



DIFFERENTIAL EXPRESSION OF INTERFERON INDUCIBLE PROTEIN: GUANYLATE BINDING PROTEIN (GBP1 & GBP2) IN SEVERE DENGUE

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ABSTRACT

Dengue virus is reported to activate endothelial cells (EC), but the precise cause for severe dengue (SD) is not known. Guanylate binding proteins (GBPs) are IFN-inducible proteins secreted by ECs and are involved in the anti-oxidant and anti-viral response. The involvement of GBPs in the pathogenesis of dengue remains under explored. We have quantified the mRNA and protein levels of GBP1 and 2 during different phases of infection and have correlated with the oxidative stress in plasma samples of different study groups. The efficacy of the proteins in predicting disease severity was done by Support Vector Machine (SVM) model. The study recorded a decreased expression of GBPs during critical phase among severe dengue. The GBP levels were found to be negatively correlated with plasma leakage and emerged as a strong predictor of disease outcome based on machine models.

AIM & OBJECTIVES

To see the role of GBPs on dengue disease progression by:

- quantifying the mRNA and protein levels of GBP1 and GBP2 in severe and non-severe forms of DENV infected patients during the course of infection
- Assessing GBPs predictive capacity as effective prognostic markers using machine-based mathematical models
- Finding the association between GBPs, oxidative stress process and severity markers during the course of DENV infection

METHODOLOGY

- 3 mL of venous blood was collected under aseptic condition at the Day of admission (1st day, DOA), Day of defervescence (4th day, DOD) and Day of convalescence (11th day, DOC) from patients.
- The PBMCs were isolated from the whole blood samples, RNA extracted using trizol method. Finally, 1 µg of RNA was converted into complementary DNA (cDNA) and the cDNA was used for qPCR analysis.
- To assess the plasma (Protein) concentration of GBP1 and 2, a quantitative human GBP1 and GBP2 sandwich ELISA kit was used.
- Plasma MDA (Lipid peroxidation) was estimated by the Kei Satoh method.
- The status of protein and DNA oxidation in the plasma samples were estimated using an advanced oxidation protein production (AOPP) and 8-hydroxy-2-deoxyguanosine (8-OHdG) ELISA kit.
- Data was collected and Statistical Analysis of Result were performed with 5% level of significance (P≤0.05).

Sample Size Used in this Study

Severe Dengue	Dengue With Warning sign	Dengue Without Warning Sign	Other Febrile Illness	Healthy Control
10	25	15	10	8

SUMMARY AND CONCLUSION

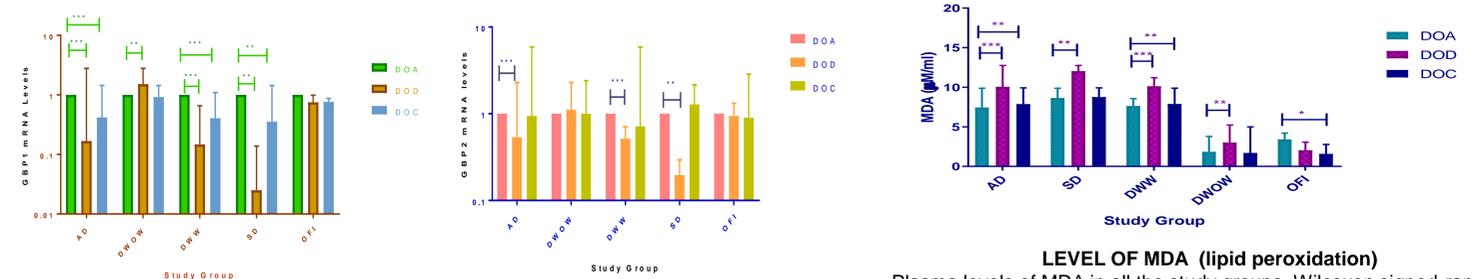
Expression pattern of GBP 1 and 2 were found to be negatively correlated with plasma leakage and elevated levels of oxidative stress is associated with the decreased expression of GBPs during the course of Dengue infection. Thus, antioxidant supplement as adjuvant therapy may regulate the expression of GBPs and disease virulence. Nevertheless, machine models found that the plasma levels of GBP1 and 2 along with routine clinical symptoms could predict the dengue disease severity with higher accuracy. A large prospective cohort study may be required to ascertain the role of GBP1 and GBP2 as effective prognostic markers of dengue severity.

The findings has been published in Free Radical Biology and Medicine, 194: 131-46, 2023.

