International Immunopharmacology 73 (2019) 270–279

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Nonstructural protein-1 (NS1) of dengue virus type-2 differentially stimulate expressions of matrix metalloproteinases in monocytes: protective effect of paracetamol

Rituraj Niranjan*, M.K. Sumitha, Thirumal Sankari, Subramanian Muthukumaravel, Purushothaman Jambulingam

Unit of Microbiology and Molecular Biology, ICMR-Vector Control Research Center, Puducherry 605006, India

ARTICLE INFO

Keywords:
Dengue viral disease
Nonstructural protein-1
Monocytes
Matrix metalloproteinases

ABSTRACT

Background: Dengue fever is a re-emerging viral disease and affects millions of population worldwide. Monocytes are involved in dengue viral disease, however, their exact role is still not clear. In the present study, we investigated, the effect of NS1 antigen of dengue virus and paracetamol on THP-1 monocytes associated to expressions of matrix metalloproteinases (MMPs) and cytokine release.

Methods: Assessment of cell morphology by bright field microscopy, cell viability by MTT assay, protein estimation by Bradford reagent were done in cells exposed to NS1 antigen in the presence and absence of paracetamol. Cytokines estimations were done by ELISA. Expression profile of matrix metalloproteinase genes was done using real-time PCR and reverse-transcriptase PCR.

Results: NS1 exposure of THP-1 monocytes cells, changed their cell morphology and activated them for release of proteins in 24 h. Expressions of MMP-2, MMP-8, MMP-9 and MMP-14 genes were upregulated by NS1 exposure. Further, exposure of NS1 to THP-1 monocytes cells increased expression profile of MMP-10 and MMP-13 genes to a lesser extent. Treatment with paracetamol (1 mg/ml and 2 mg/ml), significantly down-regulated the expression profile of MMP-2, MMP-8, MMP-9 and 14 in dose dependent manner. NS1 exposure also increased the release of cytokines IL-4, IL-6, and IL-10 but decreased the release of TNF-α and IL-15. Interestingly, paracetamol reversed NS1 induced changes in the release of these cytokine in dose dependent manner.

Conclusion: Monocytes mediated expression of MMPs participates in the development of dengue pathogenesis in the severe cases of disease and paracetamol may have a protective effect in dengue viral disease.

1. Introduction

Dengue fever is a viral disease affecting a huge number of population throughout the world [1,2]. Dengue or dengue fever has become a serious health problem globally, which still lacks proper preventions or therapeutics [3]. The incidence of dengue disease is increasing, along with its geographic spread to new countries [4,5]. Dengue is a vector borne viral disease which is caused by the bite of *Aedes aegypti* and *Aedes albopictus* [6,7]. About 70% of dengue cases are reported from Asian countries [8,9]. The present decade has witnessed the spread of the disease from urban to rural settings. Dengue virus causes havoc in the lives of millions of people around the globe and could possibly affect non endemic areas having presence of mosquito vectors [3]. Dengue is caused by four different serotypes [10]. The genome of dengue virus (DENV) codes for the three structural and a total of seven non-structural proteins [11]. The non-structural proteins range from 1 to 5 (NS1 to NS5) [11]. Dengue NS1 is a non-structural lipoprotein that can also be in a secreted form [12,13]. Its molecular weight is 50 kDa [13]. Lipoproteins also participate in coagulation pathways which in turn are linked with vascular inflammation, NS1 is thought to have multiple connotations in the disease pathogenesis [14]. The association of dengue NS1 protein was reported with the severe clinical cases of

Abbreviations: DSS, dengue shock syndrome; NS, non-structural protein; DENV, dengue virus; CD8, cluster of differentiation 8; MMPs, matrix metalloproteinases; ECM, extracellular matrix; IL-15, interleukin-15; TNF-α, tumor necrosis factor-α; IL-4, interleukin-4; IL-10, interleukin-10; ROS, reactive oxygen species; IL-6, interleukin-6; Th, T helper cells; HIV, human immunodeficiency virus; DHF, Dengue Hemorrhagic Fever; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; DCs, dendritic cells; CD4, cluster of differentiation 4

* Corresponding author at: Dr. Rituraj Niranjan, Scientist-C, Talent Search Scientist (TSS-ICMR), Unit of Microbiology and Molecular Biology, ICMR-Vector Control Research Center, Puducherry 605006, India.

E-mail address: riturajniranjan@rediffmail.com (R. Niranjan).

https://doi.org/10.1016/j.intimp.2019.05.022
Received 10 April 2019; Received in revised form 6 May 2019; Accepted 10 May 2019
Available online 21 May 2019

disease. Paranavitane et al. suggested the use of NS1 protein as a marker of dengue as the protein persisted in patients with DHF [15]. Regardless of the alarming number of infections, mortalities and increasing global distribution, the mechanism by which dengue virus alter immune system has not been explored in the recent past.

One of the key features of severe dengue disease is vascular leakage which leads to low blood pressure and sometime shock in extreme cases [16]. Till date exact mechanisms leading to severe cases of disease are not clear. It is speculated that, this is a result of complex miscellaneous interaction between the host immune response and DENV. The pathophysiology dengue disease is characterized by the activation of monocytes and T lymphocytes, along with the high levels of cytokines and some mediators of inflammation leading to increased permeability of vascular system [17,18]. Even though DENV can infect different cell types, but mainly monocytes and dendritic cells are its primary targets. Infection of immature, monocyte derived dendritic cells with DENV leads to productive viral replication, cellular activation and maturation, and increased production of cytokines [19]. Monocytes are generally present in the blood but go to dermis and then change into dendritic cells (DCs) [19]. These monocyte-derived DCs become DENV-infected during the second wave and then become the primary targets for DENV replication [14,20]. Human blood monocytes are now considered to be critical in dengue infections and designated as a main virus infected cells [21]. Monocytes are thought to be implicated in both pathogenesis and protection in dengue infection. Monocytes also secrete IFN-α in response to dengue virus. Durbin et al., 2008 identified monocytes as one of the natural hosts of DENV. Differentiation of monocytes into macrophages is found to be accelerated following DENV infection [22]. Fink et al. showed that, the depletion of monocytes had resulted in a ten-fold increase in viral load, implicating that monocytes play a crucial role in controlling the viral infection [21]. In addition to monocytes, matrix metalloproteases play an important role in the dengue pathogenesis but their exact roles are not known [23,24]. Recently, some clinical studies have shown that severity of plasma leakage is correlated to the up-regulation of matrix metalloproteases expression in this disease [25]. Matrix metalloproteinase (MMPs) are members of the metzincin family of proteases, enzymes which have conserved zinc binding motifs [26]. Initially they were thought to be solely involved in the degradation of various components of extracellular matrix, but now found to regulate extracellular tissue signaling networks [27]. Some recent studies have also shown, the modulation of MMP-2 and MMP-9 expressions in response to dengue virus infection, leaving the puzzle unanswered [26,28].

Paracetamol is an antipyretic drug and most commonly used against dengue fever, however the mechanism of its protective role is not clear [29,30]. In some cases, overdose of paracetamol has shown adverse effects linking to liver pathology [31,32]. Over this dilemma, it is interesting to know that paracetamol is still the drug of choice among clinicians [29]. The effect of paracetamol on expressions of matrix metalloprotease is not properly known and forms a gap in the understanding of dengue pathogenesis and its management [33]. From above evidences, it becomes clear that monocytes are involved in the dengue fever and play the critical role in its pathogenesis [34,35]. Monocytes also express high level of matrix metalloproteases in other diseases and mediate the disease pathogenesis [36,37]. On the other hand severe cases of dengue show an increase in the expression profile of matrix metalloproteases, however, the role of matrix metalloproteases on monocytes in dengue pathogens is not clear [25,38]. Considering these all evidences, it becomes vital to understand the role of matrix metalloproteases on monocytes in response to dengue virus. Therefore, the present study was planned to understand the role of monocytes specific matrix metalloproteases in response to dengue virus NS1 antigen.

2. Materials and methods

2.1. Cell line used

THP-1, human monocytic cell line was purchased from National Centre for Cell Science, Pune and since then it is being maintained at the ICMR-VCRC tissue culture laboratory.

2.2. Culture and maintenance of THP-1 cell line

Human monocytes cell line, THP-1 was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum. Medium was changed for cells which were growing well but were not yet confluent. The pellet was re-suspended in the fresh medium which was already brought to room temperature. The cell suspension was transferred into a sterile T-25 culture flask and was sealed. The flasks were microscopically examined and were placed at 37 °C in a CO_2 incubator for growth.

2.3. MTT assay for cell viability

MTT assay was carried out to measure cell proliferation or cell viability as per the protocol described by Mosmann et al. with required modifications [39,40]. The cell suspension was harvested by centrifugation and suspended in fresh medium. THP-1 cells at the number of 1×10^5 cells/ml were seeded in the 96 well plate. The 100 µl of cell suspension/well was loaded into 96 six well plate. Treatment with desired chemical was given for desired time intervals. After 24 h of incubation 20 µl of MTT was added to all wells including the blank. After 3 h of incubation the plate cover was removed and the absorbance was measured at 570 nm.

2.4. Exposure of cells with NS1 antigen of DENV type 2

Dengue NS1 antigen was purchased from R & D company (Cat. No. DGHU0217031). The protein was reconstituted in 100 µl of autoclaved MilliQ water. Cells from a culture flask were pelleted by centrifugation and were re-suspended in fresh medium. 1 ml of cell suspension was transferred into two wells of the six well plates. One well was marked as control and the second one was marked as test, to the latter well, the NS1 antigen was added to a final concentration of 20 µg/ml as also described earlier [16].

