Global Journal of Intellectual & Developmental Disabilities
ISSN: 2575-8586

Juniper Publishers
key to the Researchers

Mini-Review
Volume 9 Issue 1 - September 2021
DOI: 10.19080/GJIDD.2021.09.555753

Glob J Intellect Dev Disabil

MicroRNAs as a Diagnostic Biomarker in Alzheimer's Disease: Potential Roles and Characteristics

Chang Youn Lee[1], Jin Hyeob Ryu[1]* and Hyun Jeong Cho[2]*

[1]*Biorchestra Co. Ltd., Techno 4-ro, 17, Daejeon 34013, South Korea*

[2]*Department of Biomedical Laboratory Science, College of Medical Science, Konyang University, 158, Gwanjeodong-ro, Seo-gu, Daejeon 35365, South Korea*

Submission: September 2, 2021; **Published:** September 14, 2021

***Corresponding author:** Jin-Hyeob Ryu, Biorchestra Co. Ltd., Techno 4-ro, 17, Daejeon 34013, South Korea, e-mail: jhyeup@gmail.com

Hyun-Jeong Cho, Department of Biomedical Laboratory Science, College of Medical Science, Konyang University, 158, Gwanjeodong-ro, Seo-gu, Daejeon 35365, South Korea, e-mail: hjcho@konyang.ac.kr

Abstract

As the world moves towards an aging society, it faces enormous public health challenges. Among them, an increased number of patients with dementia poses a serious socioeconomic burden. Alzheimer's disease (AD) is the most important type of dementia and despite many studies, cannot be cured by modern medicine. However, the progression of the disease can be delayed; therefore, early diagnosis of AD is very important. MicroRNAs (miRNAs) are abundant in the CNS, and their profiles get altered in degenerative brain diseases. Studies analyzing the tissues, blood, and cerebrospinal fluid of AD patients have shown an up-regulation in some miRNA expression and a decrease in some miRNAs. These miRNAs are involved in modulating the production of amyloid beta, hyperphosphorylation of tau protein, and synaptic plasticity. These characteristics make miRNAs very attractive tools for disease diagnosis. Through this review, we intend to investigate the characteristics and vulnerabilities of miRNAs in AD diagnosis.

Keywords: Alzheimer's disease; Diagnosis; Biomarker; miRNA

Abbreviations: AD: Alzheimer's Disease; miRNA: microRNA; CSF: Cerebrospinal Fluid; SIRT1: Silent Information Regulator Transcript-1; NAV3: Neuron Navigator 3; BDNF: Brain-Derived Neurotrophic Factor; CDK5: Cyclin-Dependent Kinase 5

Introduction

The world is becoming an aging society, and it faces enormous challenges to public health. One of them is the increase in patients with dementia, which is a huge socio-economic burden. Alzheimer's disease (AD) is a complex neurodegenerative disease and is the most usual brain disease that causes dementia [1]. According to some reports, 10% of the population aged 65 years and older is affected by AD, and the incidence nearly doubles every 5 years [2]. This phenomenon occurs in both developed and developing countries [2].

AD usually progresses gradually, leading to cognitive impairment and memory loss. The pathological features of AD include the formation of amyloid plaques and neurofibrillary tangles caused by amyloid-β metabolism and hyperphosphorylation of tau protein, respectively [3,4]. In addition, AD shows loss of neuroplasticity and inflammatory responses [5,6]. Although the causes of AD have already been discovered, it is considered as an incurable disease in modern medicine [6]. However, early diagnosis can enable the delay of disease progression, and therefore, it becomes critical in dealing with AD patients.

MicroRNAs (miRNAs) are small non-coding RNAs that bind to complementary sequences (seed sequence) on the target RNA and regulate post-transcriptional gene expression [7]. miRNAs are abundant in the CNS and are closely involved in neural development [8,9], neuronal differentiation [10,11], and synaptic plasticity [12,13]. Studies have shown that miRNA expression is altered in neurodegenerative diseases such as AD [14,15]. In addition, several miRNAs have been identified as critical regulators in the pathogenesis of AD [16]. Based on these characteristics, miRNAs are gaining attention as promising biomarkers for the diagnosis of AD [17,18]. In this review, we have shown evidence regarding the potential of miRNAs for AD diagnosis.

