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Mini Review

An Overview of Spectroscopic and Electrochemical Methods for the Detection of Biomarkers Associated with Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that damages nerve cells, leading to the eventual paralysis of the entire body and often resulting in death. Currently, there is no treatment to reverse the damage to motor neurons or cure ALS. Early and sensitive detection of ALS can significantly improve the quality of life. The recent discovery of ALS-associated biomarkers offers hope not only for diagnosing ALS in its early and asymptomatic stages but also for providing therapeutic monitoring. Electrochemical and spectroscopic bio sensing methods show promise for simple and sensitive detection of ALS biomarkers. There is a need for increased attention to developing diagnostic methods that can accurately determine ALS and its subtypes, differentiating ALS from other neurodegenerative diseases that share common biomarkers. This overview aims to shed light on ALS biomarker detection using electrochemical and spectroscopic methods, hoping to emphasize the existing gaps in the field and the necessity to build sensing platforms that can offer more specific and accurate ALS diagnoses.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease is a late-onset motor neuron disease which was first described by Charcot and Joffroy in 1869 [1, 2]. It is a progressive paralytic disorder determined by degeneration of motor neurons in the brain and spinal cord which cause muscles atrophy, paralysis and eventually death within 5 years of onset [3]. ALS required to distinguish from other neurodegenerative disorder for which required different diagnosis methods including Electrophysiological studies (motor and sensory nerve conduction studies, electromyography, transcranial magnetic stimulation, and central motor conduction studies), Neuroimaging studies (CT scan, MRI), Muscle biopsy and neuropathological studies [4]. These diagnostics methods are expensive and require sophisticated instrumentations and required delicate procedure and the patient must go through a painful experience. Some clinical markers, like oculomotor abnormalities or saccadic eye movements, have been identified for the noninvasive diagnosis of ALS [5, 6]. However, these are physical biomarkers; therefore chemical biomarker detection is required for more accurate diagnosis and determination of physiological conditions. Discoveries of ALS associated biomarkers made it possible to prepare electrochemical and optical sensing methods, which are highly sensitive for more accurate diagnosis of ALS. Some of the biomarkers reported to diagnose ALS at early stage and asymptomatic condition and therefore provide better health management process [7].

Various ALS-related biomarkers have been identified and studied. These biomarkers are associated with gene mutations, such as the Open Reading Frame gene 72 localized on Chromosome 9 (C9orf72), the superoxide dismutase (SOD1)-producing gene, the DNA-binding protein 43 kDa (TDP-43)-producing gene (TARDBP gene), and the microtubule-associated protein tau (MAPT gene). Neurofilaments are another type of biomarker linked to ALS; these include Neurofilaments light, medium, and heavy chains (NfL, NfM, and NfH). Various biomarkers related to proteins, such as Chitinase-3-like protein (YKL-40), Glial fibrillary acidic protein (GFAP), and cystatin 3 (CST3), as well as those related to cells, such as T regulatory cells (Tregs) and urinary neurotrophins receptor p75 extracellular domain (p75ECD), and related to RNAs, such as miRNA miR-338-3p, miRNA-181a, and miRNA-181b, have been identified as ALS biomarkers [8-11].

Almost half of the familial cases, and 20% of apparently sporadic form of ALS come from GGGGCC hex nucleotide expansions in the Open Reading Framework gene 72 localized on Chromosome 9 (C9ORF72) which was discovered in 2011 [12, 13]. The discovery of C9ORF72 makes it possible for not only early-stage ALS diagnosis as well as gene therapy to treat ALS. C9ORF72 related ALS could be observed by different biomarkers present in different physiological conditions. These include clinical and neuroimaging biomarkers in C9+ ALS, circulating biomarkers in serum and cerebrospinal fluids including polydipeptide repeats [poly Glycine-Alanine (Poly-GA), poly Glycine-Proline (poly-GP), poly Glycine-Arginine (Poly-GR), poly Proline-Alanine (poly-PA) and poly Proline-Arginine (poly-PR)] [14]. Poly-GP is an important pharmacodynamic biomarker for c9ALS as it is present in C9orf72 expansion carriers and absent in noncarriers [15]. It is found in both asymptomatic and symptomatic patients with no significant difference in quantities. Poly-GP is present in high quantities in cerebrospinal fluids (CSF) and in peripheral blood mononuclear cells (PMBCs) and is considered an important pharmacodynamic biomarker [16]. The SOD1 gene encodes the enzyme superoxide dismutase, which is widely present in cells throughout the body [17]. This enzyme acts as an antioxidant, converting superoxide into molecular oxygen and hydrogen peroxide. Numerous amyotrophic lateral sclerosis (ALS)-linked SOD1 mutations constitute the second most common pathological subtype of ALS. The potential utility of SOD1 as a biomarker lies primarily in assessing the pharmacodynamics of SOD1-lowering therapies [18]. The TARDBP gene provides instructions for synthesizing a protein called transactive response DNA-binding protein 43 kDa (TDP-43). TDP-43 is an RNA/DNA-binding protein detected in serum, plasma, and cerebrospinal fluid (CSF) [19, 20]. The TARDBP gene is implicated in approximately 4% of familial ALS cases and 1.5% of sporadic ALS patients [21, 22]. The MAPT gene encodes a protein known as microtubule-associated protein tau (MAPT) [23]. In ALS, tau protein exhibits downregulation, yet its effectiveness in distinguishing ALS from other neurodegenerative diseases is limited. This suggests that tau alone is unlikely to serve as a disease-specific biomarker [24].

