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EMERGING ROLE OF CIRCULATORY MICRORNAS AS BIOMARKER IN AUTISM

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Abstract:
A heterogeneous group of complex neurodevelopmental disorder is termed autism spectrum disorder (ASD) that is characterized by a change in behaviour, deficits of communications and social skills. The neurodevelopmental transcriptional networks of the human brain are mainly regulated through small non-coding RNAs and microRNAs. The emerging role of circulatory microRNAs has been proved in previous investigations and research that open new techniques for the treatment of human disease or disorder as prognostic biomarkers in Autism. The development of biomarkers is progressively considered as a cornerstone in the medical field that shows an effective role in the diagnosis of multiple diseases and helps effectively in discovering different drugs and limits the progress of multiple diseases. Biomarkers for autism spectrum disorder have not been established yet due to its complexities. Circulatory microRNAs in cell-free conditions are considered as next-generation biomarkers for multiple pathologies such as neurodevelopmental disorders and neurological disorders. The presence of cell-free microRNAs in bio fluids is noticed to be in extraordinary stable conditions and is non-invasive. This article is based on evaluating the emerging role of circulatory microRNAs as biomarkers in Autism disorder. Clinical aspects of Autism disorder, a brief introduction of microRNAs and their functions and role of microRNAs as a biomarker to detect Autism has been provided in the context of the paper.

Keywords: Circulatory microRNAs, biomarkers, autism spectrum disorder, neurodevelopmental disorder, cell-free microRNAs.

抽象的：一组复杂的神经发育障碍被称为自闭症谱系障碍 (ASD)，**其特征是行**为改变、沟通和社交技能缺陷。人脑的神经发育转录网络主要通过小的非编码 RNA **和** microRNA 进行调节。循环 microRNA **的新**兴作用已在先前的调查和研究中得到证实，这些调查和研究开辟了治疗人类疾病或障碍的新技术，作为自闭症的预后生物标志物。生物标志物的开发逐渐被认为是医学领域的基石，它在多种疾病的诊断中显示出有效的作用，有助于有效地发现不同的药物并限制多种疾病的进展。由于其**复**杂性，尚未建立自闭症谱系障碍的生物标志物。无细胞条件下的循环 microRNA **被**认为是神经发育障碍和神经系统疾病等多种病理的下一代生物标志物。注意到生物流体中无细胞 microRNA **的存在**处于非常稳定的条件并且是非侵入性的。

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本文基于评估循环 microRNA 作为孤独症生物标志物的新兴作用。自闭症障碍的临床方面、microRNA 及其功能的简要介绍以及 microRNA 作为检测自闭症的生物标志物的作用已在本文的上下文中提供。

关键词：循环微RNA，生物标志物，自闭症谱系障碍，神经发育障碍，无细胞微RNA。

Introduction:

From a small family of nucleotides or single-stranded RNAs, microRNAs that came to play a significant role in the expression of genes. MicroRNAs affect various cellular processes such as differentiation, cell death, metabolism, proliferation, and growth. The process of molecules that are canonically preserved begins in nuclei that are transcribed into pre-miRNAs by RNA polymerase II. At the interval of 18 to 36 months, a pervasive and multifaceted disorder of neurodevelopmental changes tends to emerge itself and considered as ASD. (Salloum-Asfar et al. 2019). This disorder is very complex hence it has no clear aetiology. Pragmatic aspects in communication, lack of socialization ability, lack of empathy, indifferent to human interactions, emotional outburst, lack of expressions, restricted or repetitive behaviour are the primary symptoms of this disorder. There is no single test is available to diagnose ASD and all the available tests are possible to do only after one or two years of age of child. Biomarker molecules are found in blood or other fluids of the human body that help in detecting the abnormal or normal process of blood flow and conditions of disorder or disease. Circulatory microRNAs are considered as the most effective biomarkers of infection that are caused due to a variety of pathogens such as the Hendra virus, HIV, Tuberculosis, cancer, and even more complicated diseases. The present study is an attempt towards identifying circulatory cell free microRNAs as potential biomarkers for diagnosing ASD.

