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Original Article

A Computational Approach to Design Potential Antiviral RNA for 3'UTR Post Transcriptional Gene Silencing of Different Strains of Zika Virus

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ABSTRACT

Background: Zika virus is a flare-up mosquito-borne virus that manifests itself in sporadic human infections with Zika fever. It has a serious affect in pregnant women. Mother transmits Zika virus infection to her infant during the time of delivery which results in the birth of newborn with microcephaly and some neurological malformations. Molecular studies revealed that the 3'UTR of Zika virus genome plays a vital role in viral replication and pathogenicity. So, the 3'UTR can be a suitable target for the prevention of viral multiplications and degree of pathogenicity. The activity of the 3' UTR of Zika virus genome can be controlled by blocking or down regulating its expression through RNAi technology. RNAi works by silencing or turning off target gene expression using siRNA. Hence there arises an urgent need to design potential siRNA against the target sequence of Zika virus genome to control its replication and pathogenicity. This study is aimed to predict potential siRNA based therapeutics that might be used for the treatment of Zika virus infection. **Methods:** Designing siRNA against the target region of different strains of Zika virus is difficult due to its genetic diversity. Therefore, the work is done on the basis of rational siRNA designing method by targeting the 3'UTR of Zika virus strains. The prediction of potential siRNA was done by using various computational tools as searching target sequences, multiple sequences alignment, secondary structure prediction, siRNA-Target sequence interaction prediction and finally the evaluation of effectiveness of predicted and depicted siRNA. **Results:** Out of sixty siRNA only four potential siRNA were predicted and depicted rationally for silencing 3'UTR of 37 different Zika virus genome used in the study through RNAi technology. Conclusion**:** The outcomes of this study are four potential siRNA molecules which might be used as a potential antiviral RNA based therapeutics to suppress the Zika virus mediated infection.

Key words: Antiviral RNA, RNAi technology, siRNA, Zika virus, 3' UTR region.

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INTRODUCTION

The first Zika virus was isolated from the Rhesus monkey in 1947 and human in 1952.[1] Thereafter epidemic transmissions of Zika infections were reported in Pakistan, Malaysia, Indonesia in 1977 and 1978, Micronesia in 2007, Cambodia in 2010 and Bangladesh in 2016.[2-8] Zika virus (family *Flaviviridae*, genus *Flavivirus*) is an arthropod-borne arbovirus that is closely related to Spondweni virus, originally transmitted in Africa through a sylvatic cycle involving mainly *Aedes* vectors (*Ae. Africanus, Ae. Luteocephalus, Ae. hensilli, and Ae. Aegypti)* and nonhuman primates with humans being occasional hosts.[9]

In recent years, an outbreak of transmission in a dengue-like *Aedes*-human-*Aedes* cycle has been increasingly reported, with nonspecific clinical symptoms (influenza-, dengue-or chikungunya-like syndromes).[10,11] Furthermore, Zika virus can transmit from infected pregnant women to her infant during delivery which results in microcephaly and some neurological malformations in infant and Guillain-Barré syndrome in adults.[12-14] Till now, there is no drug against Zika virus or treatment for Zika infection.[15]

Zika virus is a positive-sense single-stranded RNA (10794kb) virus with 2 flanking non-coding regions (5' and 3' non-coding region were typically 0.09-0.1 and 0.3-0.5 kb nucleotide long, respectively).[16-18] Genome of Zika has open reading frame which encoding polyprotein associated with three structural proteins, Capsid (105 amino acids), premembrane (187 amino acids) and envelope (505 amino acids) and seven nonstructural proteins, NS1 (352 amino acids), NS2A (217 amino acids), NS2B (139 amino acids), NS3 (619 amino acids), NS4A (127 amino acids), NS4B (255 amino acids) and NS5 (905 amino acids).[19] The 5' UTR (non-coding region) of Zika virus consists of two conserved structural elements such as large stem loop (SLA) and short stem loop (SLB) which are essential for viral RNA synthesis and replication.[17,20,21] The 3' UTR (non-coding region) contain seven highly conserved secondary stem loop (SL) structure; SL-I, SL-II, SL-III, SL-IV, DB1, DB2 and CRE.[18,22] The two conserved secondary stem loop SL-II and SL-IV shield the 3'UTR from ribonuclease mediated digestion which is essential for viral induced cytopathicity, pathogenicity and multification.[22] Moreover, the conserved secondary stem loop, DB1/DB2 and CRE (Cis-acting replication element) take part in viral translation and replication, respectively.[18,23] Therefore, the 3' UTR in different strains of Zika virus, plays a vital role in viral multiplication and pathogenicity.[22]

