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ADVANCEMENT IN BIOMARKERS FOR SCHIZOPHRENIA THERANOSTIC: A REVIEW

Harsh Thakkar[1], Amit Khairnar[2], Monika Sharma[2], Rajeshwari Rathod[1*]

[1]Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research (NIPER)-Ahmedabad, Gandhinagar, Gujarat, India.

[2]Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER)-Ahmedabad, Gandhinagar, Gujarat, India.

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***Corresponding Author**
Dr. Rajeshwari Rathod
Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research (NIPER)-Ahmedabad, Gandhinagar, Gujarat, India.

ABSTRACT

Schizophrenia is a neurodevelopmental disorder and is non dividing line between reality and imagination. Though schizophrenia is multidimensional disease, there is no single convincible biomarker available for the disease. Diagnosis of schizophrenia at early stage is the most challenging task and due to misdiagnosis there are severe clinical threatening implications. Identification of biomarker is needed to diagnose or determine the response of the treatment in people with Schizophrenia. Biomarker research involves diagnostic, prognostic and theranostics approach and for that appropriate methodological strategy is required. Highly acknowledged pathophysiology and treatment approaches involved in schizophrenia associated with neurotransmitter changes, like dopamine and serotonin, though disorder cannot be prevented. Other than neurotransmitter changes, omics based approach is also most widely used. In this review, we will discuss some modernistic techniques like genomics, metabolomic, neuroinflammation based along with some symptomatic based biomarkers which could unwind new direction of biomarker research with clinical applicability. The combinatorial discovery approach will provide easy diagnosis, severity of disease and treatment outcomes without precipitating offshoots.

KEYWORDS: Schizophrenia, Biomarkers, Metablomics, Genomics, Combinatorial discovery.

1. BIOMARKER IN SCHIZOPHRENIA

According to Greek origin “skhizein” - to split and “phren”- mind, in Latin it is called as schizophrenia. It is also known as dementia praecox. It is chronic and severe mental disorder symbolized by thought, behaviour and emotional disturbance which leads to significant deterioration in the quality of life. Schizophrenia affects 21 million people globally, which is 0.5-1 % of total world population.[1] According to Diagnostic and statistical manual of mental disorder 5th edition (DSM-V) schizophrenia is categorised based on their symptoms into paranoid schizophrenia, catatonic schizophrenia, schizoaffective disorder and childhood schizophrenia.[2] Paranoid schizophrenia which is the most prominent form of schizophrenia reveals influence of auditory hallucinations or important delusional thoughts about persecution or collusion. People with paranoid-type schizophrenia may display annoyance, anxiety, and unkindness.[3] The person normally has normal intelligence functioning and utterance of affect. While the catatonia shows excessive and peculiar behaviours also called as catatonic excitements.[4] Schizoaffective disorder is a disorder in which person experiences mood changes which is psychotic symptom of schizophrenia. The less prevalent but the most severe type of schizophrenia is childhood onset schizophrenia which normally occurs during adulthood.

According to NIH (National Institute of Health) biomarker are quantifiable outcomes which are further modified by the diseases and therapeutic intervention. In narrow sense, any molecular or cellular change in the body tissue or fluid which can act as an indicator for specific disease or disease progression is called as biomarker. Ideal biomarker should be easily detectable, stable, sensitive and reproducible[5]. Other than all these characteristics most important is clinical utility, hitherto there is no biomarker in schizophrenic patients which can detect it at early stage and posses clinical utility. The current diagnosis of schizophrenia remains personalized due to its multifaceted spectrum of symptoms, and the mechanism behind the disease progress has yet to be explored.

It is not surprising that no possible biomarker has been accepted for the detection of schizophrenia as the disease has multiple causes with various biological mechanisms. Moreover schizophrenia is associated with various co-morbidities like depression, hyperglycaemia, cardiovascular disease so it is difficult to find a single biomarker for this disease.[6] In the 21st century prototypic biomarker for disease can only be identified through “omics” which includes genomics, transcriptomics and proteomics.[7] However it is possible

to identify biomarker at macroscopic scale, most schizophrenia research have concentrated on abnormal changes in brain through MRI (magnetic resonance imaging) or electrophysiological measures(EEG) but it has some limitations. Schizophrenia may originate from either organs or cells like liver, fibroblast, nasal epithelial and blood cells, respectively. After diagnosis of schizophrenia it is necessary to choose correct drug its dose and route of administration, so optimal biomarker should be used to determine severity of disease and degree of symptoms.[8]

1.1 Metabolomic as Biomarker in Schizophrenia

Scientific regulation dealing with the small molecules up to 1.5kDa molecular weight is called Metabolomics.[9] Metabolites are endogenous molecules which are synthesized in cell during the metabolic process. Various metabolites like carbohydrates, amino acids, nucleic acids, lipids, vitamins are final product of controlled cellular growth.[10] Set of all these bio molecules defines metabolome which can be determined at various levels. The metabolome comprise of all metabolites that are available in all biological samples like as blood, urine, saliva, sweat and hair follicles.[11] Levels of these metabolites get changed due to alteration in biochemical pathway in response to disease or treatment. Metabolomics represents the final output of interactions among various factors including genetic, physiological and environmental factors and suggests the functional status of human being. It also links metabolic state of individuals with environmental aspects.[12]

