Protein Biomarkers in Autistic Children: A Review

Mona Ghonaim Alharbi a*

a Department of Biological Sciences, King Abdulaziz University, Saudi Arabia.

Author’s contribution
The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information
DOI: 10.9734/AJBGMB/2022/v12i130282

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here:
https://www.sdiarticle5.com/review-history/90200

Received 01 June 2022
Accepted 04 August 2022
Published 06 August 2022

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, UQRC2 ubiquinol-cytochrome c reductase core protein 2,

*Corresponding author: E-mail: mgalharbi@kau.edu.sa;
ApoA4, Apolipoprotein A4; PON1, paraoxonase/arylesterase 1; ApoA1, Apolipoprotein A1; C3, Complement protein C3; TEGA, Tenascin-C; CP, Ceruloplasmin; TF, Transferrin; BDGF, Brain-derived neurotrophic factor; sAPPα, Secreted amyloid precursor protein alpha; PF4, Platelet factor 4; TAAR6, Trace amine-associated receptor 6; FGA, Fibrinogen alpha chain precursor; CBP2, Carboxypeptidase B2; AFP, Alpha-fetoprotein precursor; APOC1, Apolipoprotein C-1 precursor; FABP1, Fatty acid binding protein 1; SERPINA5, Plasma Serine Protease Inhibitor; EGF, Epidermal growth factor; GAD65, Glutamic acid decarboxylase 65; NFG, Nerve growth factor; Dhh, Desert hedgehog; HMGB1, High Mobility Group Box 1 Protein; α-syn, α-Synuclein; NF-kB, 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, Vanishing endoplasmic reticulum ATPase; TYK2, Non-receptor tyrosine-protein kinase TYK2; TRIP8, Thyroid hormone receptor interactor 1; NF, Tissue factor; sRAGE, receptor for advanced glycosylation end product; SHBG, Sex hormone binding globulin; SELDITOF, MS, Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry; RT-qPCR, quantitative reverse transcription polymerase chain reaction; RGDPD4, RANBP2-like and GRIP domain containing 4; RNP19, Ring finger protein 149; PTAA, PP 2A activator; reg subunit 4; PIPL3, 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; IKKα, I-kappa-B kinase 1; IGHG1, Ig gamma heavy chain; IGH1, 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, Glucuronide epimerase; FRAT1, Frequently rearranged in advanced T-cell lymphomas 1; FETUB, Fetuin B; EPO, Erythropoietin; EIF4G1, Eukaryotic translation initiation factor 4 gamma 1; Ehdo3, EH domain-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 morphogenetic protein-6; ARMC3, Armadillo repeat containing 3; APOE, Apolipoprotein E; APOC2, Apolipoprotein C2; ApoA1, Apolipoprotein A1; ADIPO, Adiponectin; Des31,32, Oxyblot; 2D-Oxyblot, 2D-Oxyblot; 2D-Oxyblot 2-DE; two-dimensional gel electrophoresis; 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 on 5th edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [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-VI, 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-Pl, 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
**Increased**: IKK-α,
**Decreased**: EIF4G1, TYK2,
Protein kinase C iota type
**Increased**: d: C3 |
| 4   | Shen et al. [27] | Proteomics | B-lymphocytes | Antibody chips | |
| 5   | Momeni et al. [86] | Proteomics | Serum | SELDI TOF MS MALDI TOF/TOF MS ESI-FTICR MS | |
| 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                                                                                                                                 |
|-----|-------------|-------------|-----------|----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| 10  | Ngounou Wetie et al. [87] | Proteomics | Saliva    | 2D-PAGE LC-MS/MS     | **Decreased:** p532, CREB-binding protein, Transferrin, Zn alpha2 glycoprotein, Cystatin D, Plasminogen, Alpha-amylase, Zymogen granule protein 16.  
**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.  
Mannan-binding lectin serine protease 2 isoform 2 precursor, IGHG1, Kininogen-1.  