2.5. Protein estimation in the culture supernatants and bright field microscopy

Protein estimation was done using Bradford's reagent. Standard BSA (Bovine Serum Albumin) solution (2 mg/ml) was made by dissolving 10 mg BSA powder in 5 ml MilliQ water. 5 µl of these standard sample was added to separate wells in the 96 well plate and to the blank 5 µl of normal saline was added. Equal amount of the unknown samples was also added in separate wells. 250 µl of Bradford's reagent was added to the wells and mixed well. It was kept for incubation at room temperature for 45 min. Absorbance was then measured in the Multiskan™ GO Microplate Spectrophotometer at 595 nm. The concentrations of unknown samples were calculated from standard samples. Bright field microscopy for the THP-1 cells was done for taking images of THP-1 cells with or without exposure with NS1 antigen using Nikon microscope.

2.6. RNA isolation and quantification

Cells were collected after centrifugation and then lysed in 1000 µl trizol by repeated pipetting (as per the protocol provided by sigma). RNA pellet was dried for 10 min. The pellet was reconstituted by adding 20 µl of autoclaved MilliQ water. Isolated RNA was quantified using

Table 1
Details of primer used in the study.

Primer	Sequence 5′-3′	Tm	Target size
MMP2 forward	CCCCAAAACGGACAAAGAG	57	314 bp
MMP2 reverse	CACGAGCAAAGGCATCATCC	60	
MMP9 forward	CACTGTCCACCCCTCAGAGC	65	263 bp
MMP9 reverse	GCCACTTGTCGGCGATAAGG	63	
MMP14 forward	CGCTACGCCATCCAGGGTCTCAAA	69	497 bp
MMP14 reverse	CGGTCATCATCGGGCAGCACAAAA	67	
GAPDH forward	TCAACGGATTTGGTCGTATTGGG	63	234 bp
GADHP reverse	TGATTTTGGAGGGATCTCGC	58	

Thermo Scientific µDrop™ Plate (Catalogue Number: N12391). 2 µl of the isolated RNA was used for quantification. The isolated RNA was stored in −20 °C.

2.7. One step reverse transcriptase polymerase chain reaction

For each reaction 20 µl of the master mix (Roche), template RNA and 3 µl of MilliQ water were used. Primer concentration was 0.4 pmol. 200 ng of RNA was used as template throughout the study. For negative control 20 µl of master mix and 5 µl of MilliQ water was used. The reaction mixture was vortexed for 10 s and then centrifuged. Tubes were kept in the thermal cycler and the programmer was run. The polymerase chain reaction was set for reverse transcriptase reaction at 50 °C for 30 min, followed by initial denaturation at 94 °C for 5 min, followed by 36 cycles involving denaturation at 94 °C for 30 s, annealing at different temperature according to the primers for 1 min and 10 s and extension at 68 °C for 2 min, and a final extension step at 68 °C for 6 min. The PCR products were subjected to agarose gel electrophoresis for separation of DNA fragments. 1.5 to 2% agarose gel was used in this study. The target genes were identified using the standard molecular size marker. The details of primers and their conditions are given in

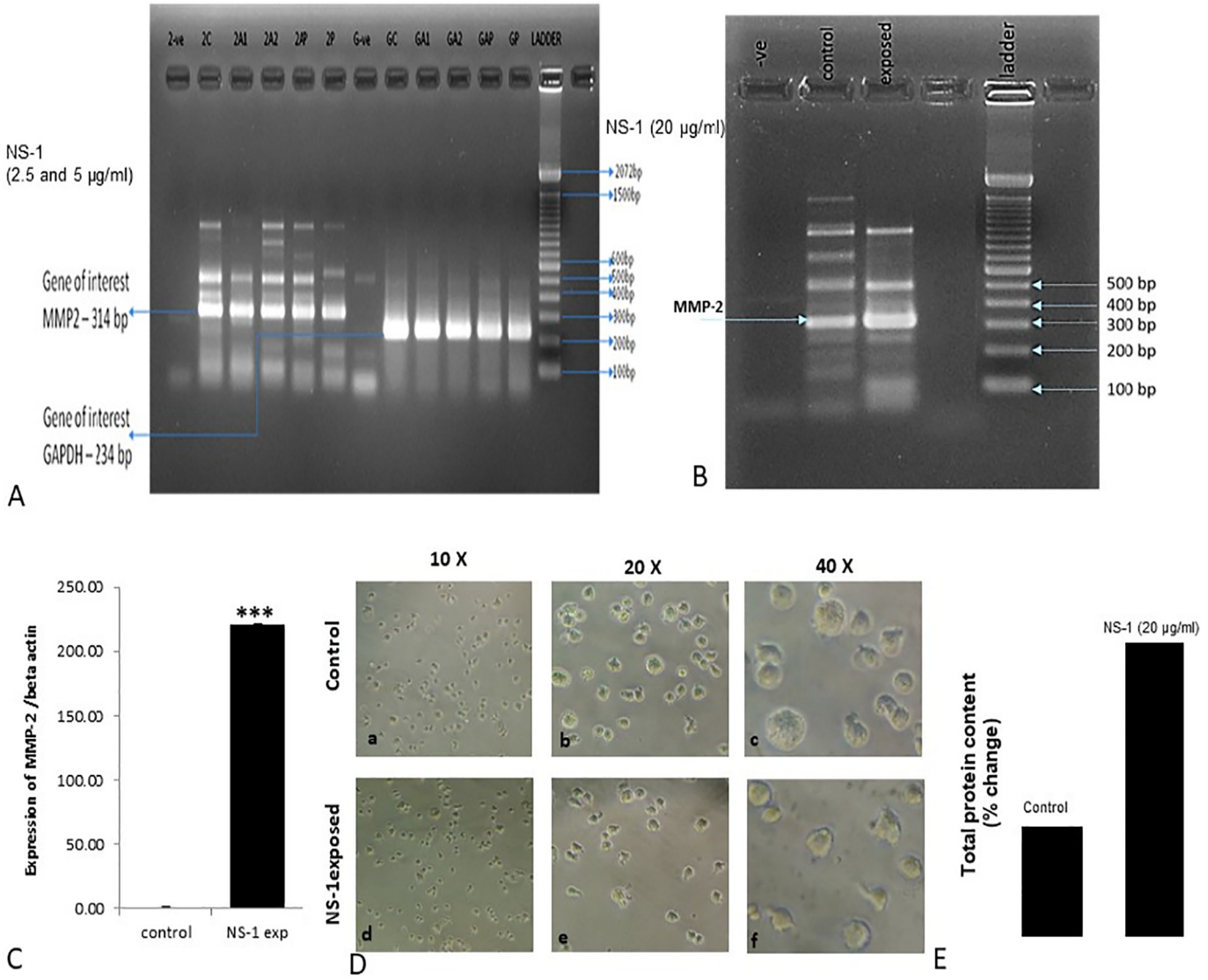

Fig. 1. A. MMP-2 gene expression profile by THP-1 monocytes after the NS1 exposure. Image of agarose gel, showing expression pattern of MMP-2 gene (314 bp). Lane: 1 = negative control, 2 = control (cell without NS1 exposure), 3 = test (cell with NS1 exposure 2.5 µg/ml), 4 = test (cell with NS1 exposure 5 µg/ml). Lane no. 7 to 10 are GAPDH, respectively. Second last = DNA ladder (marker 100–1500 bp). B. MMP-2 gene expression profile in THP-1 monocytes after NS1 (20 µg/ml) exposure. Image of agarose-gel showing MMP-2 gene expression (314 bp) in response to NS1 antigen. Lane: 1 = negative control, 2 = control (cell without NS1 exposure), 3 = test (NS1 exposed), 4 = BLANK (No sample). 5 = DNA ladder. C. MMP-2 gene expression profile in THP-1 monocytes after NS1 (20 µg/ml) exposure measured by qPCR, ***$p < 0.001$ significant with control group. D. Morphological assessment of THP-1 cells in response to NS1 antigen (a), control cells at 10× (b), control cells at 20× (c), control cells at 40× (d), cells exposed with NS1 antigen at 10× (e) cells exposed with NS1 antigen at 20× (f), cells exposed with NS1 antigen at 40×. E. Protein estimation in supernatant of THP-1 monocytes exposed with NS1 antigen.

Table 1.

2.8. Enzyme linked immunosorbent assay for the measurement of cytokines (ELISA)

ELISA was done in the culture supernatant as per the manufacture instructions (R&D systems). Amount of all cytokines were calculated and were presented compared to the control.

2.9. cDNA synthesis and real time polymerase chain reaction

Isolated RNA was converted into cDNA using reverse transcriptase PCR. 200 ng/μl of isolated RNA was used for cDNA synthesis. The obtained cDNA was quantified and used as template for real time polymerase reaction. Real time PCR was performed to measure the relative expression of selected genes in response to stimulation with DENV serotype 2 NS1 antigen. The cDNA synthesized above was made use for the reaction. SYBR Green is a dye which binds to dsDNA and emits fluorescence only when it binds to dsDNA. Fluorescence increases proportionally with increase in the amount of amplicon, that is generated in each cycle, and hence can be quantified. 5 μl of template was added to a mixture containing 10 μl of SYBR Green, 3 μl of MilliQ water and 1 μl (0.5 pmol) of each primers. A sample in which the template had been replaced with MilliQ water was used as a negative control. The samples were analyzed in duplicates. The C_T value of the gene of interest was normalized using the reference gene (GAPDH or actin), using the algorithm: Analysis of results was done using (the $2^{-\Delta\Delta CT}$ method). The GAPDH gene was taken as a reference gene for quantification of expression profile of genes.