1.1.1 Blood Based Metabolomic

Blood has several advantages as a biomarker as it contains various proteins, lipids and metabolic products which can be easily examined in serum or plasma.[13] Another major advantage of this modality is that compared to tissue based biomarkers, blood based biomarkers can be easily accessible. Because blood can be availed in sufficient quantity through standardized collection procedure which allows for repeated sampling in large cohorts. The correlation between blood- cortex and blood-cerebellum was found to be 0.66 and 0.76 respectively[14]

Alterations in biochemical pathway are the major cause for any disease condition. Homeostasis of the glucose metabolism is necessary to regulate the normal brain function. The current proteomics study found that, 87.5% of glucose metabolizing enzymes such as aldolase C, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase 1, phosphoglycerate mutase were inflated in first-episode

schizophrenic (sz) patients. They are reported to be elevated in both brain tissues and PBMCs (peripheral blood mononuclear cells) of schizophrenic patients.[15] Another study has reported elevated levels of ATP and increased gene/protein expression linked with glucose metabolism. Combined with this, it is reported that glycolysis pathway and TCA cycle may be stimulated in early stages of schizophrenia.[16] Pentose phosphate pathway (PPP) is one more important pathway for glucose metabolism, in which ribose 5-phosphate is the major product. Levels of ribose 5-phosphate were reported to increase in PBMCs.[17] Other than glucose, changes in lipid level also modulate the disease condition. Altered levels of linoleic acid (polyunsaturated fatty acid C18:2n-5) in serum which is precursor to omega-6 polyunsaturated fatty acid affect neuronal signal transduction. Myo-inositol is requisite for the production of membrane inositol phospholipids. Serum levels of myo-inositol get elevated in patients normalised by risperidone treatment, suggesting role of myo-inositol in schizophrenia.[18] This metabolite not only affects the psychosis condition, but is also responsible for other comorbidities present in schizophrenic patients. One report suggests that due to oxidative stress condition lysophosphosphatidylcholine converts to phosphotidylcholine during LDL(low density lipoproteins) oxidation process which further leads to metabolic and cardiovascular disorders.[19] As inflammation is linked with schizophrenia, abnormalities can be observed in cortisol level in paranoid sz patients compared to control and drug treated patients.[20]

For conversion into metabolite either from drug molecule or neurotransmitter, enzyme plays crucial role in the metabolic pathway. The serum levels of adenosine deaminase (ADA) is elevated in schizophrenia which is an enzyme responsible for degradation of adenosine.[21] Adenosine hinders the release of neurotransmitters such as dopamine, serotonin, and glutamate[22] thereby decreasing neuronal activity by post synaptic hyperpolarisation. There is direct correlation between ADA_{diff} (even-morn) and auditory hallucinations and a converse correlation between ADA_{diff} (even-morn) and avolition-apathy. One clinical report found that elevated ADA level is much correlated with positive symptoms compared to negative symptoms. Altered expression of glycolytic enzymes has also been reported in central and peripheral system in schizophrenic patients. Decreased levels of 1, 3 bisphosphoglycerate (1, 3 BPG) as well as increase in levels of lactate suggest shift of glycolysis towards a less efficient anaerobic respiration. Decrement in levels of citrate and α-ketoglutarate together suggest reduction in TCA cycle activity. Though blood based biomarker have several advantages, the success rate of blood based biomarker is very low because of its accuracy.

1.1.2 Brain Tissue Based Biomarker

As the neurotransmitter is produced from amino acids, change in level of amino acids in brain affects the disease condition by altering the level of neurotransmitters. In schizophrenia patients, arginine consumption and its conversion into ornithine increased due to abnormal metabolism of NO. L-arginine is a semi-essential amino acid which is metabolised in number of bioactive molecules like L-citrulline, nitric oxide(NO), ornithine and agmatine.[23] It is widely distributed in the brain tissue and converted into ornithine in presence of arginase. Accumulated ornithine further converts into glutamate, glutamine and GABA.[24] These neurotransmitters interact with number of receptor subtypes including metabotropic glutamate receptor, NMDA (N-methyl-D-Aspartate), GABAergic receptos. Out of all arginine metabolites, the major one is agmatine formed by enzyme arginine decarboxylase. To confirm the arginine metabolites in brain, tissue of grey matter of left frontal cortex were collected from 20 sz patients. After homogenization, metabolites were measured using liquid chromatography/mass spectrometry assay. They found, elevation in agmatine levels was 26% compared to control patients.[23] The same pattern changes of agmatine/ornithine can also be observed in blood. Other than this one another system known as ubiquitin proteosome system (UPS) is responsible for protein degradation is involved in neuropsychiatric disorders. Dendritic spine density mainly depends on balance between systhesis and degradation. Abnormalities in UPS system can be observed in hippocampus, prefrontal cortex and temporal gyrus which further produces synaptic dysfunction.[25] As compare to blood based biomarker, the success rate of brain based biomarker is higher, but brain based biomarker is invasive and costly.

