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Protein Biomarkers in Autistic Children: A Review

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Systematic Review Article

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ABSTRACT

Autism spectrum disorder (ASD) is a developmental and neurological disease that starts in beginning of childhood and remains all through life. ASD manifested by difficulties in social communications and interactions and constrained, repetitive actions. ASD prevalence is steadily rising globally, posing significant social and economic consequences. There is currently no medication for autism treatment because its etiology is not fully known. However, there are several behavioral therapies that can help with supplementary symptoms of autism, especially if started at a young age. Finding biomarkers for ASD is thus becoming important. Although diagnostic biomarkers have not yet been developed, investigations of immune system, inflammation, and microRNAs, as well as genomics and gene testing, proteomics, metabolomics, and transcriptomics, have all been conducted. Many proteins can serve as ASD blood biomarkers since proteomic investigations show that several proteins' levels in plasma and serum are altered in ASD. This review aimed is to focus on protein biomarkers for ASD.

Keywords: Autism spectrum disorder; diagnosis; protein biomarker; proteomics; review.

ABBREVIATIONS

2D-PAGE two-dimensional gel electrophoresis, *Tricine-PAGE* Tricine gel electrophoresis, *iTRAQ* isobaric tags for relative and absolute quantitation, *LC-MS/MS* Liquid chromatography-tandem mass spectrometry, *CALR* Calreticulin, *ATP5B* ATP synthase F1 subunit beta, *SERPINA1* alpha-1 antitrypsin, *ATP5A1* ATP synthase F1 subunit alpha, *MDH2* malate dehydrogenase 2, *FLOT1* Flotillin 1, *UQCRC2* ubiquinol-cytochrome *c* reductase core protein 2,

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ApoA4 Apolipoprotein A4, *PON1* paraoxanase/arylesterase 1, *ApoA1* Apolipoprotein A1, *C3* Complement protein C3, *TENA* Tenascin-C, *CP* Ceruloplasmin, *TF* Transferrin, *BDGF* Brain-derived neurotrophic factor, *sAPPa* Secreted amyloid precursor protein alpha, *PF4* Platelet factor 4, *TAAR6* Trace amine-associated receptor 6, *FGA* Fibrinogen alpha chain precursor, *CPB2* Carboxypeptidase B2, *AFP* Alpha-fetoprotein precursor, *APOC1* Apolipoprotein C-I precursor, *FABP1* Fatty acid binding protein 1, *SERPINA5* Plasma Serine Protease Inhibitor-Precursor, *EGF* Epidermal growth factor, *GAD65* Glutamic acid decarboxylase 65, *NGF* Nerve growth factor, *Dhh* Desert hedgehog, *HMGB1* High Mobility Group Box 1 Protein, *α-syn* α-Synuclein, *NF-κB* Nuclear Factor-Kappa B, *sAPP-α* amyloid precursor protein alpha, SOD Superoxide dismutase, *IL-8* Interleukin-8, *TSH* Thyroid-stimulating hormone, *ZC3HE* Zinc finger CCCH domain-containing protein 14*, Vcp* Transitional endoplasmic reticulum ATPase, *TYK2* Non-receptor tyrosine-protein kinase TYK2, *TRIPB* Thyroid hormone receptor interactor 11, *TNF* Tumour necrosis factor-alpha, *TLE1* Transducin-like enhancer of split 1, *TF* Tissue factor, *sRAGE* receptor for advanced glycosylation end product, *SHBG* Sex hormone binding globulin, *SELDI TOF MS* Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry, *RT-qPCR* quantitative reverse transcription polymerase chain reaction, *RGPD4* RANBP2-like and GRIP domain containing 4, *RN149* Ring finger protein 149, *PTPA* PP 2A activator, reg subunit 4, *PIP* Prolactin-inducible protein, *PAGE* Polyacrylamide gel electrophoresis, *PAP* Prostatic acid phosphatase, *MRRP1* Mitochondrial RNase P protein 1, *MRP14* Migration inhibitory factor-related protein 14, *MB-WCX* Magnetic beads cation-exchange chromatography, *MASP2* Mannan binding lectin serine protease-2 isoform-2 precursor, *MAPRE2* Microtubule-associated protein RP/EB family member 2, *LTF* lactotransferrin, *IL* Interleukin, *IKKA* I-kappa-B kinase 1, *IGHG* Ig gamma heavy chain, *IGHA1* Ig alpha-1 chain C region, *IGFALS* Insulin-like growth factor-binding protein complex acid labile subunit, *IgA* Immunoglobulin A, *ICAM1* Intracellular adhesion molecule-1, *GOT1* Serum glutamic oxaloacetic transaminase, *GLCE* Glucuronic acid epimerase, *FRAT1* Frequently rearranged in advanced T-cell lymphomas 1, *FETUB* Fetuin B, *EPO* Erythropoietin, *EIF4G1* Eukaryotic translation initiation factor 4 gamma 1, *Ehd3* EH domain-containing containing protein 3, *DMBT1* Deleted in malignant brain tumors 1, *CTGF* Connective tissue growth factor,*CLC4K* C-type lectin domain family 4 member K, *CHGA* Chromogranin A, *C5* Complement C5, *BMP6* Bone morphogenic protein-6, *ARMC3* Armadillo repeat containing 3, *APOE* Apolipoprotein E, *APOC2* Apolipoprotein C2, *APOA1* Apolipoprotein A1, *ADIPO* Adiponectin, 31, 32-PI Des31,32-proinsulin, *2D-Oxyblot* 2-DE (two-dimensional gel electrophoresis) plus Western blot analysis (WB).