2.10. Statistical analysis

Here majority of results are presented as mean ± SEM. The software GraphPad prism 5 was taken in use for analyzing data. One-way analysis of variance (ANOVA), followed by Newman-Keuls test as post-hoc test was done for the analysis of the data. The p value < 0.05 was considered statistically significant.

3. Results

3.1. Effect of NS1 antigen on mRNA expression pattern of MMP-2 gene, cell morphology and total protein release by THP-1, monocytes

MMP-2 has been involved in the majority of dengue cases but their expression profile specifically in monocytes is not known. Therefore, we measured expression profile of MMP-2 in response to NS1 antigen of dengue serotype-2 (2 μg/ml and 5 μg/ml) in THP-1 monocytes after 24 h of exposure. As shown in Fig. 1A, the expression of MMP-2 gene is not sufficiently up-regulated in the NS1 exposed cells compared with control (unexposed cells). We found that MMP-2 mRNA is not significantly high in the NS1 exposed cells as evidenced by the bands observed in the agarose gel electrophoresis. The expression profile of a reference gene or internal control gene was also checked to support the data. We have used GAPDH gene as a reference. GAPDH expression is not changed in response to NS1 antigen, as all bands looks same (Fig. 1A). Therefore, we have chosen to go high concentrations (20 μg/ml) of NS1 antigen to assess its effect on THP-1 monocytes. Next, we have tested the effect of higher concentrations of NS1 protein (20 μg/ml) on the THP-1 monocytes. We found that, higher concentrations of NS1 protein (20 μg/ml) have significantly increased the MMP-2 expression in 24 h of exposure period in THP-1 monocytes.

As cell morphology is an important feature of the immune cells in conditions of their activation towards any antigen we have assessed the cell morphology of THP-1 monocytes after NS1 antigen exposure (20 μg/ml). As seen in Fig. 1D, NS1 antigen has effectively changed the morphology of the cells in a twenty 24-hour time intervals. NS1 exposed cells look like irregular type compared to the control cells (the activated cells). The THP-1 monocytes exposed with NS1 antigen of DENV appeared to be elongated and wrinkled which may be compared with their activation state in presence of NS1 antigen. In addition to cell morphology we have also tested the total protein content released by the monocytes in response to NS1 exposure. Protein estimation was done in supernatant harvested from cells after 24 h of exposure with NS1 antigen. As seen in Fig. 1E, NS1 antigen has effectively increased the total protein content in 24-hour time intervals. We found that, supernatant in the NS1 exposed cells shows a high level of protein content (about 110%) compared with the control cells (cells without exposure).

3.2. NS1 exposure increased mRNA expression pattern of MMP-8, MMP-9, MMP-10, MMP-13 and MMP-14 genes in THP-1, monocytes

In addition to MMP-2, we have also measured the expression profile of MMP-8, MMP-9, MMP-10, MMP-13 and MMP-14 gene in NS1 (20 μg/ml) stimulated monocyte cells. In addition to one step RT-PCR, we have also measured the MMPs expression patterns by real time PCR (qPCR). Real-time PCR was done using the protocol described in the material and methods section. To achieve this, cells were exposed with the NS1 antigen (20 μg/ml) for 24 h and real time PCR was done to measure the expression profile of MMPs genes. Analysis of results was done using the $2^{-\Delta\Delta CT}$ method. The beta actin gene was taken as a reference gene for the quantification of the expression profile of MMPs genes. We found that (Fig. 2), the expressions of MMP-8, MMP-9, MMP-10, MMP-13 and MMP-14 genes were up-regulated in the NS1 exposed cells compared to unexposed cells. Among these MMP genes, the expressions of MMP-14, MMP-2, MMP-8, and MMP-9 were highly upregulated compared to the other MMPs genes. Therefore, we have taken these four genes for our further confirmation study. To support these results, one step reverse transcriptase PCR was also done to measure the expression profile of MMPs genes. As shown in Fig. 2A, the expression of MMP-14 gene is up-regulated in the NS1 exposed cells compared to the unexposed cells. As observed in Fig. 2A, we see that, the thickness of MMP-14 band of exposed cells is higher than the thickness of band of un-exposed cells. Similarly, expression of MMP-9 gene is up-regulated in the NS1 exposed cells compared to the unexposed cells (Fig. 2C).

3.3. Paracetamol significantly downregulated NS1 induced mRNA expressions of MMP-14, MMP-2, MMP-8 and MMP-9 genes

Paracetamol is a most common drug used against dengue fever but its mechanism of action in severe cases of dengue viral disease is not yet understood and need extensive study. Therefore, to understand the effect of paracetamol in dengue pathogenesis, we tested the effect of paracetamol on NS1 induced matrix metalloproteinases expressions in THP-1 monocyte cells. We found that (as shown in Fig. 3), NS1 exposure increased mRNA expressions of MMP-14, MMP-2, MMP-8 and MMP-9 genes which were reversed by paracetamol in dose dependent manner. We found that paracetamol (1 mg/ml) has significantly reversed the NS1 induced mRNA expressions of MMP-14 and MMP-8 genes (Fig. 3A and C). However, paracetamol (2 mg/ml) attenuated the mRNA expressions of MMP-2 and MMP-9 genes.

3.4. NS1 exposure increased release of interleukin-6, interleukin-10, interleukin-4, but downregulated TNF-alpha, which were reversed by paracetamol in dose dependent manner

It is now widely accepted that, cytokines like interleukin-6, interleukin-10, interleukin-4, and TNF-alpha are involved in the regulation of matrix metalloproteases however their role in dengue viral disease was not previously understood. Therefore, to understand the mechanism of MMP regulation in monocyte in response to NS1 measurements for these cytokines was done in the cell supernatant after the exposure with NS1 antigen. We found that (Fig. 4), NS1 exposure