1.1.3 Urine Based Biomarker

Urine is another major source of biomarker and one of the most important advantage is that urine based biomarkers can be obtained non-invasively in relatively larger volume. Urine based isoprostanes (IPs) which are novel prostaglandins isomers are produced through free radical-catalysed reaction from arachidonic acids. Levels of isoprostane-8-epi-prostaglandin $F_{2\alpha}$(8-isoPGF$_{2\alpha}$) were increased significantly in urine indicating free radicals induced peroxidation of unsaturated fatty acids in sz patients when compared to that of control group[26]. The normal level of isoprostane was 0.035ng/ml. But in sz patients it was significally increased to 0.055ng/ml which is 1.5 times higher than the normal patients.[26] The one of the major disadvantage of urine based marker is it's specificity as large spectrum of pathologies produces similar urinary metabolites.

1.1.4 Saliva Based

The pros of saliva based biomarker is that it can also be collected from non-cooperative patients like sz patients and just like serum, it also contains hormones, growth factors, enzymes and various metabolites. Increased levels of α-defensins which are also called HNP (human neutrophil peptide) are secreted from monocyctes, natural killer cells, and T cells. In one study, level of α-defensins 1-3 versus α-defensins 4 is reported to be in ratio of 5:1 in saliva. This increase in concentration of α-defensins 1-3 could only be correlated to elevated neutrophil activity. Also levels of S100A12 called as calgranulin C along with levels of cystatins A and B are found to be elevated in saliva of schizophrenic patients. In addition to this, one clinical study also suggests that increased level of kyenuric acid in saliva is found in schizophrenic patients.[27] First there is conversion of tryptophan to kyenurine in presence of tryptophan 2, 3-dioxygenase (TDO) and further through irreversible transamination kyenurine converts to kyenuric acid. In the schizophrenia, due to increase oxidative stress, there is increase in concentration of TDO which is major step involved in formation of kyenuric acid. This kyenuric acid acts as an NMDA antagonist. This salivary based biomarker suggests novel direction in biomarker research. Some early studies found difficulty in utilization of saliva as a diagnostic tool because the metabolites are found in less quantity compared to serum, but due to emerging techniques like LC/MS, western blotting and ELISA discovery of biomarker from saliva is comparatively easy.

1.2 Genetics Based Biomarker in Schizophrenia

A huge genetic data from schizophrenia associated research has recognized collection of genes and disturbed pathways which indicates participation of complex genetic elements in schizophrenia and other psychotic disorders. The importance of genes in the physiology of schizophrenia is augmented by advances in genetic technology and grouping studies with collective patient databases and genetic analytical techniques. The major advantage of genetic based biomarker is for phenotypic diagnosis of disease which is further useful for development of personalized medicine, but this type of diagnosis is laborious and less reproducible.

Table I: Genetics based biomarker in schizophrenia.

Genes	Names	Pathway/ Mechanism involved	Location (blood / serum/urine)	Levels of expression	Preclinical or clinical
ADSS	Adenylosuccinate synthase	Influences energy metabolism by purine nucleotide cycle and AMP-activated protein kinase (AMPK) pathway	blood derived	downreguled	clinical
S100A9	calgranulin B		blood based	upregulated	Clinical
CLC	galactin-10	Found to interact with eosinophil phospholipases and increases infectious/inflammation components in the disease	blood based	upregulated	Clinical
GAS7	growth arrest specific gene7	This gene plays an important role in actin and micro-tubule polymerization, which are essential in neurodevelopment in schizophrenia. The impaired pre-pulse inhibition (PPI) of GAS7-deficient mice displayed diseased related behaviour in mice.	In various regions of brain hippocampus,cortex	downregulation	Clinical

ACP1	acid phosphatase 1	Positive correlation between ACP1 and rate limiting enzymes involved in bio-synthesis and metabolism of serotonin and GABA.		up regulation	Clinical
FABP4	fatty acid binding protein 4	FABP4 also known as adipocytes-specific fatty acid-binding protein, they are active in fatty acid uptake, transport and metabolism.Not depend on duration of illness.	hair follicles	downregulated	clinical
SST	Somatostatin	SST is co-localized with GABA as an inhibitory neuropeptide with modulatory and inhibitory actions in the brain.		decreased in cortices of patients	Clinical
NPTX2	neuronal pentraxin 2	Deficiency of NPTX2 leads to imbalance of glutaminergic neurochemical in patients. Modulates through AMPA receptor		down regulated	Clinical
SELEN BP1	selenium-binding protein 1		In PBCs	up regulation	

1.3 Possible Epigenetics Based Biomarkers in Schizophrenia

Epigenetics is a link between environmental factors and genetic changes. In mammalian cells chromatin is made up of nucleosomes which provide packing to chromosomal DNA. Each chromosome includes octamer protein complex containing two copies each of core histone proteins H2A,H2B,H3 and H4, with 147 bp of chromosomal DNA wrapped around it.[28] Post translational modification in nucleosomal histones and DNA methylation represents epigenetic modifications. Epigenetic modification changes gene expression without producing alteration in gene sequence.[29]

