Exploring the Theranostic roles of miRNA and Epigenetics in Autoimmune Diseases - A Comprehensive Review

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Abstract

Autoimmune diseases (AD) are severe pathophysiological ailments that are stimulated by an exaggerated immunogenic response towards self-antigens, which can cause systemic or site-specific organ damage. An array of complex genetic and epigenetic facets majorly contributes to the progression of AD, thus providing significant insight into the regulatory mechanism of microRNA (miRNA). miRNAs are short, non-coding RNAs that have been identified as essential contributors to the post-transcriptional regulation of host genome expression and as crucial regulators of a myriad of biological processes such as immune homeostasis, T helper cell differentiation, central and peripheral tolerance, and immune cell development. Pertaining to the differential expression of miRNA attributed in target tissues and cellular bodies of innate and adaptive immunity, a paradigm of scientific expeditions suggests an optimistic correlation between immunogenic dysfunction and miRNA alterations. Therefore, it is not astonishing that dysregulations in miRNA expression patterns are now recognized in a wide spectrum of disorders, establishing themselves as potential biomarkers and therapeutic targets. Owing to its theranostic potencies, miRNA targets have been widely utilized in the development of biosensors and other therapeutic molecules originating from the same. This article tends to deliberate and conceptualize the brief pathogenesis and pertinent epigenetic regulatory mechanism as well as miRNA networks majorly affecting five different ADs namely Rheumatoid Arthritis (RA), Diabetes, Multiple Sclerosis (MS), Systemic Lupus Erythematosus (SLE) and Inflammatory Bowel Disorder (IBD) thereby providing novel theranostic interventions.

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Abstract

Autoimmune diseases (AD) are severe pathophysiological ailments that are stimulated by an exaggerated immunogenic response towards self-antigens, which can cause systemic or site-specific organ damage. An array of complex genetic and epigenetic facets majorly contributes to the progression of AD, thus providing significant insight into the regulatory mechanism of microRNA (miRNA). miRNAs are short, non-coding RNAs that have been identified as essential contributors to the post-transcriptional regulation of host genome expression and as crucial regulators of a myriad of biological processes such as immune homeostasis, T helper cell differentiation, central and peripheral tolerance, and immune cell development. Pertaining to the differential expression of miRNA attributed in target tissues and cellular bodies of innate and adaptive immunity, a paradigm of scientific expeditions suggests an optimistic correlation between immunogenic dysfunction and miRNA alterations. Therefore, it is not astonishing that dysregulations in miRNA expression patterns are now recognized in a wide spectrum of disorders, establishing themselves as potential biomarkers and therapeutic targets. Owing to its theranostic potencies, miRNA targets have been widely utilized in the development of biosensors and other therapeutic molecules originating from the same. This article tends to deliberate and conceptualize the brief pathogenesis and pertinent epigenetic regulatory mechanism as well as miRNA networks majorly affecting five different ADs namely Rheumatoid Arthritis (RA), Diabetes, Multiple Sclerosis (MS), Systemic Lupus Erythematosus (SLE) and Inflammatory Bowel Disorder (IBD) thereby providing novel theranostic interventions.

Keywords - Autoimmune Diseases, miRNA, Epigenetic Regulation, Diagnostics, Therapeutics

Introduction

Autoimmune diseases (ADs) are chronic and progressive ailments designated by an exaggerated self-immunogenic response, accompanied by the overproduction of self-antibodies leading to an overall systemic dysfunction and abnormalities in cellular components. Depending on various biological and physicochemical factors, ADs can bring damage to a particular organ or other biological systems. The interaction of environmental factors and genetic anomalies has a key role in showcasing the pathological effects of ADs . The involvement of B cells in the progression of ADs displays an array of different biological roles. These biological roles mainly include the entrenched secretion of self-antibodies; the presentation of self-antigens and arising complementary interactions with T cells; the release of cytokines involved in the inflammatory response; and the development of deranged specialized microstructure named as germinal centers. With the help of these cellular processes, autoimmune conditions that are often categorized as antibody-mediated or as T cell-mediated, both are considered to be controlled and affected by B cells . The maturation of T-cells in the thymus is responsible for the elimination of a large amount of auto-reactive T cells, but a bulk of T cells that have gained maturity and are able to detect autoantigens can be observed in the peripheral circulatory system of healthy people along with the people suffering from AD. While they appear to be responsible for the pathophysiology of a number of ADs in patients, these auto-reactive cells are maintained in an unresponsive condition in healthy persons. CD4+ CD25+ are considered to be T cells possessing a natural regulatory mechanism and furthermore, it is a population of T cells that have been recently discovered and is regarded to be predominantly responsible for the modulation of the activity of these auto-reactive immune cells . Recent studies suggest that in some types of autoimmunity, the interaction between the environment and the host is influenced by epigenetic alterations induced by various environmental factors, including altered DNA

methylation patterns. Due to environmental factors, it may become difficult for certain cells to maintain epigenetic homeostasis, which can result in loss of tolerance due to abnormal expression of genes. These altered cells can subsequently contribute to the onset of autoimmunity in those with a genetic predisposition . Expression of genes and cellular processes are altered by epigenetic changes, but the genomic sequence remains unaffected. The key epigenetic processes include expression of non-coding RNA, modification of amino termini of histone proteins by post-translational alterations, and CpG DNA dinucleotides methylation and/or their hydroxymethylation. Pathophysiology of ADs has been strongly connected to disease responsible for triggering gene alterations or a combination of genetic vulnerability, and epigenetic changes occurring due to the involvement of various environmental factors. Thus, it is crucial to understand how some ADs are caused by the concoction of genetic as well as epigenetic pathways . A new family of non-coding RNA known as long noncoding RNA (lncRNA) is essential for the control of both autoimmune and immunological processes, whereas, on the other hand, endogenous non-coding RNAs (ncRNAs) known as circular RNAs (circRNAs) showcases itself as the crucial immune system gene modulators and is responsible for the occurrence and progression of ADs . In addition to this, small, conserved non-coding RNA molecules called miRNAs target the 3' untranslated region (UTR) of particular messenger RNAs (mRNAs) and either promote their destruction or suppress translation. Apoptosis, differentiation, cell cycle, and immunological activities are the biological processes that miRNA is known to control. According to recent studies, miRNAs are essential for the regulatory mechanisms of immunological processes and play a key role in preventing ADs . The therapy of ADs has changed little over the past few decades due to advancements in medicine, and the mechanisms behind many of these diseases are still unknown. It's also important to understand how ADs initiate, progress, and end. Owing to its unique regulatory properties and pathogenic contributions, miRNA can legitimately serve as a potential biomarker candidate to efficaciously diagnose the progression of AD. Several daunting attempts were actualised to construct a compendium of biosensors to detect sole pathogenic miRNA candidates participating in AD pathophysiology. Due to the advent of advancing progressions in the domain of material sciences and pharmaceutical interventions, several miRNA encapsulating strategies have been formulated to enhance site-directed specific drug delivery to curb a number of ADs. Altered physiological microenvironment and physical properties are some of the characteristic hallmarks of AD that demand the application of stimuli-responsive drug delivery platforms to cater a stimulus specific to the disease. Understanding the methods through which miRNAs participate in these processes can therefore offer a new window to advance our knowledge of ADs. This article tends to provide insight into miRNA regulation and responsiveness towards the complexities of immunological cascades associated with progressive ADs, pertaining special emphasis on Rheumatoid arthritis (RA), Diabetes, Multiple Sclerosis (MS), Systemic Lupus Erythematosus (SLE) and Inflammatory bowel disorder (IBD), thereby providing optimistic deliberations on novel theragnostic interventions concerning the same. Along the same lines, it also heralds to showcase significant epigenetic modulations for the above-mentioned ADs.

