coronary Artery Disease	Apolipoprotein B
		C reactive protein
		Lipoprotein lipase

3.	Myocardial Infarction	MMP 3
		Apolipoprotein
		C type natriuretic peptide
4.	Stroke	MMP 1, 2
		Nitric oxide synthase
		Angiotensinogen

2.5.4 Sleep disorder biomarkers

Quality and amount of sleep an individual gets has direct effect on normal physiology, mental, and physical performance of body. Sleep disorders are group of disorders in which quality or quantity of sleep, or both are affected. They are among most ignored and underdiagnosed yet prevalent disorders affecting mental health and ultimately daily life of individuals. Biomarkers have been explored for diagnosis of sleep apnea. Most of the cases of sleep apnea which is one of the most common and prevalent sleep disorders goes undiagnosed and hence untreated owning to factor that diagnosis is done by polysomnography which requires sleep lab and is conducted overnight and hence time consuming apart from requirement of several monitoring equipment. Biomarkers can assist in overcoming this underdiagnosis and hence undertreatment by providing quick diagnosis from easy to derive source i.e., from biological fluids and do not require overnight sleep study. In addition, they can be detected using simple biochemical detectors. As inflammation is a manifestation of apnea, inflammatory molecules holding potential for being utilized as diagnostic indicator and hence being investigated are CRP, IL-6 and 8, and lastly elevated levels of TNF-alpha [16][17] . Apart from these, there are apnea indicators linked with oxidation associated stress and metabolism are also being investigated. and oxidation related stress. Fibrinogen it yet another has been investigated for holding potential of being biomarker for sleep apnea diagnosis [17]. Rise in its level from that in normal subjects is observed when stroke occurs in coexistence with apnea. Lastly many miRNAs are under study for holding potential to be diagnostic marker for apneas [18].

2.6 Biomarker detection technologies

A wide range of technologies have been employed or are being tested to detect vast array of diagnostic biomarkers and hence allowing for quick, early, and precise diagnosis of multiple diseases such as PCR technologies, immunoassays, flow cytometry, 2D gel electrophoresis, CRISPR technology, microarray, quantum dots, imaging technologies and other technologies [19].

PCR technologies especially real time PCR and microarray have been adopted in detection, identification, and validation of genetic markers and their expression. On the other hand, technologies such as ELISA, 2D electrophoresis and mass spectrometry have been utilized for detection of proteomic markers. Imaging technologies have been implemented in identification of imaging markers. One of the novel technologies that is being explored for its potential as diagnostic tool in detection of biomarkers is CRISPR. Nucleic acid biomarkers for a disease can be detected using CRISPR which involves identification of sequences which are specifically linked with a disease followed by cleavage of those sequences and generation of an output signal [20]. Sequences linked with specific diseases such as sequence of bacterial or viral pathogen e.g., E and N gene in SARS CoV-2 or mutated sequences linked with cancer can serve as biomarkers for COVID-19 and cancer respectively and such sequences can be detected using CRISPR technology [3][20].

2.7 Challenges

Although biomarkers offer numerous advantages over other methodologies adopted in diagnosis, there are certain challenges or limitations which may present themselves in clinical application of biomarkers. Cost, availability, ease of operation and accessibility of suitable equipment for identification and detection of biomarkers is one such factor. Even if equipment is available, they are usually expensive, sophisticated and take long time to generate result. Ideally, miniaturized biosensors which are user friendly, portable, affordable, have quick response time, high sensitivity and minimally invasive are preferred equipment for detection of biomarkers over sophisticated

equipment. Although significant progress has been made over past few decades in development of miniaturized biosensors for detection of diagnostic biosensors by making use of technologies such as Bio-MEMS, still it has not been adopted extensively [21]. Another challenge is that although many biomarkers are investigated and discovered each year, very few of them are approved post validation by agencies like FDA for application in clinical lab testing for diagnosis [22]. Lastly several biomarkers are affected in more than one condition particularly those which are associated with a clinical manifestation rather than the disease itself. Such biomarkers may assist in the course of diagnosis or as complement with other diagnostic techniques but cannot be used in confirmatory or differential diagnosis alone. For example, inflammatory biomarkers which are linked with clinical manifestation i.e., inflammation which be elevated in several diseases involving inflammation and hence are not specific for single disease. Lastly, all characteristics of an ideal biomarker as discussed in section are generally not present in single candidate and even identification of candidate having most of these characteristics if not all if difficult task.

3. CONCLUSION

Biomarkers have been widely applied for the diagnosis of number of diseases owing to number of advantages they offer over other diagnostic technologies and also in part due to vast diversity in types as well as numbers of biomarkers for a single disease. Thus, detection of more than one biomarker adds to the true positivity and true negative diagnostic accuracy over other methods that generally offer single technology-based detection and hence if such technology fails then accuracy will be affected. Also, biomarkers can be isolated with ease from biological fluids and can be detected through many technologies thus giving scope for improvement and optimization. Over past 20 years several biomarkers have been investigated for diagnostics but only few get validated and are applied in clinical diagnostics of a disease. Certain biomarkers that are associated with manifestations of disease rather than the disease itself will get affected in multiple diseases with same manifestations and thus can lead to incorrect or unspecific diagnosis. Thus, only disease specific markers are ideal for clinical diagnosis and not those based on clinical manifestations though they may assist in revelation of inherent symptoms which may assist in course of diagnosis to go for further tests. Also, for detection of biomarkers, equipment that is simple, small, gives quick results and is cost effective are ideal. Significant progress has been made in miniaturization of equipment for biomarker detection such as utilization of Bio-MEMS in biosensors for biomarker detection. Also, several technologies have been developed for biomarker detection and have improved over time thus offering diversity. Often biomarkers may be adopted in diagnosis as a complement to other methods thus providing improvement in diagnosis.

Although there are several challenges in implementation of biomarkers in diagnostics and to get a near ideal diagnostic marker for a disease, yet number of biomarkers have been successfully adopted in diagnostics including its implementation in diagnosis of emerging diseases like COVID-19 and diseases with high mortality rates namely cancer and cardiovascular diseases. Also, benefits offered by biomarkers are far greater than the limitation and challenges hindering its adoption can be bridged by conducting large scale research to identify novel biomarkers having most characteristics of ideal biomarkers. This can be realized through technological advancement to improve the process of biomarker discovery, identification, detection, and quantification.

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