Chromatin remodelling by histone modification is key mechanism for neuro-degeneration, neuronal differentiation, memory formation and psychiatric disorders. According to one report, histone modification such as up regulation of DNA acetyltrasferase and down regulation of AcH3K9(acetylated lysine 9 of histone H3) is observed in schizophrenic rats.[30] Histone acetylation can occur via histone acetyl transferase and histone deacetylase. Histone acetylation is mainly involved in learning and memory. Histone deacetylatese 5 (HDAC5) gets phosphorylated through Cam kinase II and translocated from nucleus to extranuclear space. This decrease in level of HDAC5 further decreases level of Ac-H3K9 in animals in prefrontal cortex. This suppression in Ac-H3K9 in the prefrontal cortex can be restored through clozapine treatment.[31]

Second concept is DNA methylation. Covalent modification of cytosine residues at 5' position through methylation is called as DNA methylation. During 1960-1970, Scientists observed that patients treated with L-methionine produces worsening of disease condition.[32] GAD1(glutamic acid decarboxylase) and reelin are the most important genes involved in inhibitory regulation in SZ patients, reduction in these two genes are involved due to DNA-methylation. Post-mortem report suggests that promoter methylation and reelin methylation increases as a consequence of decrease reelin expression. The possible mechanism behind decrease reelin expression is gene silencing. Hypermethylation prevents interaction between cognate recognization site from its positive trans-acting factors.[33] This is confirm through treatment of clozapine and quetiapine, which further increases the level of GAD1 and reelin by decreasing expression of DNA methylation. Another study reported that, decrease in *Shati/Nat8l* promoter CpGIs (CpG Islands) methylation in DNA which are extracted from both the brain (nucleus accumbens) and blood of mice.[34] NAc which receives dopaminergic neuronal inputs, considerable difference was observed in both the mRNA expression levels

and methylation ratio of *Shati/Nat8l* is observed. Neuronal system in NAc (Nucleus Accumbens) comprise of GABAergic neurons, thus GABAergic neurons may regulate DNA methylation of *Shati/Nat8l*. It is also suggested that over expression of DNMT1 (DNA methyltransferase 1) and DNMT3a (DNA methyltransferase 3a) in prefrontal cortex and hippocampus of sz patients which with favours chromatin conformation that is linked with decrease level of GABAergic gene expression[35]. Also decreased levels of BDNF (brain-derived neurotrophic factor) in brain and blood cells are associated with DNA hypermethylation at specific BDNF promoters. Decreased expression of GCR (glucocorticoid receptor) has been associated with elevated expression of DNMT and increased DNA methylation across GCR promoter regions in answer to stress in childhood.[36] Whereas on the contrary, hypomethylation of COMT and HTR2A has been reported in schizophrenia.[37] Main reason for DNA methylation is inflammation and hypo and hypermethylation in blood. Further studies are required to identify epigenetics changes as biomarkers to identify and treat the disorder.

1.4 Neuroimmune Based Biomarker

Inflammation is a defensive mechanism for survival but when it is extreme it is detrimental. In the earlier and acute phase of disease anti-inflammatory system is capable to fight against stimulation of pro-inflammatory system but in the late stages negative symptoms predominates and capability to counter inflammation is lost. Inflammation has been hypothesized to be a linkage between the immune response and pathogenesis of schizophrenia because of cytokines which influence multiple neurologic processes, including neurotransmitter metabolism, neuroendocrine function and neural plasticity.

In first episodic patients (FEP), there are higher levels of inerleukin-6(IL-6), interleukin-10(IL-10), TNF-alpha and TRAP (Total Radical trapping Antioxidant capacity) than healthy control which inversely correlates to lower serum activity of PON1 (Paraoxonase1). This decrease in PON1 gene increases the suicidal tendency. FEP patients treated with risperidone for eleven weeks showed elevated activity of PON1 and declined levels of IL-6, IL-10, TNF-alpha and lipid hydroperoxides.[38] While in chronically treated schizophrenic patients, inflated levels of IL-6, soluble tumour necrosis factor receptors (sTNF-R), leptin and the chemokines CCL-11(eotaxin) and CCL-3 (MIP-1alpha)were observed, whereas the levels of IL-2, IL-4, IL-10 were found to be reduced.[39] CCL-11 is a selective recruiter of eosinophils and reported to play role in aging associated impairment of both hippocampal neurogenesis

and learning memory. Abundant research study suggests that other than CCL-11, elevated level of c-reactive protein has also been found in sz patients.[40] These C - reactive protein are the acute phase protein which are involved in host cell defence and contributes to blockade of IL-6.

Dysregulation in inflammatory gene expression suggests alteration in T-cell function. T helper cells have been subdivided into two types either Th1 cells which produce markers such as IL-1 and IL-2, whereas Th2 cells which produce markers such as IL-4 and IL-10. These both subtypes counterbalance each other. Leptin is one of the inflammatory cytokine, which is synthesized in the adipocytes that is essential for Th1 and Th2 dependent immune response. The "Th1-Th2 seesaw" balance is shifted towards Th2 side in schizophrenia.[41] The anti-inflammatory cytokine IL-10 is correlated with self referential ToM (Theory of Mind) bias in affected group of patients over healthy group. Only this cytokines correlates with ToM bias in patients, the increased levels of this cytokine in blood may be used as marker for shifting for see saw balance in diseased conditions. This link between IL-10 and ToM bias suggests that immune process may play a modulating role in induction and maintenance of delusions in schizophrenia.