Growing recognition of the importance of RNA is shedding light on diseases, and on how it might be treated-particularly through a process called RNA interference (RNAi). It has become one of the most exciting frontiers in medicine, in such short order that two of its pioneers, Andrew Fire and Craig Mellow, won the 2006 Nobel Prize for Medicine, just eight years after their key work was published.[24] RNAi relies on double-stranded RNA molecules called short interfering RNAs (siRNAs), each about 21 units in length. siRNAs interfere with the activity of genes that generate the same sequence in messenger RNA, so that lower quantities of protein are produced. These induce the down regulation of gene

expression in a very sequence-specific manner by the assistance of different enzymes.[25] It can be introduced into the cell for knockdown of a gene of interest by using various methods.[26] The technique's medical potential lies in its ability to target particular genes and their protein products with great precision. RNAi can therefore be used to switch off rogue genes, of the sort that drive cancer or other disorders, without messing up the chemistry of healthy cells. As a result, siRNA may also be used for the therapeutic purposes as chemical drugs.[27] So, RNAi approach is one of the most exciting frontiers in medicines for several clinical cases: CNS disorder, human herpes simplex 1, hypercholesterolemia, hepatitis B virus, hepatitis C virus.[28-32]

In this computational approach, we have aimed to design a potential siRNA molecule to prevent the expression of 3'UTR of Zika virus on the principle of post-transcriptional gene silencing mechanism.

MATERIALS AND METHODS

Sequence retrieval

The nucleotide sequence of 3' UTR of different strains of Zika virus were retrieved from the NCBI database with the following accession numbers[33]: gi|592746858, gi|592746859, gi|592746860, gi|592746861, gi|592746862, gi|592746863, gi|592746864, gi|592746865, gi|592746866, gi|592746867, gi|592746868, gi|592746869, gi|592746870, gi|592746871, gi|592746872, gi|592746873, gi|592746874, gi|592746875, gi|592746876, gi|592746877, gi|592746878, gi|592746879, gi|592746880, gi|592746881, gi|592746882, gi|592746883, gi|592746884, gi|592746885, gi|592746886, gi|592746887, gi|592746888, gi|592746889, gi|592746890, gi|592746891, gi|592746892, gi|592746893, gi|592746894.

Forecast of siRNA with target position

Forecast of functional siRNA with target positions of these nucleotides sequences of Zika virus were carried out with the help of the siDirect 2.0[34] with the followed some rules like Ui-Tei, Amarz-guiout, Renold rules and melting temperature (T_m) should be below 21.5°C for potential siRNA duplex.[35-37]

Construction of common target position

Alignment of the selected siRNA target positions was constructed under default conditions by using Clustal W program.[38]

Analysis of off-target sequence

Analysis of any off-target sequence resemblance in human genome transcript, blast tool program was used against whole Genebank database by applying expected thresholds value 10 and BLOSUM 62 matrix as the parameter.[39]

Calculation of GC content

DNA/RNA GC content calculator tool was used to calculate the GC content of the selected siRNA.[40]

Prediction of siRNA secondary structure

Secondary structure of siRNA was predicted by using the mfold server to calculate the free energy of folding.[41]

Analysis of RNA-RNA interaction

Analysis of RNA-RNA interaction like thermodynamics of interaction between the target region and predicted siRNA with hybridization energy, RNAcofold Program was used.[42] This software program functions as an extension of McCaskill's partition function algorithm to evaluate probabilities of base pairing, realistic interaction energies and the equilibrium concentration of duplex structures.

Flow chart (Figure 1) showing the complete approaches used for prediction of effective siRNA molecules against 3' UTR region of 37 different strains of Zika virus used in this study.

