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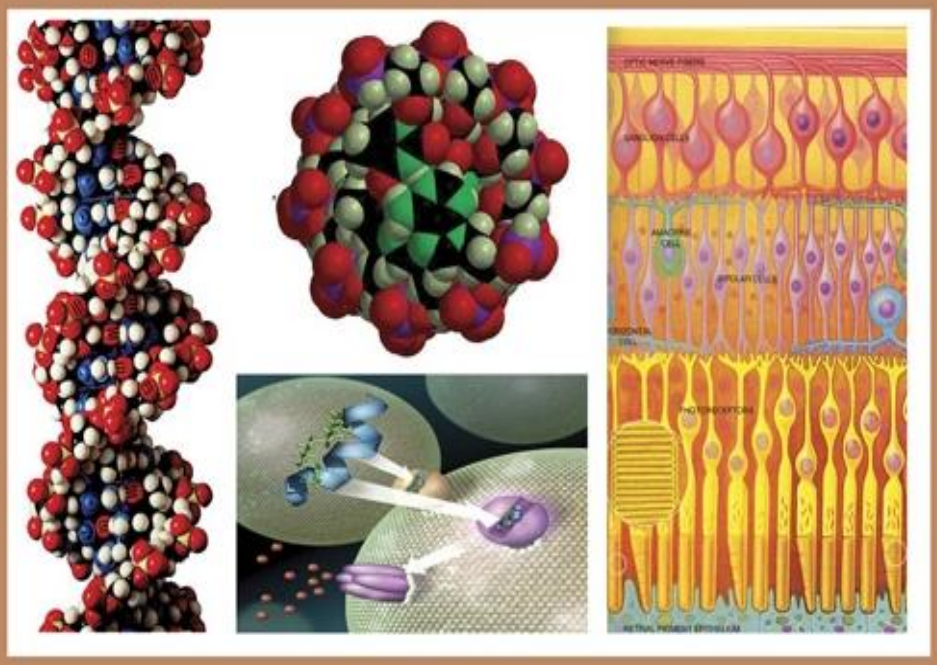

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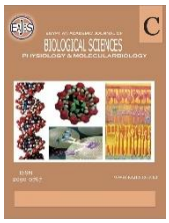

Bioinformatics Analysis of has-miR-181a in some Covid-19Vaccinated People in Al-Najaf Province

Mohammed. K. S. Alquraishi[1] and Mohammad Alzeyadi[2]
[1]Department of Pathological Analyses, Faculty of Science, University of Kufa.
[2]Department of Biology, Faculty of Science, University of Kufa.
***E-mail:** mohammedk.alquraishi@uokufa.edu.iq ; mohammed.mhawish@uokufa.edu.iq

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ABSTRACT

The angiotensin-converting enzyme 2 (ACE2) is an enzyme of the renin-angiotensin-aldosterone system (RAAS), which catalyzes the conversion of some other angiotensin. ACE2 is widely expressed in different tissues. Many researchers reported many microRNAsinvolved in ACE2 expression, hsa-mir-181a one of these microRNAs which involved in ACE2 expression.

In this study, we develop a comparative method (miR-Explore) to demonstrate that clustering of vaccinated miRNAs hsa-mir-181a of gene ACE2- alters the sensitivity and specificity of the prediction method even when a simple direct alignment of the positional secondary structure is used. The basic idea of the current approach was to generate a predictive visualization of the miRNAs hsa-mir-181a consensus structure for each vaccinated and unvaccinated miRNAs hsa-mir-181a class and use this consensus structure to perform alignment with the query sequence A set of data was taken from pre-miRNA by extracting total RNA and converting it to cDNA, then reading the DNA sequence and following up the in situ secondary structure of these sequences by means of dedicated software programs and websites and comparing them with each other and with sequences available in the microRNA database website.

INTRODUCTION

Coronaviruses are nonsegmented, enveloped, positive-sense, single-strand ribonucleic acid viruses, belonging to the Coronaviridae family (Weiss and Leibowitz 2011). Six types of coronavirus have been identified that cause human disease: four cause mild respiratory symptoms, whereas the other two, Middle East respiratory syndrome (MERS) coronavirus and severe acute respiratory syndrome (SARS) coronavirus, have caused epidemics with high mortality rates(Wang *et al.,* 2020). The virus binds to angiotensin-converting enzyme 2 (ACE2) receptors present in vascular endothelial cells, lungs, heart, brain, kidneys, intestine, liver, pharynx, and other tissue.

The mechanism for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection requires the binding of the virus to the angiotensin-converting enzyme 2 (ACE2) receptor (Jia *et al.*, 2005). It has been reported that ACE2 is the main host cell receptor of 2019-nCoV and plays a crucial role in the entry of the virus into the cell to cause the final infection (Xu *et al.*, 2020).

MicroRNA (miRNAs) is small endogenous RNAs that regulate gene expression post transcriptionally. MiRNA research in allergy is expanding because miRNAs are crucial regulators of gene expression and promising candidates for biomarker development small non-coding RNA sequences, known as microRNAs (miRNAs/miR-), may present a novel antiviral strategy. We can take advantage of their ability to modulate host-virus interactions through mediating RNA degradation or translational inhibition. Investigations into miRNA and SARS-CoV-2 interactions can reveal novel therapeutic approaches against this virus.

Many researchers reported many microRNAs involved in ACE2 expression, hsa-mir-181a one of these microRNAs which involved in ACE2 expression. Some studies also indicate that the family of the coronavirus reached its claws to the skin of genes and gene expression (Alquraishi and Alzeyadi 2022), in this study, we will discuss the problems related to the anti-Covid19 vaccine and its repercussions on the microRNA, specifically hsa-mir-181a ACE2 for receiving infection. The scientific importance of this study is to extract moral evidence of the effect that the vaccine has on the microRNA, which is related to changing its shape and thus its genetic expression, its effectiveness, or its natural function. Investigations into miRNA and SARS-CoV-2 interactions can reveal novel therapeutic approaches against this virus. But in this study, we will investigate the effect of the anti-Covid19 vaccine on the microRNA, specifically hsa-mir-181a ACE2 for receiving infection. In people who received the vaccine and compared them with people who did not receive the vaccine.