Due to inflammation in periphery and CNS, the kynurenine pathway in brain is activated. In one of its pathway, there is formation of quinilinic acid (QA) from kynurenine in microglia. While in another pathway, there is formation of kynurenic acid (KYNA) from kynurenine in astrocyctes. QA is potent agonist of NMDA receptor and responsible for cell death and excitoxicity, while KYNA is specific antagonist of the NMDA receptor as well as α7 nicotinic receptor. There are reports indicating elevated levels of KYNA in PFC and hippocampus in schizophrenic patients[42] while levels of QA remains unchanged thus providing further evidence for the TH2 shift of the TH1/TH2 seesaw due to oxidative stress and activation of astrocytes.

Plasma ICAM-1(intercellular adhesion molecule -1) levels were decreased during the initial stages of schizophrenia[43] while it increased with course of time indicating over activation of immune system. While no such consistent difference was observed between the levels of VEGF (vascular endothelial growth factor) and VCAM-1(vascular endothelial adhesion molecule -1) in schizophrenic and healthy group of people.

1.5 Neurochemical Bases for Biomarker

The key pathology involved in schizophrenia is hyperdopaminergic activity of D2 receptor but other than dopaminergic, schizophrenia is "multi neurotransmitter" pathologies which are dynamically linked with each other. An imbalance between neurotransmitters leads to various disorders including depression, schizophrenia, anxiety and addiction. Among all of them, the prominent are dopamine, serotonin and glutamate. These neurotransmitters show positive and negative symptoms based on excessive or deficient levels of them.

1.5.1 Dopamine Hypotheses

The first dopamine hypothesis was given in 1967 by Rossum. This hypothesis is base for determining the antipsychotic activity of drugs. The hypothesis suggested the hyperactive dopaminergic transmission in cortical and limbic regions in schizophrenia.[44] This hypothesis is without any articulation about positive or negative symptoms and even did not link the genetic and molecular level changes with the disease. The revised hypothesis was given by Davis in 1991, called as 'modified hypothesis for schizophrenia'.[45] This hypothesis mainly includes region specificity based on post-mortem study in brain of schizophrenia patients, metabolite identification, preclinical study and imaging data. Some data also suggests that decreased metabolites can be found in psychotic patients, this shows contradiction with the previous dopamine hypothesis. This inconsistency leads to attention towards the role of receptor specificity in schizophrenia. D1 receptors are majorly present in cortical area while D2 receptors are present in sub-cortical areas. PET imaging study shows that decreased cerebral blood flow towards prefrontal cortex leads to low CSF dopamine metabolites.[45] Lesions in dopamine neurons lead to increased level of dopamine and D2 receptor activity. This mechanism shows hypodopaminergic state due to frontal region and hyperdopaminergic state due to striatum. Although substantial evidences are available in support of 2nd dopamine hypothesis, but there are several limitations and major one is there is no direct evidence which suggests lower dopaminergic state in frontal cortex in diseased condition. Due to limitations of the second hypothesis, third version of dopamine hypothesis was proposed which further includes 3 hypothesis. This hypothesis is also called as final pathway. First is, dopamine dysregulation is associated with multiple targets not due to single pathway. Second, in this dysregulation, D2 receptor at presynaptic terminal plays a key role. Third, this dopamine dysregulation is associated with all type of psychiatric disorders and is not specific to schizophrenia. Though this hypothesis is known as final pathway, sufficient data is still not available.

Changes in the metabolite levels such as homovanillic acid (HVA) can also act as a biomarker. But, the results were controversial. Elevated level of HVA is associated with schizophrenia patients with suicidal tendency.[46] Whereas low level of HVA is linked with acute sz patients. Further study is needed to confirm its level. Progressive decrement in the level of other metabolite DOPAC (3, 4-dihydroxy phenylacetic acid) can be seen in plasma due to less metabolism of dopamine. The decrease or increases in levels of DOPAC and HVA have shown conflicting results so more study is needed to validate them as biomarkers.

1.5.2 Serotonin Hypothesis

Other than dopamine, serotonin is another neurotransmitter involved in schizophrenia. Firstly it was found that hallucinogens like Lysergic acid derivative and psilocybin produce psychomimetic effect through hyperactivation of 5-HT2a receptor.[47] This study was confirmed by 5-HT2a receptor antagonism through atypical antipsychotic such as clozapine. Some report suggests that schizophrenia patients do not respond to non-clozapine therapy even after high occupancy of D2 receptors[48], which shows that 5-HT2 receptors are as much important as D2 receptor. Various subtypes of 5-HT are involved in schizophrenia. Each of them has different function. Preclinical and clinical data has shown that 5-HT2a receptor antagonism suggests antipsychotic activity and decreases positive symptoms. 5HT1a receptor modulates activity of NMDA receptor present on prefrontal cortex and further leads to improvement in cognitive dysfunction. While 5HT6 receptor antagonist decreased MK-801 induced schizophrenia. Serotonin is produced from tryptophan and in presence of MAO is further converted into 5-hydroxyindol acetic acid (5-HIAA). Similar to dopamine study, there is decrease in level of MAO which further decreases the level of 5-HIAA in the CSF.[49] This study reports has been done in 20th century. Currently, there is no single report available supporting this, so this hypothesis needs further investigation.