Neurofilaments, including Neurofilament Heavy Chain (NfH), Neurofilament Medium Chain (NfM), and Neurofilament Light Chain (NfL), are detectable in serum, plasma, and CSF [25]. Their quantitative measurement in these fluids serves as a marker for ALS and other neurodegenerative diseases. NfL, especially in CSF, is presently considered the most effective ALS biomarker for diagnosis and predicting survival time, although the need for more specific biomarkers across neurodegenerative diseases is acknowledged [26, 27]. Chitinase-3-like protein (YKL-40) is a glycoprotein, with elevated levels in both monogenic and sporadic cases of ALS compared to asymptomatic mutation carriers and controls [28, 29]. Glial fibrillary acidic protein (GFAP), an intermediate filament protein, is also elevated in ALS CSF samples [30, 31]. However, the presence of YKL-40 and GFAP in other neurodegenerative diseases suggests they are markers of neurodegeneration related to neuroinflammatory mechanisms rather than disease-specific biomarkers [32, 33]. Cystatin C (CST3), a cysteine protease inhibitor abundant in CSF, is implicated in cell signaling, inflammation, and neuronal cell death [34, 35]. Further research is necessary to establish the use of CST3 as an ALS biomarker. T regulatory cells (Tregs) are considered a promising therapeutic target and an important pharmacodynamic biomarker [36]. The urinary neurotrophin receptor p75 extracellular domain (p75ECD) is an advantageous biomarker due to its non-invasive nature and ease of accessibility [37].

Overall, although these biomarkers have been identified for ALS, they are also associated with other neurodegenerative diseases. Therefore, more studies need to be conducted to ascertain the specificity linked to ALS based on their varying quantities in different media. The use of these biomarkers will likely be necessary in combination with other types of biomarkers for more accurate and specific ALS diagnosis and effective health management [38, 39].

ALS related biomarkers have been detected by immunoassay [40], microarray analysis [41], RNA-sequencing [42], Real-Time quantitative Polymerase Chain Reaction (RT-qPCR)[43], Enzyme-Linked Immunosorbent Assay (ELISA) [44], Magnetic Resonance Imaging (MRI) [45, 46], Positron Emission Tomography (PET) [47], Total reflection X-Ray Fluorescence (TXRF)[48], fluorescence microscopy[49], fluorescence spectroscopy[50], microfluidics[51] and electrochemical analysis [52-55]. Electrochemical and optical biosensors have several advantages over other techniques because of their high sensitivity, low detection limit, high specificity with accuracy. Most of the electrochemical and many of the optical methods offer miniature or handheld detection systems and therefore show potential for the preparation of point of care (POC) devices [56].

Electrochemical detection method involves the use of electrode modified with biorecognition element. The biorecognition element captures the target analyte specifically and changes in the electrochemical measurement at electrode-solution interface before and after target analysts is recorded. Some important electrochemical techniques used in biosensing are voltammetry [such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV) etc.], amperometry, Chronocoulometry (CC) and electrochemical impedance spectroscopy (EIS). Volta metric techniques involve studying the current response of chemical species under an applied potential difference. The techniques CV, DPV, and SWV differ based on different input voltage waveforms [57]. In cyclic voltammetry, the potential difference varies at a constant rate with time between two potentials, and the direction of the potential change is reversed when either of the limiting potentials is reached. This method can determine various electrochemical properties, including the reversibility of a reaction, formal reduction potential, and electron transfer kinetics. Differential pulse voltammetry (DPV) increases the base potential in small increments with each drop, maintaining the pulse height between 10-100 mV above the base potential. Two currents are recorded—one before the pulse and one just

before the end of the pulse—and the difference between these currents is plotted against the base potential. DPV enhances sensitivity by minimizing the effect of background charging current during analysis due to its differential pulse form. Square wave voltammetry (SWV) combines the sensitivity of pulse voltammetry with the ability to directly test products, a feature of cyclic voltammetry. Amperometry, a simple and widely used electrochemical measurement technique, is employed in various commercial sensors, such as those for glucose measurement in blood [58]. In amperometric biosensing, the concentration of the analyte is determined by the output current signal with respect to time at the given applied potential. In the case of Electrochemical impedance Spectroscopy (EIS) technique the transduction of electrochemical signal is the charge transfer resistance of redox chemical species or change in capacitance at electrode solution interface [59]. EIS is a relatively new technique compared to other electrochemical techniques and it has advantages such as it's a steady state technique which utilizes small signal analysis over a wide range of applied frequency. EIS offers highly sensitive and label free detection with low limit of detection and rapid response [60].