Biogenesis of mi-RNA and its regulatory mechanism on ADs

Small non-coding RNAs (19-21 nucleotides) called miRNAs majorly influences the post-transcriptional regulation of gene expression by either limiting messenger RNA (mRNA) translation or encouraging mRNA degradation. miRNA was first identified in the year 1993 and it remains conserved among a wide variety of species . miRNAs are the major contributing factors in the pathophysiology of multiple diseases including cancer, cardiovascular, metabolic and ADs . Animal miRNAs are encoded as mono-cistronic (individual genes), poly-cistronic (cluster of genes), or introns of host genes (intronic). Primary miRNA (pri-miRNA) transcripts with hairpins and 5' and 3' flanking sequences are produced by RNA polymerase II . As depicted in Figure 1, the processing is carried out mainly by Drosha and Dicer, two members of the RNase III family of enzymes , which work in complexes with dsRNA-binding proteins (dsRBPs), such as DGCR8 and transactivation-responsive RNA-binding protein (TRBP) in mammals, to catalyze the two steps of primary precursor (pre-miRNA) processing in the canonical pathway . The structural properties of individual pri-miRNA sequences influence the effectiveness of pri-miRNA processing. Co-transcriptional processing of pri-miRNAs results in a fast pool of 59-71-nt-long stem-loop pre-miRNAs. Exportin-5, a member of the karyopherin protein family, exports nascent pre-miRNAs to the cytoplasm in a GTP-dependent manner .

Once in the cytoplasm, the pre-miRNA is integrated into the RISC Loading Complex (RLC), where it is processed into a 21-nt-long miRNA/miRNA* duplex by the type III ribonuclease Dicer . Up to one-third of human mRNAs may be miRNA targets, and miRNA-mediated gene regulation is essential for normal physiological processes including the cell cycle, differentiation, and death. miRNAs are essential for the control of immunological processes and the avoidance of AD, as stated by the recent research . There are various checkpoints that guarantee the deletion or silencing of autoreactive T and B lymphocytes, which are produced regularly and randomly throughout lymphomagenesis. But occasionally, self-reactive lymphocytes manage to get past the checkpoints and continue to live in peripheral lymphoid tissues. When these autoreactive cells are triggered, they launch a vicious assault against self-tissues that trigger ADs . miRNAs control autoimmunity by influencing the formation, differentiation, and function of many cell types, including innate immune cells (innate immunity), adaptive immune cells (adaptive immunity), and local resident cells . Toll-like receptors (TLRs), C-type lectin-like receptors (CLRs), nucleotide-binding oligomerization domain (NOD)-like receptors, retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), are all expressed by host cells. These receptors are capable of recognizing a wide range of pathogen-associated molecular patterns (PAMPs) . These processes activate intracellular signaling pathways, resulting in the release of proinflammatory cytokines, chemokines, and interferons (IFNs) as well as the production of co-stimulatory molecules. Several investigations have demonstrated that miRNAs play critical roles in the biological processes of these adaptive immune cells in autoimmunity, miRNAs also alter/regulate a particular subgroup of T cells called regulatory T cells (Tregs) are essential for regulating the immune response, which finally results in the upkeep of self-tolerance and homeostasis . According to Husakova et al., (2016), miRNAs have an impact on the development of CD8+ T cells, Th1 cells, Th2 cells, and Thymus by affecting the levels of miRNA-155, miRNA-147, and miRNA-146a. The in-vivo application of the major miRNAs are already known. The multi-modal applications of miRNA delves into the conceptualization and understanding of various novel developmental strategies for the treatment and prevention of ADs

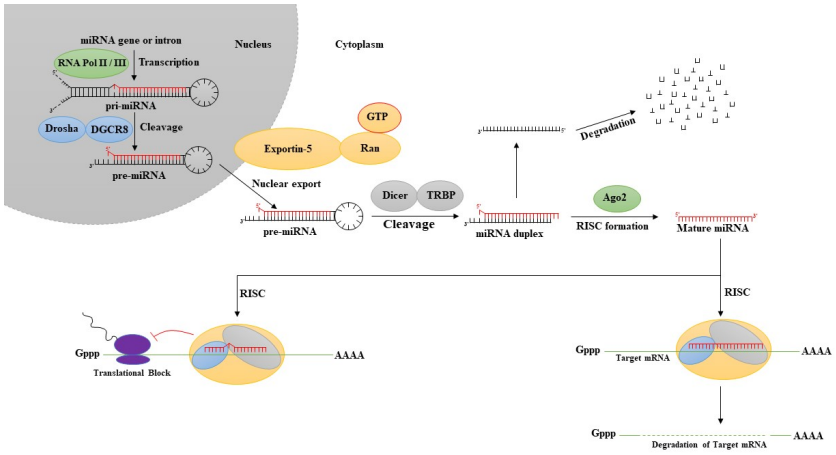

Figure 1 - *Biogenesis of miRNA. Initial steps include the formation of pre-miRNA followed by nuclear export and subsequent cleavage to form matured miRNA and further the fate of the matured miRNA is decided by the RISC complex attachment*

miRNA in the progression of ADs

miRNAs have always been considered as major contributors to the regular immune system functioning, immunological tolerance pathways and autoimmunity. Recent research from clinical investigations and animal models indicates that miRNAs play a role in the etiology of various ADs. A number of ADs in humans have a significant association between their progression and the abnormal miRNA expression . In peripheral and

central lymphoid organs, miRNAs function at a number of checkpoints to sustain immunological tolerance and combat ADs.**Figure 2 -** *Overview of role of miRNA involved in the disease pathogenesis, biomarker detection and development of miRNA-based therapeutics for different ADs*Latest evidence has now established specific miRNAs that regulate the lineage specificity and effector potentials of T helper subsets to promote immunological homeostasis .