Method:

This study is conducted by maintaining all essential methodology, which directly has an impact on the growth and development of this study. On the other hand, beliefs about the phenomenon and scope of the study are analysed through positivism research philosophy. Despite these three effective research philosophies are noticed such as pragmatism, realism, and interpretivism philosophy (Snyder 2019). Two effective intergroup are present in research philosophy such as deductive and inductive research approaches. A deductive research approach has been selected for this study. Relationships between concepts and variables are maintained successfully with the help of the deductive research approach. Deductive research approaches are also involved in maintaining the development of the research hypothesis. Mohajan (2018) stated that qualitative concepts between hypotheses are maintained with the involvement of a deductive research approach.

On the other hand, a descriptive research design has been selected by the researcher. The framework of the research method is maintained with the involvement of a descriptive research design. Overall strategy and scope of the study are maintained by this research design. Secondary and primary are two effective groups of data which has been used in this study. Qualitative data analysis process has been used to analyse collected secondary data. Secondary data was especially collected from the published

journal, article, and other sources. In addition, all ethical considerations are maintained to conduct this study (Ørngreen and Levinsen 2017).

Fresh blood sample was drawn into EDTA containing (~1.8mg EDTA per blood collection tube) Tarson tubes from 50 Autistic patients and 50 normal individuals of same age group. Tubes were inverted carefully to mix the blood with coagulant and the samples were followed by centrifugation for 10 minutes at 1000-2000 RCF at room temperature. Carefully aspirated the supernatant(plasma) at room temperature and transferred into cryovials and properly labelled for storage at -80 degree Celsius for further use (Chomczynski, P (1993)

- **Isolation of miRNA from the plasma of normal, Autistic individuals.**

0.75 ml of TRIzol LS Reagent was added for each 0.25 ml of sample and homogenized. The reagent and sample were mixed well by pipetting. The homogenized samples were incubated for 5 min at room temperature to permit the complete dissociation of nucleoprotein complexes. Added 0.2 ml of chloroform and vigorously shaken for 15 seconds, followed by incubation at room temperature for 2 to 15 min. Samples were then centrifuged at 12000 X g for 15 min at 4ºC and the aqueous phase was transferred to a clean tube. Added 0.5 ml of isopropyl alcohol to this and incubated at room temperature for 10 min before centrifuging at 12000 X g for 10 min at 4ºC for RNA precipitation. The supernatant was discarded and RNA pellet was washed with 75% ethanol. It was then centrifuged at 7500 X g for 5 min at 4ºC. The RNA pellet was then air dried for 5min dissolved in RNase-free water and incubated for 10 min at 55-60ºC. The purity of the isolated RNA was analyzed using Thermo Scientific Nanodrop by checking the 260/280 ratio. [Esther R. Berko et.al,2014) The concentration of total RNA was normalized for the cDNA preparation.

- **cDNA preparation**

The cDNA was prepared according to the S poly (T) method proposed by Kang et al (2012). Briefly in this method, microRNAs are subjected to polyadenylation and subsequent reverse transcription with microRNA specific S poly (T) RT primers. S poly(T) RT primers consists of 4 segments (in the 5'to 3'direction) a universal reverse primer sequence for PCR, a universal Taqman probe sequence and poly (dT)11, followed by 5~7 specific bases that are complementary to the 3' end of a particular mature microRNA. The sequence details of the primers used in this study are given in table 1.0

MicroRNA	SEQUENCE	FORWARD PRIMER	RT PRIMER
Hsa-miR-	AGGCAAGAUGCUGGCAUAGCU	CGGAGGCAAGATGCTGGC	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATTTTTTTTTTTAGCTAT

31-5p			
Hsa-miR-182-5P	UUUGGCAAUGGUAGAACUCACACU	GCTCGGTTTGGCAATGGTAGAACT	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATTTTTTTTTTTAGTGTG
Hsa-miR-124-3p	UAAGGCACGCGGUGAAUGCC	CGGTAAGGCACGCGGTG	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATTTTTTTTTTTGGCATT
Hsa-miR-129-5p	CUUUUUGCGGUCUGGGCUUGC	GTCGGCTTTTTGCGGTCTGG	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATTTTTTTTTTTGCAAGC
Hsa-miR-320c	UUUGCAUUAAAAAUGAGGCCUUCUCUUCCCAGUUCUUCCCAGAGUCAGGAAAAGCUGGGUUGAGAGGGUAGAAAAAAAAUGAUGUAGG	GGCTCGGAAAAGCTGGGTTGA	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATTTTTTTTTTTACCCTC
Hsa-miR-1271	CACCCAGAUCAGUGCUUGGCACCUAGCAAGCACUCAGUAAAUAUUUGUUGAGUGCCUGCUAUGUGCCAGGCAUUGUGCUGAGGGCU	CGGCTTGGCACCTAGCAAG	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATTTTTTTTTTTTGAGTG

Hsa-miR-875-5p	UAUACCUCAGUUUUAUCAGGUG	GGCTCGGTATACCTCAGTTTTAT	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATTTTTTTTTTCACCTG
Hsa-miR-8485	UCUGUGAUAUACGUGUGUGUGUGUGUAUAUAGCAUAUGUGUAUACAUACACACACACACACACACACACACACACACACACGUAU	CTCGGCACACACACACACAC	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATTTTTTTTTTATACGT

Table 1.0 Sequence of oligos used for cDNA synthesis and amplification of microRNA by RT PCR

- **Polyadenylation**

10X reaction buffer (1 µl), 10 mM ATP (1 µl), poly A polymerase (1unit),7.8 µl(953ng) total RNA were mixed and incubated at 37 ºC for 30 minutes followed by enzyme inactivation at 65 ºC for 5 minutes.

- **Reverse transcription**

Polyadenylation reaction product (1µl), 0.5 µM RT primers (1µl), 10Mm dNTP (0.5 µl), MMLV HP RT 10X reaction buffer (1 µl), MMLV High Performance Reverse Transcriptase (50 units) were mixed and incubated at 42 ºC for 60 minutes followed by enzyme inactivation at 85 ºC for 5minutes.

After cDNA synthesis, the samples were diluted 1:5 ratio with nuclease free water and stored at -20ºC until further use.

- **Real Time PCR analysis of microRNAs**

Fast start essential DNA Green Master was used for real time PCR. The RT assay mix contained SYBR Green PCR Master Mix (10µl), Universal Reverse Primer (1µl), forward primer (1µl), cDNA (2 µl) and RNase free water (6µl). Separate internal controls were taken for each sample (mostly RNU6B). All the assays were done in triplicate with appropriate non template control (NTC).

For RT analysis the PCR was performed in a Roche LC 480 RT PCR machine with the following cycling condition,

95 ºC-30sec (95 ºC-5 sec;60 ºC-30sec;72 ºC-30sec)44 followed by melt curve analysis.

- **Bioinformatics analysis**

Bioinformatics analysis were conducted for identifying candidate microRNA that target the genes deregulated in Autism, with the help of three databases microRNA .org., Diana, and

Target Scan. The various microRNAs were given a score based on the number of hits it revealed in the various databases. E.g.: MicroRNAs that found hits in all the three databases were scored 3 and those that were found in only two were scored 2.

Literature review:

Circulatory MicroRNAs and their Functions

According to Rios-Colon et al. (2019), a small single-stranded non-coding RNA molecule that consists of almost 20 to 22 nucleotides is termed as microRNA. MicroRNAs are found in animals, plants, and in some viruses too and it works for silencing RNA and post-transcriptional regulation for the expression of genes. MicroRNA or non-coding RNA was first discovered in 1993 by Ambros and Ruvkun groups as a revolutionary element in microbiology that has deeper information about human pathologies (Ishmael 2019). The effective role of microRNAs could be noticed as a contribution in detecting or prescribing personalized medicine. MicroRNAs are not capable of encoding proteins and thus show an effective role in holding structure, performing catalytic functions, and in the regulation of disease components. Circulatory microRNAs are recognized through their cellular microenvironments that emerge as important biomarkers in ASD. Workflows of microRNA are observed to be in four distinct steps such as secretion, isolation, detection, and clinical biomarkers respectively as described in figure 1.