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. |
| 12  | Suganya et al.[89] | Proteomics | Urine     | 2D-PAGE MALDI-TOF-MS | **Increased:** Kininogen-1, Alpha-1-antitrypsin, Haptoglobin, Fibrinogen beta chain, Fibrinogen gamma chain, IGHA1, IGHG, Alpha-2-macroglobulin, |
| 13  | Yang et al. [90] | Proteomics | Urine     | iTRAQ labeling, LC-MALDI-MS/MS | **Increased:** Kininogen-1, Alpha-1-antitrypsin, Haptoglobin, Fibrinogen beta chain, Fibrinogen gamma chain, IGHA1, IGHG, Alpha-2-macroglobulin, |
| 14  | Cortelazzo et al. [24] | Proteomics | Plasma    | 2D-PAGE, LC-MS/MS    | **Increased:** Kininogen-1, Alpha-1-antitrypsin, Haptoglobin, Fibrinogen beta chain, Fibrinogen gamma chain, IGHA1, IGHG, Alpha-2-macroglobulin, |
| No. | Researchers               | Types          | Samples           | Technique                  | Related Proteins                                                                                                                                                                                                 |
|-----|---------------------------|----------------|-------------------|----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 15  | Feng et al. [67]          | Proteomics     | Plasma            | 2D-Oxyblot, MALDI-TOF      | **Decreased:** Prealbumin, serum transferrin, Apolipoprotein A-I, Apolipoprotein J, and Apolipoprotein A-IV. **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-1, 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, UQRC2. (Forty-one differentially expressed proteins in total) **Increased:** Galectin-1, glutathione S-transferase P, mitochondrial Cytochrome c Oxidase Subunit 5A, Improver of basic |
| 20  | Pichitpunpong et al. [64] | Proteomics     | Lymphoblastoid cell lines | 2D-PAGE, MS |                                                                                                                                                                                                                 |
| No. | Researchers           | Types       | Samples | Technique                                      | Related Proteins                                                                                                                                                                                                 |
|-----|-----------------------|-------------|---------|-----------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 21  | Castagnola et al. [47] | Peptides    | Saliva  | HPLC-ESI-IT-MS                                | homolog, Calmodulin-140S ribosomal protein SA, an inhibitor of benzodiazipines 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). 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). Decreased: IgG1.                                                                                       |
| 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  | Biardi 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: sAPPα. Decreased: BDNF, sAPPβ, Aβ1-42, Aβ1-40.                         |
| 33  | Işeri et al. [58]               | Single protein   | Serum   | ELISA              | Decreased: BDNF, sAPPβ, Aβ1-42, Aβ1-40.                                          |
| 34  | Al-Ayadhi et al. [57]           | Single protein   | Serum   | ELISA              | Increased: EGF.                                                                   |
| 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. |
| 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 an essential role in 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 a critical role in ASD pathogenesis [77-79]. LIMK1 enhanced axonal outgrowth and synaptic plasticity [80] and related to ASD [81]. ARHGEF13 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 a 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 till 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.