1. INTRODUCTION

A series of severe neurodevelopmental diseases known as autism spectrum disorder (ASD) are manifested by defect social communications. ASD comprises childhood disintegrative disorder, autistic disorders, Asperger syndrome, and pervasive developmental disorders not otherwise specified, based upon 5th edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (PDD-NOS) [1]. ASD is serious public health issue with a negative impact. The negative ASD effects are catastrophic and multifaceted, affecting not just the affected child but also his or her siblings and parents. It greatly interferes with daily activities for a family afflicted by it [2].

The current ASD prevalence on center for disease control (CDC) prevention is 1 in 54 children [3]. ASD affects about 1–2% of people, with males to females ratio was 4–5:1 [4]. Moreover, there is no shared agreement on reasons of sharp rising in ASD prevalence. Some linked it to a rise in disease awareness and improved diagnostic criteria, while others contend that it is result of a real rise in prevalence or a confluence of all these causes.

ASD is frequency associated with comorbidities like intellectual disabilities [5], epilepsy [6], hyperactivity [7], immune dysfunction [8], and gastrointestinal abnormalities [9]. In numerous neurodevelopmental diseases, including ASD, there are no specific tests that can foretell cognitive and developmental abnormalities until early childhood. Many distinguishing traits that have been documented for early diagnosis of problems are known as "Red flags for ASD." [10] like no eye contact at age of 6 months, absence response to name-calling and absence of social referencing at age of 10 months, absence imitation and two meaningful words at age of 12 months, absence of proto-imperative and proto-declarative pointing at age of 14 months, and

absence joint attention at age of 18 months. Most ASD children like solitary play, with no pretend or symbolic play by age of 2 years, and cooperative play by age of 3 years. ASD manifestations often appear around 2 years of age [10]. ASD children interact only when necessary and exhibit confined behavioral patterns, like concentrating on only one aspect of a toy rather than playing with entire toy, which indicates limited interests. Other repetitive behaviors like hand flapping, head rocking, or toe walking [2].

ASD etiology affected by both genetic and environmental aspects. With a vast number of researches on related physiological abnormalities in ASD multifactorial disorder, environmental toxicant exposures, inflammation, oxidative stress, mitochondrial dysfunction, and immune dysregulation were all found (Fig. 1). Many ASD risk factors studied like birth complications, low birth weight, advanced parental age, prematurity, and assisted conception [4, 11-15]. The exact ASD pathogenic process is unknown, and there is no effective ASD therapy.

ASD clinical diagnosis based upon 5th edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-V) [17], that could leads to exclusion of autistic children with moderate disorders. So, accurate biomarkers that help in ASD diagnosis are required. Clinical relevance of a biological marker for ASD risk prediction, early diagnosis support, or even identification of possible treatment targets [18]. Based on current understanding of ASD, several blood-based biomarker candidates studied, mainly neurotransmitters [19], cytokines [20], mitochondrial disorders markers [18], and oxidative stress markers and disorders of methylation [21]. There is currently a lot of interest in the creation of molecular biomarkers that may be easily applied in clinical practise using conventional laboratory procedures. These biomarkers are based on the routine collection of physiological fluids like saliva, blood, or urine.