Several biochemical and structural studies have shown that SARS-CoV-2 binds the human receptor for angiotensin-converting enzyme 2 (ACE2) (Wrapp *et al.*, 2020).The surface viral protein spike, membrane, and envelope of coronavirus are embedded in host membrane-derived lipid bilayer encapsulating the helical nucleocapsid comprising viral RNA (Fig. 1) (Finlay, See and Brunham, 2004).

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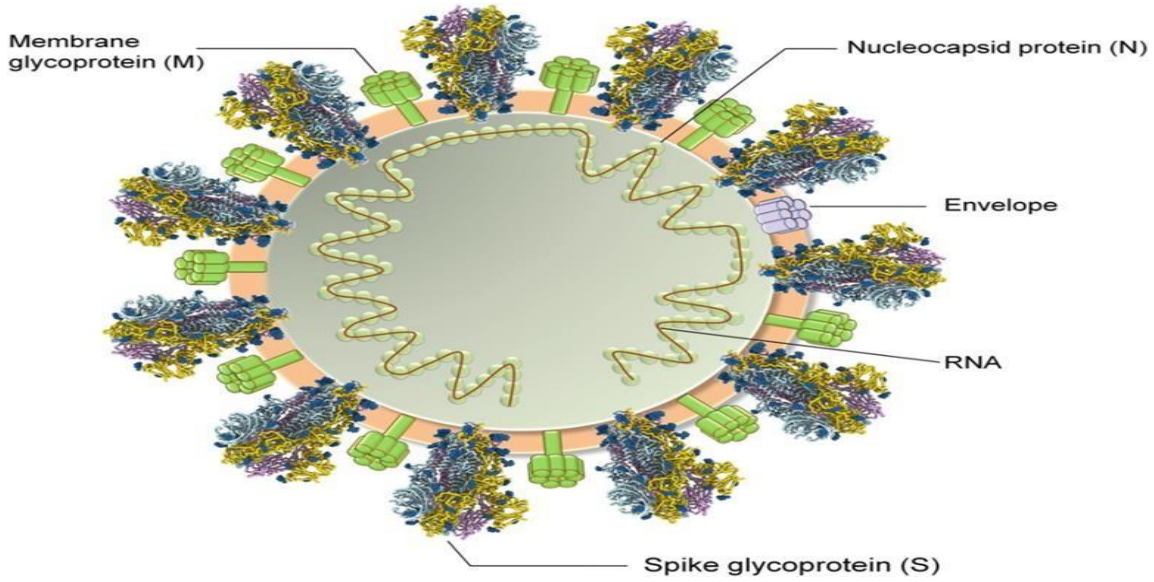

Fig. 1 Structure of SARS-CoV-2 has surface viral proteins, namely, spike glycoprotein (S), which mediates interaction with cell surface receptor ACE2. The viral membrane glycoprotein (M) and envelope (E) of SARS-CoV-2 are embedded in a host membrane-derived lipid bilayer encapsulating the helical nucleocapsid comprising viral RNA. (Finlay, See, and Brunham 2004).

Bioinformatics analyses of non-coding RNAs and transcripts in human and rodent muscle during aging have identified miR-181a as a potentially key regulator of muscle mass and function during aging (Soriano-Arroquia *et al.,* 2016). The miR-181 family of miRs includes four miRs in humans and rodents: miR-181a, b, c and d. miR-181a and b are clustered together at two genomic loci on chromosomes 1 and 9 and miR-181c and d are clustered on chromosome 19 in humans.

The COVID-19 virus is traced to death in non-survivors, and the longest virus shedding of 37 days has been observed in survivors (Zhou e*t al.,* 2020). It shows the severity of COVID-19 and its high infection rate, especially in elderly and immune-compromised or weak immunity Individuals. Several pharmaceutical R&D units and researchers are working to develop a vaccine for the SARS-CoV-2 pandemic Researchers are putting all efforts to find a solution for combating this novel coronavirus. Researchers across the globe are trying to develop SARS- CoV-2 vaccine by using approaches like whole virus vaccine, antibody vaccine, DNA vaccine, recombinant protein subunit vaccine, and mRNA vaccine. Currently, there is no approved vaccine available for SARS-CoV-2. Recently, mRNA 1273 investigational vaccine has been developed by NIAID scientists in collaboration with the biotechnology company Moderna, Inc., based in Cambridge, MA.

MATERIALS AND METHODS

Sampling:

During this study, fifty cases of females and males were examined as 25 vaccinated volunteers and 25 unvaccinated volunteers from the Najaf Governorate who were not previously infected with COVID-19. The volunteers ranged in age from 18 to 60 years. The volume of blood in each sample was 3 milliliters of blood collected in a tube containing EDTA and used to extract the total RNA**.**

Total RNA Extraction:

Extracted RNA from whole blood by using the Solarbio Total RNA Extraction Kit Mini as described by the manufacturer. RNA was used for the cDNA preparation.

Quantitative Real-time Polymerase Chain Reaction (PCR):

Total RNA was reverse transcribed into cDNA using Taqman reverse transcription reagents Universal Reverse Transcription Kit (M-MLV). All quantitative PCRs were done with SYBR Green Master Mix (Qiagen). We used primers that were described by (Tomlins *et al.,* 2009).And are specific for the fusion (hsa-mir-181a- forward F = AGAAGGGCTATCAGGCCACC and reverse R=TCCCGATAGTCCGGTGGAA T.hsa-mir-181a primers. Forward and reverse primers (10 µmol) were used and procedures were done according to the manufacturer's recommended thermocycling conditions**.** the program of PCR amplification was an initial denaturation step at 94°C for 5 min, followed by denaturation at 94°C for 30 sec, annealing at 52°C for 15 sec and extension at 72°C for 15 sec. The amount of each target gene relative to the housekeeping gene ACE2- hsa-mir-181a for each sample was determined using the comparative threshold cycle method (Applied Biosystems User Bulletin 2).