Spectroscopic techniques are non-destructive methods in which radiation from the source interacts with the material, producing signals or causing changes in the original radiation. These interactions provide information about bonding, structure, energy, dynamics, etc. [61]. Fourier-transform infrared spectroscopy (FTIR) is a vibrational spectroscopic technique in which infrared radiation interacts with vibrationally active species, such as chemical bonds, providing insights into the bonds and bonding environment. For example, the absorption peak position can predict the environment around C=O, aiding in determining if the protein is in an α-helical state, β-sheet, or a random coil [62]. Raman spectroscopy, another vibrational spectroscopy technique, differs from FTIR as it is a scattering technique. The polarizability of the molecule under consideration is a crucial condition for analysis by Raman spectroscopy [63]. Raman shifts are considered to identify related molecular groups and their surrounding environment. Nuclear magnetic resonance (NMR) utilizes a magnetic field to alter the natural magnetic field of specific atoms [64]. Resonance and related effects are considered to gather information about the entire molecular assembly. These non-destructive techniques are gaining popularity in identifying biomarkers related to various disease conditions, including ALS. This literature discusses selected examples of such applications.

ALS associated biomarkers sensing

i. Electrochemical sensing

Unlike other sophisticated techniques electrochemical techniques have potential to miniaturize and portability, they are of low cost of preparation and analysis, they do not require trained personnel to operate, they required sample volume as low as microliter and they can be adopted to wearable devices and with digital readout devices such as smartphone. Real sample analysis with fast response is an important potential feature to build commercial electrochemical detection method. These features represent a promising platform for early and robust diagnosis and healthcare management. Some of the Electrochemical methods for the detection of ALS biomarkers have been reported which are discussed here briefly. Table 1 provides a quick overview of various reported methods for biomarker detection in different matrices and their corresponding

A label-free electrochemical immunoassay has been devised for the sensitive detection of analytes in human blood serum (HS) [65]. For this purpose, a commercially available graphene screen-printed electrode (SPE) was used to establish an immunosensing platform for detecting ALS-associated neurofilament light chain (NfL) in HS samples. The approach involved electrografting the SPE with carboxyphenyl groups followed by the subsequent immobilization of anti-NfL antibodies to form biorecognition surface as shown in Figure 1. Then electrochemical impedance spectroscopy (EIS) was employed to detect label-free surface signals resulting from biolayer formation and the target analytes exposure. To evaluate the performance of the NfL detection platform, a calibration plot was generated using different concentrations of synthetic NfL spiked into commercial human serum LOD (0.087 ng/mL). Finally, sera samples from ALS patients were analyzed to assess NfL concentration through the immunoplatform and regression models. The results indicate that NfL concentration tends to rise with age in both male and female groups. Additionally, the male group exhibited a generally higher NfL level compared to the female group. An electrochemical immunosensor for the detection of phosphorylated TDP-43 protein (pTDP-43) [66]. The sensing surface is prepared by immobilizing antibodies on an Au-electrode through a multistep process followed by employing electrochemical Impedance Spectroscopy, which measures changes in impedance on the sensor surface upon binding of the target TDP-43 protein. The research aims to improve the sensitivity, selectivity, and stability of the immunosensor for the The developed immunosensor is highlighted for its potential application in the early diagnosis and monitoring of ALS. The work presents a novel approach for the detection of phosphorylated full-length TDP-43 protein. The sensor shows high sensitivity (LOD 11 ± 6 nM) and selectivity of target compared to other proteins like bovine serum albumin and tau protein. Another study assesses sudomotor function in amyotrophic lateral sclerosis (ALS) using electrochemical skin conductance (ESC) [67]. ESC measures the ability of sweat glands to release chloride ions in response to an electrical stimulus, quantified in MicroSiemens (μS) units by a Sudoscan device. The study included 31 ALS patients and 29 healthy controls, with ESC recorded from hands and feet. The results showed significantly lower ESC measurements in ALS patients compared to controls, indicating sudomotor dysfunction in both upper and lower extremities. The study demonstrated that ESC is a non-invasive method for assessing sudomotor function in ALS. A DNA-hybridization electrochemical biosensor (DNA-HEB) was developed for the detection of a specific DNA sequence related to the Cu-Zn superoxide dismutase enzyme (SOD1) associated with familial Amyotrophic lateral sclerosis (ALS) [68]. The biosensor

Table 1: Reported electrochemical detection methods for ALS associated biomarkers in given sample matrices.