REFERENCES

1. Al-Mosawi AJ. Heller syndrome in two Iraqi children. Clinical Research and Trials. 2019;5:1-3.
2. Edition F. Diagnostic and statistical manual of mental disorders. Am Psychiatric Assoc. 2013;21(21):591-643.
3. Maenner MJ, Shaw KA, Baio J. Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2016. MMWR Surveillance summaries. 2020;69(4):1.
4. Wiśniowiecka-Kowalnik B, Nowakowska BA. Genetics and epigenetics of autism spectrum disorder—current evidence in the
field. Journal of applied genetics. 2019;60(1):37-47.
5. Mpaka DM, Okitundu DLE-A, Ndjukeni AO, N’situ AM, Kinsala SY, Mukau JE, et al. Prevalence and comorbidities of autism among children referred to the outpatient clinics for neurodevelopmental disorders. The Pan African Medical Journal. 2016;25.
6. Besag FM. Epilepsy in patients with autism: links, risks and treatment challenges. Neuropsychiatric disease and treatment. 2018;14:1.
7. Bishop-Fitzpatrick L, Mazefsky CA, Eack SM, Minshew NJ. Correlates of social functioning in autism spectrum disorder: The role of social cognition. Research in Autism Spectrum Disorders. 2017;35:25-34.
8. Chen M-H, Su T-P, Chen Y-S, Hsu J-W, Huang K-L, Chang W-H, et al. Comorbidity of allergic and autoimmune diseases in patients with autism spectrum disorder: A nationwide population-based study. Research in autism spectrum disorders. 2013;7(2):205-12.
9. Chaidez V, Hansen RL, Hertz-Picciotto I. Gastrointestinal problems in children with autism, developmental delays or typical development. Journal of autism and developmental disorders. 2014;44(5):1117-27.
10. Mohan P, Paramasivam I. Feature reduction using SVM-RFE technique to detect autism spectrum disorder. Evolutionary Intelligence. 2021;14(2):989-97.
11. Tye C, Runicles AK, Whitehouse AJ, Alvarees GA. Characterizing the interplay between autism spectrum disorder and comorbid medical conditions: an integrative review. Frontiers in psychiatry. 2019;9:751.
12. Kim JY, Son MJ, Son CY, Radua J, Eisenhut M, Gressier F, et al. Environmental risk factors and biomarkers for autism spectrum disorder: an umbrella review of the evidence. The Lancet Psychiatry. 2019;6(7):590-600.
13. Sokol DK, Maloney B, Westmark CJ, Lahiri DK. Novel contribution of secreted amyloid-β precursor protein to white matter brain enlargement in autism spectrum disorder. Frontiers in psychiatry. 2019;10:165.
14. Yang Y, Tian J, Yang B. Targeting gut microbiome: A novel and potential therapy for autism. Life sciences. 2018;194:111-9.
15. Li Q, Zhou J-M. The microbiota–gut–brain axis and its potential therapeutic role in autism spectrum disorder. Neuroscience. 2016;324:131-9.
16. Shen L, Liu X, Zhang H, Lin J, Feng C, Iqbal J. Biomarkers in autism spectrum disorders: Current progress. Clinica Chimica Acta. 2020;502:41-54.
17. Kocsis RN. Book review: diagnostic and statistical manual of mental disorders: (DSM-5). Sage Publications Sage CA: Los Angeles, CA; 2013.
18. El-Ansary A, Hassan WM, Daghestani M, Al-Ayadhi L, Ben Bacha A. Preliminary evaluation of a novel nine-biomarker profile for the prediction of autism spectrum disorder. PLoS One. 2020;15(1):e0227626.
19. Aaron E, Montgomery A, Ren X, Guter S, Anderson G, Carneiro A, et al. Whole blood serotonin levels and platelet 5-HT2A binding in autism spectrum disorder. Journal of autism and developmental disorders. 2019;49(6):2417-25.
20. Heuer LS, Croen LA, Jones KL, Yoshida CK, Hansen RL, Yolken R, et al. An exploratory examination of neonatal cytokines and chemokines as predictors of autism risk: the early markers for autism study. Biological Psychiatry. 2019;86(4):255-64.
21. Howsmon DP, Kruger U, Melnyk S, James SJ, Hahn J. Classification and adaptive behavior prediction of children with autism spectrum disorder based upon multivariate data analysis of markers of oxidative stress and DNA methylation. PLoS computational biology. 2017;13(3):e1005385.
22. Aebersold R, Agar JN, Amster IJ, Baker MS, Bertozzi CR, Boja ES, et al. How many human proteoforms are there? Nature chemical biology. 2018;14(3):206-14.
23. Frantzi M, Bhat A, Latosinska A. Clinical proteomic biomarkers: relevant issues on study design & technical considerations in biomarker development. Clinical and translational medicine. 2014;3(1):1-22.
24. Cortelazzo A, De Felice C, Guerranti R, Signorini C, Leoncini S, Zollo G, et al. Expression and oxidative modifications of plasma proteins in autism spectrum disorders: Interplay between inflammatory response and lipid peroxidation. PROTEOMICS–Clinical Applications. 2016;10(11):1103-12.
Zhao R, Qin W, Qin R, Han J, Li C, Wang Y, et al. Lectin array and glycoene expression analyses of ovarian cancer cell line A2780 and its cisplatin-resistant derivate cell line A2780-cp. Clinical proteomics. 2017;14(1):1-10.

Shen L, Zhang K, Feng C, Chen Y, Li S, Iqbal J, et al. iTRAQ-based proteomic analysis reveals protein profile in plasma from children with autism. PROTEOMICSClinical Applications. 2018;12(3):1700085.

Shen C, Zhao X-I, Ju W, Zou X-b, Huo L-r, Yan W, et al. A proteomic investigation of B lymphocytes in an autistic family: a pilot study of exposure to natural rubber latex (NRL) may lead to autism. Journal of Molecular Neuroscience. 2011;43(3):443-52.

