



Original article

## *In silico* prediction and experimental validation of siRNAs targeting ORF1ab of MERS-CoV in Vero cell line

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### ABSTRACT

The Middle East Respiratory Syndrome Coronavirus is well known to cause respiratory syndrome and this virus was identified and isolated for the first time from Jeddah, Saudi Arabia in 2012 from infected patient. In this report, we have conducted the *in-silico* prediction, designing and evaluation of siRNAs targeting Middle East Respiratory Syndrome Coronavirus orf1ab gene to inhibit the virus replication. By using bioinformatics software, total twenty-one functional, off-target reduced siRNA were selected from four hundred and sixty-two siRNAs based on their greater potency and specificity. We have evaluated only seven siRNAs to analyze their performance and efficacy as antivirals by reverse transfection approach in Vero cells. There was no cytotoxicity of siRNAs at various concentrations was observed in Vero cells. Based on the real-time PCR results, better inhibition of viral replication was observed in the siRNA-1 and 4 as compared to other siRNAs. The results generated from this work provided suitable information about the efficacy of siRNAs which encouraged us to further evaluate the remaining siRNAs to determine their inhibitory effect on the virus replication. We concluded that the *in-silico* prediction and designing resulted in the screening of potential siRNAs with better efficiency, and strength. This can be used to develop oligonucleotide-based antiviral therapeutics against MERS-CoV in the near future.

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## 1. Introduction

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) presents serious health problem for human population. The first case of MERS-CoV was identified in June 2012 from Jeddah, Saudi Arabia (Zaki et al., 2012). Currently, a total of 2,562 confirmed MERS-CoV cases with 881 related deaths and spread in 27 countries (WHO, 2019). The virus is transmitted to humans through close contact with camels or infected patients (Obobo et al., 2015). The MERS-CoV causes symptoms like fever, cough, shortness of breath and in serious cases multi-organ failure

(Assiri et al., 2013). Coronaviruses belongs to family *Coronaviridae*, which is a large group of viruses cause disease in human and animals. The first human coronavirus was identified in 1960. There are four main groups of coronaviruses known as alpha, beta, gamma and delta coronaviruses. Coronaviruses genome varies in length from 25.0 to 32.0 kb with high sequences variability favoring recombination resulting into emergence of new virus strain with novel characteristics amongst the extended host species (Omrani et al., 2015).

RNA interference (RNAi) is a biological phenomenon found in multiple eukaryotes where small RNA inhibits the gene expression. The main function of short interfering RNA (siRNA) is to down regulate and silence the specific gene by degrading the mRNA after transcription. The uses of RNAi as antiviral therapeutics against many diseases including viral infections are well reported globally. The regulatory role of RNAi in gene expression has been shown in many studies. The siRNAs are short RNA sequences with length varying from 21 to 23 base pairs with 5' phosphate group and a 3' hydroxyl group. Their function is to bind to a specific protein forming RNA induced silencing complex (RISC), which further binds to specific RNA sequences and thereby degrading the RNA

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resulting into silencing of mRNA expression. As all siRNAs are not equally potent, various rules have been suggested for the computational prediction, designing, and selection of potential siRNAs. Currently, the siRNA approach has been implemented to inhibit viral replication in cell culture against many viruses like HIV (Liu et al., 2017), Flock house virus (FHV) (Taning et al., 2018), Dengue virus (Idrees and Ashfaq, 2013), Hepatitis C virus (HCV) (Shahid et al., 2017), Influenza virus (Huang et al., 2017; Tsai et al., 2018), Hepatitis B virus (HBV) (Wang et al., 2016), Human Papillomavirus (HPV) (Zeng et al., 2017) and SARS coronavirus (SARS-CoV) (Kumar et al., 2013).