Biomarker	Sample matrix	Biorecognition surface	Detection technique	Limit of detection	Linear range	Reference
NfL	Blood serum	SPCE/Carboxyphenyl/Ab/BSA	EIS	0.087 ng/mL	0.01 – 1.5 ng/mL	[65]
pTDP-43	-	Au/lipoic acid/Ab/1-butylamine/hexanethiol	EIS	11 ± 6 nM	1 – 1000 nM	[66]
Chloride ion in sweat	Sweat	Nickel electrode	ESC	-	-	[67]
SOD1 encoding DNA	Blood plasma	GCE/PANI/rGO-Sm_2O_3/ssDNA	FFT-SWV	1.3×10^{-14} M	0.1 pM – 10 nM	[68]
G4C2 repeat RNA	Cell extracts	Au/ssDNA/MCH	EIS	6.4 – 6.8 pmol	0 – 50 pmol	[52]
CoQ10	CSF	-	HPLC-EC	0.2 nM	-	[69]
miRNA-338-3p	Total exosome lysate	SPCE/ AuNP-Fe_2O_3NC	CC	100 aM	100 aM - 1 nM	[55]
Ascorbate	MEM	CFD/ RuOHCF	Amperometry	25 µM	-	[70]
TDP-43 and tau protein	Raw plasma samples and brain tissue extracts	dSPCE/p-ABA/3D-Au-PAMAM/CAbs immunosandwich IgG HRP	Amperometry	Tau: 2.3 pg/mL, TDP-43: 12.8 pg/mL	Tau: 0.008 -5.0 ng/mL TDP-43: 0.043 - 15.0 ng/mL	[71]
TDP-43	Serum	Au/SATA/Ab	DPV	0.0005 µg/mL	0.0005 - 2 µg/mL	[72]
Tau 441 protein	-	Au/lipoic acid/Ab/1-butylamine/hexanethiol	EIS	-	-	[73]

ESC = Electrochemical Skin Conductance; FFT-SWV = Fast Fourier Transform Square Wave Voltammetry; PANI = Polyaniline; rGO = Reduced Graphene Oxide; SPCE = Screen Printed Carbon Electrode; BSA = Bovine Serum Albumin; MCH = Mercaptohexanol; CoQ10 = Co enzyme; CSF = Cerebrospinal Fluid; HPLC-EC = High Performance Liquid Chromatography with Electrochemical Cell; NC = Nanocatalyst; CC = Chronocoulometry; CFD/RuOHCF = Ruthenium Oxide Hexacyanoferrate -modified Carbon Fiber Disc microelectrode; MEM = Minimum Essential Medium; dSPCE = Dual Screen Printed Carbon Electrodes; HRP = Horse Radish Peroxidase; 3D-Au-PAMAM = Gold nanoparticle-Poly(amidoamine) dendrimer nanocomposite