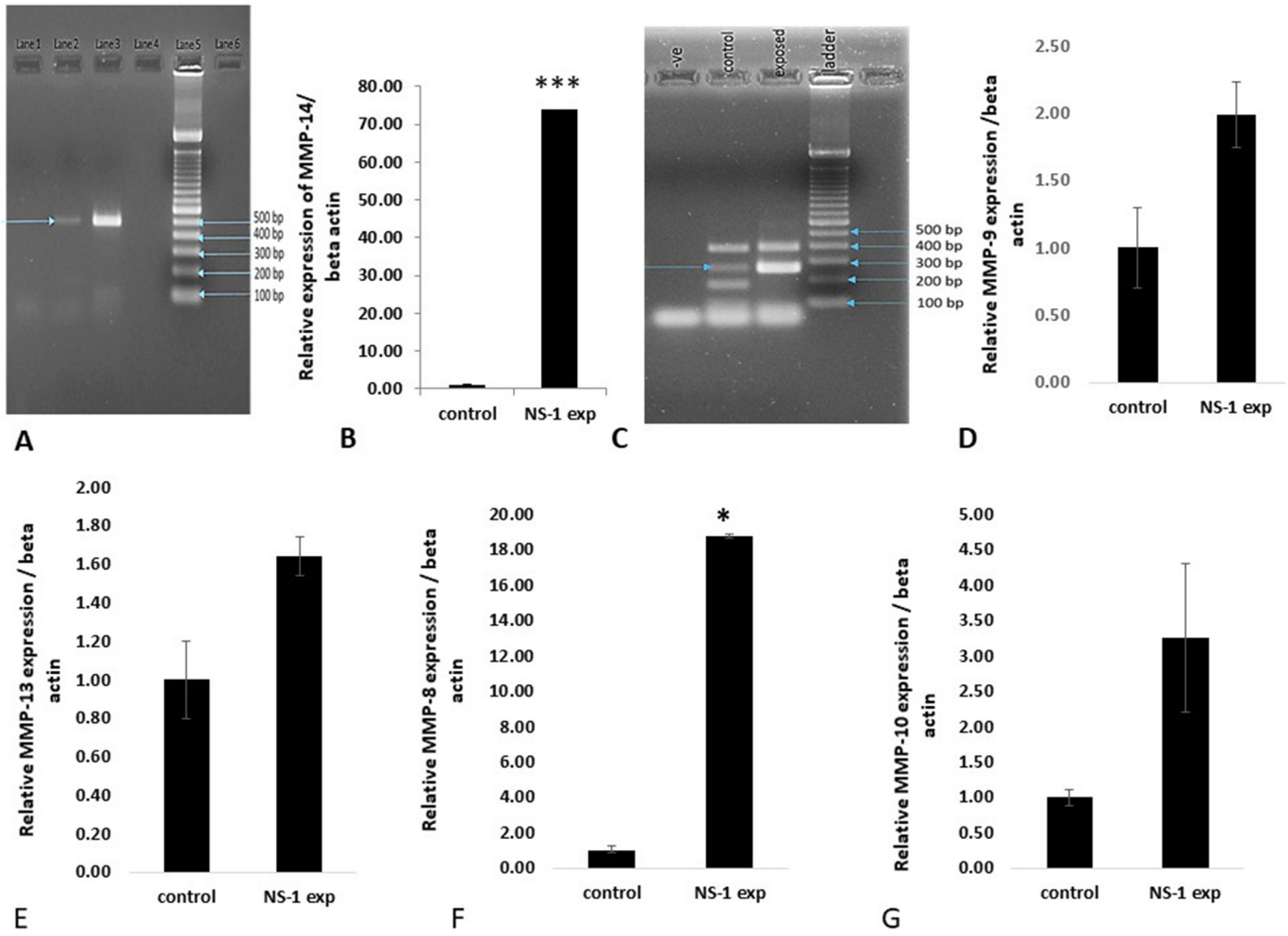

Fig. 2. Expression profile MMP-14, MMP-8, MMP-9, MMP-10 and MMP-13 genes in monocytes after exposure of NS1 antigen. A. Image of agarose gel showing MMP-14 gene expression pattern (297 bp) in response to NS1 antigen exposure. Lane: 1 = negative control, lane: 2 = control (cell without NS1 exposure), lane: 3 = Test (NS1 exposed), lane: 4 = blank (no sample). Lane: 5 = DNA ladder. B. Real time PCR measured expression profile to MMP-14 gene in response to NS1 exposure. C. Image of agarose-gel showing MMP-9 gene expression (263 bp) in response to NS1 antigen exposure. Lane: 1 = negative control, 2 = control (not NS1 exposed), 3 = test (cell with NS1 exposure), 4 = DNA ladder. D. Expression profile to MMP-9 gene by qPCR. E. Expression profile to MMP-13 gene by qPCR. F. Expression profile of MMP-8 gene by qPCR. G. Expression profile of MMP-10 gene by qPCR. Histograms represent means ± SEM of different groups showing relative mRNA expressions. *$p < 0.05$ and ***$p < 0.001$ significant with control group.

increased release of interleukin-6, interleukin-10, interleukin-4, but downregulated TNF-alpha, which were reversed by paracetamol in dose dependent manner. Paracetamol is the most common drug used against dengue fever but its mechanism of action in severe cases of dengue viral disease is not yet understood and need extensive study. Therefore, in order to understand the mechanism of action of paracetamol against this disease we have tested the effect of paracetamol in these NS1 induced THP-1 monocyte cells. We found that paracetamol (1 μg/ml and 2 μg/ml) has significantly reversed the NS1 induced release of interleukin-6, interleukin-10 and interleukin-4. Paracetamol concentration also stimulated the release of TNF-alpha in dose dependent manner.

3.5. NS1 exposure downregulated IL-15 release, which was reversed by paracetamol in dose dependent manner

Interleukin-15 is one of the important cytokine which plays a critical role in the tissue specific antivirus activities [41]. Therefore, in addition to other cytokines, we have also measured IL-15 release in response to NS1 exposure. We found that (Fig. 5), NS1 exposure downregulated IL-15 release when conpared with control. We found that paracetamol (1 μg/ml and 2 μg/ml has significantly reversed the NS1 induced downregulated release of IL-15 in dose dependent manner. Paracetamol concentration also stimulated the release of IL-15 per se.

4. Discussion

In this study, we show that, NS1 antigen of DENV-2 significantly up regulate matrix metalloproteases (MMPs) expression in monocytes. We have tested the effect of NS-1 antigen on in vitro cultured human monocytes, THP-1 cells. We found that, NS1 exposure to human monocytes significantly up regulated the expressions profile of MMP-2, MMP-9, and MMP-14 gene expression in a 24 hour exposure period. NS1 also up regulated the expressions profile of MMP-8, MMP-10, and MMP-13 gene expressions, but to a lesser extent.

A wide range of studies speculate that, MMPs expression is involved in the vascular permeability leading to plasma leakage and thus development of dengue shock syndrome [25,34]. A number of cell specific studies also describe the role of dengue virus mediated expressions of MMPs, however, the exact mechanism remains elusive [42,43]. It has also been well established that, monocytes cells are the major cells type involved in the pathogenesis of dengue fever [44]. It is also reported that, monocytes associated protein MCP-1 is highly up regulated in the dengue shock syndrome suggesting an important role of monocytes in the development of dengue shock associated dengue pathogenesis [45].

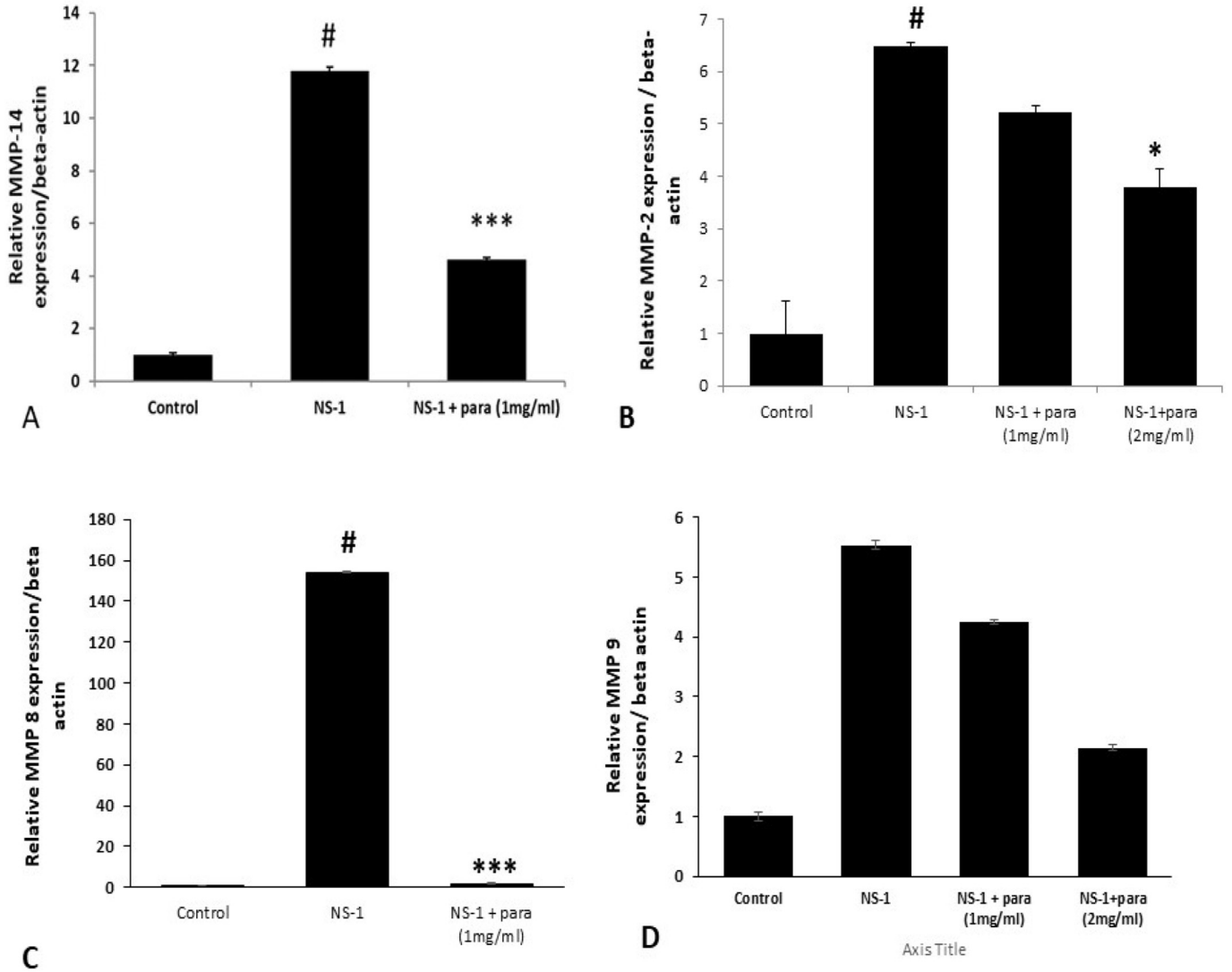

Fig. 3. Effect of paracetamol on the mRNA expression profile MMP-14, MMP-2, MMP-8 and MMP-9 genes. A. Real time PCR measured expression profile of MMP-14 gene in response to NS1 and paracetamol. B. Real time PCR measured expression profile of MMP-2 gene in response to NS1 exposure. C. Real time PCR measured expression profile of MMP-8 gene in response to NS1 and paracetamol. D. Real time PCR measured expression profile of MMP-9 gene in response to NS1 and paracetamol. Histograms represent means ± SEM of different groups showing relative mRNA expressions. #Significant with control, *$p < 0.05$ and ***$p < 0.001$ significant with NS1 treated group.

Importantly, the localization of dengue virus antigen is also found in the tissue macrophages of human biopsies affected with dengue virus suggesting an important role of monocytes in the causation of disease pathogenesis [46]. Despite a lot of upcoming research the exact role of monocytes on dengue virus associated matrix metalloproteases is not known [45].

It has already been described that, dengue virus infection up regulates expression of MMP-2 with an increase in vascular permeability leading to a fatal shock-like syndrome (DHF/DSS) [47]. It is also shown that direct infection of endothelial cells may also lead to the induction of vascular permeability by up regulation of MMP-2 expression [48]. In the present study, NS1 antigen of dengue virus type-2 has also increased the MMP-2 expression which is in accordance with the previous literature and thus emphasizes its role in the monocytes mediated pathogenesis of dengue viral disease leading to dengue shock syndrome (DSS). Similar to MMP-2, the MMP-8 and MMP-9 have also been involved in the pathogenesis of dengue shock syndrome via altering the vascular permeability leading possible plasma leakage [26,47]. We found that, NS1 antigen of dengue virus type-2 increased MMP-8 and MMP-9 expressions which supports previous findings and highlight monocytes as a key cells responsible for the development of severer dengue pathology (Fig. 2) [49].