The *in silico* designing of siRNA helps in predicting the potential siRNAs candidates with high target specificity and reduced off-target effects. Before designing of potential siRNAs, various factors should be considered; like conserved genome sequences, off-target effects, siRNA folding, thermodynamic properties, target accessibility, stability and immuno-stimulation of siRNAs. The basic rules and guidelines for the designing of siRNAs have been published in many reports (Fakhr et al., 2016; Mysara et al., 2011; Sohrab et al., 2018a). The *in vitro* efficacy of siRNA has been reported for the inhibition of other coronaviruses. The cytopathic effect (CPE) was specifically inhibited by siRNA targeting viral RNA polymerases of SARS-CoV in Vero cells. The titer and levels of viral proteins were reduced indicating the inhibition of viral replication. In a study conducted, the function of SARS-CoV-Spike protein gene was selectively silenced by DNA vector-driven siRNA in SARS-infected HEK-293 T cells. Another study showed that SARS-CoV infection and replication in fetal rhesus kidney cells was inhibited by siRNA duplexes targeting viral RNA polymerases, and spike protein gene. The siRNA duplexes had a prolonged prophylactic effect, with  $\leq 90\%$  inhibition of transcription, lasting for  $\geq 72$  h. additionally, in a study conducted the combinational use of siRNA duplexes against multiple targets in the viral genome showed  $\leq 80\%$  inhibition. Therapeutic siRNAs and miRNAs are found to be the most promising biopharmaceuticals in commercial space as oligonucleotide-based next-generation medicines (Sohrab et al., 2018a; b). Currently, miRNA- and siRNA-based candidate drugs are being evaluated in more than 20 clinical trials (Chakraborty et al., 2017).

The prediction and designing of siRNAs can be performed by using *in silico* approach and subsequently, the designed siRNA can be biologically evaluated for their effective action against the targeted pathogens. Recently, MysiRNA-Designer has been developed with better siRNAs design and workflow (Mysara et al., 2011; Fakhr et al., 2016; Sohrab et al., 2018a; b). For MERS-CoV, two studies have reported *in silico* design of siRNA and miRNA against orf1ab replicase polyprotein using computational methods for possible use as antiviral therapeutics against MERS-CoV (Hasan et al., 2014; Nur et al., 2015). Recently, we have also elaborately reviewed and published the design and delivery of potential siRNAs for MERS-CoV (Sohrab et al., 2018a; b).

In this work, we have used the integrative siRNA prediction and design including different bioinformatics tools and screened the siRNAs targeting against orf1ab region of MERS-CoV genome. In the present study, we report the *in silico* prediction, design, chemical synthesis, evaluation of cytotoxic effect and antiviral activity of only seven siRNAs targeting the orf1ab region of the MERS-CoV genome. The predicted siRNAs were chemically synthesized, and their cytotoxicity and antiviral efficacy were validated using dose-dependent experiments in Vero cells at various concentrations. The Vero cells were transfected with siRNAs at 60–70% confluency and the viral RNA was isolated to determine the inhibition of virus replication by quantitative real-time PCR (q-RT-PCR). Based on the results obtained in this study, we observed that, selected siRNAs showed good antiviral effect at low concentration (0.1 nM–50 nM) against MERS-CoV. There was no cellular cytotoxicity

was observed evaluated by MTT cytotoxicity assay suggesting that the predicted and selected siRNAs were MERS-CoV specific and have antiviral potential and can be used as oligonucleotide based antiviral therapeutics.

## 2. Materials and methods

### 2.1. Sequence selection and analysis

A total of seventeen full-genome sequences of MERS-CoVs (human and camel isolates) were collected from GenBank (NCBI-PubMed). The multiple sequence alignment was performed using the multiple sequence alignment tools in BioEdit (Version 7.2). The accession numbers and their sequence identity matrix and homology have been presented in Fig. 1 and Table 1.

### 2.2. *In silico* prediction and scoring of siRNAs

The potential siRNAs were predicted and designed and by using the MERS-CoV-orf1ab gene. The overall flow for the prediction and selection of potential siRNAs has been presented in Fig. 2. The potential siRNAs were predicted and selected by integrated bioinformatics approach using online software (Mysara et al., 2011; Nur et al., 2015; ElHefnawi et al., 2016; Sohrab et al., 2018a). The iSCORE calculates the algorithm scores for designed siRNAs (Reynolds et al., 2004). The consensus multi-score threshold filtration layer was adopted after the *in-silico* prediction by defining threshold accepted scores. Finally, adopting an integrated bioinformatics approach with different scoring tools, we screened and selected the potential siRNAs with their desired characteristics (ElHefnawi et al., 2016; Fakhr et al., 2016; Mysara et al., 2011; Nur et al., 2015; Sohrab et al., 2018a). Thermodynamic features like siRNA free energy folding calculation and overall GC% are taken into account to increase specificity and reduce the false positives. Based on the various factors and *Insilico* results using multiple scoring tools, desired and energetically favored siRNAs were selected. The characteristics and thermodynamic properties of predicted and designed siRNAs are presented in Table 2.