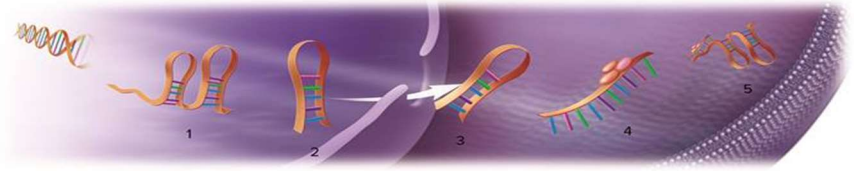

Figure 1. Function of microRNAs
(Source: Joy et al. 2018, p. No. 5)

The prime function of microRNAs in the human body is to regulate the expression of genes and undergo a series of cleavage events that create mature RNAs. The genes of microRNA are found in either intragenic regions or intergenic regions. As per Vasu et al. (2019), more than 50% of investigated microRNAs are found in intragenic regions that involve processing from introns and some exons with the coding of protein genes. The role of microRNAs is not limited to the endogenous regulation of elements as investigated in recent studies of plethora that shows microRNAs as biomarkers of Autism. Circulatory micoRNAs are detected in diverse fluids in comparison to intracellular fluids and found to be stable for more than 3 days at room temperature. Adverse conditions like low or high pH values, cycles of multi-freeze and boiling points could not affect the structure of microRNAs. The potential strength of cell-free circulatory microRNAs could be noticed in cellular communication as a biomarker for several diseases.

Circulatory MicroRNAs as Biomarkers in ASD:

Serial No.	Extraction of RNA	Validation and Profiling	Gender Ratio (M:F)	Age Group (in Years)	Type of Sample taken

		of microRNA			
1.	Exosomal or epithelial RNA isolated	Sequence of RNA	180:55	1-5	Saliva
2.	Test kit designed for blood microRNAs	qRT-PCR	25:5	3-10	Serum
3.	Plasma kit or serum made from Qiagen of microRNAs	qRT-PCR and TLDA	21:7	3-15	Serum

Table 2. Circulatory microRNAs as biomarkers in Autism
(Source: Rahat et al. 2020, P.no.10)

Table 2 describes the circulatory microRNAs as biomarkers in Autism disorder. Table 2 includes information for the RNA extracted and to check its validity and profiling for microRNA. The above table shows that the sources of sample for testing the emerging role of circulatory microRNAs as biomarkers of Autism have been taken from either serum or saliva. RNA is extracted from the isolation of exosomal or epithelial RNA, a kit designed for blood miRNAs, and a plasma kit or serum made from Qiagen of miRNAs. The gender ratio shows the ratio of male and female participants in this observation and the ratio is observed to be 80:55, 25:5, and 21:7 that validate sequences of RNA, qRT-PCR, and TLDA respectively (Lassandro et al. 2021). Observing the above table shows that samples of circulatory microRNAs could be obtained from serum and saliva from ASD individuals. The above information is based on the sample collected from saliva and serum of children from age group 1 to 5 years.

Circulatory microRNAs present in saliva work as effective biomarkers for ASD as it is detected in samples of saliva. The method for the extraction of RNA from the sample of saliva is a non-invasive technique. The method is useful in drawing required blood and avoiding the unnecessary flow of blood and reducing anxiety. The sample of human saliva is taken for a diagnostic specimen that avoids the disturbance caused by sample viability for example haemolysis of blood samples. Sample taken in the form of children's saliva is considered as prognostic, predictive, and diagnostic biomarkers in the diagnosis of Autism Spectrum Disorders. According to Rahat et al. (2020) a study of 2016 based on the observation of 25 children that are taken as participants involved in giving their saliva as a sample. The microRNAs of all district children are observed to be different, showing that circulatory microRNAs are effective in detecting individuals' disorders and providing personalized medication for them. The emerging role of microRNAs that work as biomarkers for cancer has been observed after 6 years to discover the existence of RNA and its role.

A serum sample from 50 children has been taken to screen with the disorder of Autism. The obtained result after the experiments has been obtained to be identical microRNAs for each child. As per Wu et al., (2020) diagnosis of microRNAs with serum samples is effective in

providing personalized treatment for everyone. Axon guidance, signalling for TGF-beta, actin cytoskeleton regulation, signalling for MAPK, signalling for mTOR, oxidative phosphorylation, etc are some essential neurological pathways that help in analysing the microRNAs. MicroRNAs with their abundance and high stability in different tissues and fluids made it the most effective diagnostic and prognostic biomarkers. MicroRNAs work differently for directing the issues for patients suffering from ASD and the sample obtained as serum. Quantitative reverse transcription provides validity through different expressions of microRNAs. The observed result from the serum sample of patients, the level of polymerase chain reaction or PCR is comparatively lower than healthier patients. PSA (Prostate-Specific Antigen) testing is considered a special biomarker for the development of discriminatory and specific biomarkers for ASD.