Bioinformatics Method for Analysis of Sequences of miRNA:

The Vienna RNA Webserver was used for a comprehensive collection of tools for folding, designing and analysis of RNA sequences. It provides a web interface to the most commonly used programs of the Vienna RNA package. Among them, we find folding of single and aligned sequences, prediction of RNA–RNA interactions, and design of sequences with a given structure. Additionally, we provide analysis of folding landscapes using the barriers program and structural RNA alignments using LocARNA. The web server together with

software packages for download is freely accessible at http://www.tbi.univie.ac.at. The server provides programs, web services, and databases, related to our work on RNA secondary structures .For general information and other offerings. In addition, the RNAalifold server RNAalifold predicts the consensus structure of a set of aligned DNA or RNA sequences.

Statistical Analysis:

The GraphPad Prism 7 Software Inc. software was used to detect the effect of difference factors in study parameters least significant difference – t-test was used to significantly compare between means in this study, and P-values less than 0.05 were considered statistically significant.

RESULTS

This research was done to examine fifty cases of females and males 25 volunteers vaccinated and 25 volunteers unvaccinated who are from Najaf Governorate, all of whom were not previously infected with COVID-19. The ages of volunteers were expressed as mean ± standard deviation. They were found to be 48.4 ± 10.9 years, with a minimum to a maximum of 60-18 years. Total RNA was extracted from fresh blood prepared by volunteers who were vaccinated and unvaccinated as described previously. The concentration of extracted RNA was measured by Biophotometer (Fig. 2). Results (Mean±SD) exhibited a level of 55.5 ± 13.61 µg/ml. The purity of the extracted RNA was estimated by measuring the ratio of A260/A280. It was found to be 1.93 ± 0.04 suggesting an appropriate purity.

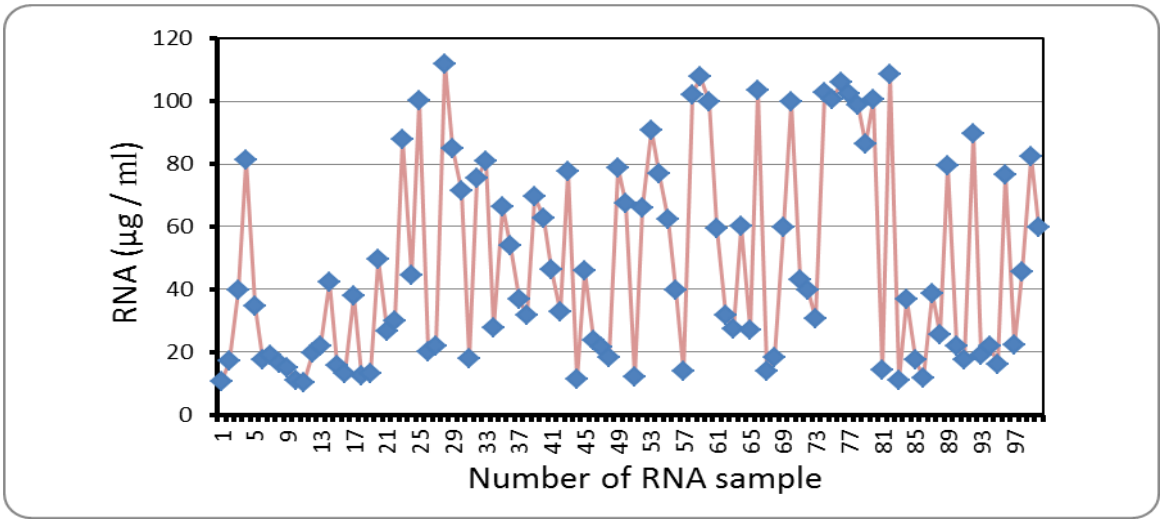

Fig. 2: Concentration of extracted mRNA measured by Bio photometer.

Extracted RNA from blood samples was converted to cDNA. The products were amplified with the use of two levels (5 and 10 pmole) of the designed primers. Both levels were observed to amplify the cDNA successfully as shown in (Figure 3) primer level of 10 pmole was considered to be the best. The specificity of the designed primer was confirmed through the monitoring of the melting curve obtained (Fig. 3).