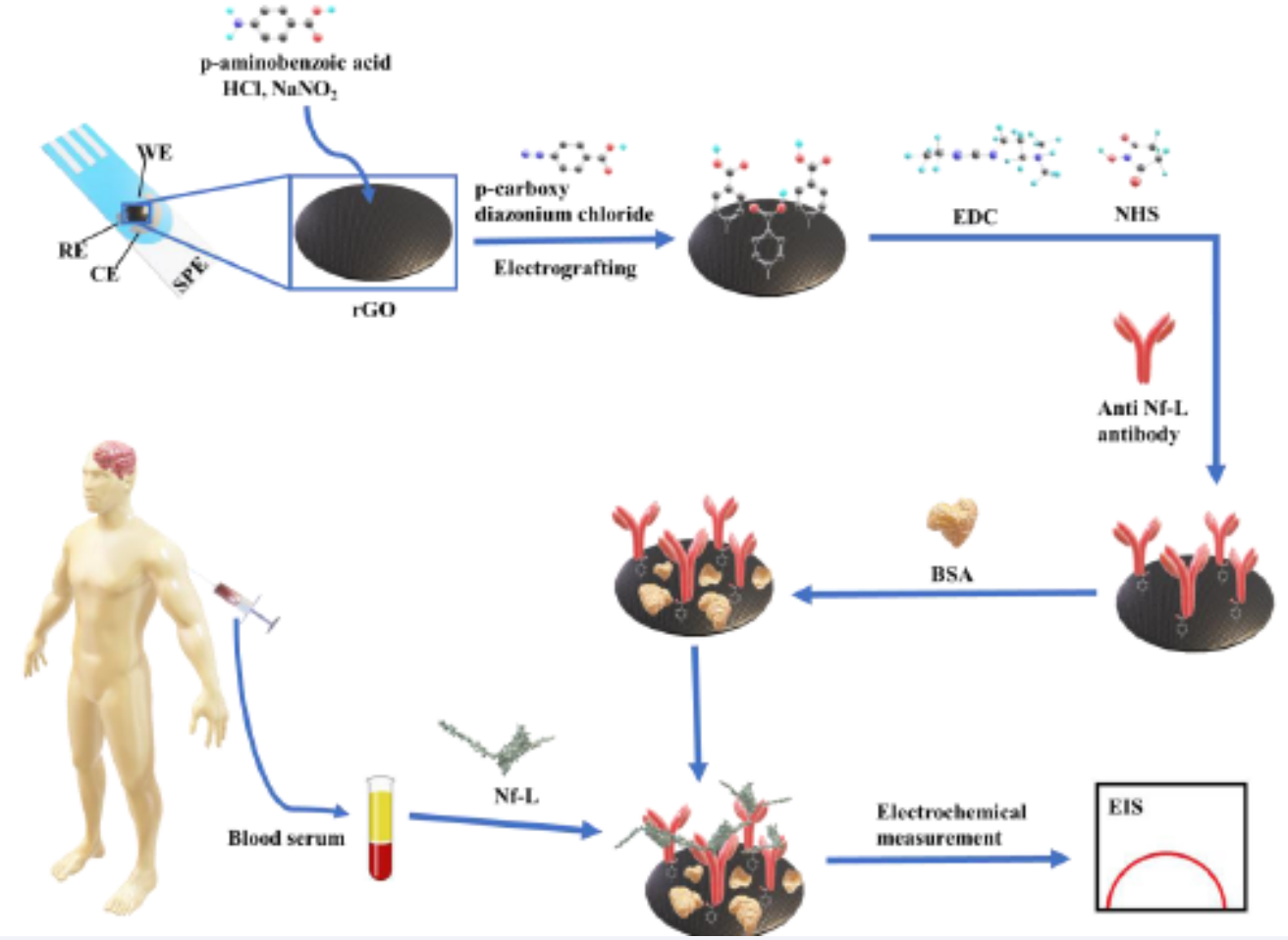

Figure 1 Schematic illustration of step-by-step preparation of the electrochemical immunosensing platform on rGO screen-printed electrodes and its use in the detection of NfL biomarker in ALS patients' serum samples. (Reproduce with permission from Ref. [65]).