Table 1 - *Different ADs and their respective miRNAs associated with disease pathogenesis*

Autoimmune Disease	**miRNA**	**Regulation**	**Mechanism**	**Reference**
Rheumatoid Arthritis	miRNA-126-3p, let-7d-5p, miRNA-431-3p, miRNA-221-3p, miRNA-24-3p, miRNA-130a-3p, miRNA-339-5p, let-7i-5p, miRNA-486-5p	Upregulated	Promotes apoptosis and proinflammatory markers Anti-proliferative effect and increases the apoptotic death of FLSs Maintains homeostasis and associated with cartilage development pathways	
	miRNA-320a, miRNA-132, miRNA-363, miRNA-498a, miRNA-124a, miRNA-140, let-7a, miR-204-5p	Downregulated		
Diabetes	miRNA-375, miRNA-29a, miRNA-29b, miRNA-200, miRNA-7, miRNA-140-3p, miRNA-574-3p, miRNA-139-5p, miRNA-106a, miRNA-17, miRNA-486-3p, miRNA16, miRNA-222, miRNA-885-5p, miRNA-197	Upregulated	Malabsorption of glucose and insulin resistance Maintains glucose homeostasis Beta cell differentiation and proliferation, regulates apoptosis Controls glucose and lipid metabolism	
	miRNA-155, miRNA-92a, miRNA-126, miRNA-34a, miRNA-214-3p miRNA-27-3p	Downregulated		

Multiple Sclerosis	miRNA-155, miRNA-146a/b, miRNA-214, miRNA-23a, miRNA-219, miRNA-338, miRNA-128, miRNA-27b, miRNA-340, miRNA-29b, miRNA-326, miRNA-301a, miRNA-17-5p	Upregulated	miRNA expression altered in CNS lesions and in the immune system, which effects gene expression and promotes the disease Contribute towards Th-17 differentiation and polarization
	miRNA-219, miRNA-338, miRNA-23b, miRNA-25, miRNA-15a, miRNA-16-1 miRNA-17-92 cluster	Downregulated	
Systemic Lupus Erythematosus	miRNA-155, miRNA-15a, miRNA-124-3p, miRNA-377-3p, miRNA-21, miRNA-7, miRNA-34a, miRNA-148a, miRNA-126,	Upregulated	Involved in Apoptotic pathway Triggers inflammation, exaggerated immunogenic response and pathogenesis
	miRNA-17, miRNA-142-3p, miRNA-146a, miRNA-125b	Downregulated	
Inflammatory Bowel Disorder	miRNA-101, miRNA-515-5p, miRNA-623, miRNA-325, miRNA-876-5, miRNA-1224-5p, miRNA-1226-5p, miRNA-1253, miRNA-455, miRNA-20a, miRNA-17-5p, miRNA-424, miRNA-16-5p, miRNA-21-5p	Upregulated	Serve as biomarkers for disease diagnosis Enhances paracellular permeability of the intestinal epithelium Boosts zonulin expression and promotes epithelial permeability Reduces the expression of aquaporin 3, resulting in a weakening of the intestinal barrier

miRNA-24, miRNA-107, miRNA-10a, miRNA-223, miRNA-9, miRNA-21, miRNA-874, miRNA-150, miRNA-125b, miRNA-17-92,	Downregulated

3.1. Rheumatoid Arthritis RA is a heterogeneous, enfeebling, systemic AD. This chronic ailment pertaining to musculoskeletal pain, prolonged morning stiffness, inflammatory alterations of the tissues of synovial joints, cartilage and bones, often resulting in erosion of bones and subsequently, complete destruction of joints, if not treated on time . The internal extra-articular organs like skin, eyes, lungs, heart, kidney, and blood vessels are also affected by this symmetrical AD . People afflicted with RA range from 0.24% to 1% globally . It is predominant in women compared to men and has increased prevalence in the age group of 50-65 years . The emergence of non-communicable diseases have become a leading cause of mortality worldwide and RA is one of them. Both developed and low-middle income countries (LMIC) are severely affected by these diseases . RA being a complex AD, the pathophysiology is regulated by several inflammatory markers and immunogenic proteins. B lymphocytes, T lymphocytes and macrophages predominantly contribute to the progression of the disease . These autoreactive B cells induce the secretion of cytokines like tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-12, IL-23, and IL-1α in the diseased tissue. Another cytokine, receptor activator of nuclear factor ϰB ligand (RANKL) has been found to show enhanced production from memory B lymphocytes in peripheral blood, synovial fluid and affected tissue of patients. This cytokine is responsible for bone resorption as it binds to its receptor, stimulates differentiation and activation of osteoclasts . B lymphocytes are also responsible for the secretion of rheumatoid factors (RF) and anti-citrullinated protein antibodies (ACPA) .**Figure 3 -** *Detailed immune regulation and miRNA networks involved in RA proliferation. Illustrative representation of multifaceted roles of mast cells secreting several proinflammatory interleukins like IL-6, IL-1 and tumor necrosis factors leading to osteoclast maturation and subsequent bone degradation*The activation of T lymphocytes are stimulated by both B cells and macrophages. Synovial T cells have been reported to secrete cytokines like, IL-15, IL-7, IL-13, IL-4, IL-2, basic fibroblast growth factor, epidermal growth factor and these cytokines have a major contribution in the etiology and pathogenesis of the ailment . Macrophages, in the early stages of the disease, are known to stimulate T cells and enhance the production of IL-1α, IL-1β, and Matrix metalloproteinases (MMPs) . The inflammatory mediators secreted by the autoreactive immune cells interact with neurons, and neuroglia and result in neurogenic inflammation . RA patients are often broadly classified into ACPA-positive and ACPA-negative. These two patient types have very different pathophysiology, ACPA-positive being a more clinically aggressive-phenotype. Citrullination is categorised as a post-translational modification which results in the synthesis of a polar (neutral) citrulline from a positively charged arginine and is also aided by Ca2+-requiring enzyme peptidyl arginine-deiminase (PAD). The genetic and epigenetic factors interact and trigger the production of ACPA in diseased individuals. Environmental factors like smoking, inhalation of nanosized silica or silica dust may trigger mucosal-TLRs which activates Ca2+-dependent PADs and also antigen presenting cells (APCs) like dendritic cells and B lymphocytes . miRNA-117-5p expression is seen to be reduced in chronic RA. This miRNA specifically targets the JAK-STAT pathway and downregulates the IL6 expression. Similarly, several miRNAs acts as a biomarker and can be detected from the synovial fluid or tissue in RA patients. miRNA-34a is regulated by NF-kB family of cell signaling molecules, which are further activated by inflammatory cytokine molecules . miRNA-29a acts as an anti-inflammatory marker in RA fibroblast-like synoviocytes (RA-FLSs). The drop in miR-199a-3p expression contributes to local hyperplasia. miRNA-375 in RA-FLSs exhibits a positive effect on synovial pathogenesis that occurred as a result of canonical Wnt signaling inactivation, thereby influencing the progression of the disease and severity

