Plasma nitric oxide and salivary oxidized LDL as early predictive biomarkers of progression to Dengue Hemorrhagic Fever.

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Introduction

Dengue is a mosquito-borne disease characterized by a mild febrile illness as dengue fever (DF) and severe stage of illness as dengue hemorrhagic fever (DHF) and dengue shock syndrome that can lead to mortality. Early clinical management is critical for preventing mortality. Virus-induced activation of phagocytes is associated with oxidative stress. Several markers of oxidative stress have been reported to differentiate between serum and plasma samples from DF and DHF patients. Nitric oxide (NO) which plays a complex and diverse physiological and pathophysiological role in viral infections may also serve as an early prognostic marker of disease severity of Dengue infections. Expression of inducible NO synthase (iNOS) results in NO biosynthesis resulting in generation of a highly reactive nitrogen oxide species, peroxynitrite, via a radical coupling reaction of NO with superoxide which in turn causes potent oxidation and nitration reactions of various biomolecules including lipids[1]. This study was carried out to assess the potential of biochemical markers of oxidative stress; NO and oxidized LDL (Ox LDL) in plasma to serve as markers of disease severity during the early stages of infection.
Saliva is a non-invasive source of biological markers. Therefore, we also measured the salivary NO levels and Ox LDL for their potential as non-invasive early prognostic markers for severity of infection.

Aim

To quantify the levels of NO and Ox LDL in plasma and saliva from dengue patients to evaluate potential to serve as prognostic markers.

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 platelets / mm^3), and HCT rise > 20%). Ethics approval was obtained from the Ethics Review Committee, Faculty of Medicine, University of Kelaniya. Plasma and saliva were stored at – 80 ^{0}C within 2 hrs from sample collection.

NO levels in plasma and saliva measured using Griess reaction

Ox LDL levels in plasma and saliva measured with human Ox LDL ELISA kit (Elabscience)

Results

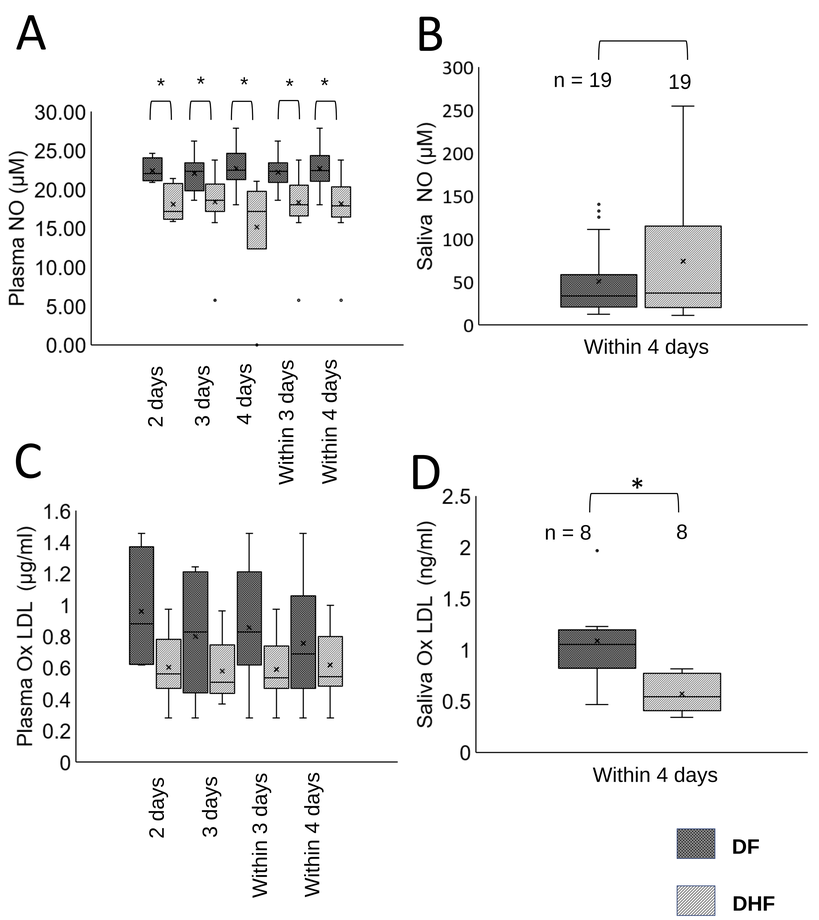

Figure 1 (A) NO concentration in plasma and **(B)** saliva from acute Dengue patients and those who later developed DHF. **(C)** Ox LDL levels in plasma and **(D)** saliva from DF patients and those who later developed DHF collected within 4 days from fever onset. * p<0.05

NO concentration in plasma in DF (n=30) patients is significantly higher (P<0.05) compared to DHF (n=29) patients within 4 days from fever onset. Further analysis showed significantly (P<0.05) high NO concentration in plasma collected from DF patients recruited on day 2 (n_{DF}=4, n_{DHF}=6), day 3 (n_{DF}=7, n_{DHF}=17), day 4 (n_{DF}=17, n_{DHF}=5), within 3 days (n_{DF}=11, n_{DHF}=23) and within 4 days (n_{DF}=30, n_{DHF}=29) from fever onset. However, NO concentration in saliva from DF (n=19) did not show a significant difference from DHF (n=19) patients due to large variation in NO concentration among the patients. This may be due to the effect of oral microbial activity and the effect of dietary infleunces on the salivary NO levels.

Ox LDL levels in plasma collected from patients recruited on day 2 (n_{DF}=4, n_{DHF}=6), day 3 (n_{DF}=7, n_{DHF}=8), day 4 (n_{DF}=5, n_{DHF}=1), within 3 days (n_{DF}=11, n_{DHF}=14) and within 4 days (n_{DF}=16, n_{DHF}=15) from fever onset showed higher mean Ox LDL levels in DF compared to DHF patients. The Ox LDL levels in saliva from DF (n=8) patients who tested positive for NS1 antigen, collected within four days from fever onset, is significantly higher (P<0.05) compared to saliva from those who later developed DHF (n=8).

Within the first four days of infection, NO and Ox LDL levels did not show a significant correlation (r=0.1) with the platelet count in patients with thrombocytopenia (platelet count < or = 100000 / mm^3),(n_{NO}=20, $n_{Ox\ LDL}$=7) compared to those who maintained a platelet count > 100000 / mm^3 (n_{NO}=39, $n_{Ox\ LDL}$=24) during the course of infection. However, the number of samples from patients presented with thrombocytopenia may not be sufficient to draw any conslusions regarding the correation between the platelet count and Ox LDL levels. In addition, only a few DHF samples have been collected from female patients to warrant evaluation of the plasma and salivary NO and Ox LDL levels in male and female patients.

Conclusions

Plasma NO levels and salivary Ox LDL may serve as biomarkers of severity of Dengue infection during the acute phase of infection. Salivary Ox LDL may have potential use as a non-invasive marker for predicting manifestation of DHF during the early onset of infection. However, our study has several limitations inlcuding relatively small sample size and a dispropotionately high number of male subjects. Therefore, samples analysis for a larger cohort is required to validate our results.

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References

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