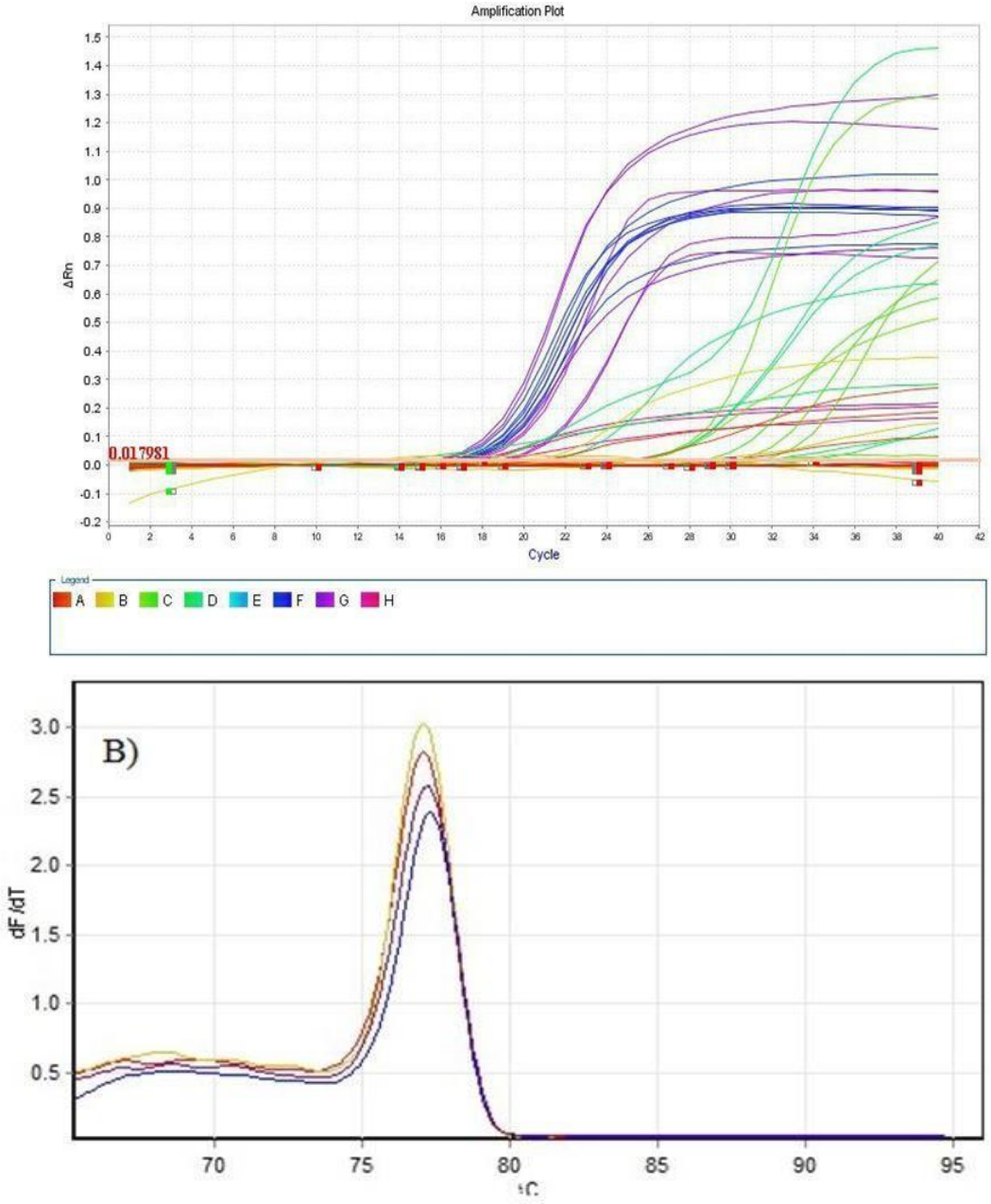

Fig. 3: A) Validation of the concentration of the specific primer for hsa-mir-181a primers; **B)** Melting curve measuredas -□F/□T versus temperature (°C) of FOXM1 gene, with two different levels, (5, 10 pmol).

PCR product of cDNA of 50 test samples was sent to the business macro gen (Koria). We opted to eliminate 40 samples 20 vaccinated and 20 unvaccinated from our study whilefor the other samples (10), we did not obtain a regular DNA sequence, perhaps due to contamination or lack of concentration by PCR amplification (Fig. 4).

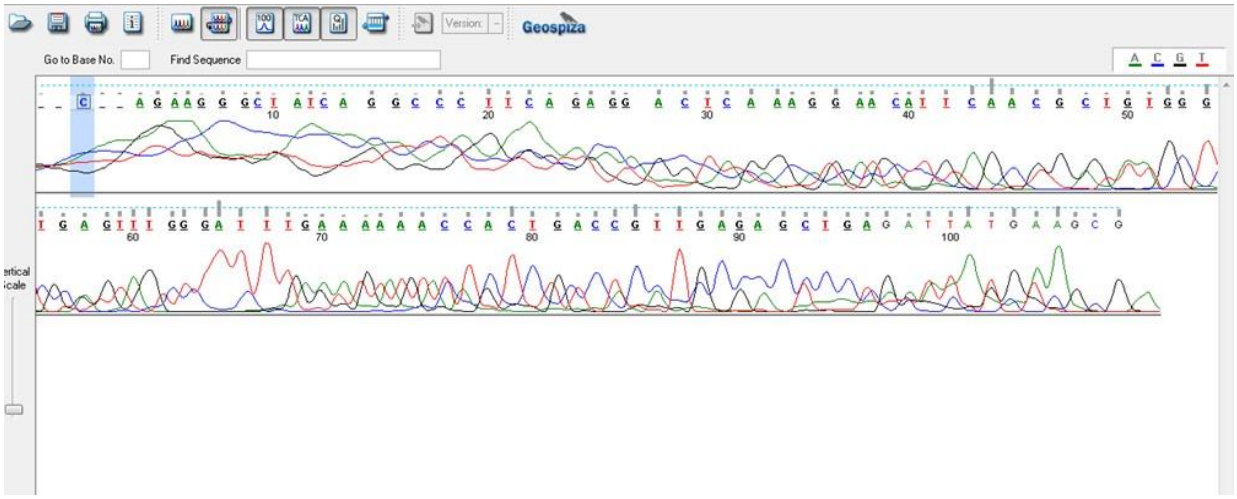

Fig. 4: shows the PCR product of cDNA of sequencing of hsa-mir-181a ACE2 by macrogen (Koria).

The Vienna RNA Webserver was used for Drawing the secondary structure of the samples taken and comparing them with the wild type, where the results showed a clear difference in the secondary structure of the vaccinated samples, as in the (Fig. 5), which represents the first sample of vaccinated, and also in the (Fig. 6) that represents sample No. 12 of the vaccinated, while no clear difference appeared between the unvaccinated samples compared to the wild type, as in the (Fig. 8) that represents The first sample of the unvaccinated and also in the (Fig. 9) that represents sample No. 11 of the unvaccinated. The RNA alif old server was used to draw the multiple sequences for a complete set of samples. It showed the secondary structure of all vaccinated samples (micro-RNA), as in the (Fig. 7), and also the secondary structure of all unvaccinated samples (micro-RNA), as in the (Fig 10).