of the condition . Exosome secreted miRNAs are important biomarkers and are vital targets for developing therapies to treat RA. According to a study by Cunningham et al. eight serum miRNAs viz., miRNA-126-3p, let-7d-5p, miRNA-431-3p, miRNA-221-3p, miRNA-24-3p, miRNA-130a-3p, miRNA-339-5p, let-7i-5p are found to have enhanced expression in RA afflicted individuals compared to that of healthy people . Exosome derived miRNA-486-5p from synovial tissue is associated with tumor initiation or suppression in a number of cancers and also pulmonary fibrosis. miRNA-486-5p secreted from the exosomes of RA Fibroblast-like synoviocytes (RA-FLS-Exo) has been reported to promote osteoblast differentiation in RA patients by decreasing the expression level of Tob1 gene and triggering the bone morphogenetic protein (BMP)/Smad pathway . Fibroblast-like synoviocytes are known to be responsible for cartilage destruction and maintenance and progression of inflammation in RA patients. Mesenchymal stem cell (MSC)-derived exosomes are evident to exhibit immunosuppressive effects like suppressing B and T lymphocytes. miRNA-320a is found to have very low expression levels in RA and is known to have anti-proliferative effect and increases the apoptotic death of FLSs by downregulating MAPK/ERK1/2 signaling pathway . There are $CD^{4+}CD^{25+}Foxp^{3+}$ T cells also called regulatory T lymphocytes or Treg cells. These cells are known to inhibit the autoimmune response found in RA. Patients non-respondent to disease-modifying anti-rheumatic drugs (DMARDs, or Methotrexate) are often treated with biological DMARDs or immunotherapy. Increasing the number of Treg cells has demonstrated a reduction in the pro-inflammatory responses. Stimulation of Treg cell-specific targeted gene proliferation induced more and more production of Treg cell which slowed the disease progression and proved itself to be an important treatment for ADs .**3.2. Diabetes** Diabetes is a complicated and persistent clinical condition requiring continuous biomedical support and preventive measures which is just not concerned about glucose management . Diabetes may be classified by three conditions namely, Type 1 Diabetes (T1D) and Ttype 2 Diabetes (T2D) and gestational diabetes . T1D out of the above is concerned with an autoimmune abnormality wherein the β-cells producing Insulin in the pancreas are destroyed . Relative insulin deficiency in T2D is predominantly impaired by β-cell activity of the pancreas and resistance to the action of insulin in specific organs . The occurrence of diabetes in adults varying in the age group of 21 to 80 over the world was predicted to shoot upto 10.6% (536.7 million) in 2021 and 12.3% (783.3 million) in 2045 . Small endogenous miRNAs are predisposed to control cellular cycles, proliferation, differentiation, and apoptosis by inhibiting the biogenic molecules that control these processes, such as RNA transcripts, or by degrading mRNA . According to various research groups, miRNA-375 was found to be upregulated, impacting the beta cells that secrete insulin along with other miRNA molecules such as miRNA-29a, miRNA-29b, miRNA-200, and miRNA-7 . A cross-sectional nested, case-control based research study was carried out by Barutta et al. to look at the differential expression of miRNAs in the blood samples of patients suffering from T1D.. A considerable upregulation of the miRNAs miRNA-140-3p, miRNA-574-3p, miRNA-139-5p, miRNA-106a, miRNA-17, miRNA-486-3p, miRNA16, miRNA-222, and miRNA-885-5p was found . Children with new-onset T1D were found to have blood samples significantly overexpressed miRNA-197. It was also claimed that miRNA-197 can accurately predict residual beta-cell functioning . miRNA-155, miRNA-92a, and miRNA-126 were all noticeably downregulated in the blood samples of T1D patients . Additionally, diabetes is frequently characterized by endothelial homeostasis, which is maintained by miRNA-126. Additionally, miRNA-126 regulates endothelial inflammation in individuals with micro/macrovascular problems related to diabetes, establishing a connection between these issues and reduced levels of miRNA-126 . Patients suffering from T2D had shown greater levels of miRNA-29a in their blood. It was determined that miRNA-29a overexpression inhibits glucose absorption by stimulating insulin, eventually leading to insulin resistance. Another study involving plasma samples from T2D patients to look into the dysregulation of miRNA-34a found that miRNA-34a was highly elevated and connected to senescence or death in pancreatic cells with a decline in SIRT1 activity. miRNA-130b was shown to be severely reduced in the blood samples that were collected from the affected individuals along with high urine albumin-to-creatinine ratios (UACR) in a research that included 327 T2D patients with different levels of UACR . Additionally, it was shown that blood levels of miRNA-130b had a bad correlation with HbA1c and HIF1- levels. Furthermore, the serum miRNA-130b level was closely correlated with insulin resistance and blood glucose levels in T2DM. Evidences suggest that miRNA-214-3p and miRNA-27-3p might serve as potential T2D biomarkers comes from reports of their downregulation in whole-blood samples from T2D patients as well as in a population