### 2.3. Screening of off-targets

We have used two different phases of selection to avoid any off-target binding of the designed siRNAs. We performed BLAST and Smith-Waterman algorithms as implemented in ParAlign to find out the near complimentary and similarity match with human mRNA sequences in NCBI-PubMed database and increase the validity percentage of the siRNAs and filtered out the siRNAs with high off-target effects. In the next phase, siRNAs were filtered through the exclusion of seed-region like human mRNA.

### 2.4. Thermodynamics, target accessibility, and secondary structure prediction

The thermodynamics and target accessibility threshold prediction were performed by using RNAs tool (<http://rna.tbi.univie.ac.at/cgi-bin/RNAs/RNAs.cgi>) and secondary structure of siRNA was predicted utilizing RNAfold server (<http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi>). This program calculates the minimum free energy and partition function of RNAs by reading the RNA sequences. The secondary structure of siRNAs was visualized in forna format by using online software (<http://rna.tbi.univie.ac.at/forna/documentation.html>). The color of the secondary structure indicates; Green: Stems (canonical helices), Red: Multiloops (junctions), Yellow: Interior



Fig. 1. Multiple sequence alignment of ORF1ab gene with selected MERS-CoV genome.

Table 1  
Sequence identity matrix of selected MERS-CoV based on full-genome.

Accession numbers	Locations	Hosts	Year	% Identity
KF958702	Jeddah	Human	2014	99.0
KU851859	Jeddah	Human	2015	99.6
KF192507	UAE	Human	2013	99.8
KT156561	Oman	Human	2013	99.7
KT861628	Jordan	Human	2014	99.7
KX034100	Korea	Human	2015	99.7
KT036372	China	Human	2015	99.6
KP223131	Florida	Human	2014	99.8
NC019843	Netherland	Human	2012	99.6
KF745068	France	Human	2013	99.7
KT225476	Thailand	Human	2015	99.7
KJ556336	Jeddah	Human	2013	92.3
KF917527	Jeddah	Camel	2013	99.9
KX108946	UAE	Camel	2015	99.6
KJ650098	Qatar	Camel	2014	99.8
KT368879	Riyadh	Camel	2015	99.7
KJ477102	Egypt	Camel	2013	99.4

Loops, Blue: Hairpin loops. The overall information have been presented in Fig. 3

2.5. Final selection and chemical synthesis of siRNAs

The predicted and designed siRNAs were finally selected based on their best parameters following the rule and suggestions of online software (Naito et al., 2009; Mysara et al., 2011; Naito and Ui-Tei, 2012; Nur et al., 2015; ElHefnawi et al., 2016; Fakhr et al., 2016; Sohrab et al., 2018a). The predicted siRNAs against the targeted orf1ab gene of MERS-CoV were chemically synthesized by Integrated DNA Technologies (USA) and further used to evaluate their efficacy in the Vero cell line.

2.6. Cell culture and siRNAs transfection

The Vero cells (ATCC CCL-81) were grown in DMEM medium at 37 °C and 5% CO<sub>2</sub> and used for siRNA reverse transfection. The Vero

cells (1 × 10<sup>4</sup>) were grown in 96-well plates (in triplicates) with 60–80% confluency and transfected by siRNAs complexed with Lipofectamine 2000 (Invitrogen, USA) as per the manufacturer instruction. Briefly, 50 μM siRNAs stocks were diluted to various concentrations (0.1–50 nM) in 100 μl Opti-MEM medium by adding Lipofectamine following incubation at room temperature for 30 min. The siRNA-lipid complex (1 μl) was added to the Vero cells at various siRNA concentrations (0.1–50 nM) and mixed gently and incubated at 37 °C for 72 h. Vero cells without siRNA, cells with Opti-MEM and cells with Lipofectamine were used as negative controls. After 72 h post-transfection, the antiviral assay was performed.

