



Bioinformatic Evaluation of the miRNAs Targeting ACE2 Gene in COVID-19

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Abstract

Introduction: Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which began in late 2019 in Wuhan, China, has become a global epidemic. Angiotensin 2 converting enzyme (ACE2) acts as a receptor for host function to cause acute coronavirus 2 acute respiratory syndrome (SARS-CoV-2). ACE2 is abundantly expressed in different cells of different human organs. In human physiology, ACE2 is a major player in the renin-angiotensin-aldosterone (RAAS) system by degrading angiotensin II. Many factors have been associated with altered ACE2 expression and the severity and progression of COVID-19, including microRNAs that may be effective in it. Identifying pathological changes due to SARS-CoV-2 infection is important because it has major implications for understanding the pathophysiology of COVID-19 and developing evidence-based treatment strategies. Currently, many intervention strategies are being explored in ongoing clinical trials.

Objective: The aim of this study is to use bioinformatics databases to find potential antiviral therapies against SARS-CoV-2 through host microRNAs (miRNAs) that can reduce viral gene expression to inhibit virus entry and replication.

Methods: Using different algorithms in TargetScan, DIANA, ENCORI and miRWalk databases, the potential microRNAs were identified that target ACE2. Then, a score table was prepared from the candidate microRNAs, based on the affinity of the seed region of microRNAs and the 3'-UTR region of the ACE2 gene. Finally, microRNAs with higher scores were chosen as candidates for practical analysis.

Results: The results of Bioinformatical analysis showed that Has-miR-200c-3p, Has-miR-29a, Has-miR-29c, and Has-miR-942 are most likely to inhibit ACE2. These microRNAs are the most potent factors that might be affected on ACE2 during virulence.

Conclusion: It seems that ACE2 is under the control of the miR-200c-3p and plays a crucial role in the pathophysiology process. Therefore, this microRNA can be considered as a suitable new candidate for experimental evaluation.

Keywords: SARS-COV-2, COVID-19, ACE2, microRNA, miRNA

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), is associated with a high risk of acute respiratory distress syndrome and mortality. The symptoms of this disease vary significantly from rare asymptomatic cases, mild symptoms of the flu including high fever, to severe respiratory disease (1). In several reports of COVID-19 patients in China, a large number of severely ill elderly suffered from diseases

such as cardiovascular diseases, diabetes mellitus, or hypertension. The potential path of the transmission of the SARS-CoV-2 virus to the respiratory and gastrointestinal tract is through angiotensin-converting enzyme 2 (ACE2), which interfaces with the external environment. ACE2 is expressed in the alveolar epithelial cells of the lung, small intestinal epithelial cells, vascular endothelial cells, and smooth muscle cells, as well as the border of the proximal tubular cells of the kidney and moderately or poorly in parietal endothelial and ovarian

cells. It was found in all studied organs. ACE2 was also present in the epidermal layer of the skin and the mucous membranes of the mouth and the nose while ACE2 was not found in the lymph tissues and bile structures of the liver (2, 3).

ACE2 is encoded by a gene on chromosome XP22 and consists of at least 18 exons and 20 introns. Its genomic DNA size is approximately 40 kb. Further, ACE2 is a homologue of the ACE that plays a major role in the renin-angiotensin-aldosterone (RAAS), which involves the regulation of blood pressure and electrolyte homeostasis (4). The produced angiotensinogen by the liver is decomposed by renin to form angiotensin I (AngI). ACE is an enzyme that catalyzes the conversion of AngI to AngII (5), which is a major and active component of RAAS and exerts its effects primarily through type 1 AngII receptors (AT1R). The major effects of AngII include vasoconstriction, renal sodium reabsorption, potassium excretion, aldosterone synthesis, hypertension, and induction of pro-fibrotic pathways. ACE2 breaks down AngII into angiotensin, which has anti-inflammatory, vasodilator, and anti-fibrotic effects through binding to the Mas receptor. In addition, it breaks down AngI to angiotensin, which, in turn, is converted to angiotensin by ACE although this mechanism is usually of less physiological importance (6, 7). Moreover, ACE2 virtually neutralizes the physiological role of ACE, and the final effect of RAAS activation depends on the ratio of ACE/ACE2 tissues, which determines the availability of different angiotensin peptides and thus the balance between inflammatory, pro-fibrotic, anti-inflammatory, and anti-fibrotic pathways (8, 9).