Junaid MA, Kowal D, Barua M, Pullarkat PS, Sklower Brooks S, Pullarkat RK. Proteomic studies identified a single nucleotide polymorphism in glyoxalase I as autism susceptibility factor. American Journal of Medical Genetics Part A. 2004;131(1):11-7.

Broek JA, Guest PC, Rahmoune H, Bahn S. Proteomic analysis of post mortem brain tissue from autism patients: evidence for opposite changes in prefrontal cortex and cerebellum in synaptic connectivity-related proteins. Molecular autism. 2014;5(1):1-8.

Zerbo O, Yoshida C, Grether JK, Van de Water J, Ashwood P, Delorenze GN, et al. Neonatal cytokines and chemokines and risk of Autism Spectrum Disorder: the Early Markers for Autism (EMA) study: a case-control study. Journal of neuroinflammation. 2014;11(1):1-9.

Krakowiak P, Goines PE, Tancredi DJ, Ashwood P, Hansen RL, Hertz-Picciotto I, et al. Neonatal Cytokine Profiles Associated With Autism Spectrum Disorder. Biol Psychiatry. 2017;81(5):442-51.

Corbett B, Kantor A, Schulman H, Walker W, Lit L, Ashwood P, et al. A proteomic study of serum from children with autism showing differential expression of apolipoproteins and complement proteins. Molecular psychiatry. 2007;12(3):292-306.

Taurines R, Dudley E, Conner AC, Grassl J, Jans T, Guderian F, et al. Serum protein profiling and proteomics in autistic spectrum disorder using magnetic bead-assisted mass spectrometry. European archives of psychiatry and clinical neuroscience. 2010;260(3):249-55.

Ramsey JM, Guest PC, Broek JA, Glennon JC, Rommelse N, Franke B, et al. Identification of an age-dependent biomarker signature in children and adolescents with autism spectrum disorders. Molecular autism. 2013;4(1):1-9.

Steeb H, Ramsey JM, Guest PC, Stocki P, Cooper JD, Rahmoune H, et al. Serum proteome analysis identifies sex-specific differences in lipid metabolism and inflammation profiles in adults diagnosed with Asperger syndrome. Molecular autism. 2014;5(1):1-10.

Ngounou Wetine AG, Wormwood K, Thome J, Dudley E, Taurines R, Gerlach M, et al. A pilot proteomic study of protein markers in autism spectrum disorder. Electrophoresis. 2014;35(14):2046-54.

Suganya V, Geetha A, Sujatha S. Urine proteome analysis to evaluate protein biomarkers in children with autism. Clinica chimica acta; international journal of clinical chemistry. 2015;450:210-9.

Yang L, Rudser, K., Golnik, A., Wey, A., Higgins, L. A., & Gourley, G. R. Urine protein biomarker candidates for autism. J Proteomics Bioinform. 2016;14:004.

Ngounou Wetine AG, Wormwood KL, Charette L, Ryan JP, Woods AG, Darie CC. Comparative two-dimensional polyacrylamide gel electrophoresis of the salivary proteome of children with autism spectrum disorder. J Cell Mol Med. 2015;19(11):2664-78.

Ngounou Wetine AG, Wormwood KL, Russell S, Ryan JP, Darie CC, Woods AG. A Pilot Proteomic Analysis of Salivary Biomarkers in Autism Spectrum Disorder. Autism research : official journal of the International Society for Autism Research. 2015;8(3):338-50.

Shen L, Feng C, Zhang K, Chen Y, Gao Y, Ke J, et al. Proteomics study of peripheral blood mononuclear cells (PBMCs) in autistic children. Frontiers in cellular neuroscience. 2019;13:105.

Shen L, Zhao Y, Zhang H, Feng C, Gao Y, Zhao D, et al. Advances in biomarker studies in autism spectrum disorders. Reviews on Biomarker Studies in Psychiatric and Neurodegenerative Disorders. 2019:207-33.

Yang J, Chen Y, Xiong X, Zhou X, Han L, Ni L, et al. Peptidome analysis reveals novel serum biomarkers for children with autism spectrum disorder in China.
Proteomics–Clinical Applications. 2018; 12(5):1700164.

44. Chen Y-N, Du H-Y, Shi Z-Y, He L, He Y-Y, Wang D. Serum proteomic profiling for autism using magnetic bead-assisted matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a pilot study. World Journal of Pediatrics. 2018;14(3):233-7.