RESULT AND DISCUSSION

In this study, we have found total 60 siRNA against 60 target sequences in 3' UTR of 37 Zika virus strains. Among them, 15 target siRNA for 15 Zika strains, 42 target siRNA for 21 Zika strains and 3 target siRNA for 1 Zika strain which fulfilled all the criteria and algorithms of Ui-Tei, Amarzguioui and Reynolds and shown in Supplementary Table. Therefore, maximum 3 siRNA target positions were found for 37 different Zika virus strains. All target sequences were divided into four target groups (a, b, c and d) on the basis of their sequence similarity and target position using multiple sequences alignment. This aided the construction of common siRNA against the Zika virus sequences analyzed in this study shown in Figure 2. To reduce off-target effect, T_m should be less than 21.5°C.[43] Based on the nearest neighbor model with the thermodynamic parameters, T_m was calculated for the seed-target duplex. In this tool, predicted siRNA with minimum T_m value at the seed region and result of siDirect defines no possibilities for off targets silencing. In the Table 1, all the four classified consensus targets were selected on the basis of their off-target similarity that can be suitable for post transcriptional gene silencing of 3' UTR of 37 different strains of Zika virus used in this study. Further, these classified consensus targets were clarified by Blast similarity search of whole the human genome where no off target found. Therefore these four targets have no chances for off target silencing.

So, four clarified consensus siRNA against 3' UTR for 37 strains of Zika Virus were selected for next study. Furthermore, we checked GC%, free energy for folding and binding of the predicted siRNA with their target sequence.

The GC content of siRNA is an important parameter that represents as the functionality of siRNA. The percentages of predicted siRNA were 42.9% GC content in consensus A, 38% GC content in consensus B, 42.9% GC content in consensus C and 42.9% GC content in consensus D respectively shown in the Table 1. siRNA with GC content within the range of 31.6% - 57.9%, is usually recommended due to its negative correlation between GC-content and RNAi activity.[44] Four predicted consensus siRNA have found within the range of recommended GC content. The free energy of folding is a significant parameter that illustrates the stability of designed siRNA. To calculate the stability of the consensus siRNA (guide strand), the minimum free energy (kcal/mol) of the optimal folding was computed by using mfold program followed by most used algorithms for the prediction of RNA secondary structure, based on the minimal free energy state for exploring effective folding of siRNA guide strand. In this study the free energy of folding of predicted four siRNA were -3.42 in siRNA A, -1.29 in siRNA B, -1.29 in siRNA C and -1.29 in siRNA D respectively at 37°C temperature shown in Table 1 and the secondary structure of these siRNA shown in the Figure 3. Earlier it was recommend that a guide strand siRNA must have smallest free energy for their stability.[45] Here, predicted siRNA of folding were having minimal free energy. Therefore, this minimal free energy of folding represents the effectiveness of these predicted siRNA and in this study our observations support the findings.

The free energy of binding with target is another parameter for siRNA efficiency. The thermodynamic of RNA-RNA interaction of these consensus siRNA with consensus target sequences was predicted using RNAcofold Program. In this study, the binding of predicted siRNA with their target was -36.00 for consensus target group A, -35.90 for consensus target group B, -37.20 for consensus target group C and -37.10 for consensus target group D shown in the Table 1. This program that recommend the algorithms of RNA folding, calculate and forecast of RNA-RNA interactions, related to our work on RNA secondary structure.[29] Therefore, these predicted siRNA represent the smallest hybridization energy for binding with their target sequence.

Table 1: Four designed siRNA molecules with GC%, free energy of folding and free energy of binding with target.

Consensus target group	Location of target	siRNA target within consensus target	Designed siRNA duplex at 37°C	GC%	Free energy of folding	Free energy of binding with target
a	271-293	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC * GCUGUAAGCACCAAUUUCAAU	42.9%	-3.42	-36.00
b	186-208	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA ** GGAUCAUAGGUGAUGAAGAAA	38%	-1.29	-35.90
c	186-208	CAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUG *** GGAUCAUAGGUGAUGAAGAAA	42.9%	-1.29	-37.20
d	186-208	ACGGATCATAGGTGATGAAGAGA	UCUUCAUCACCUAUGAUCCGU **** GGAUCAUAGGUGAUGAAGAGA	42.9%	-1.29	-37.10

Here, 4 predicted siRNA: * Consensus siRNA A, ** Consensus siRNA B, *** Consensus siRNA C and **** Consensus siRNA D respectively for 37 different strains of Zika virus used in this study.

Supplementary Table: Predicted siRNA target sequences with siRNA for 3′ UTR of 37 different strains of Zika virus.