with T2D risk factors . miRNA-26a in pancreatic beta cells has successfully reduced Type 2 Diabetes by increasing peripheral insulin sensitivity and maintaining beta cell activity . An alteration to the daily routine, furthermore following a holistic diet, biomedical or surgical therapeutic procedures, or considering a combination of these methods can lead to diabetes remission. Lifestyle changes that affect daily activities linked to nutrition and exercise have health impacts that go well beyond those that are specifically relevant to diabetes .**3.3. Multiple Sclerosis** MS is categorized as an auto-immunogenic inflammatory disease inducing persistent demyelination in the central nervous system (CNS). According to a study conducted between September 2019 and March 2020, it was statistically estimated that 2.8 million individuals across the globe suffer from MS approximately 35.9 per 100,000 population . In younger individuals, MS is the predominant contributor to atraumatic neurological impairment . The fundamental clinical features of MS include several foci which are found to be dispersed across the white matter which reverts back throughout the illness's progression time and again . The etiological factors that lead to the onset of MS include T-cell-mediated immune responses that trigger cytokine release , alter the barrier permeability between the brain and cerebrospinal fluid , axonal inflammation, gradual demyelination and a rise in the pro-inflammatory miRNAs and its relevant hallmarks. Macrophages, followed by CD8+ T-cells are significantly observed in the influx, along with a lesser number of CD4+ T cells, B cells, and plasma cells. Also, the lymphocytes that majorly contribute to the progression of MS are Th1 and Th17 . Diverse genetic, epigenetic, microbiological, and ecological variables aid the pathogenesis of MS. miRNAs are quintessential epigenetic phenomena that regulate abnormal cellular events during the prognosis of the disease. The key purpose of miRNA is to monitor and control the translation of genes, either by suppressing translation or by cleaving the target mRNA. In MS, miRNA expression profiles are altered in CNS lesions as well as in the immune system, which has significant implications on gene expression in the array of cell types and ultimately promotes the disease . In the study by Baulina et al., a range of miRNAs having altered expression during the disease prognosis from varied sources were outlined that including miRNA-155, miRNA-146a, miRNA-181c, miRNA-326, miRNA-346, miRNA-17, miRNA-320a, miRNA-34a, miRNA-340, miRNA-132, and their target sequences . Based on multiple data identified, miRNA-155 is regarded as the most potent promoter of inflammation and is crucial for the pathophysiology of the disease, by virtue of myeloid cell polarization to a morphological and active pro-inflammatory type. Upregulation of miRNA-155 has been detected in the cellular blood samples of individuals affected by MS which may be a sign of a severe disease progression. miRNA-155 modulates the MS risk genes PIK3R1 and PIK3CA which encodes for proteins belonging to the phosphoinositide 3-kinase (PI3K) family. Dysfunction of the PI3K family leads to oncogenesis, neurological and immune disorders, and demyelination in MS . Dysregulation of miRNA-3606-3p in patients of systemic sclerosis is a prominent biomarker and can lead to the reduction of TGFBR2 expression . This can lead to the severity of the diseased condition. However, an upsurge in miRNAs like miRNA-126 and miRNA-139-5p in systemic sclerosis have found a correlation to the inflammatory cytokines and signaling molecules like IFN-B . With reference to a study on miRNAs to ascertain their correlation with gadolinium-enhancing (Gd+) lesions for evaluating their utility as MS activity biomarkers, it was observed that peripheral blood mononuclear cells (PBMCs) of MS patients had overexpression of miRNA-146a/b in comparison to the control. They hypothesized that this elevation was particular to the critical stage of MS and contributed to the proliferation of Th cells, which are effective in the aggravating mechanisms that occur in the CNS of MS patients. First, activated T cells traverse through the blood-brain barrier from the periphery into the CNS. Cytotoxic CD8+ T cells can directly cause axonal damage, while CD4+ T cells aid in retaining the CNS inflamed. Furthermore, regulatory T cells (T reg), that sustain immune tolerance by inhibiting effector T cells, carry out this control through the secretion of miRNA-based exosomes, a route for gene silencing . miRNA-20a-5p and miRNA-20b-5p were shown to be key regulators of 1,000 targeted genes according to database analysis of miRNA-mRNA interactions 46, indicating that they may be key players in MS pathogenesis . By analyzing the association of the expression of miRNAs and its potential target genes, the involvement of miRNAs in the disease prognosis has been established. Nevertheless, the exact functioning of miRNA in the pathogenesis of MS is not well identified. New medications and therapeutic modalities that effects miRNAs will undoubtedly emerge with the development and use of new advances in research, providing the groundwork for the eventual eradication of different immunogenic disorders.**3.4. Systemic lupus erythematosus** SLE is identified as a persistent

inflammation associated with auto-immunogenic ailment, caused by the progressive decline of resistance to the self–antigens, stimulation of dysfunctional immune T cells and B cells, synthesis of autoantibodies (auto-Abs), and altered cytokine activity. Due to the advent of complex molecular associations between epigenetic triggers, unbalanced hormone levels, genetic susceptibility, epigenetic control, immune status, and other unknown factors, SLE is strongly related to the downregulation of the immunological responses both innate and adaptive . miRNAs are significant regulators of ADs, where miRNA-146a and miRNA-155 are demonstrated to be crucial in the pathogenesis of SLE. miRNA-146a was found to be adversely correlated with the expression of the type I IFN regulatory signaling system. The toll-like receptor (TLR)-myeloid differentiation factor 88 (MyD88) pathway, which includes IRAK1 and TRAF6, was proven to be a critical regulator of signaling pathways . miRNA-155 is verified to upregulate the alteration of Treg phenotype in MRL/lpr mice by modifying the CD62L expression . miRNA-17 is associated with the production, division and activation of immune B cells, T helper cells namely Th1, Th2, Th17 . In a study conducted by Kaga et al., miRNA-17 production was found to be more in the healthy control as compared to that of the SLE patients and it was also observed that miRNA-17 has an antagonistic relation with the interferon alpha mRNA that may play a role in the etiology of the disease . miRNA-142-3p was discovered to be considerably downregulated in SLE patients. miRNA-142-3p, which is mostly produced in hematopoietic stem cells, has been identified to be crucial for the immune response, particularly in macrophages and Regulatory T cells . miRNA-20a downregulation was linked to lupus nephritis and vascular thrombosis . miRNA-125a comprises the inflammatory chemokine pathway. Recent studies have demonstrated that miRNA-125a targets KLF13 in SLE, which results in increased production of the inflammatory chemokine RANTES . miRNA-15a specifically has a deleterious impact on the B-10 subpopulation, and miRNA-15a reduction may help treat SLE . In a scientific study designed by Yan et al., miRNA-124-3p and miRNA-377-3p were highly expressed in PBMCs and serum collected from patients affected by SLE in comparison to the healthy controls . miRNA-21 is upregulated due to the hyperactivity of immune T cells whereas miRNA-7 is related to the overproduction of B cells and autoantibodies. miRNA-34a is responsible for the collapsing of the immunological tolerance .**3.5. Inflammatory bowel disorder** The term "IBD" corresponds to a group of relapsing, chronically inflammatory gastrointestinal illnesses, notably Crohn's Disease and Ulcerative Colitis . Over the past decade, as lifestyles and dietary practices became more westernized, IBDs expanded around the world . According to experimental data, excessive consumption of certain macronutrients in the modern food habit triggers an inflammatory response in colon by preying on innate immune sensors and disrupting the metabolism of gut microbes. Even though incidence is stabilizing in western nations, the burden is still significant since prevalence exceeds 0.3%. These findings underline the need for research into IBD prevention and advances in healthcare systems to manage this difficult and expensive condition . Immunometabolism regulates vulnerability to intestinal inflammation, and the risk IBD genetically. This partially influences metabolism and stress related signaling of innate immunity . Some micro-RNAs, including miRNA-101, miRNA-515-5p, miRNA-623, miRNA-325, miRNA-876-5p, miRNA-1224-5p, miRNA-1226-5p, and miRNA-1253, have been found to invade bacterial membrane and subsequent regulate gene transcription. This promotes bacterial proliferation and mobility, which in turn affects the population and diversity of the gut microorganisms . By reducing the over-expression of inflammatory cytokine receptors like IL7R and IL17RA as well as signal proteins like GP130, miRNA-31 reduced the inflammatory response in a mouse model of Dextran Sodium Sulfate -induced colitisl, which Tian et al., 2019 found to be hyper-expressed in tissues from patients suffering from IBD. Another study using the DSS mouse model revealed that miRNA-155 binds to SHIP-1 mRNA directly, resulting in a considerable drop in SHIP-1 levels, which control cell membrane trafficking . The most often researched miRNAs in relation to IBD appear to be miRNA-21, miRNA-155, and miRNA-31 . With links between miRNA-21 and IBD being reproduced in numerous research and functional relevance being shown in mice models of IBD, miRNA-21 is arguably considered to be prevalent in IBD. miRNA223 regulates innate immunity in intestinal inflammation . miRNA 214-3p and miRNA 206 activate the NF-kB pathway and promote intestinal inflammation . Some members of the regenerative gene (REG) family, I, including (REG Iα, REG Iβ and REG IV) are expressed in Crohn's disease and ulcerative colitis and have a role in proliferative mucosal components in IBD. Through the downregulation of miRNA-24, LPS caused REG IV expression in human intestinal epithelial cells. Intestinal epithelial cells' RAGE/TLR4 receptors

controlled the LPS signal. Damaged intestinal cells were replaced under IBD circumstances by intestinal cell growth. Since REG IV is a growth factor for intestinal epithelial cells, it could help to multiply intestinal cells to restore intestinal mucosa .