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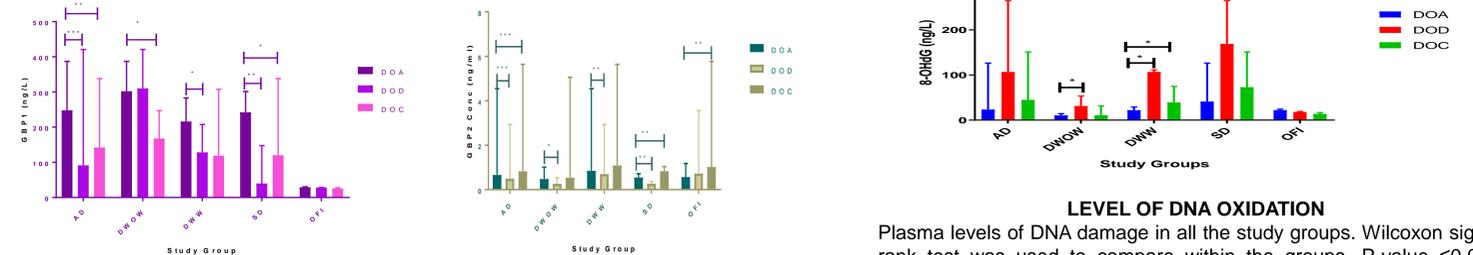
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RESULTS



mRNA levels GBP1 and GBP2

GBP1 and GBP2 expression was measured by qRT-PCR and normalized by β-actin. DOA of all the groups is taken as a baseline. P-value ≤0.05 is considered significant.



LEVEL OF MDA (lipid peroxidation)

Plasma levels of MDA in all the study groups. Wilcoxon signed-rank test was used to compare within the groups. P-value ≤0.05 is considered significant.

LEVEL OF DNA OXIDATION

Plasma levels of DNA damage in all the study groups. Wilcoxon signed-rank test was used to compare within the groups. P-value ≤0.05 is considered significant.

ELISA (PLASMA LEVEL) GBP1 and GBP2

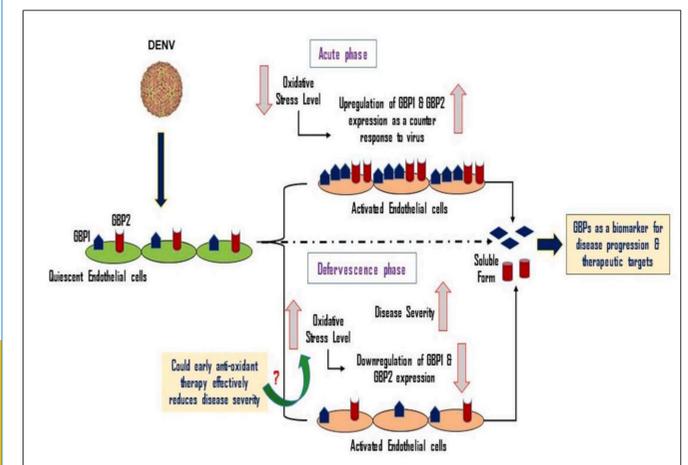
Plasma levels of GBP1 and GBP2 in all the study groups. Wilcoxon signed-rank test was used to compare within the groups. P-value ≤0.05 is considered significant.

DISCUSSION

- During the febrile phase (DOA), GBP1 showed an elevated mRNA and protein level compared to Other Febrile illness, and towards the critical phase (DOD), a significant decrease in the mRNA and protein level of both GBP1, as well as GBP2, was observed in severe Dengue (SD) cases, indicating the importance of these two molecules in disease manifestation.
- Lipid Oxidation (MDA) showed a negative correlation with GBP1 as well as GBP2 in Severe Dengue groups, particularly at the critical phase of infection.
- GBP1 and GBP2 showed a negative association with protein and DNA oxidation respectively.
- Increased oxidative stress may downregulate both GBP1 and 2 thereby enhancing disease manifestation by creating a suitable micro-environment for virus propagation accompanied by endothelial dysfunction.
- Thus, the study found that the expression pattern of GBP 1 and 2 were found to be negatively correlated with plasma leakage, and elevated levels of oxidative stress are associated with the decreased expression of GBPs during Dengue infection.

MACHINE LEARNING BY SVM MODEL

We observed that the protein levels & mRNA levels of GBP1 and GBP2 along with other clinical symptoms could predict the disease severity with an accuracy of 98% and 100% respectively. Interestingly, the SVM model showed that plasma levels of GBP-2 with clinical symptoms could predict dengue severity with 100% accuracy.



Differential Expression of GBP1 and GBP2 in response to Oxidative Stress

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