S/N	accession number	Target	Location of target position	siRNA target sequence	Designed siRNA
1	gi|592746858	Target 1	186-208 [ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293 [ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
2	gi|592746859	Target 1	186-208 [ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293 [ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
3	gi|592746860	Target 1	186-208 [ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293 [ii]	GTGCTGTAAGCACTTATTTCAAT	UGAAAUAAGUGCUUACAGCAC GCUGUAAGCACUUAUUUCAAU
4	gi|592746861	Target 1	186-208 [ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293 [ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
5	gi|592746862	Target 1	271-293 [ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
6	gi|592746863	Target 1	186-208 [ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293 [ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
7	gi|592746864	Target 1	186-208 [ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293 [ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
8	gi|592746865	Target 1	186-208 [i]	CAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUG GGAUCAUAGGUGAUGAAGAAA
9	gi|592746866	Target 1	186-208 [i]	CAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUG GGAUCAUAGGUGAUGAAGAAA
10	gi|592746867	Target 1	186-208 [i]	ACGGATCATAGGTGATGAAGAGA	UCUUCAUCACCUAUGAUCCGU GGAUCAUAGGUGAUGAAGAGA

Continue...

Supplementary Table: Cont'd

11	gi|592746868	Target 1	186-208[i]	ACGGATCATAGGTGATGAAGAGA	UCUUCAUCACCUAUGAUCCGU GGAUCAUAGGUGAUGAAGAGA
12	gi|592746869	Target 1	186-208[i]	ACGGATCATAGGTGATGAAGAGA	UCUUCAUCACCUAUGAUCCGU GGAUCAUAGGUGAUGAAGAGA
13	gi|592746870	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACTTATTTCAAT	UGAAAUAAGUGCUUACAGCAC GCUGUAAGCACUUAUUUCAAU
14	gi|592746871	Target 1	186-208[i]	ACGGATCATAGGTGATGAAGAGA	UCUUCAUCACCUAUGAUCCGU GGAUCAUAGGUGAUGAAGAGA
15	gi|592746872	Target 1	186-208[i]	ACGGATCATAGGTGATGAAGAGA	UCUUCAUCACCUAUGAUCCGU GGAUCAUAGGUGAUGAAGAGA
16	gi|592746873	Target 1	186-208[i]	ACGGATCATAGGTGATGAAGAGA	UCUUCAUCACCUAUGAUCCGU GGAUCAUAGGUGAUGAAGAGA
17	gi|592746874	Target 1	271-293[i]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
18	gi|592746875	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
19	gi|592746876	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
20	gi|592746877	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
21	gi|592746878	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
22	gi|592746879	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
23	gi|592746880	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
24	gi|592746881	Target 1	186-208[iii]	CAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUG GGAUCAUAGGUGAUGAAGAAA
		Target 2	269-291[iii]	GAGTGTTGTAAGCACCAATTTCA	AAAUUGGUGCUUACAACACUC GUGUUGUAAGCACCAAUUUCA
		Target 3	271-293[iii]	GTGTTGTAAGCACCAATTTCAGT	UGAAAUUGGUGCUUACAACAC GUUGUAAGCACCAAUUUCAGU
25	gi|592746882	Target 1	186-208[i]	CAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUG GGAUCAUAGGUGAUGAAGAAA
26	gi|592746883	Target 1	186-208[i]	CAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUG GGAUCAUAGGUGAUGAAGAAA

Supplementary Table: Cont'd

27	gi|592746884	Target 1	186-208[i]	CAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUG GGAUCAUAGGUGAUGAAGAAA
28	gi|592746885	Target 1	186-208[i]	CAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUG GGAUCAUAGGUGAUGAAGAAA
29	gi|592746886	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTCTAGT	UAGAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUCUAGU
30	gi|592746887	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTCTAGT	UAGAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUCUAGU
31	gi|592746888	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTCTAGT	UAGAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUCUAGU
32	gi|592746889	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU
33	gi|592746890	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTCTAGT	UAGAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUCUAGU
34	gi|592746891	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTCTAGT	UAGAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUCUAGU
35	gi|592746892	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTCTAGT	UAGAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUCUAGU
36	gi|592746893	Target 1	186-208[i]	ACGGATCATAGGTGATGAAGAGA	UCUUCAUCACCUAUGAUCCGU GGAUCAUAGGUGAUGAAGAGA
37	gi|592746894	Target 1	186-208[ii]	TAGGATCATAGGTGATGAAGAAA	UCUUCAUCACCUAUGAUCCUA GGAUCAUAGGUGAUGAAGAAA
		Target 2	271-293[ii]	GTGCTGTAAGCACCAATTTCAAT	UGAAAUUGGUGCUUACAGCAC GCUGUAAGCACCAAUUUCAAU

Here, Predicted maximum 3 siRNA target positions of 37different strains of Zika virus used in this study: 1 target position, 2 target positions and 3 target positions were represented by 3 characters: i, ii, and iii respectively.