45. Zaman S, Yazdani U, Deng Y, Li W, Gadad BS, Hynan L, et al. A search for blood biomarkers for autism: peptoids. Scientific reports. 2016;6(1):1-8.

46. Momeni N, Bergquist J, Brudin L, Behnia F, Sivberg B, Joghataei M, et al. A novel blood-based biomarker for detection of autism spectrum disorders. Translational psychiatry. 2012;2(3):e91-e.

47. Castagnola M, Messana I, Inzitari R, Fanali C, Cabras T, Morelli A, et al. Hypophosphorylation of salivary peptidome as a clue to the molecular pathogenesis of autism spectrum disorders. Journal of Proteome Research. 2008;7(12):5327-32.

48. Husarova V, Lakatosova S, Pivovarceva A, Bakos J, Durdiakova J, Kubranska A, et al. Brief report: Plasma oxytocin is lower in children with Asperger syndrome and associated with autistic trait attention to detail. Open Journal of Psychiatry. 2013:2013.

49. Wang H, Xu H, Wang X, Zhou A, Wu M, Liu J, et al. Amyloid precursor protein associates with autism spectrum disorder: a potential candidate biomarker for early screening. International Journal of Clinical & Experimental Medicine. 2016;9(11).

50. Abdel-Salam OM, Youness ER, Mohammed NA, Elhamed WAA. Nuclear factor-kappa B and other oxidative stress biomarkers in serum of autistic children. Open Journal of Molecular and Integrative Physiology. 2015;5(01):18.

51. Kadak MT, Celin I, Tarakcioglu MC, Ozer OF, Kacsar S, Cimen B. Low serum level α-synuclein and tau protein in autism spectrum disorder compared to controls. Neuropediatrics. 2015;46(06): 410-5.

52. Babinska K, Bucova M, Durmanova V, Lakatosova S, Janosikova D, Bakos J, et al. Increased plasma levels of the high mobility group box 1 protein (HMGB1) are associated with a higher score of gastrointestinal dysfunction in individuals with autism. Physiol Res. 2014;63(Suppl 4):S613-S8.

53. Dinçel N, Ünalp A, Kutlu A, Öztürk A, Uran N, Ulusoy S. Serum nerve growth factor levels in autistic children in Turkish population: a preliminary study. The Indian journal of medical research. 2013;138(6):900.

54. Bashir S, Halepoto DM, Al-Ayadhi L. Serum level of desert hedgehog protein in autism spectrum disorder: preliminary results. Medical Principles and Practice. 2014;23(1):14-7.

55. Husarova VM, Lakatosova S, Pivovarceva A, Babinska K, Bakos J, Durdiakova J, et al. Plasma oxytocin in children with autism and its correlations with behavioral parameters in children and parents. Psychiatry investigation. 2016;13(2):174.

56. Rout UK, Mungan NK, Dhossche DM. Presence of GAD65 autoantibodies in the serum of children with autism or ADHD. European child & adolescent psychiatry. 2012;21(3):141-7.

57. Al-Ayadhi LY, Mostafa GA. A lack of association between elevated serum levels of S100B protein and autoimmunity in autistic children. Journal of neuroinflammation. 2012;9(1):1-8.

58. İşeri E, Güney E, Ceylan MF, Yücel A, Aral A, Bodur Ş, et al. Increased serum levels of epidermal growth factor in children with autism. Journal of autism and developmental disorders. 2011;41(2):237-41.

59. Ray B, Long JM, Sokol DK, Lahiri DK. Increased secreted amyloid precursor protein-α (sAPPα) in severe autism: proposal of a specific, anabolic pathway and putative biomarker. PloS one. 2011;6(6):e20405.

60. Correia C, Coutinho A, Sequeira A, Sousa I, Lourenco Venda L, Almeida J, et al. Increased BDNF levels and NTRK2 gene association suggest a disruption of BDNF/TRKB signaling in autism. Genes, Brain and behavior. 2010;9(7):841-8.

61. Blardi P, de Lalla A, Ceccatelli L, Vanessa G, Auteri A, Hayek J. Variations of plasma leptin and adiponectin levels in autistic patients. Neuroscience letters. 2010;479(1):54-7.