Epigenetic networks underlying AD progression

Epigenetic parameters have a great influence on cell signaling, differentiation, gene expression and morphogenesis of cellular development in an organism. Hence, they have an essential role to play in the onset of a disorder and its related genetic background . There are various epigenetic factors which are common in various disorders, due to their long lasting effect on the nature and progression of the genetic regulation in a diseased condition. The mechanisms like DNA methylation and modification of histone proteins can contribute to the pathogenesis of various ADs . These epigenetic parameters can correspond to the initiation and prolongation of inflammation inADs. It has been observed in various studies that miRNAs play a centric role as epigenetic regulators and prevent the progress of inflammation . DNA methylation is a process in which the methyl group is removed from the methyl cytosine structures, which results in the loss of structural and functional entities in various metabolic pathways. Ten-eleven Translocation (TET) enzymes act as DNA methylases and take part in the process of hypo or hyper methylation . Histone modifications act as major epigenetic markers. They are involved in transcriptional upregulation or downregulation, chromosomal compaction and DNA repair techniques. One of the major histone modifying enzyme inhibitors, is histone deacetylase inhibitor (HDACi) which is involved in the changes that contribute to the growth and advancement of ADs . The interplay and corresponding effects of the epigenetic regulation of miRNA is an area of ongoing research. Epigenetic therapies is a new area of clinical advancement that helps in the reversal of epigenetic alteration or aberrations . Corresponding researches are being done in the line of the development of epigenetic modifying drugs in conjugation with adjuvant therapy. This can be used in T cell polarization and control of the inflammatory processes.**4.1. Rheumatoid Arthritis**RA is a disease caused by loss of autoimmunity, that encompasses synovial fluid hyperplasia and inflammatory joint degradation . The inflammation in RA is mediated by several factors like ACPAs which further result in structural damage of the bone joints . The dynamic expression of the diseased condition in the patients is due to the epigenetic regulation of the genes directly associated with the immune system. The synovial fibroblasts consist of a unique methylation pattern, which varies during the course of gene progression and consequently affects its severity . L1 (LINE) is hypomethylated and indirectly induces the surge of cytokine, growth factors, receptor and inflammatory co factors . Local hyperinflammation spike is noticed in the RA patients due to the hypomethylation of the CpG islands associated with the IL6 promote gene. This leads to the overexpression of IL6 and related proinflammatory cytokines . Recent research has verified that epigenetically regulated gene networks in the peripheral blood mononuclear cells consequently result in the severity regulation of RA . The synovial cells modify themselves in such a way that they start to show tolerance toward the apoptosis. This in turn triggers the inflammatory cascades in the pathogenesis pathway of RA development. This is majorly induced by the histone modification and hypermethylation of the NFkB promoter gene . The histone modifications like acetylation, methylation, citrullination, phosphorylation and ubiquitination contribute to the changes in the transcriptional factors, making the chromatin available for gene expression. The different histones that take part in the RA pathogenesis and histone modification are - H3K9, H3K14, H4K5, and H4K16 (for acetylation), H2BK5, H3K4, H3K36, and H3K79 (for methylation), H3S10 and H3S28, H4S1 (for phosphorylation) and H2BK120 (for ubiquitination) . These developments can in turn be used as biomarkers and contribute to the therapeutic application of this disease.**4.2. Diabetes**Autoimmune dysfunction of pancreatic β cells leads to the development of type I diabetes. Its progression is monitored on a wider scale as it is widely affected by the epigenetic regulation of the various related genes . Glucose and insulin levels in the blood, affect the degree of methylation. They regulate the homocysteine metabolism, which further modulates the DNA methylation by DNMTs . These epigenetic factors contribute in the glucose intolerance, due to the alteration in the methionine metabolism. The immunogenic response is seen to be related to the DNA methylation of several regulator genes. FOXP3 promoter region of the CD4+ is hypermethylated in the case of latent autoimmune diabetes in adults (LADA) patients . The INS gene promoter in diabetic patients is seen to be hypomethylated, which has consequently culminated in the release

of inflammatory cytokines like TNF-β , IFNγ, IL6 and IL-1B . The histone modifications are mainly regulated by histone-acetyltransferases (HATs) and histone-deacetylases (HDACs), which regulate the process as transcription coactivators. RNA modification and ncRNA regulation are also noticed in the aged group of diabetic patients, which also attributes in cellular senescence and post transcriptional modification . Miao et al have documented the histone modification of H3K9Ac, H4K16Ac, H3K4me3, H3K9me2,3, H3K27me3 genes in the diabetic pathway . As these genes are closely located to the DQB1 and DRB1 genes. This leads to a surge in the transcription process in the monocytic cell line, thereby triggering the immunogenic pathway . One of the most significant autoimmune markers of the progression type I diabetic condition is the reduced expression of the H3 histone acetylation that develops a consequent the expression of GAD autoantibodies .**4.3. Sys**MS is a neurodegenerative, inflammatory disease that results in physical and cognitive impairment. The pathogenesis primarily roots from the self intolerance towards the neuronal antigens. Most patients develop relapsing-remitting MS (RRMS) and others develop primary progressive MS (PPMS). In case of PPMS, DNA methylation of the primary epigenetic regulation that controls the progression of MS. Studies are being conducted to identify and analyze the differentially methylated CpG sites of CD4+ T lymphocytes genome . The progression and mortality of the disease depends upon the interaction and interplay of the genetic and environmental factors, combined . The T cell exhibits autoreactivity that causes demyelination which consequently leads to an inflammatory cascade in the central nervous system. Studies have shown that the hypomethylation of promoter region peptidyl arginine deaminase (PAD) -II contributes in the citrullination of myelin basic protein (MBP) . This may lead to irreversible molecular changes that can cause insatiable and chronic inflammation. *ATXN1* gene which encodes for polyglutamine protein ataxin-1, which in turn protects the system from the demyelination event. Studies have shown that in the event of hypomyelination of *ATXN1* gene, the pathogenetic mechanism scales up in the cellular and molecular regulation of MS . Thelper (Th) 17 and T regulatory (Treg) molecular balance has an important role to play in the progression and dynamics of MS, due to their epigenetic dynamic nature. Histone modification of forkhead Box P3 (FOXP3)- cell-type-specific regulatory regions (CSRs) and RAR related orphan receptor C (RORC)-CSRs are polarized Th17 cells are regulated by estrogen in pregnant women suffering with MS, during their third trimester. A recent study shows the correlation of epigenetics and disease progression via epigenomic and transcriptomic profiling, where they have tried to compare demyelinated MS lesions and normal-appearing white matter (NAWM). Human-iPSC-derived oligodendrocytes were epigenetically edited to understand the region-dependent hypermethylation of MBP which is responsible for the myelination and axonal development . During the pathogenesis of MS, antibodies are formed against different histone proteins like H2b, H1, H3, H4, MBP, and DNA, that result in the hydrolysis of H2A histone. This cross reactivity between the abzymes and histone proteins can result in the aggravation of the disease condition . Pedre et al. reported the increased expression of H3 histone acetylation in chronic MS patients, which has also been seen as a side effect to in turn increase the transcription pattern of the inhibitors of oligodendrocytes and histone acetyltransferase (HAT) gene expression in MS patients . The acetylation and deacetylation balance is hindered during the early phase of MS progression, which can further modulate that severity of the disease .**4.4. Systemic Lupus Erythematosus**Autoantibodies and dysfunctional antigen presenting cells are the classical characteristics of SLE. The pathogenetic pathway of SLE is still a matter of debate, and has been seen to be largely affected by the epigenetic and environmental regulation over the patient's immune system . The large network in the immune system contributes to lupus pathogenesis. Regulatory factor X (RFX) 1 acts as an immune-suppressor and aggrevates the DNA methylation of the promoter region of CD70 and CD11a . This promotes autoimmune response . According to Hedrich et al, CpG-DNA methylation patterns are highly conserved and close to the promoter region of IL7F. It is observed that the SLE patients show low degree of methylation in the T- lymphocytes, as drawn parallels with the study on healthy individuals . In other recent findings, it has been seen that there is an increment in the levels of 5-hydroxymethylxytosine (5-hmC). This is due to the upregulation of ten eleven translocation (TET)-2 and TET-3 factors, which are one of the prominent DNA methylases and contribute in the enzymatic conversion of 5-methylcytosine (5-mc) into 5-hmC . In several SLE patients, DNA hydroxymethylation pattern is observed in the signaling pathway genes, which are then consequently related to the immune response genes and factors like SOCS1, NRF2F6 and IL15RA. The methylation product - 5-hmC and its aberrant regulation may contribute in the

