Story of Young Innovators in Health Sciences

Differential expression levels of miR-150, enhancer of zeste homolog 2 (EZH2), inducible Nitric Oxide Synthase(iNOS), oxidized low-density lipoprotein levels (oxLDL) and Nitric oxide(NO) in bio fluids as early prognostic markers for severe dengue

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Dengue fever is a mosquito borne viral infection, caused by a *flavivirus*. It is rapidly spreading in the tropical and sub-tropical regions of the world. Dengue infected patients demonstrate several clinical manifestations from mild dengue fever (DF) to more serious severe dengue fever (SD).The common symptoms of dengue fever are nausea, vomiting, red rash, eye pain, muscle, joint, or bone pain or can be even asymptomatic. SD can be distinctly identified by the symptoms of plasma leakage, elevated hematocrit and pleural effusions mostly after 3-5days of fever. However, SD and DF patients show similar symptoms at the early stage of the infection. Considering the fact that not having proper antiviral treatment and administration of recently discovered vaccine could lead to SD manifestation, it is utterly important to distinguish SD from DF at the early stage of the infection. Early diagnosis of SD can help to provide special attention to SD patients among DF patients during dengue out breaks. The national hospital system accommodates dengue patients beyond the capacity during the out brakes. Therefore, identifying SD patients at the early stage is more important for the disease management in the hospital setting. Detection of the Immunoglobulin Proteins G, M and NS1 antigen in serum, plasma and whole blood are the commonly used methods for dengue diagnosis. Apart from that, elevated hematocrit level(HCT) and sudden drop of platelet count in blood can be used as warning signs of SD. However, our data suggest that there is no significant difference in platelet count and

HCT levels at the early stage of the infection between DF and SD patients. Therefore, we investigated the expression changes of miRNA and their putative target genes in blood and saliva samples collected form NS1 positive patients within 4 days from fever onset to find potential biomarkers to distinguish SD from DF at the early stage of the infection. We also, investigated the changes of oxLDL and NO levels between same samples.

Findings- miR-150 micro RNA showed significant ($P < 0.01$) upregulation in SD patient samples collected on day 3 and in all samples collected within 3 days (day 2 with day 3) from fever onset. EZH2 expression showed significant down regulation ($P < 0.01$) in samples collected from patients who later developed SD compared to that in DF patients on day 4 and within 4 days from fever onset. SD patients showed significantly ($P < 0:05$) lower iNOS expression compared to the DF patients within 4 days from fever onset. Furthermore, iNOS expression in SD patients admitted on day 3 from fever onset was also significantly ($P < 0:05$) low compared to that of DF patients. Median plasma NO concentration within 4 days from fever onset in patients who later developed SD is significantly ($P < 0:05$) lower than that of DF group. A significant decrease in median NO levels ($P < 0:05$) in the SD patients was observed in samples collected at admission on day 2, day 3, day 4, and within 3 days from fever onset. The median oxLDL levels in the SD group showed a significantly ($P < 0:05$) low oxLDL levels in plasma collected within 3 days from fever onset compared to patients who did not develop SD. Median salivary oxLDL in SD patients within 4 days from fever onset is significantly lower ($P < 0:05$) than that of DF patients. This data also consistent within three days from fever onset samples.

Conclusion- Differential expression of miR-150, EZH2, iNOS, plasma NO, and salivary oxLDL levels may serve as reliable early biomarkers to predict the development of SD.

Practical Implications- We analyzed the expression changes of miRNAs and their target genes in PBC samples collected from NS1 positive patients earlier as within 4 days from fever onset. We found that miR-150, EZH2 and iNOS are differentially expressed in SD patients compared to DF patients before demonstrating sever symptoms. OxLDL level in plasma and saliva samples collected from dengue positive patients who later developed in to SD showed lower levels compared to DF patient. SD plasma samples showed lower NO levels compared to DF samples collected within 4 days from fever onset. Therefore, expression changes of these biomarkers can be used as early prognostic markers for severe dengue.

Novelty – miRNA levels found to be changing with the disease severity in many diseases including cancer. Recent studies suggest that it is remarkably stable in bio fluids. We discovered that miR150, EZH2 and iNOS levels change with the disease severity in blood samples of dengue patients. We also found that significantly lower level of oxLDL and NO in saliva and plasma of SD patients compared to DF patients. These changes were detectable within 4 days from fever and were found in easily accessible samples from patients such as blood, plasma, PBC and saliva. Therefore, these markers can be considered as novel noninvasive markers for SD.

Benefit to the Society-Severe dengue patients show similar symptoms as dengue fever patients at the early stage of the infection. The prominent symptoms of sever dengue such as plasma leakage, elevated hematocrit and pleural effusions mostly can be detected after 3-5days of fever.

Therefore, diagnosis of sever dengue at the early stage of the infection is important to reduce the fatality of SD.

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