Discussion: miRNA as diagnostic AD Biomarker Candidates

Increased miRNAs in AD

Studies have shown that miRNAs regulate Aβ metabolism. Gaurav et al. [19] identified miRNAs, such as miR-27a-3p, miR-30a-5p, and miR-34c, as biomarkers for ADs and reported that these miRNAs get increased in patients with AD [19]. These miRNAs have been shown to play roles in memory function and neurodegeneration in previous studies.

Yu et al. [20] revealed that miR-485-3p can be used as a biomarker and therapeutic target of AD, as they found increased miR-485-3p expression level in the serum of AD patients. In addition, they showed that miR-485-3p affects neuroinflammation by targeting AKT3 [20].

Studies have shown increased expression of miR-34 family in patients with AD [21,22]. MiR-34 is involved in neuronal synaptic deficiency, resting-state network activity, and energy metabolism [19,21]. Sarkar et al. [21] reported that miR-34, by inhibiting SIRT, increases Aβ production in AD patients [21]. In addition, Shephali et al. [22] reported that the expression of miR-34a, miR-34b, and miR-34c is increased in the plasma of AD patients, with miR-34c showing a significantly increased level in patients with sporadic AD [22].

Increased expression of the miR-200 family has been reported in previous studies [23, 24]. Zhang et al. [23] reported that miR-200a-3p upregulates Aβ-induced neuronal apoptosis by inhibiting the silent information regulator transcript-1 (SIRT1) in APP/PSEN1 mice [23]. In addition, Higaki et al. [24] reported that expression of the miR-200 family (miR-200a, -141, -429, -200b, -200c) is increased in the initial phases of AD. Moreover, this study showed that miR-200b/c increases Aβ accumulation in Tg2576 transgenic mice by regulating insulin signaling [24].

MiR-146a is increased in the brains of patients with AD and is involved in neuroinflammation and aging [25,26]. Dysregulation of miR-146a causes abnormal tau hyperphosphorylation and Aβ deposition in patients with AD. Li et al. [27] reported that miR-146a promotes amyloidogenesis by regulating the expression of IRAK1, TRAF6, TSPAN12 (tetraspanin12), and ADAM10 [27]. Wang et al. [28] further revealed that inhibition of miR-146a suppresses hyperphosphorylation and restores memory function by suppressing ROCK1 in 5x FAD mice [28].

Studies have now shown that miRNAs can also regulate abnormal phosphorylation of tau in AD patients. MiR-125b is up-regulated in AD patients, which induces apoptosis and hyperphosphorylation of tau in AD patients [29,30]. Ma et al. [29] found that miR-125b induces neuronal cell death and abnormal tau phosphorylation by regulating cyclin-dependent kinase 5 (CDK5) and p35/25 [29]. Banzhaf-Strathmann et al. [30] showed similar results where miR-125b affects tau phosphorylation by regulating p35, CKD5, and MAPK signaling in AD. Moreover, these authors showed that increased miR-125 impairs learning and memory, induces neurotoxicity, and expedites AD progression in patients [30].

MiR-206 is elevated in the brains of AD patients [31,32]. Moon et al. [31] reported that miR-206 levels were increased specifically in patients with olfactory mucosal AD. The study suggested that memory impairment in patients is caused by decreased expression of brain-derived neurotrophic factor (BDNF) by miR-206. Moreover, the study suggested that miR-206 has the potential to be used as an early diagnostic marker of AD. Kenny et al. [32] also reported that miR-206 is increased in the plasma of patients with AD. They suggested that the measurement of change in miR-206 expression in plasma may be used to predict cognitive decline and memory loss [32].

Decreased miRNAs in AD

miRNAs control key physiological parameters, such as the number and strength of synaptic inputs and intrinsic excitability, to maintain homeostatic plasticity [39]. However, alterations in miRNA expression that are caused by the pathological environment may contribute to disease progression as well as homeostatic failure.