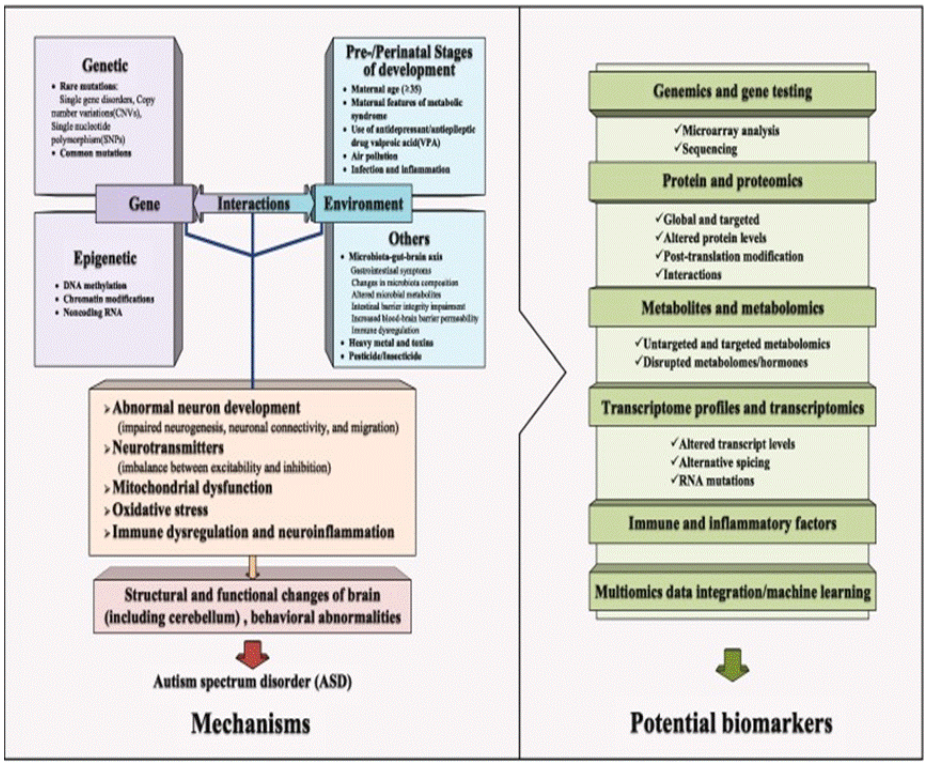

Fig. 1. Mechanisms and potential biomarkers linked to autism spectrum disorder. ASD is a multifactorial disease that involves interactions of environmental and genetic factors. Genetic factors include epigenetic and genetics factors, like chromatin modifications, mutations, DNA methylation, and noncoding RNA. Environmental factors include pre-/permanent stages of development, heavy metals and toxins, microbiota-guts-brain axis, and pesticides. These factors lead to changes in the structure and function of brain, resulting in ASD [16]

This review will discuss new and exciting protein biomarkers which might improve ASD diagnosis and therapy. We anticipate that reader will learn more about potential protein biomarkers for ASD patients as well as the drawbacks and restrictions of such biomarkers after reading this review.

1.1 Search Strategy

We reviewed PUBMED, Web of Science, Google Scholar, Scopus, Ovid Medline, and ERIC databases, covering publications from inception through June 2022, to find investigations of protein biomarkers in ASD. In this review, phrases like "Protein biomarkers" and "Autism" were combined to find researches. The development of specialized protein biomarkers for the various study fields was also examined in papers outlining specific pathophysiological processes related to ASD.

1.2 Study Selection

Researches considered if they satisfied certain requirements, like (I) reporting a direct clinical biomarker as an outcome; (II) being human randomized controlled trials; and (III) being non-randomized studies. If the animal research backed up the clinical trials being discussed, they were included. DSM-5 confirmation of ASD at the time of testing, 0 to 6 years old is the age range. Studies with promising results were included in this review after a review of the discovered studies, and these studies had to assess protein biomarkers using well-known validated methodologies and diagnose ASD using methods that were generally accepted. Studies that simply used genetic analysis and those that included other neurodevelopmental diseases including intellectual developmental disorders (IDD) and attention deficit hyperactivity disorder (ADHD) were not included in the review.

2. RESULTS

In this study, the author review 60 manuscript, of them 49 researches were included as they met inclusion criteria and 11 researches were excluded as they did not used valid method for diagnosis of ASD or used simple genetic analysis or included children with other neurological or psychological disorders or experimental studies and results were not conclusive. Several researches made proteome analyses of ASD samples, as plasma, lymphocytes, postmortem brain tissues, neonatal blood, serum, urine, and saliva. Some researchers studied peripheral blood mononuclear cells (PBMCs) and B lymphocytes. Six studies explored peptide-based biomarkers. Other researches stressed upon studying proteins that linked with ASD. These studies are shown in table (1).