1.5.3 Glutamate Hypothesis

Even after 50 years of antipsychotic drug development, there is no single drug which can treat this catastrophic disorder. For the First time, role of glutamate in sz was shown in 1949, where sz patients were treated with glutamatic acid. Based on this data, Olney and farber used NMDA antagonist like phencyclidine, MK-801 and ketamine to induce schizophrenic symptoms in animals models.[50] Similar effect can be observed in humans. NMDA antagonist exaggerates the symptoms of disease in SZ patients for longer time. NMDA receptors are classified into two types according to their location: post synaptic receptors

(mGlut1 and mGLut5) and presynaptic receptors (other than mGlut1 and mGLut5). NMDA receptor antagonist produce reduction in GABAergic interneuron activity and this further leads to disinhibition of pyramidal cell firing. In two clinical studies, reduction in glutamate and NAA(N-acetyl aspartate) level were found in schizophrenic patients[51] which can act as a biomarker. After disease progression, metabolite levels start decreasing due to cortical gray matter losses in the temporal lobes. 1H-MRS study reports found that, not all first episodic sz patients with high glutamate level progress to chronic sz, but 73% patients needed follow-up diagnosis

1.6 Symptomatic Based Biomarker in Schizophrenia

Symptom is a subjective indication that indicates presence of physical or mental disorders. Symptoms based biomarker is low cost and non invasive potential diagnostic biomarker. In this review, we have mentioned the visual impairment, sleep deprivation and facial disturbances as important biomarkers for schizophrenia. The major advantage of symptomatic based biomarker is that it can be easily translated into bench to bedside approach.

1.6.1 Eye

Eye movements play a very important role in neurological disorders. From mid 19th century ocular motor research is mainly concentrated on disorders of cerebellum and brain stem. Recently, eye movements have been assumed as a quantitative marker of cognitive and behaviour neuroscience including reward, attention, planning, prediction, and disorder like autism and schizophrenia.[52] Patients with schizophrenia have several visual abnormalities including eye tracking dysfunction, visual distortions and decreased contrast sensitivity.[53] Eye movement is valuable biomarker in neurological and psychiatric disease progression, aging personality traits and quickly fluctuating state of neurological function and genetic phenotyping. A report suggests that retinal layer changes measurement using spectra domain optical coherence tomography (SD-OCT) may open possible window for its use as a biomarker. The authors have shown that photoreceptor complex thinning particularly of the inner segment layer (ISL) and the outer nuclear layer (ONL) shows distinct association with negative symptoms severity. The pathogenesis of negative symptoms are due to NMDA glutamatergic receptor hypoactivity and this same receptor also mediates glutamatergic photoreceptors bipolar cell pathway therefore photo receptor thinning may suggest NMDA dysfunction.[54] These photoreceptors, rods for night vision and cones for day vision convert

light signals to neural signals. OCT is also useful to assess retina nerve fibre layer thickness (RNFL), macular thickness and macular volume.[55] Retina nerve fibre layer thinning is observed in schizophrenia patients. RNFL is made up of unmyelinated retina cell axons so decrease in this layer thickness reflects axonal loss this can be observed through several neuroimaging studies. To give support of this study, one clinical study showed that out of 30 schizophrenic patients, 62% people were suffering from retinal layer abnormalities (lee, 2013). This inner segment layer thinning may also link mitochondria and further suggests mitochondria abnormality in schizophrenia. These retinal layer abnormalities were studied functionally using flash electroretinogram (ERG). This ERG records electrical potential of the retina in response to light stimulus. ERG wave forms have two major components the 'a' wave, negative deflection which represents hyperpolarisation of photoreceptor and positive 'b' wave represents muller cell activity these are the cells which supports retina of the eye. In schizophrenic patients reduction in both 'a' and 'b' wave can be observed.[56] The visual system is mainly working through two pathways, first is Mangocellular pathway/ M pathway which is stimulated by low contrast images (blurry images) and motion, the second one is P pathway, which gets stimulated by high contrast images (detailed images). P pathway abnormality is observed in schizophrenia patients as well as patients with bipolar and Alzheimer's diseases but M pathway abnormality is specific to schizophrenic patients.[57] In the M pathway, two processes (motion integration and motion discrimination) have been investigated. In motion discrimination test, patients have to identify direction of motion of objects moving at different velocities and in another task they have to detect direction of targeted dots intermixed among distracted dots moving in the opposite direction. Schizophrenia patients show complete impairment in these tasks, which shows impairment in M pathway.