Regarding the critical role of ACE2 in the entry of SARS-CoV-2 cells, the population of ACE2-expressing cells in the above-mentioned organs can potentially become infected, and this raises the therapeutic role by affecting the ACE2 gene. One of these strategies is to reduce the expression of ACE2 in the body, which prevents SARS-CoV-2 from entering the cell. In recent years, a new network of regulatory cycles at the mRNA level has been considered, including a class of non-coding RNAs called microRNA. microRNA is an endogenous, non-coding, small and single-stranded molecule with a regulatory role that is distributed throughout the mammalian genome, and the human genome can encode about 1000 species of microRNAs in eukaryotes with about 22-24 nucleotides long (10). Their main task is to adjust after transcription by interacting with mRNA and silencing the target gene. MicroRNAs are mostly the products of the internal regions of other genes and these genes are mainly transcribed by RNA polymerase II (11). According to the study results, the expression changes in microRNAs converge with the severity of disease progression, and therefore, microRNA changes can be considered a marker for determining the extent of disease, prognosis, treatment, and perhaps even

as a potential drug target.

Today, identifying the presence of mi-RNA in specific tissues or the relationship between each mi-RNA and specific diseases and genes is possible using techniques such as northern blotting, microarray, and real-time polymerase chain reaction (12). Among the mentioned techniques, Microarray has the ability to provide a complete expression profile for each mi-RNA. Hence, a microarray is used as a standard method for the general study of genes affected by miRNAs (13). However, due to the extremely high cost of the device and micro slides and the need for operator expertise, its use is impossible in many scientific centers, especially in developing countries. On the other hand, with the development of bioinformatics algorithms and computer modeling systems, bioinformatics methods have been expanded with the ability to predict the targets of mi-RNAs. Thus, for this purpose, the use of bioinformatics software saves time and cost, and identifies miRNA targets. There are several computer programs with different algorithms for predicting mi-RNA binding targets. The commonly applied point in all these software is based on the complementarity of the SID region in mi-RNA with the target mRNA and the calculation of the thermodynamic stability of the bond between mRNA and mi-RNA. However, the algorithms for calculating the mentioned criteria in each software are different and specific. Hence, the purpose of this study was to investigate the effect of different miRNAs on the ACE2 gene in the process of the virus entering the cell using different and specific software. To this end, it was attempted to identify miRNAs that have the highest potential for the repression of this gene based on modeling data by various algorithms to identify the most likely role of miRNA in practice and through various specific tests (Figure 1).

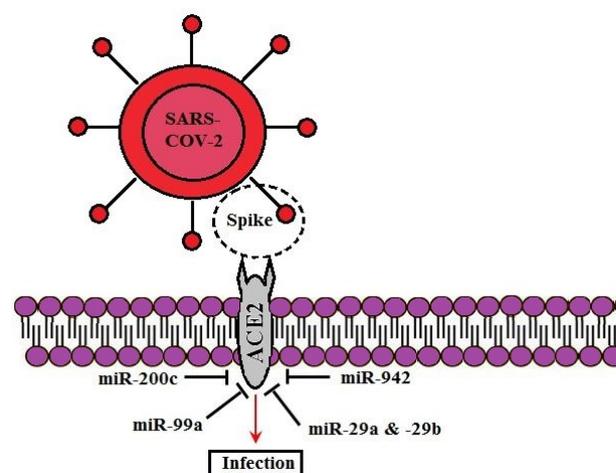


Figure 1. A model for the process of SARS-CoV-2 entering host cells in the lungs and attacking other organs and the role of microRNAs in controlling it.

Materials and Methods

Bioinformatics Analysis of Various miRNA Targets on the ACE2 Gene

This study is based on the bioinformatics theory. At this stage, using different available bioinformatics algorithms in TargetScan, Diana, miRWalk, and StarBase databases, different miRNAs on the ACE2 gene were investigated, and then a scoreboard was prepared from the candidate miRNAs. In this way, miRNAs were scored based on variables such as the binding strength of the nucleus region related to miRs and the number of repeats of the target region in the 3'UTR part of the gene. Finally, miRNAs, which have the highest score and are common among the studied databases, were selected as candidates for a practical review.