In a 2008 report by Wang et al. [33] 70 miRNAs were selected through miRNA array analysis using brain tissues from early AD, severe AD, and control groups [33]. Among them, miR-107 was significantly reduced in the early stages of AD. miR-107 binds to BACE1 mRNA 3UTP and inhibits β-secretase1 expression. Therefore, decreased miR-107 expression in patients with early-stage AD is closely related to Aβ production by increased β-secretase1 levels.

In addition, it has been reported that miR-29a is significantly reduced in brain tissue obtained from patients with degenerative brain disease. This study revealed that miR-29a targets neuron navigator 3 (NAV3), whose expression was increased in degenerating pyramidal neurons in the cerebral cortex of patients with AD. These results revealed that the reduction of miR-29a expression promotes neurodegenerative diseases by enhancing NAV3 expression in neurons [34].

In a 2017 report, Haea et al. [35] investigated miRNAs in the serum of 45 people (control group: 18 people, AD group: 27 people), and found that the expression of miR-501-3p was reduced in the AD group. Consequently, a significant correlation was established using the Mini-Mental State Examination score. In addition, overexpression of miR-501-3p in cells revealed that miR-501-3p was able to suppress 128 genes regulating DNA replication and mitosis. Therefore, the reduced expression of miR-501-3p could be used as a biomarker for AD diagnosis [35].

In addition, some studies revealed that the levels of miR-34a and miR-146a in plasma and miR-34a, miR-125b, and miR-146a in cerebrospinal fluid (CSF) were significantly reduced in AD patients compared to controls [21,37]. As such, miRNAs that are significantly down-regulatd in AD patients have the potential to be developed as new biomarkers for AD diagnosis (Table 1).

How to cite this article: Chang Y L, Jin H R, Hyun J C. MicroRNAs as a Diagnostic Biomarker in Alzheimer's Disease: Potential Roles and Characteristics. Glob J Intellect Dev Disabil, 2021; 9(1): 555753. DOI: 10.19080/GJIDD.2021.09.555753

Table 1: miRNAs associated with AD pathophysiological features.

Direction of changes	miRNA	Targets	Responses	Reference
Up-regulated	miR-27a-3p	-	neurodegeneration	[19]
	miR-30a-5p	-		
	miR-34c	-		
	miR-485-3p	AKT3	neuroinflammation	[20]
	miR-34	SIRT1	Aβ production	[19, 21]
	miR-34a,b,c		neuropeptide Y signaling stress-related diseases	[21, 22]
	miR-200a	SIRT1	Aβ production insulin signaling	[23, 24]
	miR-200b	-		
	miR-200c	S6K1		
	miR-141	-		
	miR-429	-		
	miR-146a	ADAM10	amyloidogenesis	[25, 26]
	miR-125b	CDK5, p35/25	neuronal cell death abnormal Tau phosphorylation	[29, 30]
	miR-206	BDNF		[31, 32]
Down-regulated	miR-107	BACE1	Aβ production	[33]
	miR-29a	NAV3	-	[34]
	miR-501-3p	-	DNA replication, mitosis	[35]
	miR-34a	Bcl-2	-	[36-38]
	miR-146a	-	neuroinflammation	
	miR-125b	-	neuroinflammation	

Conclusion

miRNAs are powerful molecules for the evaluation of clinical samples. Recent studies have suggested their novel potential as a diagnostic biomarker of cancer [40,41], obesity [42,43], brain disease [44,45], and heart disease [46-49]. However, using miRNAs for clinical applications still poses challenges that need to be solved. First, miRNAs are single-stranded small RNA molecules and can be quite unstable depending on the sampling method and sample storage environment [50]. Second, some miRNAs may have different profiles in the tissues, blood, and CSF [51]. Therefore, clear criteria for sampling and sufficient evidence for miRNA selection are required. Third, non-standardized RNA extraction methods and variability in analysis may cause confusion in the interpretation of results [52,53]. Lastly, evidence regarding miRNAs as biomarkers has not yet been sufficiently reported. Although miRNAs are very attractive as biomarkers, there is insufficient evidence to overcome their limitations, which is required for the development of more reliable diagnostic technologies. In the future, if more research is conducted and miRNA detection and analysis methods are standardized, it will lead to the development of new biomarkers.

Acknowledgement

We would like to thank all participants.