1.6.2 Sleep Deprivation

Another behavioural parameter is sleep. Insomnia reveals mental and physical health problem. This is first symptomatic sign of relapse of schizophrenia. There is relationship between disease severity and sleep alteration like reduced slow wave sleep suggests negative symptoms and rapid eye latency suggests positive symptoms.[58] Electroencephalography suggests that this night deficit in sleep spindles is generated by thalamic reticular nucleus and further modulated by thalamocortical and cortico-thalamic connections.[59] But exact molecular mechanism is yet to be explored. This can only be observed in schizophrenia but not in depression. Sleep deprivation further produces various brain dysfunctions including

task related fronto parietal activation and compensatory activation. In schizophrenia, sleep deprivation is not due to stress, as anxiolytic like lorazepam is ineffective in reversing this. One recent study suggests that even partial sleep deprivation (2-7hr) also leads to change in eye movements which are major symptomatic based biomarker. This has been confirmed by two studies. One clinical study found decreased spindle density and amplitude in early psychosis patients.[60] Whereas in another study, they have used hd-EEG and automated spindle detection algorithm in drug-naive and drug-treated patients. It was shown that early schizophrenia disorders show reduced spindle density mainly in centro-parietal and temporal cluster. First generation antipsychotics like haloperidol have shown less effect on slow wave sleep, but second generation antipsychotics like, clozapine have shown extensive increased in slow wave sleep in patients.[61] Similar results can be observed in quetiapine, aripiprazole and risperidone and olanzapine therapy. An early analysis reveals that out of 12, 83% of early schizophrenic patients and 47% of 17 chronic psychosis patients were suffering from one or another type of sleep disturbances includes difficulty in falling asleep, less sound sleep and early morning awakening. Compared to chronic schizophrenic patients, early schizophrenic patients faces more sleep deprivation, so this can act as potential biomarker. Though there are several advantages of sleep deprivation as biomarker, it has major limitation that high level of heterogeneity exists in sleep architectures.

1.6.3 Facial Expression

Additionally, facial emotion processing deficiency is also used for exploring neurobiology of schizophrenia. In addition to this, it is known that patients have more difficulty in identifying negative facial expression rather than positive. In one clinical study, they have measured three things , relative intellectual intactness, reorganization of six different emotions and degree of identification of facial expressions.[62] And it was they have concluded that there was no significant difference can be found in first task but in the second task identification was found to be slow compared to controls and in third task patients continuously ignored intensity. The ability to identify emotional expression is part of working memory. Due to cognitive dysfunction in schizophrenia patients, it further produces verbal and social impairments. Deficiency in emotion recognition comes under social impairments which is due to changes involved in superior temporal sulcus and limbic area.[63] As compared to control patients, schizophrenic patients can better identify anger expression. Functional magnetic resonance imaging study shows that this benefit is due to activation of globus pallidus. In one another study, they have used EEG for detection of facial affect

discrimination task. In support to this symptomatic behaviour, one another study found that compared to control group, schizophrenic patients (n=15) shows 60% worst facial recognition. Symptom based biomarker is non-invasive technique, but due to less reproducibility it can no longer be recognized as a biomarker.

1.7 Neuroimaging Based Biomarker

Neuroimaging is the type of brain imaging through magnetic resonance imaging or tomography through which images of brain structure can be obtained or brain activity can be measured. It is the best tool for quantification and identification of abnormality involved in different neurodegenerative disorders. If we talk about alteration of brain connectivity in sz, it shows progressive dysregulation. In association with genetic alteration, environmental factors plays most significant role in worsening of disease condition. In comparison of healthy control, sz patients showed remarkably decrease in the functional connectivity in the left fronto-pariental network.[64] This network is mainly associated with cognition, language processing, visual working memory and episodic memory retrival. Particularly, left lingual gyrus in fronto-parieto-occipital network doesn't show much more changes during activation of healthy control(HC) to early illness schizophrenic patients(ESZ), while right superior frontal gyrus in fronto-parieto-occipital network suggests significant alteration during HC to ESZ[65].This progressive dysconnectivity further develops psychosis like symptoms and then completely converted into sz patients. Abnormality in left Heschl's gyrus suggests auditory hallucination. Various fMRI studies illustrated reduced cluster activation in frontal and parietal nodes of functional area. These functional abnormalities can act as an endophenotypic changes, which are easily detectable and changes which further gives idea about inherited vulnaribility. Overall, it can be suggested that compared to control (10.2%) and first degree sz patients (28.1%), chronic sz patients (39.3%) shows more vulnerability.

Several clinical studies suggested that gray matter alterations in prefrontal cortex, temporal cortex, amygdala, parietal cortex and insula during early phase illness may provide useful biomarker in sz patients. This gray matter changes mainly depend on two factors. Grey matter volume (GMV) and cerebral blood flow (CBF). If reduction occurs in both GMV and CBF, it shows functional abnormality whereas normal CBF and decreased GMV suggest structural impairment with preserved function.[66] On the contrary, increased CBF in putamen can be observed in sz patients. This increased CBF activates more DAergic neurons and resulted in hallucinations. Changes in CBF are associated with glucose utilization and oxygen

consumption. Vascular theory of sz postulates that impairment in microvascular system leads to impairment of blood supply and produces metabolic abnormalities. Whereas gray matter volume and thickness mainly depends on allostatic load which is influenced by elevation of immune markers and stress hormones.[67] A meta-analysis report suggests that 66% of first episodic sz patients out of 341 patients showed progressive loss of cortical gray matter. This neuroanatomical changes contribute to decrease in cognitive function.