3. Evaluation of MERS-CoV replication inhibition in Vero cells

The predicted siRNAs were chemically synthesized and delivered by Lipofectamine 2000 mediated delivery to Vero cells following manufacturer’s instructions. Briefly, the Vero cells

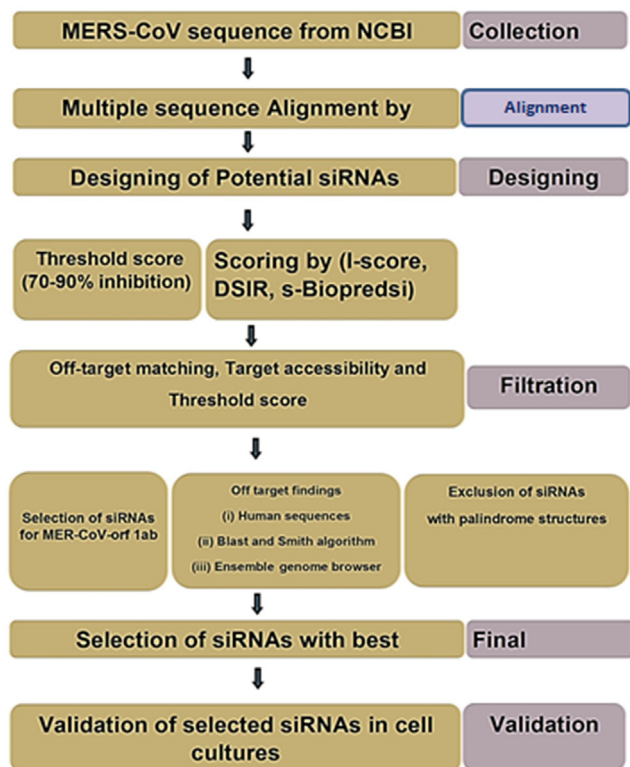


Fig. 2. Flow diagram of *in silico* prediction and design of siRNAs against MERS-CoV orf1ab gene.

(1 × 10<sup>4</sup>) were plated into 96-well plates (in triplicates) with 60–80% confluency and transfected with siRNAs at various concentrations (0.1–50 nM) and final incubation at 37 °C 72 h. All the experiments were performed in triplicates.

### 3.1. Virus inoculation

The siRNAs transfected Vero cells (1 × 10<sup>4</sup>) were inoculated with MERS-CoV as described previously (Azhar et al., 2014). The cytopathic effect in Vero cells was observed daily. After full cytopathic effect, the cells lysate and supernatants were collected for purification of viral RNA and real-time RT-PCR was performed protocol in published paper (Azhar et al., 2014).

Table 2

List of predicted siRNAs from MERS-CoV orf1ab gene (Accession NumberKF958702).

S. #	Position of siRNAs in the genome (Start-End)	Target sequence	Predicted potential RNA oligo sequences Guide/Passenger strand (5'→3')	Free energy folding	Seed-duplex stability (Tm/°C) Guide/Pass	Target accessibility position
1	791–813	AGCAATCTATTTTACTATTAAT	UAAUAGUAAAAUAGAUUGCU CAAUCUAUUUUACUUAUAAU	0.84	6.3/6.6	110
2	1615–1637	ATGGATAATGCTATTAATGTTGG	AACAUUAAUAGCAUUUACCAU GGAUAAUGCUUUUAAUGUUGG	0.75	6.9/8.7	32
3	1910–1932	GCGACTTTATGTCTACAATTATT	UAAUUGUAGACAUAAAGUCGC GACUUUUGUCUACAUAUUUUU	0.81	6.9/4.6	1184
4	4018–4040	GACACTTTAGATGATATCTTACA	UAAGAUUAUCAUAAAAGUGUC CACUUUAGAUUAUCUUACA	0.70	6.6/9.8	180
5	5597–5619	ATGCTATTAGTTTGAGTTTAAAT	UAAAACUCAAAAUUAGCAU GCUAUUAGUUUGAGUUUUAAU	0.78	13.3/2.8	1230
6	5598–5620	TGCTATTAGTTTGAGTTTAAATA	UUAAAACUCAAAAUUAGCA CUAUUAGUUUGAGUUUUAAUA	0.81	4.9/6.3	291
7	5819–5841	GAGCTAGTTTGCCTCAAATTTT	AAAUUUGACGCAAACUAGCUC GCUAGUUUGCGUCAAUUUUUU	0.70	7.4/9.8	5167

### 3.2. Total RNA extraction and real time PCR

The cell lysate and supernatant were used to isolate and purify the total RNA by using QIAampViral RNA Mini Kit (Qiagen). The purified RNA was used for the MERS-CoV open reading frame region orf1a and orf1b using quantitative real-time RT-PCR (Azhar et al., 2014). The antiviral effect of the siRNAs was calculated as percentage of the positive control.