Results

ACE2 Gene Analysis at TargetScan Database

TargetScan (<http://www.TargetScan>) is one of the applied software for predicting the targets of mammalian miRNAs. The software identifies seven or eight nucleotide regions that complement the mi-RNA nucleus region and investigates its thermodynamic stability (the investigation of secondary structures and the intermolecular and intramolecular interactions of nucleic acid). In this site, the predicted targets are ranked based on a factor called protected targeting probability, which indicates the possibility of targeting a region protected by a particular miRNA. In other words, Pct represents that it is not connected by chance, and the value of this factor is between 0 and 1 as a better measure of specific and correct miRNA binding to its target region (13). According to the results of different miRNAs on the ACE2 gene, miRNAs listed in Table 1 are among the most protected ones with the highest score as the main possible targets in this database for binding to this gene in addition to suppressing its expression and preventing the virus entering the cell.

ACE2 Gene Analysis in DIANA-MicroT-CDS Database

This webserver (<http://www.microrna.gr/microT-CDS>) is dedicated to the purpose of predicting and analyzing miRNA function and has been widely used in the scientific community since its initial launch in 2009. DIANA-microT-CDS is a new version of microT server that has been significantly improved using the predicted algorithm. In this database, the algorithm for identifying mRNA targets is based on several parameters that are

separately calculated for each miRNA. The scores of the protected and unprotected regions are then combined to provide the total score that indicates changes in target mRNA expression. Examples of the parameters studied in this database are the total miTG score (in fact, the final prediction score) and the threshold score. The higher miTG values indicate that the criterion prediction is closer to reality. Table 2 presents the possible predicted

Table 2. . miRNAs studied in DIANA Tools - MicroT-CDS for ACE2 (ENSG00000130234)

	miRNAs	miTG score
1	hsa-miR-3908	0.970948070950572
2	hsa-miR-4773	0.960097858524034
3	hsa-miR-4520-2-3p	0.929849254721604
4	hsa-miR-3065-5p	0.926768264357135
5	hsa-miR-632	0.923676991174487
6	hsa-miR-3529-3p	0.922569267082663
7	hsa-miR-362-5p	0.914304513088346
8	hsa-miR-7850-5p	0.909573464332962
9	hsa-miR-4668-3p	0.904346472767298
10	hsa-miR-4270	0.900162992376378
11	hsa-miR-4288	0.888228287378736
12	hsa-miR-6755-3p	0.882158428573902
13	hsa-miR-8063	0.880486276831588
14	hsa-miR-4666a-3p	0.877683906719009
15	hsa-miR-500b-5p	0.873786900387578
16	hsa-miR-1305	0.873114044684197
17	hsa-miR-421	0.867896854094691
18	hsa-miR-7110-3p	0.85927935631591
19	hsa-miR-3909	0.858910411194038
20	hsa-miR-942-5p	0.839640344317192
21	hsa-miR-6515-3p	0.837571937832175
22	hsa-miR-574-5p	0.83572516838789
23	hsa-miR-4729	0.833309772500181
24	hsa-miR-6873-3p	0.828347129973172
25	hsa-miR-345-3p	0.827726577654248
26	hsa-miR-6852-3p	0.822009223488199
27	hsa-miR-4463	0.821861524092668
28	hsa-miR-2113	0.817723806405707
29	hsa-miR-200c-3p	0.811147785562637
30	hsa-miR-3658	0.809914802460576

Table 1. miRNAs studied in targetscan software

miRNAs	Position in the UTR	seed match	conserved branch length	Pct
hsa-miR-200b-3p	179-185	7mer-m8	3.164	0.40
hsa-miR-200c-3p	179-185	7mer-m8	3.164	0.40
hsa-miR-429	179-185	7mer-m8	3.164	0.40

Watson and Crick's rule, and when a 7-nucleotide region of the nucleus is complemented with a target, that region extends from both ends until reaching a mismatch. After reaching the mismatch, the elongation is stopped, and finally, the results are analyzed, the seven complementary nucleotide regions are shown based on the binding site and the number of the involved open pairs, and the results are obtained based on a specific score. Table 3 provides some of the obtained miRNAs from this database with the highest scores.