References

1. Jagust W (2018) Imaging the evolution and pathophysiology of Alzheimer disease. Nature Reviews Neuroscience 19(11): 687-700.
2. Qiu C, M Kivipelto and E von Strauss (2009) Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. Dialogues in Clinical Neuroscience 11(2): 111-128.
3. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological alterations in Alzheimer disease. Cold Spring Harbor Perspectives in Medicine 1: a006189.
4. Iqbal K, Alonso Adel C, Chen S, Chohan MO, El Akkad E, et al. (2005) Tau pathology in Alzheimer disease and other tauopathies. Biochimica et Biophysica Acta 1739(2-3): 198-210.

How to cite this article: Chang Y L, Jin H R, Hyun J C. MicroRNAs as a Diagnostic Biomarker in Alzheimer's Disease: Potential Roles and Characteristics. Glob J Intellect Dev Disabil, 2021; 9(1): 555753. DOI: 10.19080/GJIDD.2021.09.555753

5. Mesulam MM (1999) Neuroplasticity failure in Alzheimer's disease: bridging the gap between plaques and tangles. Neuron 24(3) 521-529.
6. Holmes C, Cunningham C, Zotova E, Woolford J, Dean C, et al. (2009) Systemic inflammation and disease progression in Alzheimer disease. Neurology 73(10): 768-774.
7. O'Brien J, Hayder H, Zayed Y, Peng C (2018) Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front Endocrinology 9: 402.
8. Yoo AS, Staahl BT, Chen L, Crabtree GR (2009) MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. Nature 460(7255): 642-646.
9. Fineberg SK, KS Kosik and BL Davidson (2009) MicroRNAs potentiate neural development. Neuron 64(3): 303-309.
10. Sempere LF, Freemantle S, Pitha Rowe I, Moss E, Dmitrovsky E, et al. (2004) Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biology 5(3): R13.
11. Makeyev EV, Zhang J, Carrasco MA, Maniatis T (2007) The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Molecular and Cellular 27(3): 435-448.
12. Smalheiser NR, G Lugli (2009) microRNA regulation of synaptic plasticity. NeuroMolecular Medicine 11(3): 133-140.
13. Scott HL, Tamagnini F, Narduzzo KE, Howarth JL, Lee YB, et al. (2012) MicroRNA-132 regulates recognition memory and synaptic plasticity in the perirhinal cortex. European Journal of Neuroscience 36(7): 2941-2948.
14. Schonrock N, Ke YD, Humphreys D, Staufenbiel M, Ittner LM, et al. (2010) Neuronal microRNA deregulation in response to Alzheimer's disease amyloid-beta. PLoS One 5(6): e11070.
15. Hebert SS, B De Strooper (2009) Alterations of the microRNA network cause neurodegenerative disease. Trends in Neurosciences 32(4): 199-206.
16. Lau P, B de Strooper (2010) Dysregulated microRNAs in neurodegenerative disorders. Seminars in Cell and Developmental Biology 21(7): 768-773.
17. Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, et al. (2008) Serum microRNAs are promising novel biomarkers. PLoS One 3(9): e3148.
18. Hornung S, S Dutta, G Bitan (2020) CNS-Derived Blood Exosomes as a Promising Source of Biomarkers: Opportunities and Challenges. Frontiers in Molecular Neuroscience 13: 38.
19. Jain G, Stuendl A, Rao P, Berulava T, Pena Centeno T, et al. (2019) A combined miRNA-piRNA signature to detect Alzheimer's disease. Translational Psychiatry 9(1): 250.
20. Yu L, Li H, Liu W, Zhang L, Tian Q, et al. (2021) MiR-485-3p serves as a biomarker and therapeutic target of Alzheimer's disease via regulating neuronal cell viability and neuroinflammation by targeting AKT3. Molecular Genetics & Genomic Medicine 9: e1548.
21. Sarkar S, Jun S, Rellick S, Quintana DD, Cavendish JZ, et al. (2016) Expression of microRNA-34a in Alzheimer's disease brain targets genes linked to synaptic plasticity, energy metabolism, and resting state network activity. Brain Research 1646: 139-151.