therapeutic application of SLE . The Th17 cell maturation takes place through the epigenetic modification if the transcriptional factors associated with it, in the SLE patients. The STAT3 pathway plays a pathbreaking role in the modification and modulation in T cell maturation and progression of SLE pathogenesis . Histone modifications in the SLE is reported in the form of trimethylation of histone H3 in H3K27me3, which contributes to the increase of H3K27me3 in the CD4+ T cells of the SLE patients. This trimethylation is made possible with the help of enhancer of zeste 2 polycomb repressive complex 2 subunit (Ezh2). The cumulative effect of these histone modifications may lead to the t cell lineage development in the SLE patients, which may attribute to the pathogenesis of the disease . Recent research and studies have shown and possibility of 3D genome alteration with respect to the progression of disease. The study included testing the viability of cells when subjected to histone modification of H3K27ac, H3K4me1, H3K4me3, SP11 knockdown and transcription factor motif enhancement. These studies indicate the indirect effect of epigenetic regulation over the genome structure and function .**4.5. Inflammatory Bowel Disease**IBD is an autoimmune, chronic, recurrent gastrointestinal disorder. Several factors like environmental factors, gut microbiota, immune dysregulation contribute to the advancement and cause of this disorder. Pathogenesis of this disease can not be clearly elucidated as it can be manifested into colorectal cancer, fistula development or stenosis syndrome . The IBD has two twin forms- ulcerative colitis (UC) and Crohn's disease (CD). IBD related genes undergo methylation and histone modification for reshaping the disease progression . Mucosal methylation of HRAP2, FANCC, GBGT1, DOK2 and TNFSF4 in the progressive IBD, may cause a significant progression and severity of the disease . There are counter active findings which are noticed in the case of CD patients where GBGT1, IGFBP4, FAM10A4 genes are hypermethylated and in case of UC patients, IFITM1 is hypomethylated. This helps us to regulate and differentiate between the two subtypes. However, there is also the case of leukocyte methylation in the CD sub type of IBD . At the molecular level, most methylations occurring in the case of this disease, has a close proximity to GWAS risk genes, like CARD9, CDH1, ICAM3 . According to a group of British scientists, the region of the gut that is more prone to DNA methylation of the intestinal region. Major histone modifications are found to be abundant in the following histone proteins - H2BK5ac, H3K36me1, H3K4me3, macroH2A and Rme2sym, in the case of CD patients, as compared to the healthy people. These modifications can be consequently analyzed with the presence of natural killer (NK) . This accounts for the epigenetic biomarkers for the progressive and detectable approach of IBD .

Diagnostic Measures using miRNA as a Biomarker for AD

Globally, AD affect nearly 8% of the population, which include approximately 80 disorders and exhibit several geo-epidemiological variations; making it the fourth leading cause of mortality worldwide, after cancer and heart disease . It has been found that women are 2.7 times more susceptible for developing AD as compared to men . Hence, rapid and early diagnosis of AD is gaining prime importance in the light of improving the quality of patients' lives . Traditionally, the diagnosis of AD has been limited to the detection of autoantibodies in patients' samples through western blotting, indirect immunofluorescence and ELISA-based commercial assays . However, the pitfalls of such techniques include expensive antibodies, longer incubation time, the need for sophisticated instruments and complex procedures . Moreover, the accuracy and sensitivity of these techniques is not upto the mark . To overcome these limitations, a more sensitive and reliable technique is needed for the diagnosis of AD. Recently, miRNAs have proven to be effective non-invasive biomarkers for the diagnosis and prognosis of a disease, to study its progression and analyse the drug responses following treatment . This is because the abnormal miRNA expression profile is strongly correlated with diseased states and research shows that they are present in almost all biofluids . Despite the advent of high-throughput technologies, the detection of miRNA comes with a number of predicaments. Firstly, they are of short length (˜15-25 nt), making it difficult to develop a highly specific probe. Secondly, distinct miRNAs harbour homologous sequences which are prone to give false-positive results due to cross hybridization . Conventional approaches detecting miRNA include qRT-PCR,, northern blotting, enzymatic assays, oligonucleotide microarrays, cloning and sequencing which come with several drawbacks such as limited selectivity, low sensitivity, and ineffectiveness in detecting extremely low miRNA concentrations in blood samples . All these molecular techniques lack an integrated transducer element and are amplification-based . In addition to being time-consuming and laborious, these techniques do not take into account