According to the bioinformatics study, which is summarized as Venn diagram (Figure 2), Has-miR-200c-3p, Has-miR-29a, Has-miR-29c, and Has-miR-942 are most likely to inhibit these receptors and affect their downstream. In addition, the role of these common microRNAs in various cancers and their association with COVID-19 (Table 4) represents their importance. The prediction of the optimal secondary structure of these common microRNAs obtained with the least free energy was designed by RNAfold webserver (from the Vienna package) available at <http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi>, the result of which is shown in Figure 3.

Discussion

ACE2, which is a negative regulator of angiotensin II in the renin-angiotensin system, has been reported to have a necessary role in acute lung injury (ALI). The down-regulation of ACE2 is always related to ALI or ARDS induced by the avian influenza virus, coronavirus, severe

acute respiratory syndrome, respiratory syncytial virus, and sepsis. However, the molecular mechanism of the decreased expression of ACE2 in ALI is unclear (14, 15). Although these microRNAs all serve as the highest-scoring targets among the four databases, the order of results varies slightly between different databases largely due to various algorithms and minor pattern changes in scoring among these bases. As reported by previous studies, angiotensin-converting enzyme inhibitors, which reduce the production of Ang II and Ang II receptor blockers blocking the function of Ang II, do have some beneficial effects on pneumonia-related clinical outcomes (16, 17). The inhibition of miR-200c-3p may also produce a positive clinical outcome for pneumonia. In addition, previous research reported that the miR-200c-3p level was positively related to the severity of interstitial lung disease (18). Nonetheless, further studies are needed to explore whether the plasma levels of miR-200c-3p or Ang II can serve as a biomarker or a therapeutic target for severe pneumonia. In recent years, miRNA-based strategies for exploring novel therapeutic drugs have experienced rapid developments. Several miRNA-targeting drugs have now entered clinical testing or are even close to gaining access to markets (19).

Lu et al investigated the effect of miR-200c on the ACE2 gene in mouse cardiomyocytes and concluded that miR-200c could regulate ACE2 expression in heart cells. They further stated that miR-200c-based treatment may help you as long as an effective COVID-19 vaccine is available worldwide (20), which is consistent with our study results.