3. DISCUSSION

Numerous ASD-related gene products, like those involved in chromatin remodeling, synapse function, and brain development, have comparable roles or contribute to disease through widespread signal transduction. The Central Rule of Genetics states that proteins carry out biological processes while genetic levels predict disease manifestation. As a result, modifications to proteins may much accurately reflect the onset and disease progression. In addition, proteins make up the majority of illness biomarkers and therapeutic targets. When examined collectively, ASD may be researched from protein viewpoint to comprehend pathophysiology and discover biomarkers or aimed specific therapy.

In search for reliable biomarkers for early ASD diagnosis, many researches stressed upon usage of peptide-based and protein- based biomarkers (Table 1). Proteomics was an extensive study of how proteins are expressed in tissues and cells. Through alternate splicing of RNA transcripts, genetic variants as post-translational modifications and coding SNPs or mutation, and, proteomics can reflect various main protein structures, often known as "proteoforms." [22]. It is an effective tool for studying biological systems and has the potential to be helpful in locating biomarkers for diagnosis, screening, overseeing therapy, and learning about the pathogenetic pathways of diseases [23].

Several studies made proteome analyses of ASD samples, as plasma [24-26], lymphocytes [27], postmortem brain tissues [28, 29], neonatal blood [30, 31], serum [32-36], urine [37, 38], and saliva [39, 40].

In proteomics researches study techniques, gel-based methods (as 2D-DIGE and 2D-PAGE) utilized in former researches [23]. Moreover, recently gel-free methods, i.e., liquid chromatography with tandem mass spectrometry (LC-MS/MS) utilized [26, 41, 42]. Moreover, six researches explored peptide-based biomarkers [33, 43-47]. Other researches stressed upon

Table 1. Researches based upon peptides and proteins biomarkers

No.	Researchers	Types	Samples	Technique	Related Proteins
1	Junaid et al. [28]	Proteomics	Brain (gray matter)	2D-PAGE	**Decreased:** Glyoxalase Ia
2	Corbett et al. [32]	Proteomics	Serum	LC-MS/MS	**Increased:** Complement C1q, FHR1, B-100, Fibronectin and Apolipoprotein
3	Schwarz et al. [85]	Proteomics	Serum	Immunoassay	**Males** **Increased:** Factor-VII, ENA-78, Fatty acid binding protein, Erythropoietin, Connective Tissue Growth Factor, Chromogranin A, Granulocyte colony-stimulating factor, IL-1B, IL-12p40, IL-12p70, IL-18, IL-3, IL-4, IL-5, IL-10, IL-1B, and molecule 1 of intercellular adhesion, Neuronal cell adhesion molecule, Tissue factor, Stem cell factor, Thrombopoietin, Tissue factor, Sortilin 1, Tumor necrosis factor-α **Decreased:** TENA, GOT1 **Females** **Increased:** Insulin, 31, 32-PI, IL-1B, Free androgen index, IL-7, IL-12p40, LH hormone, NMDA receptor regulated 1, TENA, Brain-derived neurotrophic factor, Tissue factor. **Decreased:** Apolipoprotein-CIII, Immunoglobulin M, Endothelin-1, Apo-A1, Growth hormone, GOT1, Eotaxin-3, sRAGE
4	Shen et al. [27]	Proteomics	B-lymphocytes	Antibody chips	**Increased:** IKK-α, **Decreased:** EIF4G1, TYK2, Protein kinase C iota type
5	Momeni et al. [86]	Proteomics	Serum	SELDI TOF MS MALDI TOF/TOF MS ESI-FTICR MS	**Increased:** d: C3
6	Ngounou Wetie et al. [36]	Proteomics	Serum	Tricine-PAGE, LC-MS/ MS	**Increased:** ApoA4, PON1, ApoA1.