utilized a glassy carbon electrode modified with a nanocomposite of reduced graphene oxide and samarium oxide nanoparticles, creating a matrix for label-free immobilization of single-stranded DNA (ssDNA). Target DNA hybridized with probe DNA to form double stranded DNA (dsDNA) which was detected by Fast Fourier Transform Square Wave Voltammetry (FFT-SWV). The biosensor uses reduced graphene oxide and samarium oxide nanoparticles which enhances the sensitivity (LOD = 1.3×10^{-14} M) and selectivity in diluted blood plasma samples. A novel label-free strategy was used for the detection of ALS and frontotemporal dementia (FTD) associated RNA with hexanucleotide GGGGCC repeats sequence [52]. The detection technique employed is electrochemical impedance spectroscopy. Initially ssDNA probe of complimentary sequence to target was immobilize on gold electrode surface by thiol-gold bond formation followed by covering the remaining surface with 6-mercaptohexanol to avoid nonspecific adsorption. Two different probes (P1 with single C4G2 repeats and P2 with three C4G2 repeats) have been used and their performance has been compared. The target is captured, and the hybridization was detected by measuring charge transfer signal. Both probes show a linear range of 0 – 50 pmol while P2 shows higher sensitivity (LOD 6.4 pM) compared to P1 (LOD = 6.8 pmol). The method shows simple, label free detection of cell extracted RNA of GGGGCC repeats offering potential applications in the diagnosis of neurodegenerative diseases. High performance liquid chromatography with an electrochemical detector was reported to measure the concentrations of the reduced and oxidized forms of coenzyme Q10 (CoQ10) in the cerebrospinal fluid (CSF) [69]. The study investigated the potential contribution of mitochondrial oxidative damage to the pathogenesis of sporadic amyotrophic lateral sclerosis (sALS) by observing 30 sALS patients and 17 age-matched controls. The percentage of oxidized CoQ10 in the CSF of sALS patients was found significantly higher than in controls. The method shows detection of CoQ10 as low as 0.2 nM. In patients, the concentration was found negatively correlated with the duration of illness. The study suggests that mitochondrial oxidative damage is implicated in the early stages of sALS and may contribute to its onset. An enzymatic amplification-free electrochemical detection method was reported for exosomal microRNA (miR-338-3p) associated with ALS [55]. The target analyte, miR-338-3p, is extracted from exosomes obtained from preconditioned media of motor neurons in both ALS patients and healthy controls. The detection technique involves a three-step strategy: First isolation and purification of exosomal miR-338-3p using biotinylated complementary capture probes, second, direct adsorption of target miR-338-3p onto a gold-nanoparticles with ferric oxide nanocatalyst (AuNP-Fe_2O_3NC) through affinity interaction, and third, electrocatalytic signal amplification monitored by chronocoulometry using methylene blue-ferricyanide (MB^+/$[Fe(CN)_6]^{3-}$)redox cycling. The method shows high sensitivity, detecting miR-338-3p as low as 100 aM in spiked buffer samples. The study shows successful detection from ALS patient samples. An amperometric monitoring of ascorbate uptake by neuroblastoma cells was carried out to observe the relationship between ascorbate dynamics and oxidative stress levels. A ruthenium oxide hexacyanoferrate (RuOHCF)-modified carbon fiber disc (CFD) microelectrode was used to determine intercellular and intracellular ascorbate in sample matrix Minimum Essential Medium (MEM) [70]. Electrocatalytic oxidation of ascorbate at the RuOHCF-modified CFD microelectrode causes change in current signal that are monitored amperometrically. The sensing surface shows high sensitivity (LOD = 25 µM) and stability with 10% signal decrement over 120 hours. A multiplex detection of two different biomarkers tau protein and TDP-43 was reported using a sandwich-type immunoassay on the surface of dual screen-printed carbon electrodes (dSPCEs) [71]. Both working electrodes were electrografted with p-aminobenzoic acid (p-ABA) followed by covalent immobilization of a gold nanoparticle-poly(amidoamine) (PAMAM) dendrimer nanocomposite (3D-Au-PAMAM). After that each electrode was modified separately with capture antibodies specific to each of the targets. The detection mechanism involves amperometric measurements using the H_2O_2/hydroquinone (HQ) system generated by immunosandwich containing anti-IgG-HRP antibodies. The figures of merit for detection include high sensitivity and selectivity, with detection limits of 2.3 pg/mL for tau and 12.8 pg/mL for TDP-43. The method shows simultaneous determination of multiple biomarkers in raw plasma samples and brain tissue extracts from neurodegenerative disorder-diagnosed patients. A simple, cost-effective, and single-use in vitro biosensor for detecting TDP-43 in undiluted human serum was reported [72]. The biorecognition surface was prepared by binding N-succinimidyl S-acetylthioacetate (SATA) modified linked anti-TDP 43 antibodies on gold electrode by thiol-gold bond formation. The prepared sensor successfully determines human TDP-43 peptide in undiluted human serum using differential pulse voltammetry with high sensitivity and selectivity. The biosensor's bio conjugation approach eliminated the need for tedious gold surface modification, simplifying the process and reducing fabrication costs to less than $3 per biosensor. An electrochemical immunoassay was developed to detect full-length tau441 protein on immune modified gold electrode [73]. The surface was prepared by immobilizing various anti-tau antibodies, including D-8, A-10, and Tau-46, on Au electrodes. Target detection was observed by electrochemical impedance spectroscopy which shows change in charge-transfer resistance (R_{CT}) observed upon tau441 binding to the anti-tau antibody-modified Au surface. The study reports screening various anti-tau antibodies and after optimization the sensor achieves high specificity and sensitivity ranging from micromolar to nanomolar. The study reports direct detection of non-phosphorylated full-length tau441 protein, offering a sensitive and selective approach for neurodegenerative biomarker detection.

ii. Spectroscopic sensing

Various target biomarkers associated with ALS are determined by different spectroscopic methods these includes Raman spectroscopy [74-76], infrared spectroscopy [77, 78], NMR [79, 80], etc. These techniques have advantages of detecting

analyte in complex biomatrices in trace amount. Furthermore, these techniques diagnose ALS by analyzing raw bio-samples even in the absence of reliable or specific biomarker(s). Table 2 provides an overview of some reported methods for biomarker detection in different matrices.

Table 2: Reported spectroscopic methods for ALS associated biomarkers in given sample matrix.