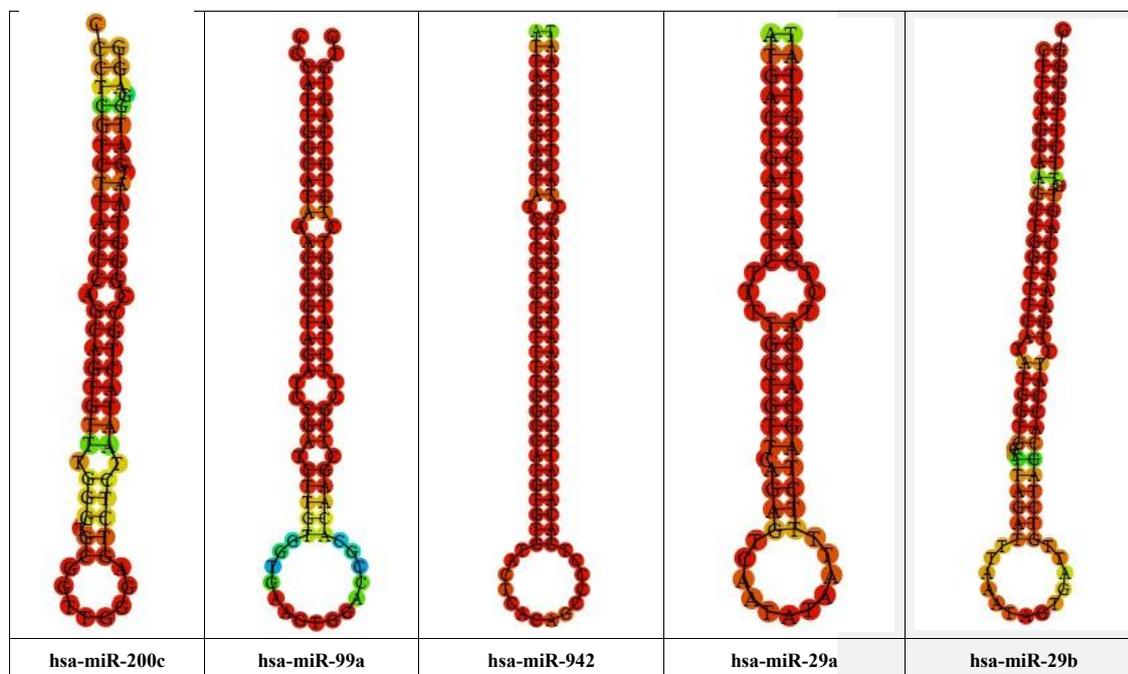


Figure 3. Prediction of Optimal Secondary structure of the hsa-miR-200c, hsa-miR-99a, hsa-miR-942, hsa-miR-29a, hsa-miR-29b (EPS format) with using the Rfold web server.

Table 4. Summary of common microRNAs obtained

microRNAs	Role in Covid	Cancers	Sequence	Location	Length	Accession N.
miR-200c	acute respiratory distress syndrome, Remodeling and fibrosis	Bladder cancer, Breast cancer, Colorectal cancer, Endometrial cancer, Esophageal cancer, Gastric cancer, Head and neck cancer, Leiomyomas, Liver cancer, Lung cancer, Lymphoma, Melanoma, Oral squamous carcinoma, Ovarian cancer, Pancreatic cancer, Prostate cancer, Renal cancer, Thyroid carcinoma, Wilms tumor, Prolactinoma cancer, Malignant pleural mesothelioma	5'CCCTCGTCTTACCACAGAGTGTGGGTGCGGGTTGGGAGTCTTAATACTGCCGGGTAATGATGAGGG3'	12p13.31	68	MIMAT0000617
miR-99a	Relate to Respiratory system	bladder cancer, gastric cancer, ovarian cancer, nasopharyngeal carcinoma, Breast cancer, retinoblastoma, Prostate cancer, cervical cancer, hepatocellular carcinoma, Renal cancer, Oral cancer, Endometrial carcinoma, squamous cell carcinoma, non small cell lung cancer, Thyroid cancer, cholangiocarcinoma, Osteosarcoma	5'CCCAATGGCATAAACCCGTAGATCCGATCTTGTGGTGAAGTGGACCGCACAGCTCGCTTCTATCGGTCTGTGTCAAGTGTG3'	21q21.1	81	MIMAT0000097
miR-942	Relate to Respiratory system	colorectal cancer, Hepatocellular carcinoma, breast cancer, non small cell lung cancer	5'ATTAGGAGAGTATCTCTCTGTTTGGCCATGTGTACTCACAGCCCTCACACATGGCCGAAACAGAG AAGTACTTTCTTAAT 3'	1p13.1	86	MIMAT0004985
miR-29	Respiratory system, Promotion of fibrosis and cardiovascular remodeling	Lung cancer, Non-small cell lung cancer, Lung adenocarcinoma, Esophageal carcinoma, Stomach cancer, Hepatocellular carcinoma, Cholangiocarcinoma, Colon cancer, Glioblastomas, Osteoblastoma, Rhabdomyosarcoma, Neuroblastoma, Bladder cancer, Prostate cancer, Renal carcinoma, Ovarian cancer, Endometrial cancer, Breast cancer, Basal cell carcinoma, Nasopharyngeal carcinomas, Malignant pleural mesothelioma	5'ATGACTGATTTCTTTGGTGTTCAGAGTCAATAATAATTTTCTAGCACCATCTGAAATCCGTTAT3'	7q32.3	64	MIMAT0000086

Similarly, Stewart et al found that miR-200 is an established regulator of epithelial-to-mesenchymal transition EMT and directly regulates ACE2 expression in SARS-CoV-2 disease (21). In another study, Liu et al indicated that the inhibition of miR-200c-3p ameliorated the ALI induced by H5N1 virus infection *in vivo*, representing a potential therapeutic target. Therefore, they identified a shared mechanism of viral and bacterial lung infection-induced ALI/ARDS via the NF- κ B-dependent upregulation of miR-200c-3p to reduce ACE2 levels, leading to increased angiotensin II levels and thus lung injury (22).