In this computational approach all the parameters and tools used for the designing potential antiviral RNA against the 37 different Strains of Zika virus supports the efficiency of antiviral RNA against their target sequences. This study successfully designed four consensus siRNA against four consensus target group which fulfill all the criteria of a siRNA molecule as therapeutic agent. So, these potential siRNA molecules might be used as potential RNA based therapeutics in advanced RNAi technology for the treatment of Zika virus infection.

CONCLUSION

This work supports the hypothesis that rate of viral replication and degree of pathogenicity in Zika virus infection can be reduced by predicted siRNA in this study. Apart from this, present investigation will help scientists toward understanding the prediction of antiviral RNA based therapeutics. However, experimental approaches and validation will be required for establishing this hypothesis.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests.

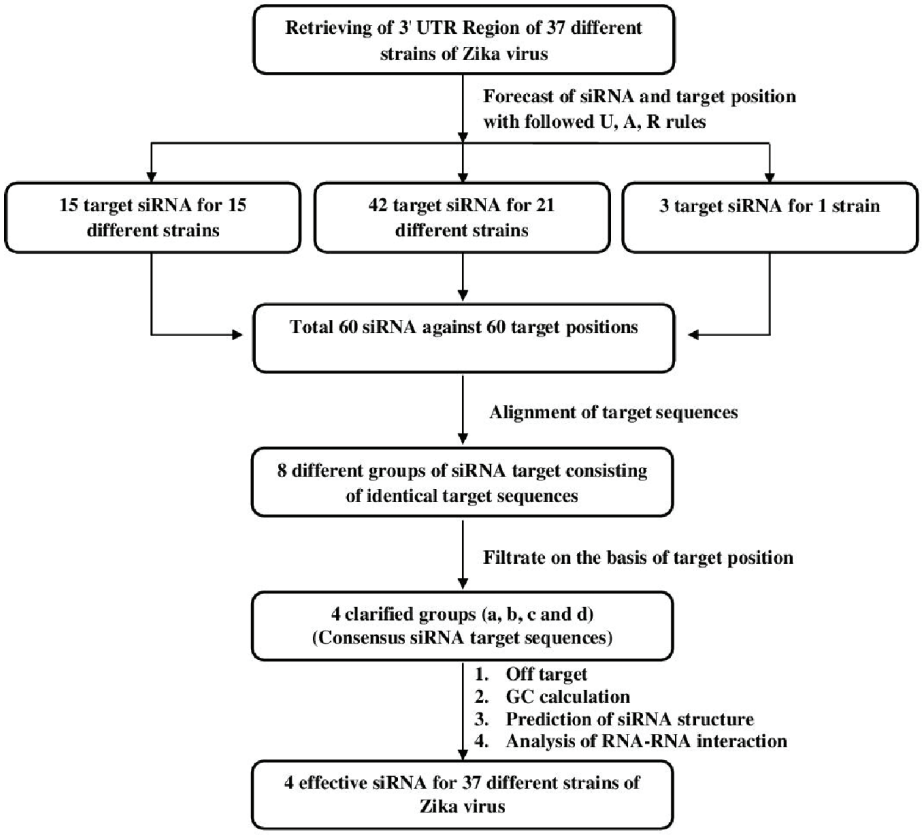

Figure 1: Flowchart showing the complete approaches used for screening of effective siRNA molecules against 3′UTR region of 37 different strains of Zika virus in this study.

Consensus target group – a

gil592746858 Target2	GTGCTGTAAGCACCAATTTCAAT
gil592746859 Target2	GTGCTGTAAGCACCAATTTCAAT
gil592746861 Target2	GTGCTGTAAGCACCAATTTCAAT
gil592746862 Target1	GTGCTGTAAGCACCAATTTCAAT
gil592746863 Target2	GTGCTGTAAGCACCAATTTCAAT
gil592746864 Target2	GTGCTGTAAGCACCAATTTCAAT
gil592746874 Target1	GTGCTGTAAGCACCAATTTCAAT
gil592746875 Target2	GTGCTGTAAGCACCAATTTCAAT
gil592746876 Target2	GTGCTGTAAGCACCAATTTCAAT
gil592746877 Target2	GTGCTGTAAGCACCAATTTCAAT
gil592746878 Target2	GTGCTGTAAGCACCAATTTCAAT
gil592746879 Target2	GTGCTGTAAGCACCAATTTCAAT
gil592746880 Target2	GTGCTGTAAGCACCAATTTCAAT
gil592746889 Target2	GTGCTGTAAGCACCAATTTCAAT
gil592746894 Target2	GTGCTGTAAGCACCAATTTCAAT