In addition to MMP-2 and MMP-9, the role of MMP-14 in dengue pathogenesis has not been elucidated before. It is seen that, matrix metalloproteinase-14 significantly help in the development of lung cancer via modifying heparin-binding EGF-like growth factor [50]. It is believed that, MMP 14 (Membrane Type 1 Matrix Metalloproteinase) induces T cell infiltration in the inflamed area and therefore it can be considered that NS1 antigen mediated MMP-14 response may be required to recruit T cells in dengue pathogenesis [51]. Another study also described that, MMP-14 regulate migration of monocytes and destruction of collagen in tuberculosis [52]. In the present study NS1 antigen of dengue virus serotype-2 have also increased the MMP-14 expression which is in accordance with the previous literature and thus emphasize on the role of monocytes in the pathogenesis of dengue virus lead dengue shock syndrome (DSS) [49].

Further it becomes vital to understand that how the MMPs regulate endothelium and induces plasma leakage responsible for the development of dengue shock syndrome [53]. It is widely understood that MMP-2 and MMP-9 regulate angiogenesis in many diseases [54].

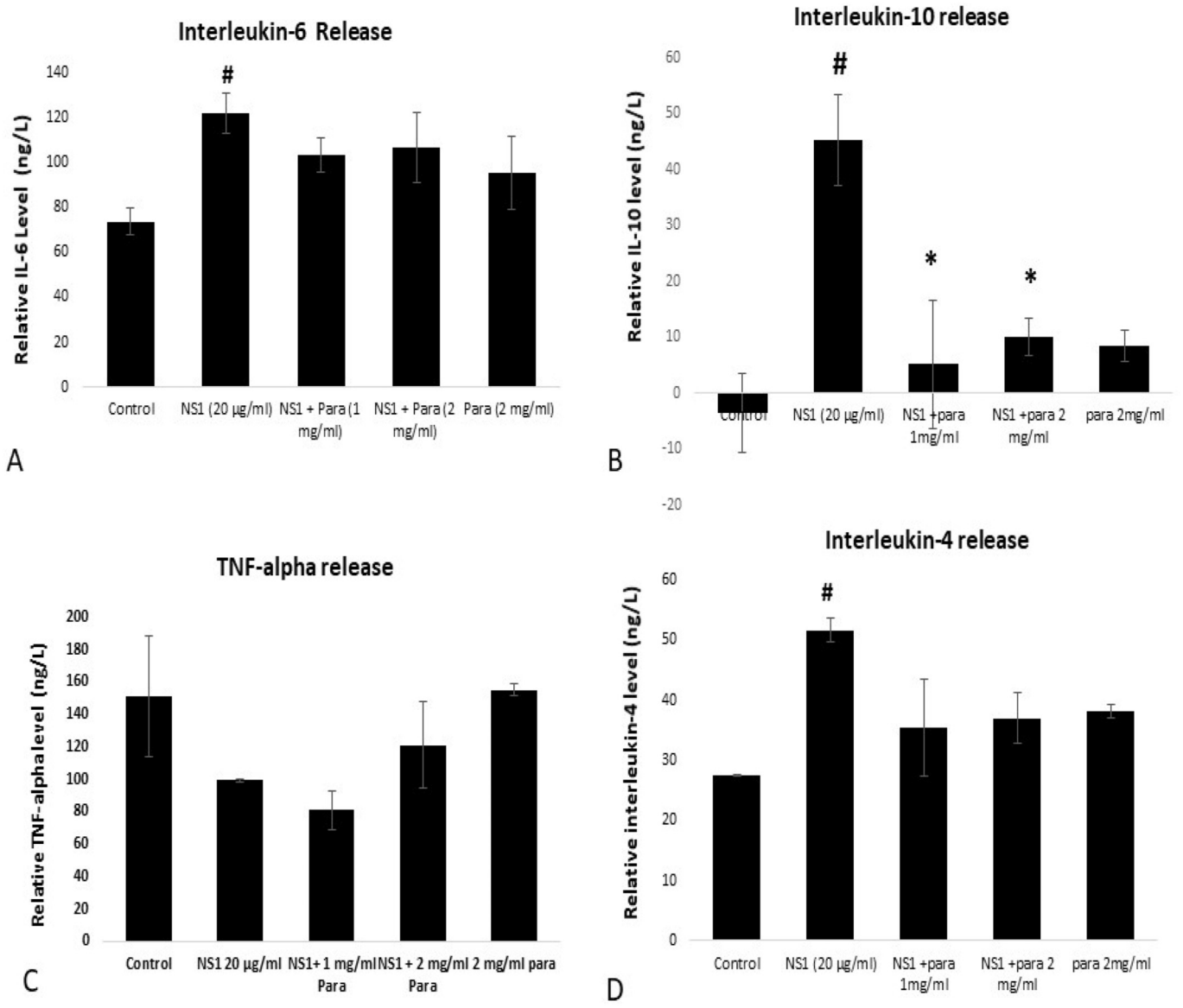

Fig. 4. Effect of NS1 and paracetamol on the release of cytokines by THP-1 monocyte. A. Interleukin-6 release in response to NS1 antigen and effect of paracetamol. B. Interleukin-10 release in response to NS1 alone and in presence of paracetamol. C. TNF-alpha release in response to NS1 antigen and paracetamol. D. Interleukin-4 release in response to NS1 antigen alone and in presence of paracetamol. Histograms represent means ± SEM of different groups showing relative change. #Significant with control, *$p < 0.05$ significant with NS1 treated group.

Recently it was proved that inhibition of MMP-2 and MMP-9 down-regulates the migration of cells and formation of blood vessels in retinoblastoma [55]. Therefore, MMP-2 upregulated in this study might effect on the endothelium cells making them dysfunctional and responsible for development of severe pathology of dengue disease. It was found that, the protease MT1-MMP/MMP-14 is involved and control a combinatorial proteolytic program in activated endothelial cells [53]. Therefore, upregulation of monocytes may be inducing endothelial cells and thus inducing dysfunctional endothelial cells responsible of plasma leakage which may be further responsible for the development of dengue shock syndrome. It is further evidence that MT1-MMP/MMP-14 regulates pericellular proteolysis which may be involved in the development of endothelium and other cells MMP-2 activation and thus amplification of MMP-14 and other matric metalloproteases. MMP-14 is also involved in the regulation of angiogenic activity [56,57]. Further it is evident that MMP-2 and MMP-14 produce its effects via exosomes mediated mechanisms [58]. There it is speculated that monocytes may be regulating the endothelium of the blood vessels causing them responsible for plasma leakage in the lethal cases of dengue shock syndrome (DSS). Paracetamol has reversed these NS1 induced changes in MMPs expressions in dose dependent manner depicting its protective effects (Fig. 3).

The expression pattern of cytokines has been a key priority in the pathogenesis of dengue viral disease, however, their exact roles were not known. It was understood that, Interleuki-6 is a key regulatory cytokine and controls the expression pattern of MMP-2 and MMP-9 [59]. The disease outcome in nasopharyngeal carcinoma patients, MMP-2 and MMP-9 activity was significantly affected by IL-6/NOS2 inflammatory signals [60]. In the present study, NS1 has significantly upregulated the release of interleukin-6 which indirectly may have increased the expression pattern of these matrix metalloproteases. Similarly, IL-4 produces a T helper type-1 response and increases T cell immunity. In this study, monocyte significantly increased the IL-4 release which is responsible for the development of T cell immunity. Paracetamol has reversed these NS1 induced changes in dose dependent manner showing its protective effects.

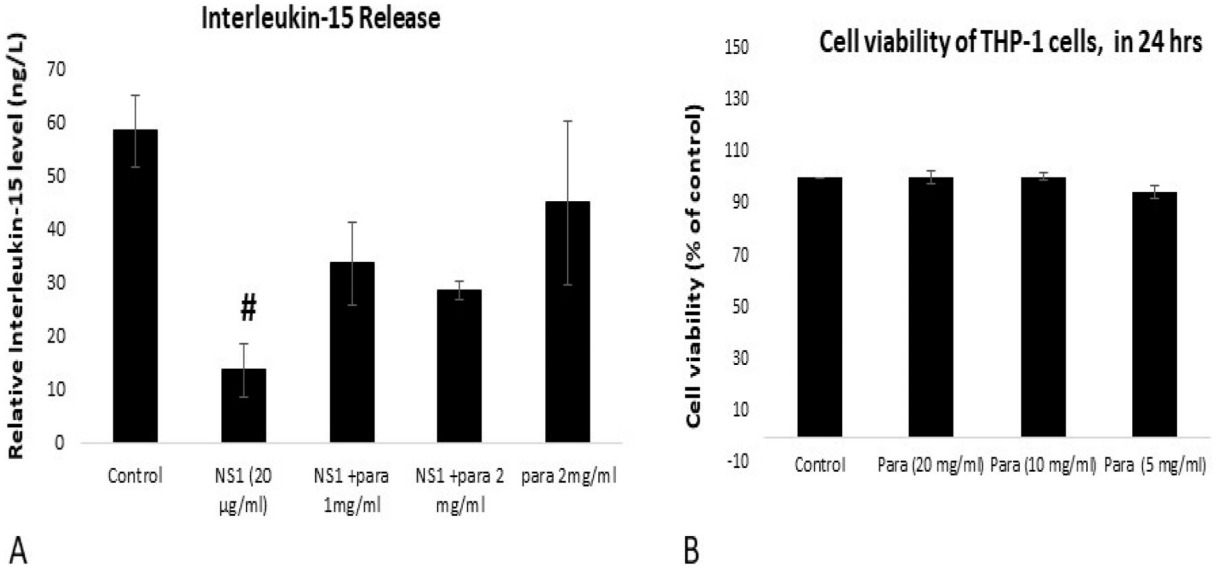

Fig. 5. A. Effect of NS1 and paracetamol on the release of cytokine IL-15 by THP-1 monocytes. Histograms represent means ± SEM of different groups showing relative change. #Significant with control. B. Cell viability of paracetamol on the THP-1 monocyte cells in in 24-hour duration.