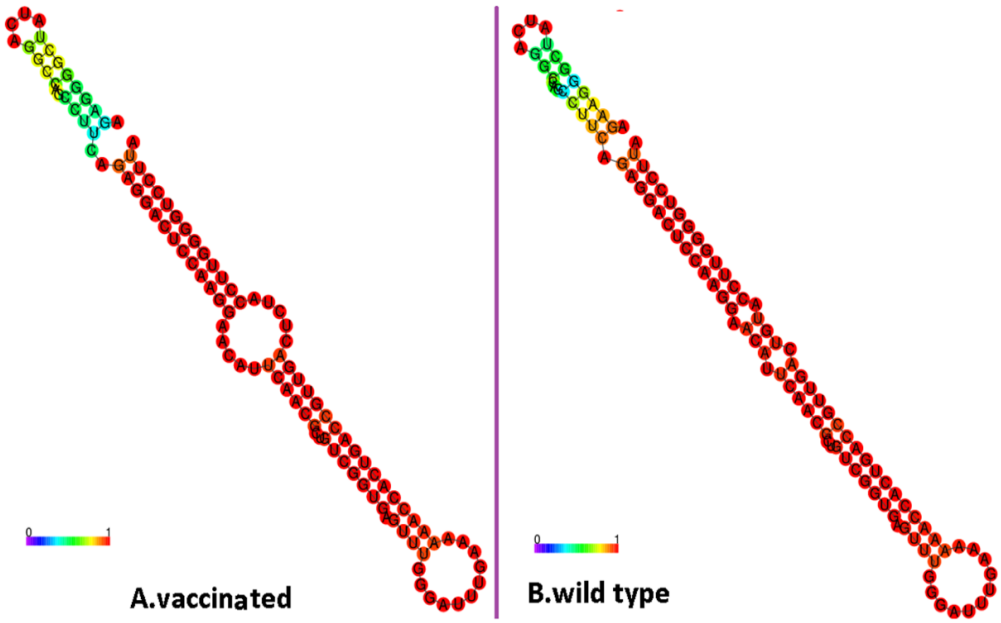

Fig. 5: The Graphical drawing of the secondary structure of hsa-mir-181a ACE2 sample 1 of vaccinated.

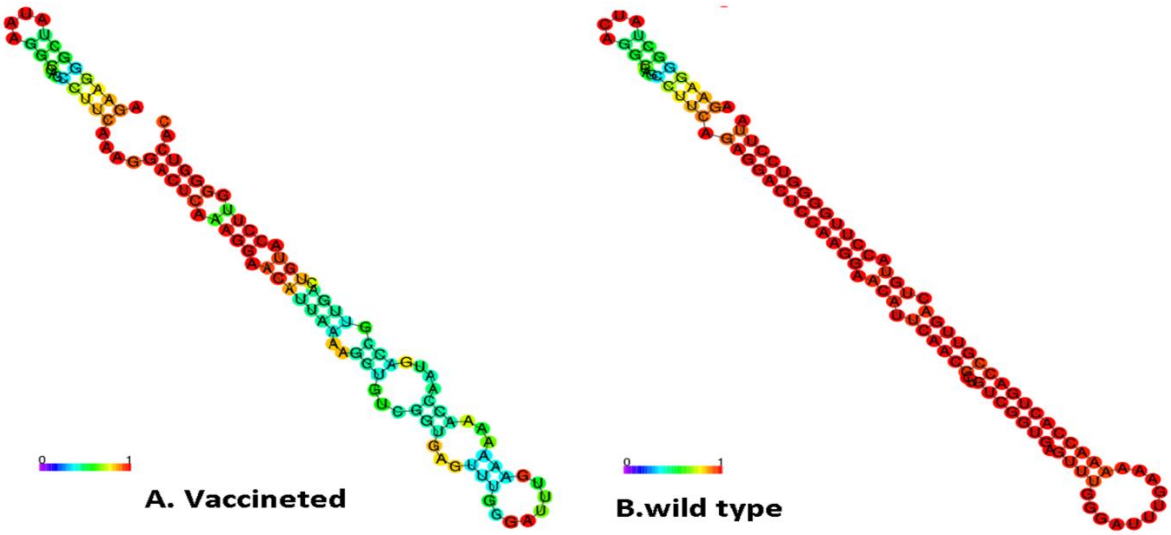

Fig. 6: The Graphical drawing of the secondary structure of hsa-mir-181a ACE2 sample 12 of vaccinated.

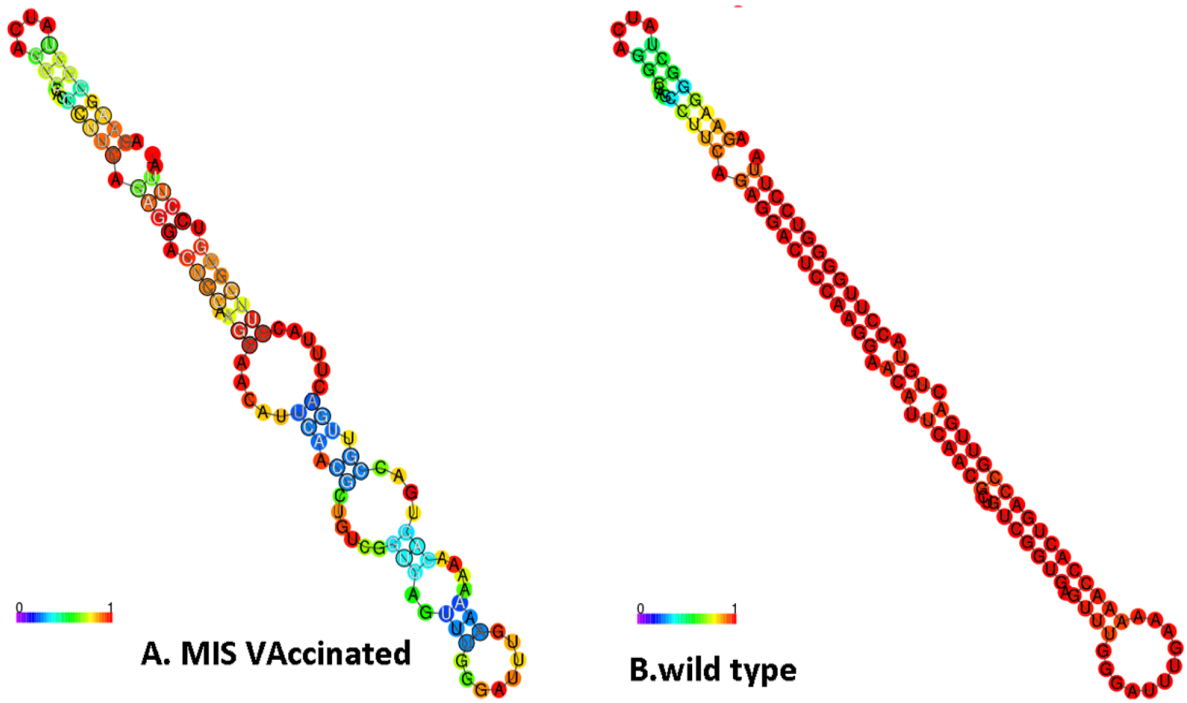

Fig. 7: The Graphical drawing of the secondary structure of hsa-mir-181a ACE2 the multiple sequence alignment of all micro-RNA sequences of 20 vaccinated samples.