No.	Researchers	Types	Samples	Technique	Related Proteins
7	Steeb et al. [35]	Proteomics	Serum	Immunoassay, LC-MS/MS	**Males** **Increased:** EPO, BMP6, CHGA, ICAM1, IL-12p70, IL-3, TENA, IL-16, CTGF, SHBG, TF, PAP, TNF-α **Decreased:** RGPD4 **Females** **Increased:** APOE, APOC2, GLCE, ARMC3, CLC4K, FETUB, RN149, TLE1, TRIPB, PTPA, ZC3HE **Decreased:** SHBG, ADIPO, APOA1, CHGA, PAP, EPO, IgA, IL-3, MRRP1, TENA
8	Broek et al. [29]	Proteomics	Brain (Prefrontal cortex, cerebellum)	LC-MS/MS	**Prefrontal cortex: Increased:** Myelin basic protein, Glial fibrillary acidic protein, Synapsin-2, Myelin-associated glycoprotein, Myelin oligodendrocyte glycoprotein, Myelin proteolipid protein. **Decreased:** Creatine kinase B-type, Syntaxin-1A, Protein kinase C casein kinase substrate 1, Synaptotagmin-1, Vimentin **Cerebellum:** **Increased:** Glial fibrillary acidic protein, Creatine kinase B-type, Synapsin-2, Synaptotagmin-1, Syntaxin-1A **Decreased:** Myelin basic protein, Myelin-associated glycoprotein, Myelin proteolipid protein, Myelin-oligodendrocyte glycoprotein, Protein kinase C casein kinase substrate 1, Vimentin
9	Ngounou Wetie et al. [87]	Proteomics	Saliva	2D-PAGE, LC-MS/MS	**Increased:** Integrin alpha6 subunit, Kinesin family member 14, Growth hormone regulated TBC protein 1, Parotid secretory protein, FRAT1, MRP14, Mucin-16, Prolactin-inducible

No.	Researchers	Types	Samples	Technique	Related Proteins
					protein precursor. **Decreased:** p532, CREB-binding protein, Transferrin, Zn alpha2 glycoprotein, Cystatin D, Plasminogen, Alpha-amylase, Zymogen granule protein 16.
10	Ngounou Wetie et al. [87]	Proteomics	Saliva	2D-PAGE LC-MS/MS	**Increased:** Growth hormone regulated TBC protein 1, FRAT1, Integrin alpha 6 subunit, MRP14, Parotid secretory protein, Mucin-16, Prolactin-inducible protein precursor, Kinesin family member 14. **Decreased:** Alpha-amylase, CREB-binding protein, Cystatin D, p532, Transferrin, Plasminogen, Zn alpha2 glycoprotein, Zymogen granule protein 16
11	Ngounou Wetie et al. [88]	Proteomics	Saliva	LC-MS/MS	**Increased:** DMBT1, Ig gamma-1 chain C region, LTF, Neutrophil elastase, Ig lambda-2 chain C regions, Ig kappa chain C region, PIP, Polymeric immunoglobulin receptor. **Decreased:** Histatin-1, Statherin, Acidic proline-rich phosphoprotein.
12	Suganya et al.[89]	Proteomics	Urine	2D-PAGE MALDI-TOF-MS	Mannan-binding lectin serine protease 2 isoform 2 precursor, IGHG1, Kininogen-1.
13	Yang et al. [90]	Proteomics	Urine	iTRAQ labeling, LC-MALDI-MS/MS	kininogen-1 isoform 2, leucine-rich alpha-2-glycoprotein 1, prostaglandin-H2 D-isomerase, alpha-1-acid glycoprotein, and immunoglobulin fragment Fab Alpha-2-glycoprotein 1, zinc, new lambda light chain, vitelline membrane outer layer 1 homolog, isoform CRAb, lithostathine-1-alpha, collagen alpha-1(XII) chain long isoform, inter-alpha-trypsin inhibitor heavy chain H4 isoform 1, and collagen alpha-1(XII) chain.
14	Cortelazzo et al. [24]	Proteomics	Plasma	2D-PAGE, LC-MS/MS	**Increased:** Alpha-1-antitrypsin, Haptoglobin, Fibrinogen beta chain, Fibrinogen gamma chain, IGHA1, IGHG, Alpha-2-macroglobulin,