Biomarker	Matrix	Surface	Method	Reference
Lipid, Protein	TES	CaF_2	FTIR	[77]
Lipid, Protein	tear	CaF_2	FTIR	[78]
Proteins, Lipid	Saliva	-	Raman	[78]
Formate, Acetate, β-hydroxybutyrate	CSF	-	^{1}H NMR	[86]

TES = Tissue-engineered invitro skin

Raman spectroscopy is used to differentiate between Parkinson's and Alzheimer's disease by M. Bedoni's group [75]. Differences between different ALS disease and healthy saliva samples show marked differences to diagnose them correctly and quickly. Surface Enhanced Raman Scattering (SERS) finds peaks at 430, 500, 576, 833, 890, 951, 1021, 1120, 1251, 1470, 1540 and 1670 cm^{-1} (Δ Intensity ≥ 0.1) of the saliva samples of different groups. The major changes are at 500, 833, 890, 923, 1021, 1445 cm^{-1}, indicating the change is related to the ALS condition. Each band shows the presence of different markers, and the correlation of such bands indicates certain conditions. Raman spectroscopic analysis of ALS and healthy tear samples [78] showing well defined peaks in between 900 and 1800 cm^{-1} range as shown in Figure 2 a & b. Raman response from protein

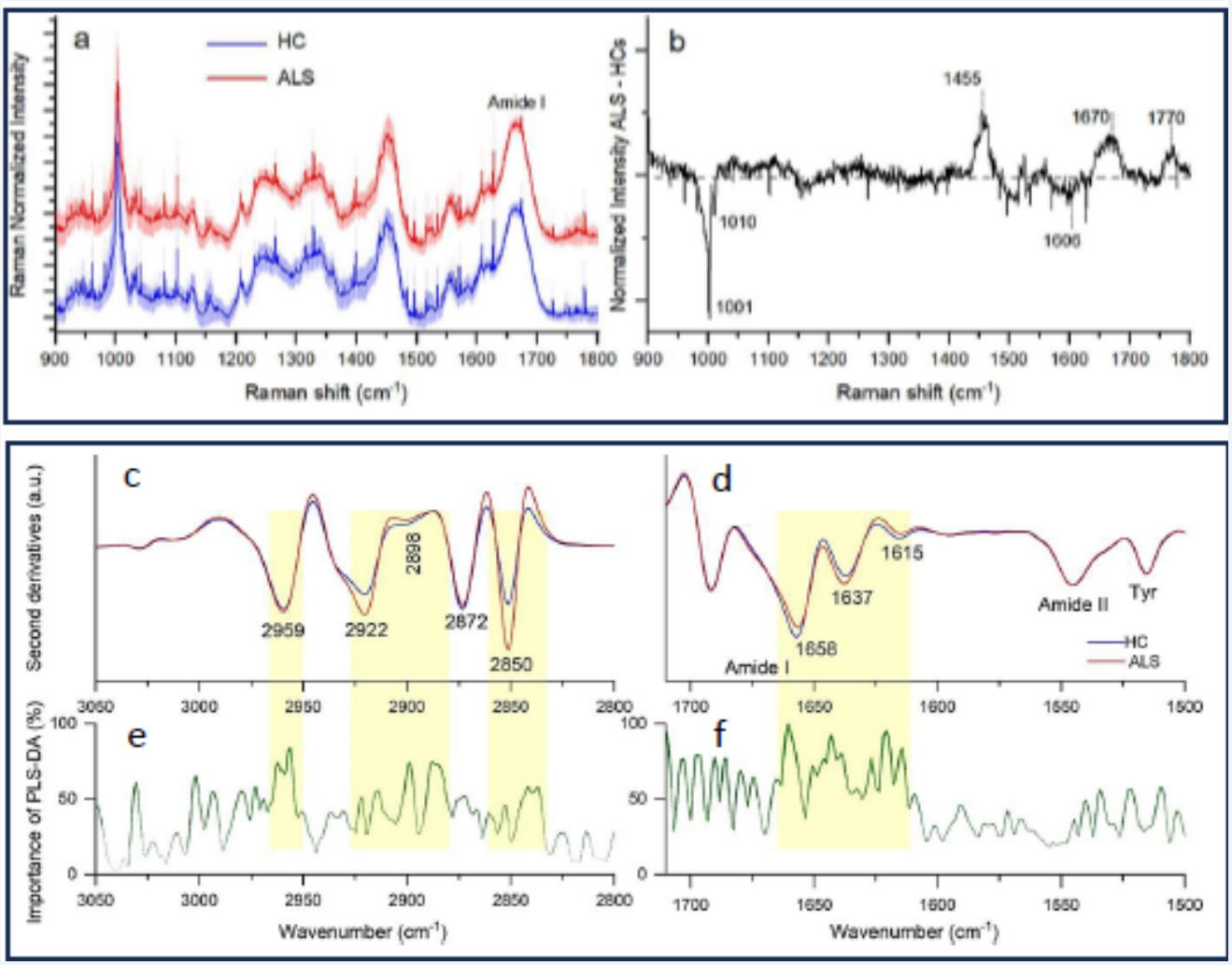

Figure 2 Vibrational spectroscopic analysis of ALS positive and healthy control (HCs) tear samples. a) Comparison of mean Raman spectra obtained by considering all the measured tears from ALS patients and HCs. Shadowed area refers to the standard deviation of the data.
b) Spectrally resolved differential average Raman spectra of the two investigated groups.
c) Average second derivative spectra of ALS-positive samples and HCs in the CHx stretching range and d) amide I and amide II bands.
e) Wavenumber importance (domain 0-100) for the PLS-DA method in the CHx stretching range and f) amide I and amide II bands. (Reproduce with permission from Ref. [78]).