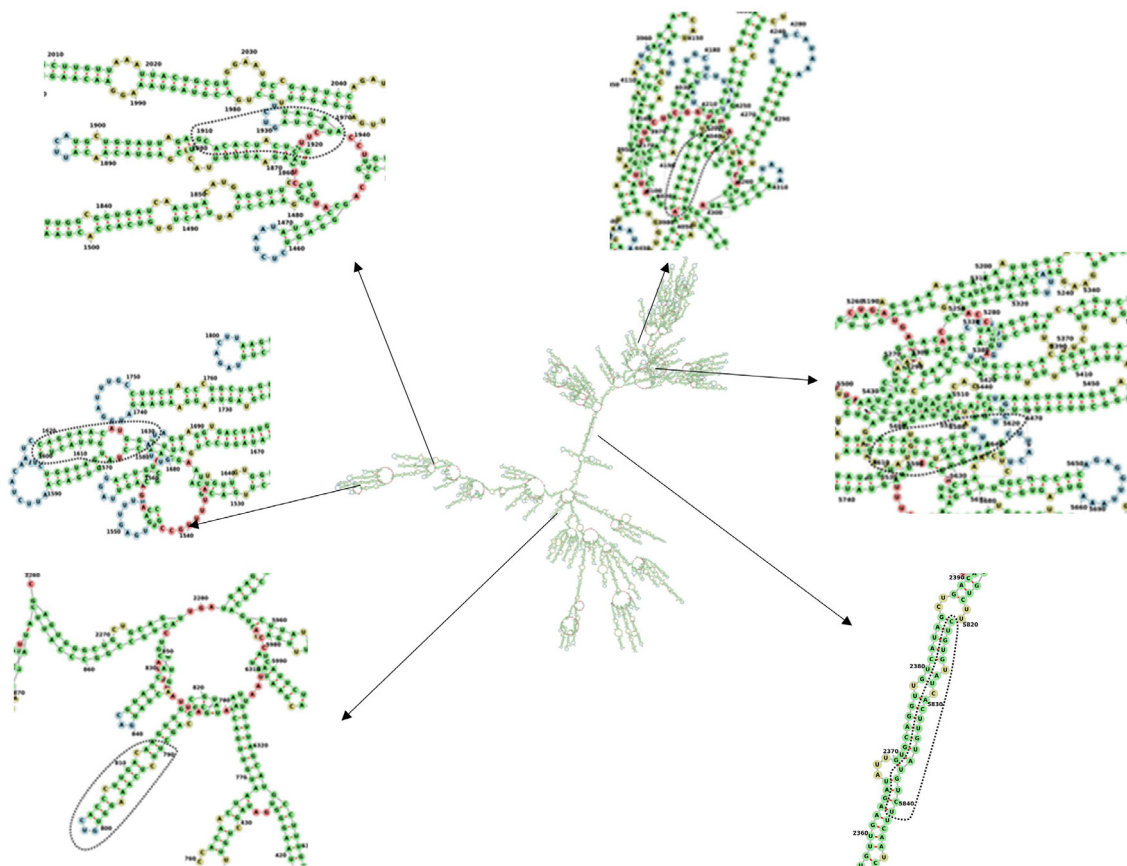
## 4. Results

### 4.1. MERS-CoV genome analysis, in silico prediction and selection of potential siRNAs

After performing the whole genome sequence analysis of MERS-CoV, we selected the orf1ab gene as a target region for further sequence analysis and siRNAs designing and selection. The multiple sequence alignment results showed high conservation among all the sequences analyzed Fig. 1. The potential siRNAs with no off-target effect and matches with any human mRNA sequences were filtered and finally selected for synthesis. During *in silico* prediction and designing, many siRNAs were found to fulfill the less favorable criteria targeting a region in the MERS-CoV orf1ab genome. By using the stringent selection and filtration criteria and multiple strategies, we selected a total of twenty one functional, off-targets reduced siRNAs from four hundred and sixty two siRNAs and further narrowed down to only seven siRNA. These siRNAs were further screened initially for their *in vitro* antiviral activity in cell culture following the guidelines of Ui-Tei, Reynolds and Amarzguoui (Naito et al., 2009; Mysara et al., 2011; Naito and Ui-Tei, 2012; Sohrab et al., 2018a). The predicted siRNAs are expected to be highly specific and potent against orf1ab gene of MERS-CoV.