By assuming that the overexpression of coronavirus-specific microRNAs in the lung epithelium may protect against infection and virus spread while low expression may cause susceptibility to infection, Chow et al reported that miR-29b significantly increased expression in SARS-CoV-2-infected Calu3 cells (23), which corroborates with the results of our bioinformatics studies. Peng et al also investigated several microRNAs in influenza patients and considered miR-29a with diagnostic value in this disease (24). By investigating the profiles of differently expressed miRNAs during H1N1 and H5N1 infection, they concluded that a subset of four miRNAs (i.e., miR-141, miR-200c, miR-21-3p, and miR-29b-1) for self-regulating was compatible during influenza A virus infection. Interestingly, they stated that avian influenza could regulate cellular miRNAs more strongly (25), which may also be possible for the SARS-CoV-2 virus. Samad et al also found that ACE2 is the key to understanding the mechanism of SARS-CoV-2 infection, noting the importance of has-mir-26b-5p and miR-99a-5p in this pathway in studies on many miRNAs (26).

Likewise, Jafarnejad-Farsangi et al demonstrated that the miR-29 family had the most binding sites (11 sites) on the SARS-CoV-2 genome (27). Direct binding of miR-29a to the 3' UTR region of the human immunodeficiency virus (HIV) genome increased the transport of virus to p-bodies and reduction of HIV replication. Ahluwalia et al also reported that the inhibitory impact of miR-29a on HIV infection is mediated through binding to the accessory viral protein negative factor (Nef), which is critical for viral persistence and release (28). Therefore, miR-29a has been considered as a potential therapeutic target for HIV eradication (29). According to the results of the present study, miR-29 binding sites were predicted in ACE2. Considering the high levels of miR-29s in the lungs of healthy adults and the better response of these people to SARS CoV-2 compared to those with respiratory diseases with low levels of miR-29s, some studies suggested the probable role of miR-29s in modulating SARS-CoV-2 infection (27, 30).

According to recent research, miR-99a regulates cell growth and cell cycle progression by targeting mTOR, AKT1, and FGFR3 (31). However, the underlying mechanisms involved in the modulation of invasion and migration by miR-99a remain elusive. Many studies

have reported the role of miR-99a in lung cancer (32-35). Additionally, Lin et al demonstrated that the miR-99 family promotes hepatitis B virus replication post-transcriptionally through IGF-1R/PI3K/Akt/mTOR/ULK1 signaling-induced autophagy (36).

Interestingly, no miR-942-related study has focused on SARS-CoV-2. One limitation of our study is that it focused on microRNAs expression. There may exist factors which function at the posttranscriptional level on ACE2. Moreover, Srivastava et al have recently indicated that SARS-CoV-2 infection induces alterations in the posttranscriptional regulatory networks in human tissues through the function of RNA-binding proteins and micro-RNAs (37). Eventually, another limitation of this study is the lack of any investigation on the effect of these microRNAs on upstream and downstream regulatory factors of the ACE2 gene.

Conclusion

The results of our study indicated that a single microRNA can directly regulate at least one important cellular pathway that includes the alteration of ACE2 expression levels. Although this work represents preliminary findings *in silico*, further studies are required to assess the implications of such findings *in vivo*. The potential effect of the reported large-scale microRNA changes and presents a daunting prospect from a therapeutic viewpoint by the extension of the alteration to mRNA and protein targets. This study and previous work indicated that the miR-200c degrades only a small number of its predicted targets although the question remains how many of the remaining target predictions are translationally regulated by miR-200c.

Finally, as a diagnostic and interventional approach, miRNAs can be used to identify regulatory networks for new and unconventional therapeutic targets against SARS-CoV-2 infection. In addition, the use of anti-sensor oligonucleotides to prevent the entry of the viral genome is considered as the most important way to achieve new therapeutic strategies. In this study, potential miRNAs, which may be involved in controlling SARS-CoV-2 entry by regulating ACE2 gene expression, were investigated and summarized using miR-related databases. Ultimately, it is believed that the results of the present study are a first step in directing attention to miRNAs as critical factors in the development of diagnosis, biomarkers, and therapeutic targets for COVID-19.

Conflict of Interests Disclosure

The authors declare that they have no conflict of interests.

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conclusions of this paper.

Ethical Statement

Not applicable.

Authors' Contributions

MR wrote the manuscript. AR, MA, SK, and AN collected the data, revised the literature, and contributed to the conception and design of the study. Eventually, all authors contributed to the critical revision, edition, and final approval of the manuscript.

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Informed Consent

Not applicable.

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