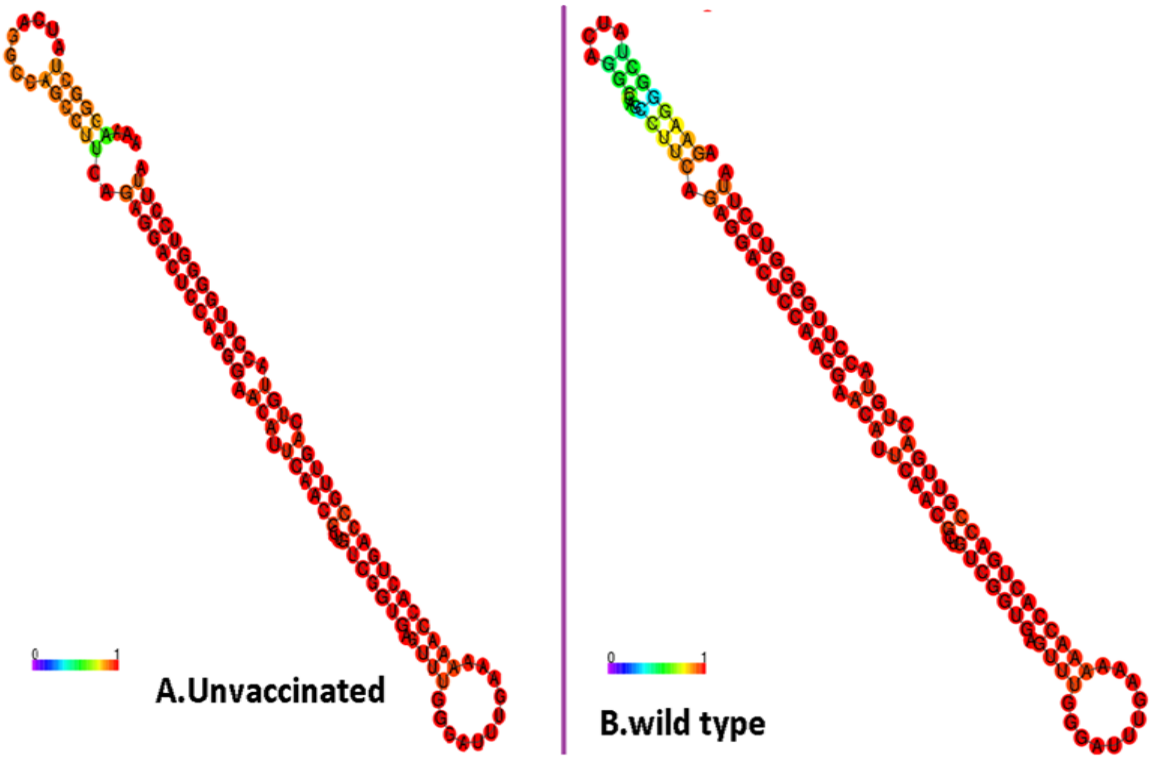

Fig. 8: The Graphical drawing of the secondary structure of hsa-mir-181a ACE2 sample 1 of unvaccinated.

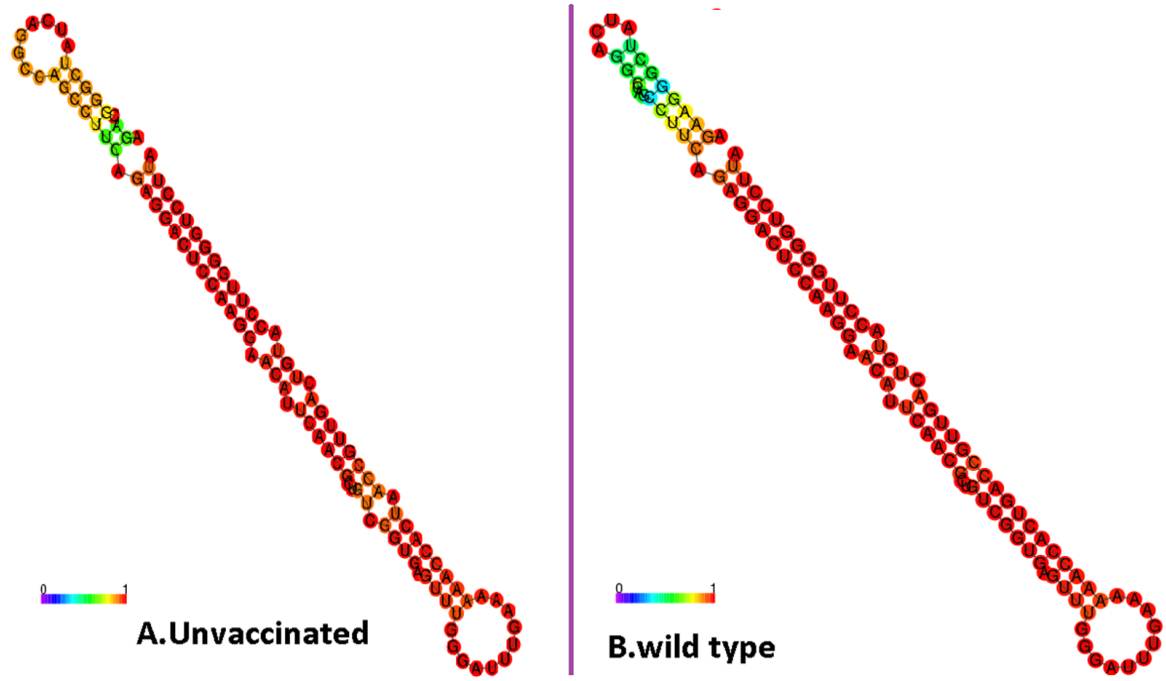

Fig. 9: The Graphical drawing of the secondary structure of hsa-mir-181a ACE2 sample 11 of unvaccinated.