### 4.2. Target accessibility and secondary structure prediction

The thermodynamic properties and target accessibility were performed using RNAXs tool. The target accessibility analysis provided the binding position of each siRNAs at starting point along the orf1ab gene of the viral genome such as siRNA-1:110, siRNA-2:32, siRNA-3:1184, siRNA-4:180, siRNA-5:1230, siRNA-6:291, siRNA-7:5167 of the viral genome. The secondary structure prediction was performed using RNAfold software. Online software showed that the minimum free energy was observed to be –2508.09 kcal/mol and the ensemble diversity was 1611.05 for partial orf1ab genome of MERS-CoV. The secondary structure and binding positions of each siRNAs have been presented in Fig. 3.



**Fig. 3.** Secondary structure generated by RNAfold in forna format and binding position of potential siRNAs. To their specific targets in the orf1ab gene, bases highlighted in green indicate stem, bases highlighted in red indicate Multiloops (junctions) while bases highlighted in blue indicate Hairpin loops, Yellow color indicates Interior Loops and orange color indicates 5' and 3' unpaired region.

#### 4.3. Evaluation of MERS-CoV replication inhibition

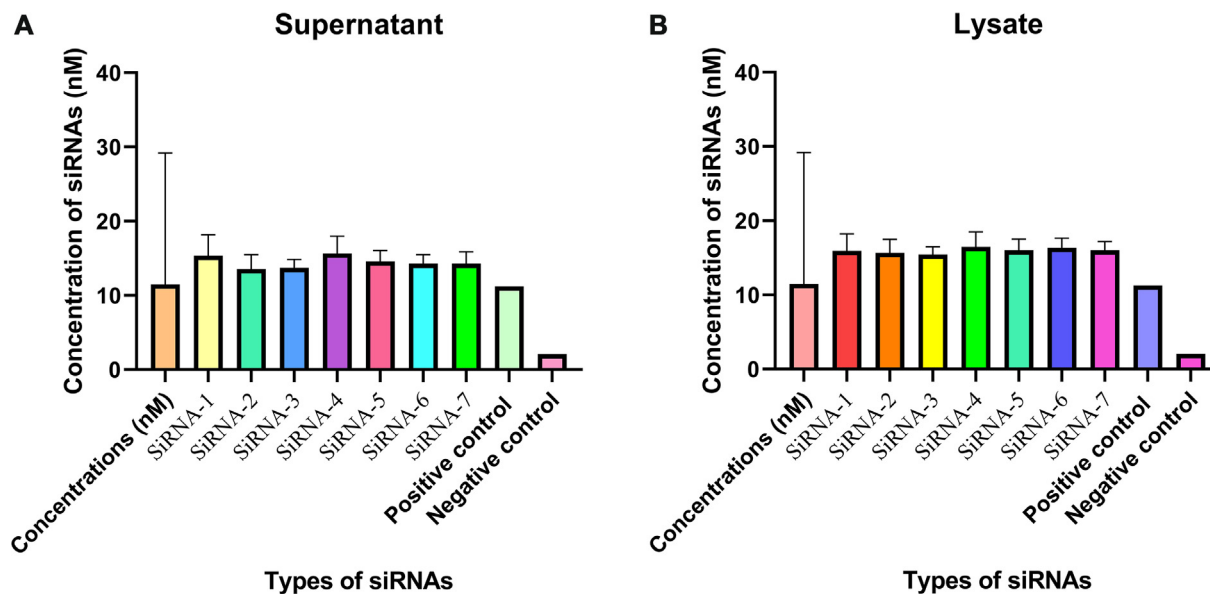
To validate the dose dependent efficacy of *in-silico* predicted and designed siRNAs, we performed the experimental evaluation of siRNAs transfection at variable doses to Vero cells followed by virus inoculation and quantitative real-time PCR analysis. The inhibition of virus replication were determined 72 h post inoculation by using both supernatant and cell lysate. Briefly, the transfection of all tested siRNAs showed reduction of virus replication in Vero cells as per variable doses. The ct values of quantitative Real-time PCR generated variable and comparable slopes of inhibition for each siRNAs at various concentrations. Notably, the inhibition was observed by siRNA, 1, 2, 4, 5 and 6 as compared to others but the siRNA 1 and 4 were observed to be the best at 0.1 nM, 5.0 and 10 nM respectively in cell lysate as compared to supernatant. The positive sample was used as cell lysate and supernatant collected from MERS-CoV infected Vero cells. While some siRNAs observed to show better inhibition at 5–10 nM concentration in supernatant. The quantitative real-time PCR result of each siRNAs has been presented in Table 3, Fig. 4 A and B. To evaluate the variable doses, we further increased the concentration of siRNAs (1.0, 2.5, 5.0, 10.0, 50.0, 100, 500 nM) respectively. The quantitative real-time PCR results indicated that the 5.0 nM to 100 nM provided inhibition of virus replication as compared to others. However, the higher concentration (250 nM and 500 nM) did not showed the significant inhibition (data not shown).