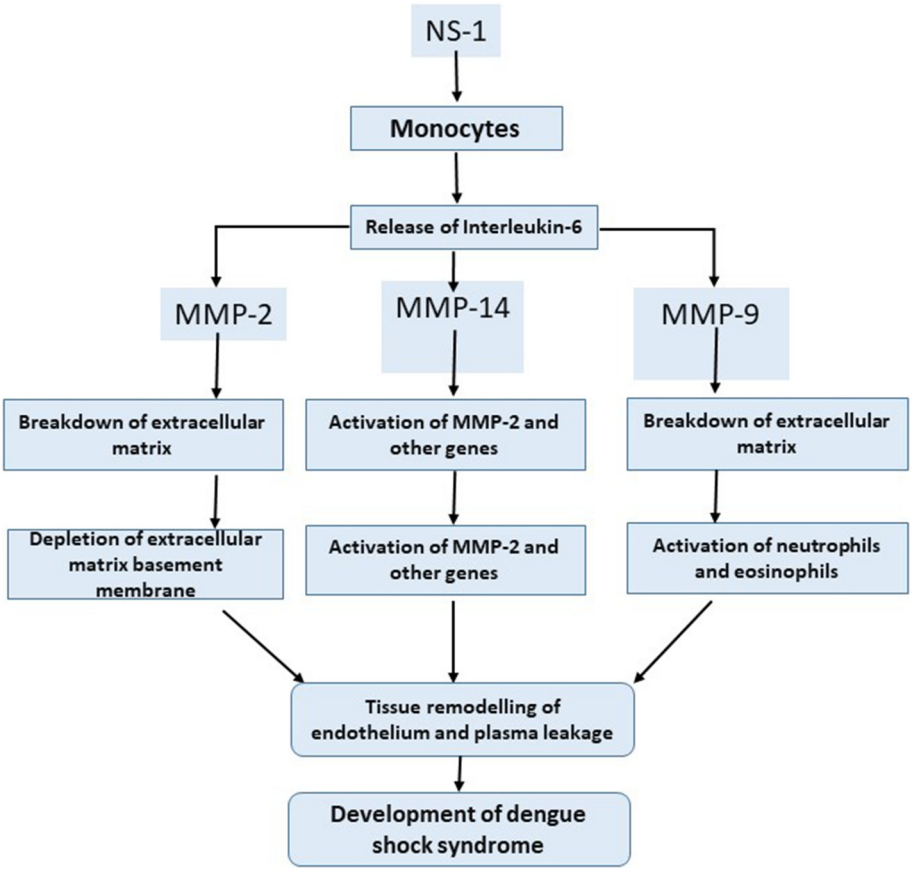

Fig. 6. Diagrammatic representation of the mechanism of NS1 induced MMPs. In this study we found, that NS1 induces release of interleukin-6. This interleukin indirectly regulates MMP-2, MMP-9 and MMP-14 expressions. These MMPs subsequently modulate endothelial functions and thus inducing the plasma leakage and may be involved in the severe form of dengue pathogenesis.

Interleukin-10 has been found in many clinical cases of dengue patients however its exact role is not still clear [61]. It was also proposed that IL-10 is only upregulated in the severe cases of dengue fever and can be a good biomarker for the dengue hemorrhagic fever or for the dengue shock syndrome [61,62]. However, it was still a question why IL-10 s upregulated in these severe cases of dengue virus disease and what it exactly does. In the present study it was found that NS1 exposure increases the release of interleukin-10 by the monocytes. It was found that, a drug azilsartan increases levels of IL-10 but on the other hand decreases the expression of MMP-2, MMP-9 thus showing an inverse correlation [63]. A similar in vitro study showed that interleukin-10 down regulated MMP-2 and MMP-9 expressions in explant culture [64]. Another study also showed that myocardial expressions of MMP-2, MMP-9 were significantly downregulated by interleukin-10 [65]. We speculate that interleukin-10 release is upregulated to counter the adverse effects of virus. Paracetamol has been the drug of choice in dengue viral disease however how does it effect on the severe cases of disease is not properly known [66]. In some cases it was found that,

overdoses of paracetamol significantly cause adverse effects on the patients [32]. In this study, we have seen that, paracetamol down-regulates the MMPs expression producing beneficial effects. However, paracetamol also decresed the release of interleukin-10, which might not be beneficial as IL-10 is involved in the countering the virus effect and to maintain the correct immune response.

NS1 exposure has also decreased the release of TNF-alpha and interleukin-15 in THP-1 monocytes. Both, TNF-alpha and IL-15 have many functions and modulate immune responses [67]. TNF-alpha helps differentiate $CD14^+$ monocytes into $CD70^+$ dendritic cells [68]. In this way NS-1 might has limited the differentiation of monocyte cells into dendritic cells. On the other hand IL-15 is involved in the activation of tissue specific immunity by activating Mucosal-associated invariant T (MAIT) cells during viral infections [41]. In the present study, NS1 has reduced the release of IL-15 thus limiting tissue specific anti-viral activity. Paracetamol treatment has restored the NS1 mediated decreased release of TNF-alpha and IL-15 showing improved defence mechanism against dengue virus antigen (Fig. 4).

5. Conclusion

In conclusion, it can be said that, monocytes carry overexpressed MMPs which participate in the development of dengue pathogenesis especially in the case of dengue shock syndrome (Fig. 6). NS1 exposure to monocytes, upregulated expression profiles of MMP-2, MMP-8, MMP-9 and MMP-14 which may be responsible for vascular permeability and thus development of dengue shock syndrome. However how these MMPs affect endothelial dysfunctions still remains a big question. Paracetamol has significantly reversed the expression profiles of these matrix metalloproteinases showing protective effects. Further, paracetamol significantly reversed, NS1 induced release of cytokines IL-6, IL-10, IL-4, in dose dependent manner. However, TNF-α and IL-15 were upregulated by paracetamol. This study suggests that, NS1 proteins of virus remain in the blood stream even after the clearance of the virus and keep stimulating immune system to produce different kinds of matrix metalloproteinases. These matrix metalloproteinases in turn may regulate the endothelium functions and results in plasma leakage, which occurs in the lethal cases of dengue shock syndrome. The current results are encouraging but further in depth studies are needed on these aspects for their possible implications in the disease pathogenesis.

Declaration of Competing Interest

None.

Acknowledgement

Intramural grant of ICMR-VCRC, Puducherry, India (grant No. IM-1802) to principal investigator Dr. Rituraj Niranjan is gratefully acknowledged.