Drawbacks of Using Circulatory-MicroRNAs as the Biomarkers of Autism Spectrum Disorder.

Circulatory microRNA as biomarker of Autism Spectrum Disorder (ASD) is effective to detect the problem and provide personalized treatment to patients. With multiple advantages, there are few limitations of using microRNA as the biomarkers of ASD. The effectiveness of microRNA profiling from bio fluids in ASD is in the nascent stages. The prime drawbacks of using circulatory microRNAs are based on methodology and consequences of lack of functional microRNAs (Taleb et al. 2021). Different groups have used a different methodology that provides the conclusion that microRNAs obtained from serum samples result in some critical technical problems. The techniques used for the serum sample in the detection of health disorders required more reviews by experts. The limitation of using microRNAs as biomarkers in Autism is observed to be the lack of protocolized methods for the extraction of blood. The main failure of the technique is the detection of ASD while it is not truly present, yet the symptoms are similar. Issues of ASD occurred as persistent issues in communicating and iterating socially. Observation of body language, facial expression, empathy, and eye contact of children could be a great way to detect the disorder of Autism. Signs and symptoms are two major concerns of ASD, patients have multiple issues regarding social, communicational, and emotional skills.

According to Modak et al. (2019), the level of salivary microRNAs could be altered by multiple environmental factors such as a change in weather, the occurrence of cold or cough, specks of dirt, etc. The difference in oral hygiene could also affect the sample of saliva taken for observing the stage of Autism disorder in patients. Lack of experimental validation for the studies based on the circulatory microRNAs is considered a major drawback. Consistency of using the same amount of starting material results in causing another major limitation of using microRNAs as biomarkers of Autism disorder those results in the discrepancy of materials.

Result:

Most of the pathological conditions are underlined by a variation in the gene expression pattern, and so is the case with Autism. Apart from coding genes, the levels of secretory microRNAs that target the deregulated genes vary in pathological conditions. In the present study, the gene FAM134B which is down regulated was selected and its target microRNAs were identified. Another gene NRXN1 with deletion in region exon1-5 was selected and microRNA originating from this region was also

identified through bioinformatics analysis. Further considering that the stability of the circulatory microRNAs and its ability to be differentially regulated under conditions of physiology and pathology, the identified candidate microRNAs were tested in wet lab for their validity to be used as a faithful biomarker for Autism.

The targeting microRNAs of FAM134B were identified by using target prediction databases (e.g.: MIRANDA, DIANA, TARGETSCAN) and microRNAs were selected with high scores which include microRNA-1271, microRNA-31-5p, miR-129-5p, miR-182-5p, miR-124-3p, miR-875-5p and miR-320c. In the case of NRXN1 gene a deletion in exon-1-5 region were identified and microRNA originating from this region include microRNA-8485. Expression levels of each microRNA from Autistic and normal samples were tested. Based on the results, microRNA -124-3p and microRNA-182-5p were selected because it shows significant difference among Autistic patients and normal subjects.

A role for overlapping MicroRNAs in Autism

Identification of various neurodevelopmental disorders is maintained properly with the help of microRNAs. MicroRNAs are represented successfully through miR-146a. Common pathogenic mechanisms are identified successfully through microRNAs. High expression of miR-146a is noticed in the cortex, amygdala, and hippocampus, as well and higher cognitive functioning is also noticed in this case. The presence of potassium in two-pore domain channels can deregulate MiR-146a, which is also related to neuron excitation. On the other hand, it is important to collect proper and effective information about the expression of neuron-specific phenotypes. It is important to learn about the key role of neural excitability. MicroRNAs should be implemented as a marker to collect proper information about the identification of neuroinflammation in the brain of Autism subjects. The higher level of Autism in children is analysed successfully through microRNAs. The binding of protein is an effective aspect, bio marking of protein bond is maintained through MicroRNAs (Tonacci et al. 2019).