#### 5. Discussion

Since the discovery of the MERS-CoV in 2012, tremendous research has been made globally to fill the gaps in existing knowledge and have provided detailed and advanced information about the MERS-CoV. Further research is still needed, especially in the area of specific antivirals, therapeutic and preventive vaccines, and targeted drug discovery. The treatment of various diseases using RNAi-based therapeutics has shown promising results and continuous development with improved technologies are being evolved continuously. Several studies have investigated the important role of RNAi technology towards the fight against viral diseases (Jeang, 2012; Javier et al., 2016; Sohrab et al., 2018a; Ahmad et al., 2018). The RNAi technology provides a very specific route to silence the function of the desired gene and is being used to develop oligonucleotides based therapeutics against many diseases (Chakraborty et al., 2017). Earlier studies hampered by obstacles like off-target binding, delivery, and stability and stimulation of immune responses which significantly contributed to hampering the use of RNAi technology as therapeutics. But continuous research has successfully overcome these obstacles and the technology is now in clinical trials for use as therapeutics against several infectious diseases (Carneiro et al., 2015; ElHefnawi et al., 2016; Mandal et al., 2016; Moon et al., 2016; Braga et al., 2017). The specificity and potency of predicted siRNAs can be improved using efficient software (Mysara et al., 2011; Naito et al., 2009). Recently, multiple siRNAs against HCV 5'-NTR have been designed and tested. The results showed that HCV321, HCV353, HCV258

**Table 3**  
CT value of qRT-PCR results of siRNAs at various concentrations.

siRNAs Conc (nM)	Si-1	Si-2	Si-3	Si-4	Si-5	Si-6	Si-7
50 nM	12.83/14.48	11.56/12.95	12.99/13.84	13.75/14.41	13.52/14.75	13.13/14.21	12.78/14.67
25 nM	15.21/15.98	14.65/16.47	15.41/16.12	15.82/16.12	14.43/16.34	14.65/15.75	15.56/16.55
10 nM	18.99/18.85	16.57/17.75	14.54/15.95	18.61/19.46	15.99/17.89	15.21/17.51	15.97/17.67
5 nM	17.95/17.89	15.95/17.87	14.81/16.83	17.84/17.95	16.75/17.91	14.12/17.96	16.78/16.98
1 nM	13.11/14.11	13.21/15.61	13.19/14.54	13.65/14.61	13.15/14.31	13.78/16.78	13.34/15.84
0.5 nM	12.91/13.42	12.36/13.54	13.85/14.96	13.21/14.32	13.45/14.64	13.72/14.91	13.8/14.69
0.25 M	12.78/13.56	12.01/13.65	12.78/13.97	13.91/14.87	13.25/14.83	13.09/14.68	12.56/14.53
0.1 nM	18.91/17.81	12.01/14.95	12.21/15.89	18.49/17.94	16.11/16.23	16.64/16.95	13.35/15.87
Positive control	11.23/11.28						
Negative positive control	2.08/2.04						



**Fig. 4.** Graphical representation of Ct value of RT-PCR result (A: Supernatant; B: Lysate).

siRNA were the most efficient siRNAs against HCV replication (ElHefnawi et al., 2016).

The MERS-CoV replication process is initiated by the binding of the viral particle with cellular receptors through the S-protein. The orf1ab region includes two-thirds of the coronaviruses genome and encodes non-structural proteins. In this study, the MERS-CoV-orf1ab region was selected as a target, based on the multiple factors; first it is long enough to design and select the multiple siRNAs candidate. Secondly, its role in viral transcription and replication makes it a possible target to control the virus replication and lastly because it is highly conserved and showed high similarities (>99% based on multiple sequence alignment) amongst various MERS-CoV isolates retrieved from GenBank. Earlier studies have reported only the design of siRNAs and miRNAs against MERS-CoV orf1ab gene using bioinformatics tools (Hasan et al., 2014; Nur et al., 2015) but their cytotoxicity and antiviral activities have not been evaluated.