References

[1] H. Kosasih, B. Alisjahbana, Q. de Mast Nurhayati, I.F. Rudiman, S. Widjaja, U. Antonjaya, H. Novriani, N.H. Susanto, H. Jusuf, A. van der Ven, C.G. Beckett, P.J. Blair, T.H. Burgess, M. Williams, K.R. Porter, The epidemiology, virology and clinical findings of dengue virus infections in a cohort of Indonesian adults in Western Java, PLoS Negl. Trop. Dis. 10 (2) (2016) e0004390.
[2] U. Raheel, M. Faheem, M.N. Riaz, N. Kanwal, F. Javed, N. Zaidi, I. Qadri, Dengue fever in the Indian Subcontinent: an overview, Journal of infection in developing countries 5 (4) (2011) 239–247.
[3] R.W. Byard, Lethal dengue virus infection: a forensic overview, Am J Forensic Med Pathol 37 (2) (2016) 74–78.
[4] S.A. Alshammari, Y.S. Alamri, F.S. Rabhan, A.A. Alabdullah, N.A. Alsanie, F.A. Almarshad, A.N. Alhaqbani, Overview of dengue and Zika virus similarity, what can we learn from the Saudi experience with dengue fever? Int J Health Sci (Qassim) 12 (1) (2018) 77–82.
[5] C.H. Calisher, Persistent emergence of dengue, Emerg. Infect. Dis. 11 (5) (2005) 738–739.
[6] A. Alhaeli, S. Bahkali, A. Ali, M.S. Househ, A.A. El-Metwally, The epidemiology of dengue fever in Saudi Arabia: a systematic review, J Infect Public Health 9 (2) (2016) 117–124.
[7] L.C. Katzelnick, J. Coloma, E. Harris, Dengue: knowledge gaps, unmet needs, and research priorities, Lancet Infect. Dis. 17 (3) (2017) e88–e100.
[8] R. Chen, G.Z. Han, Dengue in China: comprehensive phylogenetic evaluation reveals evidence of endemicity and complex genetic diversity, Am J Trop Med Hyg 94 (1) (2016) 198–202.
[9] M. Hemungkorn, U. Thisyakorn, C. Thisyakorn, Dengue infection: a growing global health threat, Bioscience trends 1 (2) (2007) 90–96.
[10] I. Kawaguchi, A. Sasaki, M. Boots, Why are dengue virus serotypes so distantly related? Enhancement and limiting serotype similarity between dengue virus strains, Proceedings. Biological sciences 270 (1530) (2003) 2241–2247.
[11] Y.S. Tian, Y. Zhou, T. Takagi, M. Kameoka, N. Kawashita, Dengue virus and its inhibitors: a brief review, Chem Pharm Bull (Tokyo) 66 (3) (2018) 191–206.
[12] Q. Xie, B. Zhang, J. Yu, Q. Wu, F. Yang, H. Cao, W. Zhao, Structure and function of the non-structural protein of dengue virus and its applications in antiviral therapy, Curr. Top. Med. Chem. 17 (3) (2017) 371–380.
[13] H.R. Chen, Y.C. Lai, T.M. Yeh, Dengue virus non-structural protein 1: a pathogenic factor, therapeutic target, and vaccine candidate, J. Biomed. Sci. 25 (1) (2018) 58.
[14] T.N. Adikari, L. Gomes, N. Wickramasinghe, M. Salimi, N. Wijesiriwardana, A. Kamaladasa, N.L. Shyamali, G.S. Ogg, G.N. Malavige, Dengue NS1 antigen contributes to disease severity by inducing interleukin (IL)-10 by monocytes, Clin. Exp. Immunol. 184 (1) (2016) 90–100.
[15] S.A. Paranavitane, L. Gomes, A. Kamaladasa, T.N. Adikari, N. Wickramasinghe, C. Jeewandara, N.L. Shyamali, G.S. Ogg, G.N. Malavige, Dengue NS1 antigen as a marker of severe clinical disease, BMC Infect. Dis. 14 (2014) 570.
[16] H.R. Chen, Y.C. Chuang, Y.S. Lin, H.S. Liu, C.C. Liu, G.C. Perng, T.M. Yeh, Dengue virus nonstructural protein 1 induces vascular leakage through macrophage migration inhibitory factor and autophagy, PLoS Negl. Trop. Dis. 10 (7) (2016) e0004828.
[17] J. An, D.S. Zhou, J.L. Zhang, H. Morida, J.L. Wang, K. Yasui, Dengue-specific CD8+ T cells have both protective and pathogenic roles in dengue virus infection, Immunol. Lett. 95 (2) (2004) 167–174.
[18] S.B. Halstead, Pathogenesis of dengue: dawn of a new era, F1000Res 4 (2015).
[19] M.A. Schmid, M.S. Diamond, E. Harris, Dendritic cells in dengue virus infection: targets of virus replication and mediators of immunity, Front. Immunol. 5 (2014) 647.
[20] M.A. Schmid, E. Harris, Monocyte recruitment to the dermis and differentiation to dendritic cells increases the targets for dengue virus replication, PLoS Pathog. 10 (12) (2014) e1004541.
[21] K. Fink, C. Ng, C. Nkenfou, S.G. Vasudevan, N. van Rooijen, W. Schul, Depletion of macrophages in mice results in higher dengue virus titers and highlights the role of macrophages for virus control, Eur. J. Immunol. 39 (10) (2009) 2809–2821.
[22] E.L. Azeredo, P.C. Neves-Souza, A.R. Alvarenga, S.R. Reis, A. Torrentes-Carvalho, S.M. Zagne, R.M. Nogueira, L.M. Oliveira-Pinto, C.F. Kubelka, Differential regulation of toll-like receptor-2, toll-like receptor-4, CD16 and human leucocyte antigen-DR on peripheral blood monocytes during mild and severe dengue fever, Immunology 130 (2) (2010) 202–216.
[23] X. Zuo, W. Pan, T. Feng, X. Shi, J. Dai, Matrix metalloproteinase 3 promotes cellular anti-dengue virus response via interaction with transcription factor NFkappaB in cell nucleus, PLoS One 9 (1) (2014) e84748.
[24] R.S. Bhatt, S.T. Kothari, D.J. Gohil, M. D'Souza, A.S. Chowdhary, Novel evidence of microglial immune response in impairment of Dengue infection of CNS, Immunobiology 220 (10) (2015) 1170–1176.
[25] Z. Her, Y.W. Kam, V.C. Gan, B. Lee, T.L. Thein, J.J. Tan, L.K. Lee, K. Fink, D.C. Lye, L. Renia, Y.S. Leo, L.F. Ng, Severity of plasma leakage is associated with high levels of interferon gamma-inducible protein 10, hepatocyte growth factor, matrix metalloproteinase 2 (MMP-2), and MMP-9 during dengue virus infection, J. Infect. Dis. 215 (1) (2017) 42–51.
[26] P. Leaungwutiwong, J.F. Kelley, A. Sachair, A. Jittmittraphap, N. Luplertlop, Relationship between MMP expression and virulence of dengue virus type-2 in infected mosquito and mammalian cells, Jpn. J. Infect. Dis. 69 (1) (2016) 45–50.
[27] M. Fanjul-Fernandez, A.R. Folgueras, S. Cabrera, C. Lopez-Otin, Matrix metalloproteinases: evolution, gene regulation and functional analysis in mouse models, Biochim. Biophys. Acta 1803 (1) (2010) 3–19.
[28] A.S. Yeo, N.A. Azhar, W. Yeow, C.C. Talbot Jr., M.A. Khan, E.M. Shankar, A. Rathakrishnan, A. Azizan, S.M. Wang, S.K. Lee, M.Y. Fong, R. Manikam, S. Devi Sekaran, Lack of clinical manifestations in asymptomatic dengue infection is attributed to broad down-regulation and selective up-regulation of host defence response genes, PLoS One 9 (4) (2014) e92240.
[29] M. Shahen, Z. Guo, A.H. Shar, R. Ebaid, Q. Tao, W. Zhang, Z. Wu, Y. Bai, Y. Fu, C. Zheng, H. Wang, P.A. Shar, J. Liu, Z. Wang, W. Xiao, Y. Wang, Dengue virus causes changes of MicroRNA-genes regulatory network revealing potential targets for antiviral drugs, BMC Syst. Biol. 12 (1) (2018) 2.
[30] A. Kaushik, C. Pineda, H. Kest, Diagnosis and management of dengue fever in children, Pediatr. Rev. 31 (4) (2010) e28–e35.
[31] K. Yoshifuji, T. Oshina, S. Sonokawa, Y. Noguchi, S. Suzuki, K. Tanaka, T. Kumagai, Domestic dengue infection with hemophagocytic lymphohistiocytosis successfully treated by early steroid therapy, Rinsho Ketsueki 57 (7) (2016) 864–868.
[32] D. Pandejpong, P. Saengsuri, R. Rattarittamrong, T. Rujipattanakul, C. Chouriyagune, Is excessive acetaminophen intake associated with transaminitis in adult patients with dengue fever? Intern. Med. J. 45 (6) (2015) 653–658.
[33] S.K. Kabra, Y. Jain, T. Singhal, V.H. Ratageri, Dengue hemorrhagic fever: clinical manifestations and management, Indian J. Pediatr. 66 (1) (1999) 93–101.
[34] H.Y. Lei, T.M. Yeh, H.S. Liu, Y.S. Lin, S.H. Chen, C.C. Liu, Immunopathogenesis of