In ASD neurological damage is analysed through the implementation of microRNAs. Involvement of this aspect can justify measurement of the immune system; elicitation of the oxidative process is analysed through this aspect. However, MicroRNAs are unable to collect proper and effective information about autoimmunity and maternal antibodies. Hu et al. (2017) stated that the pathogenesis of language and social development abnormalities is increased due to this deficiency. To show the pathogenic role of RNA in Autism it is postulated by other autoantibodies. On the other hand, microRNA is involved in the justification of the pathogenic role of autoantibodies in protecting against Autism. Along with this, it is important to collect proper and effective information about the index of autoimmunity in the brain. Implementation of MicroRNA can play a crucial role in high-functioning Autism spectrum disease.

With the help of a few effective pieces of information, it is noticed that Mi6126 is deregulated in Autism disease. In addition, these microRNAs are correlated with the severity of social deficits (Hicks et al. 2020).

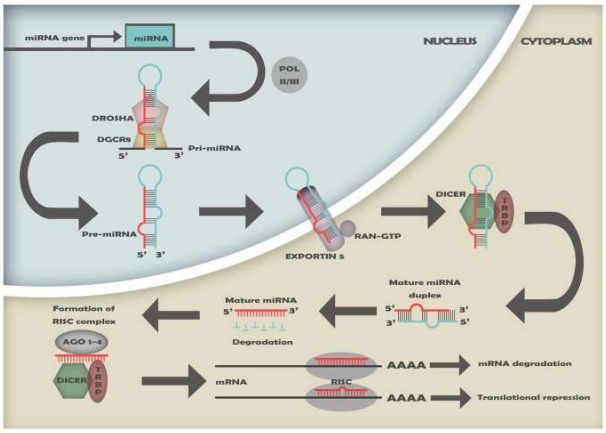

Figure 2: Role for overlapping of MicroRNAs in Autism
(Source: Hicks et al. 2020, p. 223)

MicroRNAs Biogenesis and functions

MicroRNAs are identified normally from inter or intragenic regions. However, most of the Annotated microRNAs are noticed in the intragenic region. Other miRNAs are identified successfully from intragenic regions. On the other hand, independent transcription of genes is noticed in this case. Several mutational steps are present in maintaining the formation of microRNA, functional activity of microRNA is maintained with the involvement of these mutational steps. Transcription of microRNA is maintained through RNA polymerase. Shen et al. (2018) stated that this factor can play a crucial role in maintaining the beginning of microRNA. One long polycistronic transcription pre-microRNA is involved in maintaining transcription of almost 25% microRNA from the cluster. On the other hand, it is also noticed that approximately 70 nucleotides are present in Pre-miRNA. Along with this, an important schematic biogenesis pathway is present in maintaining the formation of mature miRNA.

In addition, alternative non-canonical and canonical biogenesis factors are involved in maintaining the use of miRNA as well as independent generation is also noticed in mature miRNA strands. However, it is important to collect proper information about disruption in the biogenesis pathway(Geng et al. 2020). These characteristics of microRNA can play a crucial role in maintaining gene expression control. The presence of this efficiency can make microRNA an important biomarker of Autism disease. Few effectively derived blood organs are present in microRNAs such as plasma and serum. Along with this, it is also noticed that miR-6126 is also used as an effective indicator that can provide all essential information about the use of quantitative reverse transcription-polymerase chain reaction that can identify Autism disorder. This process can provide all essential information about the ASD status in blood. On the other hand, significant down regulation is also noticed in miR-6126. Dahariya et al. (2019) stated that this miRNA can provide effective information about the significant difference between ASD and other groups.

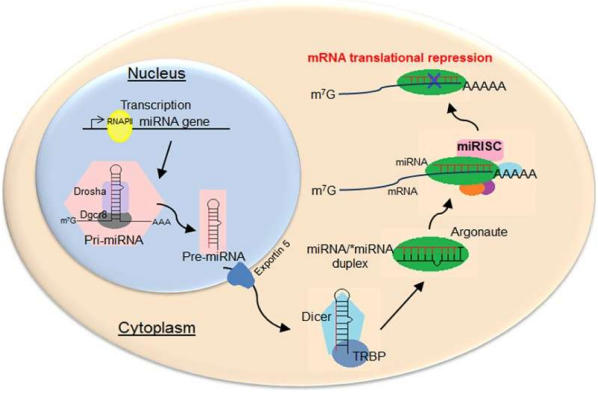

Figure 3: microRNA biogenesis
(Source: Dahariya et al. 2019, p. 322)

Discussion:

Autism is a neurodevelopmental disorder characterized by impaired social interaction, communication, and restricted and repetitive behaviour. Autistic patients are always isolated from society because of their impairments in social interaction even though they have a good

IQ level. If identified at prenatal stage of birth, the family can be counselled to take necessary early training engagements which will improves the life conditions of Autistic patients. There are currently no reliable plasma biomarkers for Autism. Therefore, search for new biomarker is necessary in order to improve prediction, diagnosis and monitoring of Autistic complications. Circulating microRNAs have been extensively investigated as Novel and non-invasive diagnostic and prognostic markers.