In our study we have designed and evaluated the cytotoxicity and antiviral activities of selected siRNAs in Vero cells. The potential of RNAi technology for the treatment of viral infections has encouraged us to investigate the potential effect of siRNA as antiviral therapy candidates against MERS-CoV. The selection of potential siRNA starts with a particular target region to generate siRNA 1-nt shifted overlapping 21-nt siRNAs representing putative guide sequences, excluding 21-nt siRNAs. Amongst rest, active siRNA molecules are calculated, screened and predicted based on various sets of parameters (like base-preference rules, duplex-related issues). Otherwise, if few promising or nil candidates get predicted,

more antisense siRNAs can be generated. Based on the empirical scoring functions, the selected candidate sequences are finally ranked. Binding of siRNAs can occur only at positions free from prior intermolecular base pairs (Tafer, 2014). SiRNA design tools (OligoWalk, Sirna, RNAs) perform siRNA design aided by target accessibility criteria. Recently, some siRNAs against HCV have been evaluated and found to be very efficient to inhibit the virus replication (ElHefnawi et al., 2016). Generally, for designing the siRNA against MERS-CoV has been reported by using only one tool (Hasan et al., 2014; Nur et al., 2015). The site specific delivery of siRNAs without any adverse effect observed to be a major hurdle but this hurdle have been solved by using the gold nanoparticles delivery (Shaath et al., 2016). As a preliminary test for the proof of concept, our primary objective was to predict and design the potential siRNAs against orf1ab gene as a target region of MERS-CoV. The secondary objective was to evaluate experimentally the efficacy of predicted siRNAs into Vero cells by quantitative real-time PCR.

In this study, we have predicted and designed the potential siRNAs targeting the orf1ab gene of MERS-CoV using automated online software by filtering and excluding the off-target effects and considering various factors and stringent rules (Naito et al., 2009; Mysara et al., 2011; Naito and Ui-Tei, 2012; Fakhr et al., 2016; Sohrab et al., 2018a). Based on the extensive bioinformatics analysis, the outputs resulted into four hundred and seventy two potential siRNAs against the target orf1ab gene of MERS-CoV, and by using further selection and filtrations criteria, total twenty one siRNAs were finally selected for chemical synthesis and evaluation

in the selected cell lines. But in this study we have selected only seven siRNAs with improved target accessibility and expected high antiviral potency with no off-target effect as a preliminary biological test of the designed siRNA. The efficacy of predicted siRNAs was found to be effective against virus replication at variable doses. The efficacy of predicted siRNAs was evaluated by using quantitative real-time PCR at various concentrations. The Ct value of quantitative real-time PCR results showed that siRNAs-1 and 4 showed the better inhibition with high efficacy than the other siRNAs and might provide promising candidates for inhibition of MERS-CoV *in vitro*. Taken together, our results provide the first preliminary experimental evidence for possible evaluation of the predicted siRNA as potential candidates for the inhibition of MERS-CoV replication *in vivo*. Further investigations are needed to investigate the effect of combining more than one siRNA to synergize their antiviral effects.

## 6. Conclusions

In conclusion, the *in silico* predicted and designed potential siRNAs against a specific target of MERS-CoV orf1ab gene can be utilized to develop oligonucleotide-based antiviral therapeutics without off-target effects, minimal cytotoxicity and high efficiency. The quantitative real-time PCR results generated in this study has provided preliminary information to evaluate the efficacy of predicted siRNAs towards the virus inhibitory effect in cell culture systems against MERS-CoV replication. The designed potential siRNAs were found to be very effective to inhibit the virus replication at various concentrations in both cell lysate and supernatant. The siRNA-1 and 4 were observed to be better in inhibiting the virus replication as compared to other siRNAs at various concentrations. Future studies will include investigating the efficacy of the designed siRNAs into other cell lines to make a comparative study for siRNAs in different cell lines so that the effect of the siRNAs can be evaluated in other cell types. This novel technology for the prediction and evaluation of potential siRNA can be utilized against other viruses to generate new antiviral therapeutics. The recent developments with improved technology to use therapeutic miRNA and siRNA open the doors to evaluate and incorporate these significant breakthroughs as therapeutic molecules against multiple diseases.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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