dengue virus infection, J. Biomed. Sci. 8 (5) (2001) 377–388.
[35] M. Abraham, S. Shapiro, N. Lahat, A. Miller, The role of IL-18 and IL-12 in the modulation of matrix metalloproteinases and their tissue inhibitors in monocytic cells, Int. Immunol. 14 (12) (2002) 1449–1457.
[36] F. Al-Rashed, S. Kochumon, S. Usmani, S. Sindhu, R. Ahmad, Pam3CSK4 induces MMP-9 expression in human monocytic THP-1 cells, Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology 41 (5) (2017) 1993–2003.
[37] A. Abbas, P. Aukrust, D. Russell, K. Krohg-Sorensen, T. Almas, D. Bundgaard, V. Bjerkeli, E.L. Sagen, A.E. Michelsen, T.B. Dahl, S. Holm, T. Ueland, M. Skjelland, B. Halvorsen, Matrix metalloproteinase 7 is associated with symptomatic lesions and adverse events in patients with carotid atherosclerosis, PLoS One 9 (1) (2014) e84935.
[38] C.A. van de Weg, C.S. Pannuti, H.J. van den Ham, E.S. de Araujo, L.S. Boas, A.C. Felix, K.I. Carvalho, J.E. Levi, C.M. Romano, C.C. Centrone, C.L. Rodrigues, E. Luna, E.C. van Gorp, A.D. Osterhaus, E.G. Kallas, B.E. Martina, Serum angiopoietin-2 and soluble VEGF receptor 2 are surrogate markers for plasma leakage in patients with acute dengue virus infection, J. Clin. Virol. 60 (4) (2014) 328–335.
[39] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1–2) (1983) 55–63.
[40] M. Ferrari, M.C. Fornasiero, A.M. Isetta, MTT colorimetric assay for testing macrophage cytotoxic activity in vitro, J. Immunol. Methods 131 (2) (1990) 165–172.
[41] B. van Wilgenburg, I. Scherwitzl, E.C. Hutchinson, T. Leng, A. Kurioka, C. Kulicke, C. de Lara, S. Cole, S. Vasanawathana, W. Limpitikul, P. Malasit, D. Young, L. Denney, S.-H. consortium, M.D. Moore, P. Fabris, M.T. Giordani, Y.H. Oo, S.M. Laidlaw, L.B. Dustin, L.P. Ho, F.M. Thompson, N. Ramamurthy, J. Mongkolsapaya, C.B. Willberg, G.R. Screaton, P. Klenerman, MAIT cells are activated during human viral infections, Nat. Commun. 7 (2016) 11653.
[42] P. Seanpong, C. Srisaowakarn, A. Thammaporn, V. Leardkamolkarn, S. Kumkate, Different responses in MMP/TIMP expression of U937 and HepG2 cells to dengue virus infection, Jpn. J. Infect. Dis. 68 (3) (2015) 221–229.
[43] J. Morrison, A.P.S. Rathore, C.K. Mantri, S.A.B. Aman, A. Nishida, A.L. St John, Transcriptional profiling confirms the therapeutic effects of mast cell stabilization in a dengue disease model, J. Virol. 91 (18) (2017).
[44] V.V. Costa, W. Ye, Q. Chen, M.M. Teixeira, P. Preiser, E.E. Ooi, J. Chen, Dengue virus-infected dendritic cells, but not monocytes, activate natural killer cells through a contact-dependent mechanism involving adhesion molecules, MBio 8 (4) (2017).
[45] Y.R. Lee, M.T. Liu, H.Y. Lei, C.C. Liu, J.M. Wu, Y.C. Tung, Y.S. Lin, T.M. Yeh, S.H. Chen, H.S. Liu, MCP-1, a highly expressed chemokine in dengue haemorrhagic fever/dengue shock syndrome patients, may cause permeability change, possibly through reduced tight junctions of vascular endothelium cells, The Journal of general virology 87 (Pt 12) (2006) 3623–3630.
[46] K. Jessie, M.Y. Fong, S. Devi, S.K. Lam, K.T. Wong, Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and in situ hybridization, J. Infect. Dis. 189 (8) (2004) 1411–1418.
[47] N. Luplertlop, D. Misse, D. Bray, V. Deleuze, J.P. Gonzalez, V. Leardkamolkarn, H. Yssel, F. Veas, Dengue-virus-infected dendritic cells trigger vascular leakage through metalloproteinase overproduction, EMBO Rep. 7 (11) (2006) 1176–1181.
[48] N. Luplertlop, D. Misse, MMP cellular responses to dengue virus infection-induced vascular leakage, Jpn. J. Infect. Dis. 61 (4) (2008) 298–301.
[49] E.S. Gan, W.F. Cheong, K.R. Chan, E.Z. Ong, X. Chai, H.C. Tan, S. Ghosh, M.R. Wenk, E.E. Ooi, Hypoxia enhances antibody-dependent dengue virus infection, EMBO J. 36 (10) (2017) 1348–1363.
[50] M. Stawowczyk, M.D. Wellenstein, S.B. Lee, S. Yomtoubian, A. Durrans, H. Choi, N. Narula, N.K. Altorki, D. Gao, V. Mittal, Matrix metalloproteinase 14 promotes lung cancer by cleavage of heparin-binding EGF-like growth factor, Neoplasia 19 (2) (2017) 55–64.
[51] A. Klose, P. Zigrino, C. Mauch, Monocyte/macrophage MMP-14 modulates cell infiltration and T-cell attraction in contact dermatitis but not in murine wound healing, Am. J. Pathol. 182 (3) (2013) 755–764.
[52] T. Sathyamoorthy, L.B. Tezera, N.F. Walker, S. Brilha, L. Saraiva, F.A. Mauri, R.J. Wilkinson, J.S. Friedland, P.T. Elkington, Membrane type 1 matrix metalloproteinase regulates monocyte migration and collagen destruction in tuberculosis, J. Immunol. 195 (3) (2015) 882–891.
[53] A. Koziol, P. Gonzalo, A. Mota, A. Pollan, C. Lorenzo, N. Colome, D. Montaner, J. Dopazo, J. Arribas, F. Canals, A.G. Arroyo, The protease MT1-MMP drives a combinatorial proteolytic program in activated endothelial cells, FASEB J. 26 (11) (2012) 4481–4494.
[54] Y. Liu, H. Zhang, L. Yan, W. Du, M. Zhang, H. Chen, L. Zhang, G. Li, J. Li, Y. Dong, D. Zhu, MMP-2 and MMP-9 contribute to the angiogenic effect produced by hypoxia/15-HETE in pulmonary endothelial cells, J. Mol. Cell. Cardiol. 121 (2018) 36–50.
[55] A.H. Webb, B.T. Gao, Z.K. Goldsmith, A.S. Irvine, N. Saleh, R.P. Lee, J.B. Lendermon, R. Bheemreddy, Q. Zhang, R.C. Brennan, D. Johnson, J.J. Steinle, M.W. Wilson, V.M. Morales-Tirado, Inhibition of MMP-2 and MMP-9 decreases cellular migration, and angiogenesis in in vitro models of retinoblastoma, BMC Cancer 17 (1) (2017) 434.
[56] A.M. Abu El-Asrar, G. Mohammad, E. Allegaert, A. Ahmad, M.M. Siddiquei, K. Alam, P.W. Gikandi, G. De Hertogh, G. Opdenakker, Matrix metalloproteinase-14 is a biomarker of angiogenic activity in proliferative diabetic retinopathy, Mol. Vis. 24 (2018) 394–406.
[57] K.Y. Han, J. Dugas-Ford, H. Lee, J.H. Chang, D.T. Azar, MMP14 cleavage of VEGFR1 in the cornea leads to a VEGF-trap antiangiogenic effect, Invest. Ophthalmol. Vis. Sci. 56 (9) (2015) 5450–5456.
[58] K.Y. Han, J. Dugas-Ford, M. Seiki, J.H. Chang, D.T. Azar, Evidence for the involvement of MMP14 in MMP2 processing and recruitment in exosomes of corneal fibroblasts, Invest. Ophthalmol. Vis. Sci. 56 (9) (2015) 5323–5329.
[59] M. Zou, X. Zhang, C. Xu, IL6-induced metastasis modulators p-STAT3, MMP-2 and MMP-9 are targets of 3,3′-diindolylmethane in ovarian cancer cells, Cell Oncol (Dordr) 39 (1) (2016) 47–57.
[60] A.A. Zergoun, A. Zebboudj, S.L. Sellam, N. Kariche, D. Djennaoui, S. Ouraghi, E. Kerboua, Z.C. Amir-Tidadini, D. Chilla, F. Asselah, C. Touil-Boukoffa, T. Merghoub, M. Bourouba, IL-6/NOS2 inflammatory signals regulate MMP-9 and MMP-2 activity and disease outcome in nasopharyngeal carcinoma patients, Tumour Biol. 37 (3) (2016) 3505–3514.
[61] L.K. Flores-Mendoza, T. Estrada-Jimenez, V. Sedeno-Monge, M. Moreno, M.D.C. Manjarrez, G. Gonzalez-Ochoa, L. Millan-Perez Pena, J. Reyes-Leyva, IL-10 and socs3 are predictive biomarkers of dengue hemorrhagic fever, Mediat. Inflamm. 2017 (2017) 5197592.
[62] G.N. Malavige, L. Gomes, L. Alles, T. Chang, M. Salimi, S. Fernando, K.D. Nanayakkara, S. Jayaratne, G.S. Ogg, Serum IL-10 as a marker of severe dengue infection, BMC Infect. Dis. 13 (2013) 341.
[63] A.A. Araujo, H. Varela, G.A. Brito, C.A. Medeiros, S. Araujo Lde, J.H. do Nascimento, R.F. de Araujo Junior, Azilsartan increases levels of IL-10, down-regulates MMP-2, MMP-9, RANKL/RANK, Cathepsin K and up-regulates OPG in an experimental periodontitis model, PLoS One 9 (5) (2014) e96750.
[64] S.J. Fortunato, R. Menon, S.J. Lombardi, B. LaFleur, Interleukin-10 inhibition of gelatinases in fetal membranes: therapeutic implications in preterm premature rupture of membranes, Obstet. Gynecol. 98 (2) (2001) 284–288.
[65] C.Y. Hu, W.H. Ding, X.N. Han, S.Y. Chu, Y.J. Hao, D.F. Bu, In vivo interleukin-10 gene transfer down-regulates myocardial matrix metalloproteinase and myocardial collagen expressions in rats with acute myocardial infarction, Zhonghua Xin Xue Guan Bing Za Zhi 36 (3) (2008) 243–248.
[66] D. Kellstein, L. Fernandes, Symptomatic treatment of dengue: should the NSAID contraindication be reconsidered? Postgrad. Med. 131 (2) (2019) 109–116.
[67] S. Inyoo, A. Suttitheptumrong, S.N. Pattanakitsakul, Synergistic effect of TNF-alpha and dengue virus infection on adhesion molecule reorganization in human endothelial cells, Jpn. J. Infect. Dis. 70 (2) (2017) 186–191.
[68] S. Iwamoto, S. Iwai, K. Tsujiyama, C. Kurahashi, K. Takeshita, M. Naoe, A. Masunaga, Y. Ogawa, K. Oguchi, A. Miyazaki, TNF-alpha drives human CD14+ monocytes to differentiate into CD70+ dendritic cells evoking Th1 and Th17 responses, J. Immunol. 179 (3) (2007) 1449–1457.