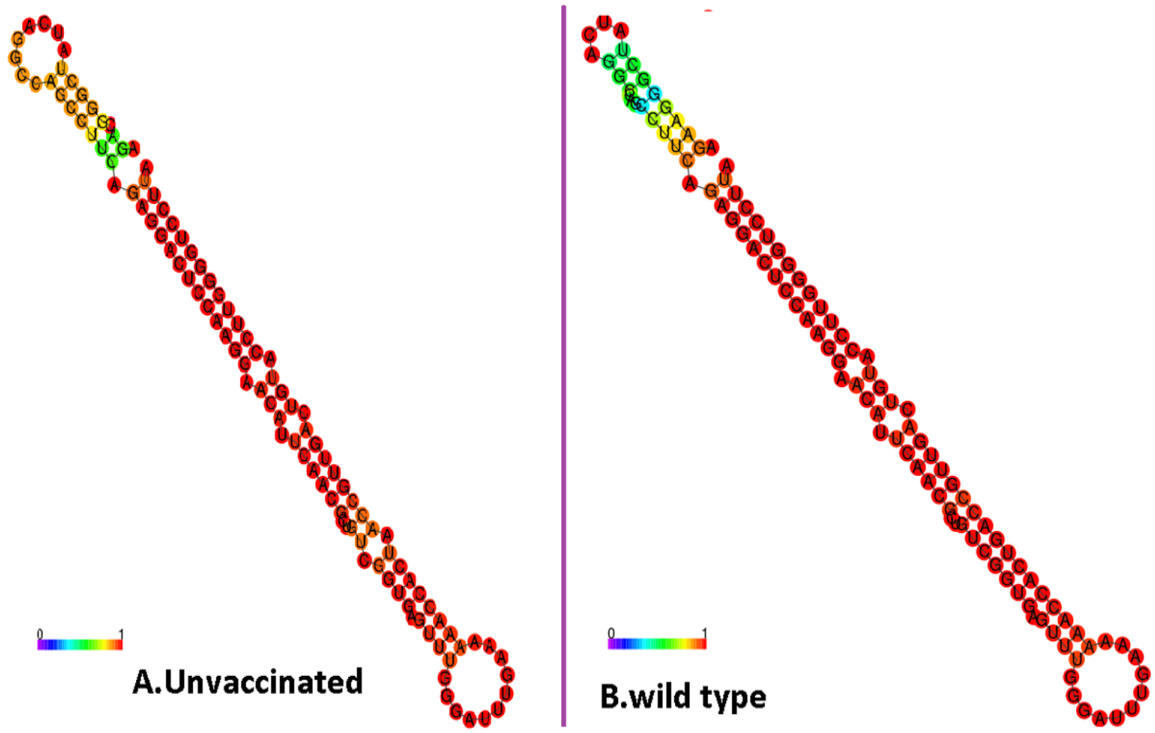

Fig. 10: The Graphical drawing of the secondary structure of hsa-mir-181a ACE2 the multiple sequence alignment of all micro-RNA sequences of 20 unvaccinated sample

DISCUSSION

Through our study, we found a clear significant relationship in the effect of the vaccine on the microRNA of hsa-mir-181a ACE2, This indicates that maybe the vaccine has a direct effect on the shape of the secondary structure of the microRNA, and since changing the secondary structure shape of the microRNA has an effective and important role on the gene expression of all types of genes, including of hsa-mir-181a ACE2 gene .Which is the direct key to receiving the spike protein of the vaccine or Covid19. This means that the vaccine may have a negative future role and is ineffective if it causes modifications in the gene expression of the cellular receptors. Due to the high prevalence of hsa-mir-181a ACE2 in vaccinated and its association with higher chances of recurrence and poorer prognosis, we hypothesized that hsa-mir-181a a miRNA previously linked to ACE2 gene expirations and recurrence in humans may be associated with the fusion gene.

One of the first antisense oligonucleotides to enter clinical trials was the locked nucleic acid (LNA-)–modified oligonucleotide complementary to 5′ end of hsa-mir-181a (miravirsen) for the treatment of some disease (Rupaimoole and Slack, 2017). Unlike widely known functions of miRNAs in gene silencing, hsa-mir-181a advances the replication through binding 5′ non-coding regions of the HCV RNA genome. The binding of the miR-18a to 5′ noncoding regions of some viral genome increases its stability in which viral RNA genome acts as a miRNA sponge for hsa- mir-181a, leading to a reduction in hsa-mir-181a abundance at the site of infection and deregulation of liver homeostasis. Studies showed binding of hsa-mir-181a serves as a 5′ cap and protects the viral RNA genome against Xrn1 exoribonuclease-mediated cleavage (Thibault *et al.,* 2015).

The basic idea of the current approach was to create a consensus structure of the pre-miRNA for each miRNA class and use this consensus structure to perform alignment with the query sequence. In light of the story of hsa-mir-181a in vaccinated and unvaccinated replication, we will also benefit from miRNA

therapies that target the receptors of SARS-CoV-2 host cells ACE2.

Fulzele *et al.* (2020), explained through his study, miRNAs play a vital role in the pathogenesis of various diseases, including viral infections, disease progression, and inhibition (Guo *et al.,* 2013). MicroRNAs are small noncoding RNAs, that bind to 3'UTR of mRNA, and inhibit translation or induce degradation of mRNAs (Song *et al.,* 2010). Recent studies suggested that host cellular miRNAs can directly target both viral 3'UTR and the coding region of the viral genome to induce antiviral effects (Song *et al.,* 2010). For example, the number of groups previously reported that host miRNAs (miR-323, miR-491, miR-485, miR-654, and miR-3145) bind to influenza PB1gene coding region degrade RNA and inhibit viral translation and reduce the accumulationof viral particles (Ingle *et al.,* 2015). Furthermore, the host cellular miRNA-29a inhibits Human immunodeficiency virus type 1 (HIV-1) nef protein expression and thus, inhibits viral replication. On the contrary, some groups also suggested the positive effect of host miRNAs on viral replication. For example, miR-122 binding to 3' and 5' UTR of hepatotropic virus RNA and increasing viral RNA stability leads to viral propagation (Henke *et al.,* 2008).

Based on the above reports, we did *in silico* analysis of miRNAs targeting SARS and COVID-19 (recent isolates from different regions) to understand the pathophysiology and identify novel therapeutic targets.