Consensus target group – b

gil592746858 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746859 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746860 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746861 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746863 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746864 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746870 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746875 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746876 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746877 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746878 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746879 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746880 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746886 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746887 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746888 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746889 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746890 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746891 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746892 Target1	TAGGATCATAGGTGATGAAGAAA
gil592746894 Target1	TAGGATCATAGGTGATGAAGAAA

Consensus target group – c

gil592746865 Target1	CAGGATCATAGGTGATGAAGAAA
gil592746866 Target1	CAGGATCATAGGTGATGAAGAAA
gil592746881 Target1	CAGGATCATAGGTGATGAAGAAA
gil592746882 Target1	CAGGATCATAGGTGATGAAGAAA
gil592746883 Target1	CAGGATCATAGGTGATGAAGAAA
gil592746884 Target1	CAGGATCATAGGTGATGAAGAAA
gil592746885 Target1	CAGGATCATAGGTGATGAAGAAA

Consensus target group – d

gil592746867 Target1	ACGGATCATAGGTGATGAAGAGA
gil592746868 Target1	ACGGATCATAGGTGATGAAGAGA
gil592746869 Target1	ACGGATCATAGGTGATGAAGAGA
gil592746871 Target1	ACGGATCATAGGTGATGAAGAGA
gil592746872 Target1	ACGGATCATAGGTGATGAAGAGA
gil592746873 Target1	ACGGATCATAGGTGATGAAGAGA
gil592746893 Target1	ACGGATCATAGGTGATGAAGAGA

Figure 2: Predicted 4 different consensus siRNA target sequences (a, b, c and d) by Clustal W

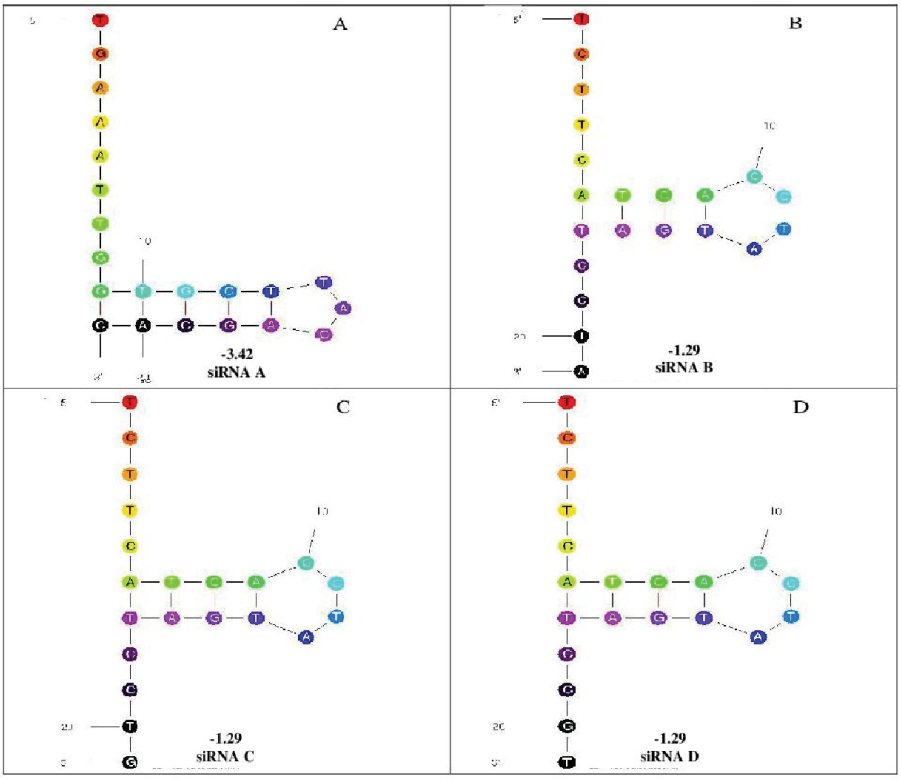

Figure 3: Predicted 4 siRNA (A, B, C and D) secondary structures with possible folding and minimum free energy.

ABBREVIATION USED

M.A: Mohammad Abul; **Md:** Mohammad; **USTC:** University of Science and Technology Chittagong.

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