is detected due to backbone C=O stretch around 1657 and 1670 cm^{-1} [81]. Weak signal around 1770 cm^{-1} is from lipid C=O stretching [82]. Differential average spectra of ALS and healthy samples shows reduction of the Raman band at around 1001 & 1010 cm^{-1} and around 1606 cm^{-1}, indicating global reduction of the phenylalanine level in the tear sample. In short, protein, lipid, reduction in amount of phenylalanine, etc. in the tear sample can be used as indicative of ALS, which can be detected, by using Raman spectroscopy.

Fourier Transform Infrared Spectroscopy is another vibrational technique used to detect ALS or related markers. Different peaks in the FTIR spectra can be designated to specific bond vibration arising from biomolecules/functional groups. Tissue-engineered in vitro skin (TES) samples from healthy individuals and ALS patients were analyzed and found to be different in IR absorption profile [77]. Authors made the differentiation between ALS and healthy TES by the difference spectrum of those after normalization in the range of 1149 and 1473 cm^{-1}. These changes arise due to disruption of phospholipid, extracellular matrix remodeling or cholesterol accumulation. A second derivative of the FTIR spectra observed the fingerprint region, where peak around 1148 cm^{-1} may arise from C-O stretch [78], 1203 cm^{-1} may arise from tryptophan side chain or collagen organization [83], 1238 cm^{-1} arises from PO^{2-} asymmetric stretch [84], and so on. When those differences in the absorption profile is analyzed by hierarchical cluster analysis (HCA) clear distinction between different groups (diseased and normal conditions) can be made [77].

Similarly, Full spectrum of the tear samples from the ALS patient was compared to the healthy individual to differentiate between them [78]. In this study, authors took consideration of full FTIR spectra divided into different parts. The spectral region of 2800-3000 cm^{-1} is an identification region for lipid and protein, lipid also absorbs around 1200-1500 cm^{-1}. As shown in [**Figure 2 c, d, e & f]**, Difference in absorption profile in those two regions of FTIR gives differentiation between ALS and healthy condition. CH_2 bands are observed around 2922, 2850 cm^{-1} [85], both the absorptions are intense in ALS patient tear samples than healthy tear. Similarly, in the lower frequency range major difference is observed around CH_2 and CH_3 peaks, both arising from lipids. Protein absorption range 1500-1700 cm^{-1} also shows major difference around 1615cm^{-1} indicating higher concentration of beta-sheet in ALS samples as beta-sheet absorbs around 1615 cm^{-1} [83]. Multivariate analysis of the second derivative of FTIR spectra supports the differentiation.

Proton nuclear magnetic resonance spectroscopy has emerged as a handy tool to discover biomarkers for ALS and related disease conditions. ^1H NMR was used to diagnose early stage of ALS [86]. Different metabolites in cerebrospinal fluid were considered as markers for ALS. Individual concentrations and regular superposition of the spectra does not indicate strong meaningful conclusion. Principle component analysis (PCA) of the NMR data regarding 17 metabolites from ALS and non-ALS samples suggested the high concentration of pyruvate, ascorbate, and acetone is found in ALS patients making them potential biomarker whereas high concentration of formate, acetate and beta-hydroxybutyrate in non-ALS individuals. Search for more sensitive and specific ALS biomarkers is ongoing process.

From those experiments, proteins, lipids, DNA, RNA, cholesterol, etc. with their related concentration in the respective bio-fluids which are found even in very low concentration, can be used to detect the ALS condition in patients with non-destructive and easy spectroscopic methods. Development of other techniques to target such markers can be very useful in easy and quick use for the diagnosis of ALS and related diseases.

CONCLUSION

The discovery of ALS-linked biomarkers opens opportunities to develop new analytical methods for more accurate diagnosis and therapeutic efficacy monitoring. Compared to other ALS diagnostic methods, electrochemical and spectroscopic biosensing methods are fast, simple, and sensitive, allowing for the detection of biomarkers in both complex invasive and noninvasive media. Since many biomarkers associated with ALS overlap with other neurodegenerative diseases, there is a need to develop detection methods capable of identifying multiple biomarkers to differentiate ALS and its subtypes from other neurodegenerative diseases. Spectroscopic and electrochemical biosensors are promising in this regard due to their potential for sensitive multiplex biomarker detection.

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