No.	Researchers	Types	Samples	Technique	Related Proteins
					Alpha-1-antitrypsin. **Decreased:** Prealbumin, serum transferrin, Apolipoprotein A-I, Apolipoprotein J, and Apolipoprotein A-IV.
15	Feng et al. [67]	Proteomics	Plasma	2D-Oxyblot, MALDI-TOF	**Increased:** The carbonyl values of Complement component C8 alpha chain, Ig kappa chain C.
16	Singh S et al. [91]	Proteomics	Serum	Immunoassay	**Decreased:** TSH hormone **Increased:** IL-8
17	Yang et al. [43]	Proteomics	Serum	MB-WCX MALDI-TOF MS LC-ESI-MS/MS	**Increased:** d: precursor of alpha-fetoprotein, precursor of apolipoprotein C-I, the alpha chain of fibrinogen, carboxypeptidase B2, Plasma precursor of the serine protease inhibitor, Trace amine-associated receptor 6, platelet factor 4, and fatty acid binding protein 1.
18	Shen et al. [26]	Proteomics	Plasma	iTRAQ labeling, LC-MS/MS	**Increased:** Vitronectin alpha-1 Complement C5, Apolipoprotein E, Complement C3, Ehd3, Angiotensinogen, Antitrypsin, Complement C5 (C5), Fibronectin, Fibulin-1, and IGFALS. **Decreased:** Calmodulin, Alpha-actinin-1, Calreticulin, Ehd3, Talin-1, Thrombospondin-1, Fermitin family homolog 3, Integrin Alpha-IIb, MAPRE2, Vcp, Vinculin, Actin, Cytoplasmic 2, Alpha-enolase, and Beta-parvin.
19	Shen et al. [41]	Proteomics	PBMCs	iTRAQ labeling, LC-MS/MS	**Increased:** CALR, ATP5B, SERPINA1, ATP5A1, and MDH2, IL-1, IFN-γ, IL-6, IL-1β, TNF-α. **Decreased:** FLOT1, UQCRC2. (Forty-one differentially expressed proteins in total)
20	Pichitpunpong et al. [64]	Proteomics	Lymphoblastoid cell lines	2D-PAGE, MS	**Increased:** Galectin-1, glutathione S-transferase P, mitochondrial Cytochrome c Oxidase Subunit 5A, Improver of basic

No.	Researchers	Types	Samples	Technique	Related Proteins
					homolog, Calmodulin-140S ribosomal protein SA, an inhibitor of benzodiazepines binding. **Decreased:** Dihydrolipoyl dehydrogenase and Isocitrate Dehydrogenase [NADP] (Mitochondrial) (mitochondrial), tumour protein that is translationally controlled, T-complex protein 1 subunit epsilon, Annexin A5, beta chain of tubulin, Alpha-2-HS-glycoprotein, Alpha-enolase Clathrin light chain A, Peptidyl-prolyl cis-trans isomerase A, and Phosphoglycerate Mutase 1 (brain isoform).
21	Castagnola et al.[47]	Peptides	Saliva	HPLC-ESI-IT-MS	Acidic proline-rich proteins, Histatin 1 Statherin. (Phosphorylation value decreased in autistic patients).
22	Momeni et al. [46]	Peptides	Plasma	SELDI-TOF MS, MALDI-TOF/TOF MS, ESI-FTICR MS	**Increased:** C3f (2020.1 Da), C3f-des-arginine (1864.2 Da). **Decreased:** C3f-modified arginine (1978.1 Da).
23	Zaman et al. [45]	Peptides	Serum	On-bead magnetic screening, tandem mass spectrometry, ELISA, Gel electrophoresis and Coomassie Blue staining	**Decreased:** IgG1.
24	Yang et al. [43]	Peptides	Serum	ELISA + MALDI-TOF MS	**Increased:** PF4, TAAR6, FGA, CPB2, AFP, APOC1, FABP1, and SERPINA5.
25	Warren et al. [63]	Single protein	Plasma	ELISA	**Decreased:** C4B complement protein.
26	Modahl et al. [92]	Single protein	Plasma	Radioimmunoassay (RIA)	**Decreased:** Oxytocin.
27	Green et al.[93]	Single protein	Plasma	RIA	**Decreased:** Oxytocin.
28	Chauhan et al. [94]	Two proteins	Serum	Nephelometric method	**Decreased:** CP, TF.
29	Ashwood et al. [62]	Single protein	Plasma	ELISA	**Increased:** Leptin.
30	Blardi et al. [61]	Single protein	Plasma	ELISA	**Increased:** Leptin.