62. Ashwood P, Kwong C, Hansen R, Hertz-Picciotto I, Croen L, Krakowiak P, et al. Brief report: plasma leptin levels are elevated in autism: association with early onset phenotype? Journal of autism and developmental disorders. 2008;38(1):169-75.
63. Warren RP, Burger RA, Odell D, Torres AR, Warren WL. Decreased plasma concentrations of the C4B complement protein in autism. Archives of pediatrics & adolescent medicine. 1994;148(2):180-3.

64. Pichitpunpong C, Thongkorn S, Kanlayaprasit S, Yuwattana W, Plaingam W, Sangsuthum S, et al. Phenotypic subgroups and multi-omics analyses reveal reduced diazepam-binding inhibitor (DBI) protein levels in autism spectrum disorder with severe language impairment. PLoS one. 2019;14(3):e0214198.

65. Abraham J, Szoko N, Natowicz MR. Proteomic investigations of autism spectrum disorder: past findings, current challenges, and future prospects. Reviews on Biomarker Studies in Psychiatric and Neurodegenerative Disorders. 2019:235-52.

66. Szoko N, McShane AJ, Natowicz MR. Proteomic explorations of autism spectrum disorder. Autism Research. 2017;10(9):1460-9.

67. Feng C, Chen Y, Pan J, Yang A, Niu L, Min J, et al. Redox proteomic identification of carbonylated proteins in autism plasma: insight into oxidative stress and its related biomarkers in autism. Clinical Proteomics. 2017;14(1):1-8.

68. Qin Y, Chen Y, Yang J, Wu F, Zhao L, Yang F, et al. Serum glycopattern and Maackia amurensis lectin-II binding glycoproteins in autism spectrum disorder. Scientific reports. 2017;7(1):1-11.

69. Wolf NJ, Salomons GS, Rodenburg RJ, Poutjes PJ, Schieving JH, Derks TG, et al. Mutations in RARS cause hypomyelination. Annals of neurology. 2014;76(1):134-9.

70. Krupp DR, Barnard RA, Duffourd Y, Evans SA, Mulqueen RM, Bernier R, et al. Exonic mosaic mutations contribute risk for autism spectrum disorder. The American Journal of Human Genetics. 2017;101(3):369-90.

71. Jewkes R, Sikwewiyia Y, Morrell R, Dunkle K. Gender inequitable masculinity and sexual entitlement in rape perpetration South Africa: findings of a cross-sectional study. PLoS one. 2011;6(12):e29590.

72. Buxbaum J, Silverman J, Keddache M, Smith C, Holland E, Ramoz N, et al. Linkage analysis for autism in a subset families with obsessive–compulsive behaviors: evidence for an autism susceptibility gene on chromosome 1 and further support for susceptibility genes on chromosome 6 and 19. Molecular psychiatry. 2004;9(2):144-50.

73. Ylisaukko-oja T, Alarcón M, Cantor RM, Auranen M, Vanhala R, Kempas E, et al. Search for autism loci by combined analysis of Autism Genetic Resource Exchange and Finnish families. Annals of neurology. 2006;59(1):145-55.

74. Cho S-C, Yoo HJ, Park M, Cho IH, Kim B-N, Kim J-W, et al. Genome-wide association scan of Korean autism spectrum disorders with language delay: a preliminary study. Psychiatry Investigation. 2011;8(1):61.

75. Dong S, Walker M, Carriero N, DiCola M, Willsey A, Ye A, et al. State MW, Wei L, Sanders SJ. De novo insertions and deletions of predominantly paternal origin are associated with autism spectrum disorder Cell Rep. 2014;9(1):16-23.

76. Napolioli V, Persico AM, Porcelli V, Palmieri L. The mitochondrial aspartate/glutamate carrier AGC1 and calcium homeostasis: physiological links and abnormalities in autism. Molecular Neurobiology. 2011;44(1):83-92.

77. Kim S-J, Silva RM, Flores CG, Jacob S, Guter S, Valcante G, et al. A quantitative association study of SLC25A12 and restricted repetitive behavior traits in autism spectrum disorders. Molecular autism. 2011;2(1):1-13.

78. Carayol J, Schellenberg GD, Dombroski B, Genin E, Rousseau F, Dawson G. Autism risk assessment in siblings of affected children using sex-specific genetic scores. Molecular Autism. 2011;2(1):1-8.

79. Jiao Y, Chen R, Ke X, Cheng L, Chu K, Lu Z, et al. Single nucleotide polymorphisms predict symptom severity of autism spectrum disorder. Journal of autism and developmental disorders. 2012;42(6):971-83.