With the help of these results, it is noticed that the use of MicroRNAs can play a crucial role in maintaining the identification of Autism disease. MicroRNAs also helps to collect proper information about the role of other auto antibodies in developing treatment and diagnosis of Autism disease. The gene down regulated in Autism that is FAM 134B was selected for predicting candidate microRNAs that target them. Another gene that is NRXN1, in which exon 1-5 deletion occurring in Autistic condition were also selected and microRNA originating from that region was predicted. FAM134B is the cis-Golgi transmembrane protein, and its gene is in chromosome 5. The main function of this gene is implicated in survival of autonomic and nociceptive ganglion neurons. Down regulation of this gene leads to Autism. NRXN1 is the synaptic adhesion proteins, and its gene is in chromosome 2. NRXN1 proteins are located on the presynaptic membrane and bind to post synaptic counterpart. Deletion in NRXN1 gene leads to Autism [Hyung-Goo Kim et. al 2008]

Also based on these results we are of opinion that miR-6126 is used as an effective indicator of Autism disease. Effective biogenesis criteria are also present in this biomarker, which is analysed in this result. Along with this, it is important to collect proper and effective information about the relationship between the clinical severity of ASD symptoms and the expression of miR-6126. A negative correlation is present between these two factors, which is evaluated through this result. This factor can help to collect information about the effectiveness of microRNA as a biomarker. On the other hand, it is noticed that representative of ASD is also included in this disease. Targeted genes of ASD disease are also highlighted successfully with the involvement of microRNA as a biomarker. Overlapping of microRNA can play a crucial role in maintaining the treatment and diagnosis of Autism disease. This process is involved in enclosing neurons successfully, which is treated as a main and effective identification ability of miRNA. Analysis of critical ASD is maintained properly with the help of deregulation of miR-6126. Along with this, it is important to collect proper information about neuronal dendritic arborization. This approach is involved in the justification of synaptic transmission. On the other hand, it is also noticed that microRNA is also used as a biomarker to maintain various neurodevelopmental diseases.

Conclusion:

The study concluded that circulatory microRNAs emerged as effective biomarkers in Autism. MicroRNAs play an important role in detecting an individual's RNA for observing the stage of Autism and providing personalized treatment. The detection of Autism in children from age 1 to 15 could be performed by taking a sample of serum and saliva. Blood vessels with circulating microRNA for the serum and saliva sample are observed to be up regulated and downregulated. Nucleic Acid Stabilizing Swab is used for testing samples of saliva, the regulatory level of blood vessels is lower for patients than a healthy

person. The study concluded that the disorder of Autism is quite complex and a form of neurodevelopmental disorder that has different levels of symptom severity. The present clinical assessment for Autism could be observed through subjective evaluation and observing the challenges of diagonals. Secretion, isolation, detection, and clinical biomarkers are four major functions of microRNAs in Autism. Attractive opinions for the regulation of gene expression have been noticed after 6 years of discovering the microRNAs. Social and environmental ethical considerations have been maintained throughout the process of conducting the present study.

Recommendation:

The recommendation of the study helps to fulfil the present knowledge gap of the study and for the development of this study. Following are some recommendations that should be followed to achieve the development of the paper:

- Researchers and scholars of a further study based on the effective methods for the treatment of Autism Spectrum Disorder (ASD) through microRNAs, the importance of microRNAs in treatment and diagnosis of multiple diseases, etc.
- The study could provide existing literature for future work based on the functions of circulatory microRNAs, and the advantages and disadvantages of microRNAs as biomarkers in Autism.
- The present knowledge gap of the study could be fulfilled with a comparative analysis of two effective techniques for the diagnosis of Autism. The validity and reliability of the paper improved with a comparative study.

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