No.	Researchers	Types	Samples	Technique	Related Proteins
31	Correia et al. [60]	Single protein	Plasma	ELISA	**Increased:** BDNF.
32	Ray et al. [59]	Five proteins	Plasma	ELISA, WB	**Increased:** sAPPa. **Decreased:** BDNF, sAPPβ, Aβ1-42, Aβ1-40.
33	Işeri et al. [58]	Single protein	Serum	ELISA	**Increased:** EGF.
34	Al-Ayadhi et al. [57]	Single protein	Serum	ELISA	**Increased:** S100B protein.
35	Momeni et al. [95]	Single protein	Plasma	The hydrolysis of fluorogenic substrates	High activity of Complement factor I in ASD group.
36	Rout et al. [56]	Single protein	Serum	ELISA	Presence of GAD65 autoantibodies.
37	Essa et al. [96]	Two proteins	Plasma	Commercially available kit	**Decreased:** Transferrin, Ceruloplasmin.
38	Xu et al. [97]	Three proteins	Plasma	Enzyme immunoassay, RIA	**Increased:** Testosterone. **Decreased:** Oxytocin, Arg-Vasopressin (Autistic Children Mothers).
39	Dincel et al. [53]	Single protein	Serum	ELISA	**Increased:** NGF.
40	Husarova et al. [48]	Single protein	Plasma	ELISA	**Decreased:** Oxytocin.
41	Bashir et al. [54]	Single protein	Serum	ELISA	**Decreased:** Dhh.
42	Babinská et al. [52]	Single protein	Plasma	ELISA	**Increased:** HMGB1.
43	Kadak et al. [51]	Two proteins	Serum	ELISA	**Decreased:** tau proteins, α-syn.
44	Abdel-Salam et al. [50]	Single protein	Serum	ELISA	**Increased:** NF-κB.
45	Husarova et al. [55]	Single protein	Plasma	ELISA	**Decreased:** Oxytocin.
46	Wang et al. [49]	Single protein	Plasma	ELISA	**Increased:** Secreted sAPP-α.
47	Wang et al. [98]	Single protein	Serum	Colorimetry	**Decreased:** SOD.
48	Shen et al. [27]	Multiple proteins	B-lymphocytes	Antibody chips	**Decreased:** tyrosine kinase 2, Protein kinase C iota type, Eukaryotic translation initiation factor 4 gamma 1. **Increased:** I Kappa K alpha.
49	Singh et al. [91]	Multiple proteins	Serum	RBM platform + MSD platform	**Increased:** IL-8. **Decreased:** TSH.

a: Expression changes in ASD versus control. b: Top ten proteins based upon multiplicative alteration in abundance between autism and control. c: Lipid peroxidation-derived aldehyde measured in this study d: Identified peptides linked with proteins e: Phosphorylation value decline in autistic patients f: Oxidatively modified (protein carbonylation)

studying proteins that linked with ASD utilizing immunoassays like enzyme-linked immunosorbent assay (ELISA) to study changes between ASD patients and control persons [48-63].

As blood contains large number of proteins related with disease pathophysiology, it can be used to find disease biomarkers. Most researches focused upon fluids of body, as urine, serum, plasma, and saliva. Two additional researchers studied PBMCs [41] and B lymphocytes [27].

Table 1 showed that there is little to no consistency in the levels of candidate proteins across different cohorts. Moreover, bioinformatics study showed that the majority of these proteins were linked to coagulation and complement cascades, focal adhesions, vitamin digestion and absorption, immunological responses, inflammation reactions, activations of the platelets, cholesterol and lipid metabolism, oxidative stress, and energy metabolism [64-66,41,42].

Importantly, assessments of the levels of three proteins (C3, CALR, and SERPINA1) in PBMCs and plasma may boost biomarkers specificity because they were simultaneously changes in cells and blood of patients with autism [41]. Furthermore, several researches investigated posttranslational changes of ASD proteins and peptides values, as carbonylation [67], glycosylation [68], and phosphorylation [47].

Indeed, simultaneous analysis of posttranslational modifications and expression values of proteins may raise markers accuracy and specificity [68]. Followed literature survey and functional assay, six proteins chose for changes in ASD plasma samples, and five successfully detected, as SLC25A12, RARS, ACTL6B, PRKAA1, and LIMK1. RARS is enzyme important for translation of RNA and has essential for myelination of neurons [69]. *ACTL6B* detected as risk gene for ASD with functions of neuron-specific chromatin remodeling and neurodevelopment [70]. PRKAA1, a catalytic subunit of protein kinase A (PKA) regulates cellular energy metabolism. Regression in ASD linked with decline in PKA-mediated proteins phosphorylation and abnormalities in cellular pathway [71]. PRKAA1 recorded in many researches linked to autism and/or ASD as linkage researches [72, 73], NGS *de novo* mutation researches [74], and genome-wide association researches [75]. *SLC25A12* proposed as a candidate gene for ASD due to its important role in ATP formation and mitochondrial function [76]. Single nucleotide polymorphism in *SLC25A12* may significantly linked with ASD risk [77]. *SLC25A12* has critical role in ASD pathogenesis [77-79]. *LIMK1* enhanced axonal outgrowth and synaptic plasticity [80] and related to ASD [81]. *ARHGEF* linked with copy number variants (CNVs) in ASD children [80, 82]. ROC curve analyses revealed that area under curve (AUCs) of SLC25A12, LIMK1, and RARS were over 0.85, indicating that they are powerful in distinguishing ASD samples from healthy controls and could act as new potential ASD protein biomarkers in blood.