The major differentiating region involved are default mode network (DMN), central executive network, visual and language network and salience. Anomalous functional connectivity can be observed in sz patients.[68] This DMN network includes medial prefrontal cortex, posterior cingulate cortex, mesial and inferior temporal lobes and adjacent precunueus. This network is more activated during resting condition compared to performing task. Different brain regions affect different functions. Functional connectivity of PCC to right superior gyrus and ACC is correlated with negative symptoms whereas decreased connectivity between PCC and mPFC, parietal regions suggest positive symptoms.[69] Thus DMN acts as a treatment biomarker. Due to novel brain imaging techniques like fMRI, magnetic resonance spectroscopy, flourodeoxyglucose tomography (FDG PET), substantial and robust data can be produced. The major limitation is, this abnormality can not be directly linked with peripheral biomarkers and is costlier compared to metabolomics and symptomatic based biomarker discovery.

1.8 Suggested Combinational Biomarker Approaches for Early Diagnosis of Disease

For any disease, biomarker identification by measuring only single pathological change is not so informative, so combinational approach is required. Schizophrenia is the disease in which pathophysiology is unknown, so fingerprint biomarker identification is hard. From 1967, dopamine hypothesis is the only concluding finding for schizophrenia disease identification. All drugs for the treatment of disease are purely dopamine and serotonin antagonist, though we are not able to prevent the disease. Based on previous research study, here we have given some combinational approaches like brain scan, metabolomics and nonbiological changes by which it may possible to identify. If we will see the pathophysiology of any disease, we can found that oxidative stress is the prime reason behind that, whether it is cancer, diabetes or any neuronal disorders. Same like other disease, in the sz there is also increase in the level of oxidative stress in the brain, this oxidative stress further activates kynurenine pathway and the major metabolite produces from this pathway is kynurenic acid (KYNA) from kynurenine

in astrocyctes. This kynurenic acid is potent antagonist of NMDA receptor. Due to inhibition of NMDA receptor, reduced level of glutamate and N-acetyl aspartate levels can be observed. One MRI study was found that decrease in level of these metabolites further decreases the level of cingulate gyrus, gray matter volume and other brain regions abnormalities.[70] Another reason behind the decreased level of glutamate was ornithine accumulation. In the brain, there is conversion of ornithine from arginine, which further converts into various neurotransmitters. There is less conversion into neurotransmitter and accumulation of ornithine in the brain was found. Another major metabolite was agmatine in the brain, in the sz patients there in increase in conversion from arginine into agmatine. These increase level was identified in the plasma of schizophrenic patients. Further moving towards symptomatic based biomarker, several clinical studies found that in the sz patients, there was thining of photoreceptor complex particularly in inner segment layer (ISL) and the outer nuclear layer (ONL) which was associated with negative symptoms. This is also due to NMDA receptor hypofunction. Other than symptomatic study, genetic study was shown that, there is decrease level of glutamate decarboxylase gene1. The epigenetic change behind this was increase promoter methylation of DNA or we can say hypermethylation. Starting from changes in the neurotransmitter level to epigenetic, if we can combined the diagnosis approaches, it may found novel direction of research with high success rate and less economic burden.

2 CONCLUSION

Biomarker acts as a best theranostics tool. From 1965, there are more than 2000 research articles are available on biomarkers in schizophrenia, but till now no potent biomarker is available because of nonreproducible clinical utility. This review includes some potential biomarkers which are found using modernistic approach. Monitoring neurochemical changes is the most renowned approach in schizophrenia. Currently all treatment for szh is only built upon neurochemical changes and primary one is dopamine. But second line of treatment also involves serotonin antagonism along with dopamine antagonism. Other than these two, glutamate also plays major role in pathology of schizophrenia. Till now, only neurochemical changes propose the disease condition, but recent research commends peripheral changes identification in blood because these directly link with neurotransmitter changes in brain. Decreased levels of HVA and HIAA have been observed in acute sz patients. Whereas on the opposite, level of N-acetyl aspartate was increased.

Another important tool to detect biomarker is "omics" based research whether it is metabolomics or genomics. Most of the diseases are associated with changes in biochemical pathway. Metabolomics could be used to identify pathological mechanism as well as side effects. This includes metabolite identification involved in blood, urine, saliva or changes in brain tissue. Most of the metabolites associated with glucose or lipid metabolism and also includes alteration in enzymes level. In the sz, key metabolite changes are due to oxidative-nitrosative stress. It is due to imbalance between pro-inflammatory and anti-inflammatory mediators. The second part of omics includes genomics. In sz, till now no etiology is known for the disease so genetics play major role in this. Other than genomics, epigenetics also plays promising role which elucidates interaction between genes and environmental factors. Histone modification involves up regulation of DNA acetyltrasferase and down regulation in AcH3K9, while DNA methylation is linked with increased or decreased level of enzyme DNA methyltransferase.

In the research of biomarker discovery, easiest one is symptomatic based research. It is very easy to detect and non-invasive. DSM- guidelines for schizophrenia includes positive, negative and cognitive symptoms but other than that visual impairment, sleep deprivation and low ability of emotion detection processing can also provide strong base for biomarker detection. It is recognised that schizophrenia is multidimensional disorder and no single causes identified for that so biomarker discovery for sz is most difficult task. This review includes some substantial research which can act as a roadmap for finding biomarker which can also be clinically utilizable. Prevention is better than cure, based on this if biomarker identification is possible than we can treat the disease in better way.

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4 Conflict of Interest

The authors have no conflict of interest to declare.

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