22. Bhatnagar S, Chertkow H, Schipper HM, Yuan Z, Shetty V, et al. (2014) Increased microRNA-34c abundance in Alzheimer's disease circulating blood plasma. Frontiers in Molecular Neuroscience 7: 2.
23. Zhang QS, W Liu, GX Lu (2017) miR-200a-3p promotes b-Amyloid-induced neuronal apoptosis through down-regulation of SIRT1 in Alzheimer's disease. Journal of Biosciences 42(3): 397-404.
24. Higaki S, Muramatsu M, Matsuda A, Matsumoto K, Satoh JI, et al. (2018) Defensive effect of microRNA-200b/c against amyloid-beta peptide-induced toxicity in Alzheimer's disease models. PLoS One 13(5): e0196929.
25. Maffioletti E, Milanesi E, Ansari A, Zanetti O, Galluzzi S, et al. (2019) miR-146a Plasma Levels Are Not Altered in Alzheimer's Disease but Correlate With Age and Illness Severity. Frontiers in Aging Neuroscience 11: 366.
26. Mai H, Fan W, Wang Y, Cai Y, Li X, et al. (2019) Intranasal Administration of miR-146a Agomir Rescued the Pathological Process and Cognitive Impairment in an AD Mouse Model. Molecular Therapy-Nucleic Acids 18: 681-695.
27. Li YY, Cui JG, Dua P, Pogue AI, Bhattacharjee S, et al. (2011) Differential expression of miRNA-146a-regulated inflammatory genes in human primary neural, astroglial and microglial cells. Neuroscience Letters 499(2): 109-113.
28. Wang G, Huang Y, Wang LL, Zhang YF, Xu J, et al. (2016) MicroRNA-146a suppresses ROCK1 allowing hyperphosphorylation of tau in Alzheimer's disease. Scientific Reports 6: 26697.
29. Ma X, L Liu, J Meng (2017) MicroRNA-125b promotes neurons cell apoptosis and Tau phosphorylation in Alzheimer's disease. Neuroscience Letters 661: 57-62.
30. Banzhaf-Strathmann J, Benito E, May S, Arzberger T, Tahirovic S, et al. (2014) MicroRNA-125b induces tau hyperphosphorylation and cognitive deficits in Alzheimer's disease. The EMBO Journal 33(15): 1667-1680.
31. Moon J, Lee ST, Kong IG, Byun JI, Sunwoo JS, et al. (2016) Early diagnosis of Alzheimer's disease from elevated olfactory mucosal miR-206 level. Scientific Reports 6: 20364.
32. Kenny A, McArdle H, Calero M, Rabano A, Madden SF, et al. (2019) Elevated Plasma microRNA-206 Levels Predict Cognitive Decline and Progression to Dementia from Mild Cognitive Impairment. Biomolecules. 9(11): 734.
33. Wang WX, Rajeev BW, Stromberg AJ, Ren N, Tang G, et al. (2008) The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1. The Journal of Neuroscience 28(5): 1213-1223.
34. Shioya M, Obayashi S, Tabunoki H, Arima K, Saito Y, et al. (2010) Aberrant microRNA expression in the brains of neurodegenerative diseases: miR-29a decreased in Alzheimer disease brains targets neurone navigator 3. Neuropathology and Applied Neurobiology 36(4): 320-330.
35. Hara N, Kikuchi M, Miyashita A, Hatsuta H, Saito Y, et al. (2017) Serum microRNA miR-501-3p as a potential biomarker related to the progression of Alzheimer's disease. Acta Neuropathologica Communications 5(1): 10.
36. Wang X, Liu P, Zhu H, Xu Y, Ma C, et al. (2009) miR-34a, a microRNA up-regulated in a double transgenic mouse model of Alzheimer's disease, inhibits bcl2 translation. Brain Research Bulletin 80(4-5): 268-273.
37. Kiko T, Nakagawa K, Tsuduki T, Furukawa K, Arai H, et al. (2014) MicroRNAs in plasma and cerebrospinal fluid as potential markers for Alzheimer's disease. Journal of Alzheimer's Disease 39(2): 253-259.
38. Bhaumik D, Scott GK, Schokrpur S, Patil CK, Orjalo AV, et al. (2009) MicroRNAs miR-146a/b negatively modulate the senescence-associated inflammatory mediators IL-6 and IL-8. Aging (Albany NY) 1(4): 402-411.
39. Dubes S, Favereaux A, Thoumine O, Letellier M (2019) miRNA-Dependent Control of Homeostatic Plasticity in Neurons. Frontiers in Cellular Neuroscience 13: 536.