80. Cuberos H, Vallée B, Vourc'h P, Tastet J, Andres C, Bénédett H. Roles of LIM kinases in central nervous system function and dysfunction. FEBS letters. 2015;589(24):3795-806.

81. Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, et al. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature. 2011;474(7351):380-4.

82. Eriksson MA, Liedén A, Westerlund J, Bremer A, Wincent J, Sahlin E, et al. Rare copy number variants are common in young children with autism spectrum
disorder. Acta Paediatrica. 2015;104(6): 610-8.
83. Yao F, Zhang K, Zhang Y, Guo Y, Li A, Xiao S, et al. Identification of blood biomarkers for Alzheimer's disease through computational prediction and experimental validation. Frontiers in neurology. 2019;9:1158.
84. Liu Y, Buil A, Collins BC, Gillet LC, Blum LC, Cheng LY, et al. Quantitative variability of 342 plasma proteins in a human twin population. Molecular systems biology. 2015;11(2):786.
85. Bahn S, Schwarz E, Guest P, Rahmoune H, Wang L, Ingudomnukul E, et al. Sex-Specific Serum Biomarker Patterns in Adults with Asperger Syndrome. Molecular Psychiatry; 2010.
86. Momeni N, Bergquist J, Brudin L, Behnia F, Sivberg B, Joghataei M, et al. Bio-marqueurs découverts pour l'Autisme Découverte de bio-marqueurs pour l'autisme. Translational Psychiatry. 2012 ;2:e91.
87. Ngounou Wetie AG, Wormwood KL, Charette L, Ryan JP, Woods AG, Darie CC. Comparative two-dimensional polyacrylamide gel electrophoresis of the salivary proteome of children with autism spectrum disorder. Journal of cellular and molecular medicine. 2015;19(11):2664-78.
88. Ngounou Wetie AG, Wormwood KL, Russell S, Ryan JP, Darie CC, Woods AG. A pilot proteomic analysis of salivary biomarkers in autism spectrum disorder. Autism Research. 2015;8(3):338-50.
89. Suganya V, Geetha A, Sujatha S. Urine proteome analysis to evaluate protein biomarkers in children with autism. Clinica Chimica Acta. 2015;450:210-9.
90. Yang L, Rudser K, Golnik A, Wey A, Higgins L, Gourley G. Urine protein biomarker candidates for autism. J Proteomics Bioinform. 2016;14:004.
91. Singh S, Yazdani U, Gadad B, Zaman S, Hynan LS, Roatch N, et al. Serum thyroid-stimulating hormone and interleukin-8 levels in boys with autism spectrum disorder. Journal of Neuroinflammation. 2017;14(1):1-7.
92. Modahl C, Green LA, Fein D, Morris M, Waterhouse L, Feinstein C, et al. Plasma oxytocin levels in autistic children. Biological psychiatry. 1998;43(4):270-7.
93. Green LA, Doyev S, Fryer Jr GE. It takes a balanced health care system to get it right.(Commentary). Journal of Family Practice. 2001;50(12):1038-40.
94. Chauhan A, Chauhan V, Brown WT, Cohen I. Oxidative stress in autism: Increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin-the antioxidant proteins. Life sciences. 2004;75(21):2539-49.
95. Momeni N, Brudin L, Behnia F, Nordström B, Yosefi-Oudarji A, Sivberg B, et al. High complement factor I activity in the plasma of children with autism spectrum disorders. Autism Research and Treatment. 2012; 2012.
96. Essa MM, Guillemin GJ, Waly MI, Al-Sharbati MM, Al-Farsi YM, Hakkim FL, et al. Reduced levels of antioxidant proteins in children with autism in Oman. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2012;2(1):53.
97. Xu X-J, Shou X-J, Li J, Jia M-X, Zhang J-S, Guo Y, et al. Mothers of autistic children: lower plasma levels of oxytocin and Arg-vasopressin and a higher level of testosterone. PLoS One. 2013;8(9): e74849.
98. Wang L, Jia J, Zhang J, Li K. Serum levels of SOD and risk of autism spectrum disorder: a case-control study. International Journal of Developmental Neuroscience. 2016;51:12-6.

© 2022 Alharbi; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here: https://www.sdiarticle5.com/review-history/90200