the susceptibility of miRNA to degradation and hence give rise to bias, unreliable results . Furthermore, these detection techniques do not encompass multiplexed analysis, in vivo analysis, detecting circulating miRNAs and specificity towards single-nucleotide, all of which are indispensable in clinical settings . With the breakthrough of nanotechnology in medicine, amplification-free biosensors have drawn considerable attention from the academia as well as the industry . A biosensor is an integrated analytical device consisting of mainly three components: (i) a bio-recognition element such as enzymes, antibodies, DNA, RNA, aptamers,etc. (ii) a transducer element which detects a biological response and converts it into an electrical signal and (iii) a signal- processing system that consists of an amplifier, a processor and a display unit . This analytical device is highly effective in quantitative or semi-quantitative detection of an analyte . It incorporates the use of specific DNA probes which have complementary sequence with target miRNA with highly adaptable transducer sensing system . This enhances their multiplexing potential, allowing them to provide rapid, label-free, extremely selective as well as sensitive real-time detection of miRNA with respect to point-of-care (POC) aims for clinical applications . On the basis of the type of signal transducers used, biosensors for detecting miRNA are broadly classified into optical and electrochemical biosensors . Optical biosensors are highly effective in detecting biomarkers as they transduce the absorbance or fluorescence signals of an optically-active reporter linked to a nucleic acid probe when hybridized to the target miRNA . This class of biosensors have a fairly simple and feasible design, wherein the bio-recognition elements are immobilized on the surface of a signal detection platform by either physical adsorption, covalent or electrostatic bonding . The detection platforms are fabricated with nanomaterials such as graphene oxide (GO), gold nanoparticles (AuNP), and quantum dots (QD) . AuNPs are highly compatible with nucleic acids as well as proteins, provide adequate surface area to volume ratio, have excellent fluorophore quenching ability and exhibit good LSPR absorption in the visible range . Surface Plasmon resonance (SPR) is another robust and sophisticated optical method for fast and direct miRNA detection . It measures the shifts in refractive index when an analyte forms a complex on the surface of the electrode . Surface plasmon resonance Imaging (SPRi) is a label free hybridization-based method and can measure miRNA concentration for as low as 2 pM in less than 30 minutes . Their limit of detection (LOD) is low as far as complex biological samples are concerned . Several strategies are employed to enhance its sensitivity such as the use of nanoparticles like AuNPs coupled with DNA sandwich, GO–AuNPs hybrids, hybridization chain reaction method, hairpin assembly, streptavidin-biotin approach, etc . Surface-enhanced Raman scattering (SERS) is employable for rapid and accurate detection of miRNA but is not suitable for medical diagnostics due to low sensitivity . Another biosensing strategy under the optical methods is fluorescence detection. Examples include Ag nanocluster DNA probes, GO with dye-labelled probes, carbon nanoparticles, magnetic beads, etc . Polyaniline-gold (PANI-Au) nanomaterial and ruthenium (Ru)-based electrochemiluminescence immunosensors have also been designed for label free, highly sensitive quantification of miRNA . Overall, SPR-based biosensors are commonly employed optical methods for diagnosis of AD due to their high selectivity, high efficacy, affordability, and reliability. However, biosensors working on the principle of electrochemiluminescence demonstrate highest sensitivity . Electrochemical biosensors are the most predominantly used sensors for the diagnosis of AD . The transducer is a solid electrode which is sensitive to changes in electrode properties caused by the hybridization between the immobilized nucleotide probe and the complementary sequence . In these, the transduction element is often Au, indium tin oxide, glassy carbon, and graphite, the sensitivity of which is enhanced by incorporation of a variety of nanoparticles, nanowires and enzymes . In contrast to optical biosensors, electrochemical biosensors have simpler electronic design, are inexpensive, and serve as excellent platforms for point-of-care tests because of their easy-to-use miniaturized portable integrated systems . This allows them to provide fast, accurate and real-time quantification of analyte. These are further classified into amperometric, impedimetric and voltammetric methods . The amperometric and voltametric techniques measure changes in the current when a target miRNA is hybridized to its complementary sequence; with the only difference being that the current is measured at a fixed potential value in amperometry, while the current is measured in a certain potential ramp in voltammetry . Cyclic voltammetry (CV), linear sweep voltammetry (LSV), differential pulse voltammetry (DPV) , and square wave voltammetry (SWV) are different techniques under voltammetry . These are further categorized as label-free biosensors if the redox reaction is generated due to an electroactive nucleic acid base (adenine or guanine) signals or

enzymes such as duplex-specific nuclease; or as label-based biosensors if the electrochemically active reporter species are nanoparticles (Eg. Au,Ag, OsO_2, or Ruthenium NPs) or hybrid nanoparticles (Eg. GO–AuNPs, MoS_2 microcubes, Au@NPFe2O3 nanocubes . Electrochemical impedance spectroscopy (EIS) is another robust, label-free, and well-established technique for quantification of miRNA . It is the most practical method to study reaction mechanisms, biofunctionalization, nanostructure formation and hybridization . EIS is more efficient and biocompatible as compared to amperometric and voltammetric methods since it is less destructive and more sensitive .**Figure 4** – *Illustrative depiction of biosensor construction deploying miRNA signature molecules corresponding to respective ADs using several biorecognition elements along with assistive nanomaterials for eliciting robust signal amplification to detect pathogenic miRNA indices.*Both optical and electrochemical methods have their pros and cons in detecting miRNA with some giving a much lower limit of detection and hence higher sensitivity than others . However, selecting an optical or an electrochemical biosensor would depend upon the experimental aim which would be different for research and clinical purposes. In conclusion, biosensors offer unique advantages over the use of traditional assays which makes them highly suitable POC devices in the clinical diagnosis of AD . The incorporation of nanoparticles in these biosensors have significantly brought down the LOD to pM and fM range which is instrumental in the rapid and early-stage diagnosis of AD. Further innovation is needed to develop in vivo sensing platforms for AD . Table 2 lists down the different optical and electrochemical techniques used for detecting miRNAs in RA, Diabetes, MS, SLE and IBD, along with their references.**Table 2 -** *Different techniques, their range and mechanism of detection of miRNAs in different autoimmune diseases*

microRNA	**Optical technique**	**Linear dynamic range**	**Disease**	**M**
miRNA-21	Fluorescence	50 pM–1 nM	RA	In
let-7a	Fluorescence	10 fM–2 pM	RA	H
miRNA-155	Absorbance	100 aM–100 fM	RA	D
miRNA-15a	SPR (Surface Plasmon Resonance)	5 fM–0.5 nM	RA	Is
miRNA-21, miRNA-155	SPR (Surface Plasmon Resonance)	10 aM–10 pM	RA	S
miRNA-155	SERS (Surface Enhanced Raman Spectroscopy)	1 fM–10 nM	RA	S
miRNA-21	square wave voltammetry (SWV); electrochemical	200 pM–1 fM	T2DM	st
miRNA-155	differential pulse voltammetry (DPV); electrochemical	0.1 fM–1.0 nM	T2DM	M
miRNA-375	square wave voltammetry (SWV); electrochemical	10–30 fM	T2DM	In
miRNA-34a	electrochemical impedance spectroscopy (EIS)	2–10 μg/mL	T2DM	In
miRNA-145	Fluorescence spectrophotometry	N/A	MS	H
miRNA- 23a,				