How to cite this article: Chang Y L, Jin H R, Hyun J C. MicroRNAs as a Diagnostic Biomarker in Alzheimer's Disease: Potential Roles and Characteristics. Glob J Intellect Dev Disabil, 2021; 9(1): 555753. DOI: 10.19080/GJIDD.2021.09.555753

40. Di Leva G and CM Croce (2013) miRNA profiling of cancer. Current Opinion in Genetics & Development 23: 3-11.

41. Fu SW, L Chen, YG Man (2011) miRNA Biomarkers in Breast Cancer Detection and Management. Journal of Cancer 2: 116-122.

42. Pescador N, Pérez Barba M, Ibarra JM, Corbatón A, Martínez Larrad MT, et al. (2013) Serum circulating microRNA profiling for identification of potential type 2 diabetes and obesity biomarkers. PLoS One 8(10): e77251.

43. Ortega FJ, Mercader JM, Catalán V, Moreno Navarrete JM, Pueyo N, et al. (2013) Targeting the circulating microRNA signature of obesity. Clinical Chemistry 59: 781-792.

44. Swarbrick S, Wragg N, Ghosh S, Stolzing A (2019) Systematic Review of miRNA as Biomarkers in Alzheimer's Disease. Molecular Neurobiology 56(9): 6156-6167.

45. Mushtaq G, Greig NH, Anwar F, Zamzami MA, Choudhry H, et al. (2016) miRNAs as Circulating Biomarkers for Alzheimer's Disease and Parkinson's Disease. Journal of Medicinal Chemistry 12(3): 217-225.

46. Ikeda S, Kong SW, Lu J, Bisping E, Zhang H, et al. (2007) Altered microRNA expression in human heart disease. Physiological Genomics 31: 367-373.

47. Chen W, S Li (2017) Circulating microRNA as a Novel Biomarker for Pulmonary Arterial Hypertension Due to Congenital Heart Disease. Pediatric Cardiology 38(1): 86-94.

48. Lee CY, Lee S, Jeong S, Lee J, Seo HH, et al. (2021) Suppressing Pyroptosis Augments Post-Transplant Survival of Stem Cells and Cardiac Function Following Ischemic Injury. International Journal of Molecular Sciences 22(15): 7946.

49. Seo HH, Lee S, Lee CY, Lee J, Shin S, et al. (2019) Multipoint targeting of TGF-beta/Wnt transactivation circuit with microRNA 384-5p for cardiac fibrosis. Cell Death & Differentiation 26(6): 1107-1123.

50. Glinge C, Clauss S, Boddum K, Jabbari R, Jabbari J, et al. (2017) Stability of Circulating Blood-Based MicroRNAs - Pre-Analytic Methodological Considerations. PLoS One 12(2): e0167969.

51. Takousis P, Sadlon A, Schulz J, Wohlers I, Dobricic V, et al. (2019) Differential expression of microRNAs in Alzheimer's disease brain, blood, and cerebrospinal fluid. Alzheimer's & Dementia Journal 15(11): 1468-1477.

52. Gustafson D, K Tyryshkin, N Renwick (2016) microRNA-guided diagnostics in clinical samples. Best Practice & Research: Clinical Endocrinology & Metabolism 30(5): 563-575.

53. Binderup HG, Madsen JS, Heegaard NHH, Houlind K, Andersen RF, et al. (2018) Quantification of microRNA levels in plasma - Impact of preanalytical and analytical conditions. PLoS One 13: e0201069.

DOI: 10.19080/GJIDD.2021.09.555753

How to cite this article: Chang Y L, Jin H R, Hyun J C. MicroRNAs as a Diagnostic Biomarker in Alzheimer's Disease: Potential Roles and Characteristics. Glob J Intellect Dev Disabil, 2021; 9(1): 555753. DOI: 10.19080/GJIDD.2021.09.555753