Differential expression of miRNA in peripheral blood cells from acute dengue and dengue hemorrhagic fever patients.

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Introduction

Dengue is the most prevalent arboviral disease transmitted by mosquitoes common in tropical areas of the world. Lack of proper medication or vaccines for dengue fever and inability to distinguish severe cases of dengue fever (DF), dengue hemorrhagic fever (DHF) during the early stages of infection, renders this disease life threatening for people living in endemic areas.

Early symptoms of DHF are similar to those of non-life threatening DF. DHF patients manifest plasma leakage, elevated hematocrit and pleural effusions after 3-5days of fever. Early diagnosis and disease management can alleviate DHF related complications. Therefore, biomarkers that distinguish DHF at acute phase of infection can reduce mortality. Due to their role in post-transcriptional regulation of cellular gene expression and remarkable stability, altered expression of miRNA can serve as clinically relevant biomarkers. Differential expression of several miRNA in DENV virus infected cultured cells has been previously reported[1,2]. Augmented expression of hsa-miR-150-5p has also been reported in DENV2 positive DHF pateints[3].

Aim

Evaluate the expression of five miRNA targets in Peripheral Blood Cells (PBC) collected from 20 DF and 20 DHF positive patients within four days from fever onset by qRT-PCR to determine their potential as early markers of disease severity in Dengue patients.

Methodology

Patients presented with clinical symptoms according to 2012 WHO Dengue case classification (fever, with two of the following criteria: headache, retro-orbital pain , myalgia, arthralgia, rash, hemorrhagic manifestations with no plasma leakage, and following laboratory findings leucopenia, thrombocytopenia, rising hematocrit with no evidence of plasma loss) within 4 days from fever onset who tested positive for onsite NS1 rapid test (SD Bioline) were recruited for the study from the North Colombo Teaching Hospital, Ragama with informed consent. Patients who later develop DHF (fever and hemorrhagic manifestation (positive tourniquet test) with evidence of plasma leakage (portable ultrasound), spontaneous bleeding, circulatory failure, profound shock with undetectable BP and pulse, thrombocytopenia < 100000 cells / mm^3, and HCT rise > 20%). Ethics approval was obtained from the Ethics Review Committee, Faculty of Medicine, University of Kelaniya.

Extract total RNA from PBC Isolated from 2.5 mL whole blood.

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Polyadenylation of RNA followed by cDNA synthesis and RT-PCR for miRNA expression analysis in DF and DHF samples.

Results

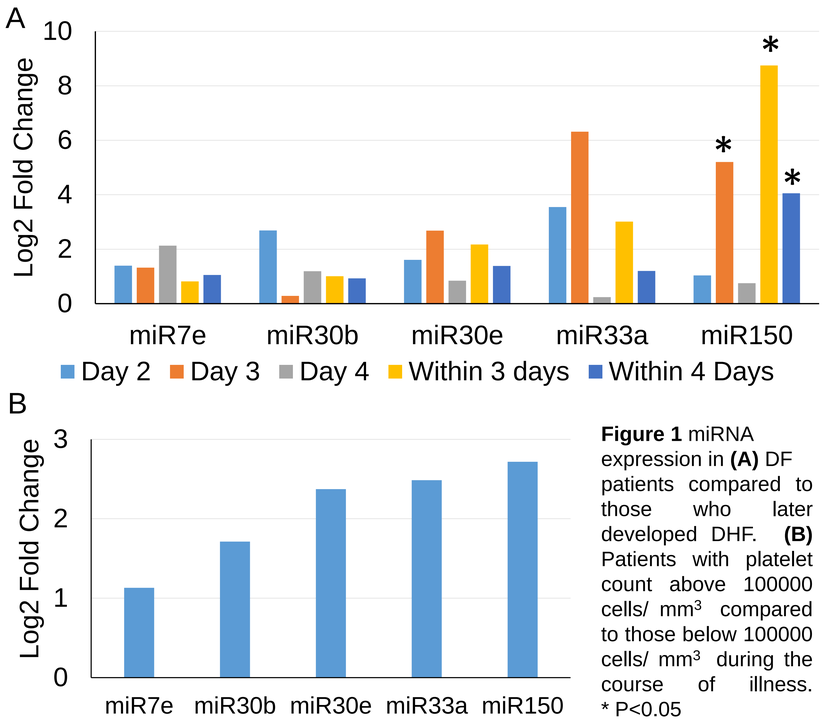

Figure 1 miRNA expression in **(A)** DF patients compared to those who later developed DHF. **(B)** Patients with platelet count above 100000 cells/ mm^3 compared to those below 100000 cells/ mm^3 during the course of illness. * P<0.05

In order to evaluate the potential of selected differentially expressed miRNA from these studies as early biomarkers of disease severity in Dengue patients, relative expression of hsa-let-7e, hsa-miR-30b, hsa-miR-30e-3p, hsa-mir-33a, and hsa-miR-150-5p were evaluated against the geometric mean of hsa-miR-103a-3p and hsa-miR-16-5p as reference genes in DF (n=20) patients who tested positive for NS1 antigen and those who later developed DHF (n=20) within four days from fever onset. Fold change based on ΔΔCt values presented as log values to the base 2, Fold change >1.5 considered as up regulation and ≤0.5 considered as down regulation, with P<0.05 considered statistically significant. hsa-miR-150-5p showed significant (P<0.05) upregulation in DHF (n=20) patients compared to DF (n=20) patients within 4 days from fever onset. Evaluation of expression of the miRNA in samples collected from patients recruited on, day 2 (n_{DF}=2, n_{DHF}=3), day 3 (n_{DF}=6, n_{DHF}=12), day 4 (n_{DF}=11, n_{DHF}=5) and within 3 days (n_{DF}=8, n_{DHF}=15) from fever onset showed significant (P<0.05) upregulation of hsa-miR-150-5p in DHF samples from patients recruited on day 3 from fever onset and within 3 days from fever onset while the samples collected on day2 and day 4 from fever onset failed to show differential expression. Only few samples were collected from patients recruited within 2 days from fever onset to warrant drawing any conclusions.

Expression of hsa-miR-150-5p is also upregulated over 2 fold in samples collected from DF patients recruited on day 4 from fever onset compared to the samples collected from DF patients recruited within 3 days from fever onset while the expression of hsa-miR-150-5p is significantly downregulated over 8 fold in samples collected from DHF patients recruited on day 4 from fever onset compared to the samples collected from DHF patients recruited within 3 days from fever onset (P<0.05). Therefore, on day 4 from fever onset, there is no differential expression observed for hsa-miR-150-5p between DF and DHF.

Within the first four days of infection, hsa-miR-30e-3p, hsa-mir-33a, and hsa-miR-150-5p did not show significant (P<0.05) upregulation of expression in patients with platelet count </=100000 /mm^3 (n=31) (thrombocytopenia) compared to those who maintained a platelet count > 100000 / mm^3 (n=9) during the course of infection. Sufficient number of DHF samples have not been collected from female patients to evaluate the differential expression of these miRNA targets in male and female patients.

Conclusions

hsa-miR-150-5p may serve as an early biomarker of patients within 3 days from fever onset. In addition, augmented expression of hsa-miR-30e-3p, hsa-mir-33a, and hsa-miR-150-5p may serve as early biomarkers of severity of infection as marked by thrombocytopenia.

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References

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