Given the intricacy of ASD pathophysiology, combining numerous biomarkers may be a potent method for diagnosing ASD [26]. E.g., former researches revealed that gather of 5 proteins (GC, C5, C3, ITGA2B, and TLN1) verified ASD children from controls with high AUC [26]. There are also novel approaches that can be used, as integrating computational prediction with experimental verification. This method determines if a gene-encoded protein can enter peripheral circulation from brain using a blood-secreting protein prediction software [83]. In addition, new high-throughput techniques like Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra (SWATH-MS) methodology can be used to test potential protein markers [84], and multiomics analyses[64]. As blood values of these proteins are usually low, highly sensitive detection methodology may developed and used [42].

4. LIMITATIONS OF THE STUDY

The use of comparison groups is one of the research projects' significant drawbacks when using protein biomarkers. Numerous studies compare people with ASD to unrelated, typically developing (TD) controls. This comparison has some significant drawbacks when taking into account the usage of biomarkers in the real clinical setting, although being scientifically credible. In the clinic, it is important to determine whether a kid with developmental delays or odd behavior might have an ASD diagnosis rather than whether a child is fully normal in development. Another crucial concern is whether sibling of ASD child will also get condition because they are more likely to. So, although TD unrelated controls could be sufficient for initially developing protein biomarkers, validation

researches required to utilize clinically relevant controls. Biological validity of several biomarkers, especially diagnostic biomarkers, is another significant drawback. For instance, suggested scientific physiological mechanism of action for FRAA is consistent with its capacity to predict the clinical response to therapy with leucovorin. However, there is a chance that a biomarker could reflect an epiphenomenon of a disease process if it does not represent a fundamental biological illness process. The information offered on the underlying illness process may thus be constrained, even if biomarker reliably and differentiate groups of individuals.

5. CONCLUSIONS

As ASD causes and pathogenesis are unknown, objectives, effective and specific early diagnostic biomarkers and therapy for ASD are not available. Given rising incidence of ASD, research into diagnostic indicators has drawn a lot of attention. Genes, proteins, peptides, metabolites, cytokines, and inflammatory agents are the focus of biomarker research in peripheral bodily fluids (blood, urine, saliva). A stress upon one or more biomarkers or their related signal transduction pathways is a feature of new methods as omics approaches, like genomics, metabolomics, proteomics, and transcriptomics; some advancement has been made in these fields of study. A specific proteins, genes, or metabolites with specific usage potential are the focus of targeting technology. Research on metabolites has advanced quickly in recent years, particularly in relation to metabolites of the gut flora. Additionally, it's critical to look for proteins, genes, or metabolites that display recurring changes throughout numerous studies with sizable sample sizes. The combination of experimental validation and computational prediction to detect blood protein biomarkers for ASD must be widely applied.

6. THE PROMISE OF UTILIZING BIOMARKERS IN THE FUTURE

Although the behavior associated with ASD might not be fully established il late by age of 2 years, in certain circumstances neuropathology link with ASD might start prenatally. As a result, biomarkers might help to detect cases that are at risk of having ASD before diagnostic symptoms are undeniably present. Despite the identification of potential biomarkers, few have had their predictive abilities examined. Maternal fetal brain autoantibodies seem to have good specificity for an offspring who developed ASD, making them the most promising prenatal biomarker. Despite the fact that this antibody panel may seem to be very adaptable as a clinical tool, the lack of any established related treatments dampened interest in creating a widely utilized commercial test. As diagnostic tools, several biomarkers are currently being developed. Numerous researches are still in the early stages, and the majority of biomarkers perform inadequately. Large samples of ASD patients as well as large, carefully chosen clinically relevant control groups would be required in order to verify biomarkers because of the enormous variation in the etiology of ASD. Any diagnostic biomarker that is created must evaluate biological processes, and it is likely that it will work best when combined with other behavioral tests and clinically pertinent data. It may be highly beneficial to use biomarkers that can separate patients into distinct etiological groupings to prescribe the best treatments and determine prognosis. Although it appears to be the most advanced element of biomarkers, research is still in its early phases. Due to the wide variation in how each individual responds to ASD treatment, biomarkers that could predict response to treatment may be very beneficial for enhancing personalized therapy regimens and enabling a personalized precision medicine approach. In the future, we might discover how to employ biomarkers in concert and how to relate them to certain significant symptoms or therapeutic effects.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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