Researchers it was demonstrated the members of micro-RNA -200.family is shown to be miRNAs, especially miR-200c-3p, AND hsa-mir-181a are strong candidate targets for ACE2 regulation in respiratory cells. As it was determined several other miRNA targets for post-transcriptional regulation ACE2 in cells. Our results indicate that human miRNAs could be the best candidates for the development of miRNA-based therapies in the Management of SARS-CoV-2 infection. Studies can also focus on miRNAs directly targeting the genomic RNA of SARS-CoV-2. Lately, it has been suggested that the SARS-CoV-2 genome can be targeted by a mixture of several molecules of RNA and these molecules can be delivered Using more convenient, patient-friendly and highly effective delivery systems such as polymer-based carriers, liposome-like exosomes, or inorganic nanoparticles (Al-Nabi *et al.,* 2020) Host cellular miRNAs are known to play an antiviral role in the number of published studies (Henke *et al.,* 2008). In this study, we performed *in silico* analysis of human cellular miRNAs targeting the SARS and COVID-19 (isolates) genome and identified some novel miRNAs. We identified a number (558) of common human cellular miRNAs targeting both SARS and COVID-19 genomes. The top 10 common miRNAs have a target score of ≥95, and each miRNA contains more than at least ten sites in the targeting viral genome.

Indicating possible antiviral properties for coronavirus infection (for both SARS and COVID-19). It will be interesting to verify these miRNA *in-vitro* and *in-vivo* animal models to use as a therapeutic target in the future. A cocktail of multiple miRNAs mimics through the intranasal route will be useful in coronavirus infection. These human miRNAs targeting coronavirus can be useful to combat any future outbreaks. In a previous report, host cellular miRNAs-181 bind to the ORF-4 region at the viral genome of the porcine reproductive and respiratory syndrome virus (PRRSV) to inhibit its replication (Guo *et al.,* 2013). One step further, Guo *et al.* (2013) delivered intranasal inhalation of miR-181 mimics to slow down the progression of PRRSV in an experimental porcine model.

This study by (Fulzele *et al.,* 2020) agrees that our results indicate that human miRNAs could be the best candidates for the development of miRNA-based therapies in the Management of SARS-CoV-2 infection. Studies can also focus on miRNAs directly targeting the genomic RNA of SARS-CoV-2. Also clarified the role of miRNAs-181 in the link to the virus genome and how it plays multiple roles through receiving infection, which acts as a positive molecule to stop it as an antidote that defends and protects against

the virus or as a negative molecule in increasing the stability of the virus and increasing its replication.

Schetter *et al.* (2009), explained through his study, the Expression of inflammatory genes was associated with miR-21 expression. The association of IL-6 and IL-12a expression was statistically significant in both the tumor and noncancerous tissues, separately. IL-6 is thought to drive the expression of miR-21 in a STAT3-dependent mechanism. Our results are consistent with that model and provide evidence that this mechanism may be relevant to colon cancer. There is also a predicted binding site for miR-21 in the 3′ untranslated regionof IL-12a as indicated by Targetscan 5.0 (Friedman et al. 2009) and miRanda (Betel *et al.,* 2008).IL-12a has a negative correlation with miR-21, which is consistent with a pattern for a miR-21 target. Based on this finding, mechanistic studies should be done to determine if IL-12a is a target of miR-21. The interaction between miR-21 and inflammatory genes may play an important role in colon carcinogenesis. Although the associations between miR-21, IL-6, and IL-12a were significant, the regression models indicated that much of the variability was explained by these models. This indicates that other mechanisms for gene regulation Contribute to the expression of these genes.

This study conducted by (Schetter *et al.,* 2009) demonstrated that there are some factors that are associated with microRNA, specifically miR-21, whose expression is associated with it and affects it. Also, the interaction between miR-21 and inflammatory genes may play an important role in colon carcinogenesis. This explains that there are factors that negatively affect the microRNA, which change its behavior and function, and whose gene expression and mechanism of action will certainly change naturally. This proves that any change in the microRNA changes its work and genetic expression.

Becker *et al.,* (2016), his study was, Inflammatory bowel disease (IBD) may develop due to an inflammatory response to commensal gut macrobiotics triggered by environmental factors in a genetically susceptible host. Isotretinoin (acne therapy) has been inconsistently associated with IBD onset and flares but prior treatment with antibiotics, also associated with IBD development, complicates the confirmation of this association. Here we studied in mice whether doxycycline, metronidazole, or isotretinoin induce epigenetic modifications, and consequently change T-cell mRNA expression and/or function directly after treatment and after a 4-week recovery period. Isotretinoin-induced IL-10 signaling in Tregs and naive T-cells directly after treatment and reduced effector T-cell proliferation alone and in co-culture with Tregs. Metronidazole activated processes associated with anti-inflammatory pathways in both T-cell subsets directly after the treatment period whereas doxycycline induced an immediate proinflammatory expression profile that resolved after the recovery period. Long-term changes indicated inhibition of proliferation by doxycycline and induction of beneficial immune and metabolic pathways by metronidazole. Persistent alterations in microRNA and mRNA expression profiles after the recovery period indicate that all three medications may Induce long-term epigenetic modifications in both T-cell subsets. In the present study, we show differential effects of doxycycline, metronidazole and isotretinoin on microRNA and mRNA expression in murine Tregs and naive T-cells with different expression patterns directly after the treatment and at the end of the recovery period.

This study was presented by (Becker *et al.,* 2016), which agrees in principle with our study and clarified the effect of the drugs and antibiotics used in the treatment and the extent of their effect on the biomarker (micro-RNA) and its genetic expression. In both cases, whether increase or decrease, the goal is to prove the change that occurred after receiving the disease, treatment, or antibiotic. This study agreed with what we found that the change in the microRNA after treatment, disease, or taking medication and antibiotics leads to the variation that results in a change in gene expression**.**

Conclusion

1. The study of bioinformatics is one of the important studies for predicting genetic diagnosis and gene expression.
2. We hypothesized that hsa-mir-181a a miRNA previously linked to ACE2 gene expirations.
3. Through our study, we found a clear significant relationship in the effect of the vaccine on the change of the secondary structure of microRNA of hsa-mir-181a ACE2.
4. It Maybe the vaccine has a direct effect on the shape of the secondary structure of the microRNA, and since changing the secondary structure shape of the microRNA.
5. This change in the secondary structure of microRNA of hsa-mir-181a ACE2 will certainly change the gene expression that it regulates.
6. Such changes that occur may contribute to the emergence of genetic mutations.

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