ABSTRACT

As found in another countries, dengue infection still a problem that has not been fully solved in Indonesia. Primary pathology of severe dengue disease is characterized by the increase of acute vascular permeability causing plasma leakage. The change of vascular permeability will affect directly to the hemodynamic, especially the blood velocity in accordace to the wall shear stress (WSS) and the blood viscosity. Recent study characterize the permeability of infected cells in static and dynamics condition using four serotypes of dengue viruses. Computational fluid dynamic (CFD) is also used to simulate the fluid flow over the infected cells and fluid leakage through the increased permeability of the infected cells.

In the *in vitro* experiment of static condition, vero cell line (CCL-81, ATCC) were cultured on the 24 mm transwell polyester permeable membrane with pore size of 3 micrometer. The cell cultures were grouped into five groups and infected by DENV-1, -2, -3, -4 and control, respectively. The leakage fluid were collected at 4, 24, 48, 72 and 80 h post infection. The protein in the sample of leakage fluid was analysed using ELISA, while the virus was detected using one step RT-PCR method. The procedure were applied for low and high titer dengue virus. In the dynamic condition, the characteristics of fluid leakage of the DENV infected- vero cell culture were analyzed through the variation of fluid velocity, variation of channel height and variation of fluid viscosity. Variation of fluid velocity refers to flow rate of 1, 2, 5, 7.5 and 10 ml/minute of culture media over the cell surface. Those flow rate correspond to the WSS of 1.38, 2.56, 6.39, 9.59, and 12.78 dynes/cm^2, respectively (using channel height of 100 micrometer). Those flow rates are also correspond to the value of WSS of 0.32, 0.64, 1.60, 2.40, and 3.20 dynes/cm^2, respectively (using channel height of 200 micrometer). The variation of fluid viscosity was performed by the application of M199, F12K and RPMI as culture media for the parallel plate flow chamber. The simulations were developed in the models contained one and four cells that cultured on the permeable membrane with pore size of 3 micrometer.

The result shows that, in static condition, using two ways-ANOVA, the serotype of dengue virus affects the intensity of the leakage fluid of dengue virus infected – vero cells ($P<0.05$). In 80 hours of observation, the total leakage fluid resulted by the infected vero cell, from the highest are DENV-2, DENV-3, DENV-4 and DENV-1 with the values of 6895, 6880, 6720 and 6395 microliter, respectively. In static condition, it is also found that the higher the titer of DENV, the higher the intensity of the fluid leakage from the DENV infected-vero cell line. Using two ways-ANOVA, it is also found that interaction of dengue titer and acquisition time affect the volume of leakage fluid($P<0.05$). In dynamic condition, using two ways-ANOVA, it is found that the intensity of the fluid leakage is also affected by the serotype of dengue virus, fluid velocity and the interaction between the dengue virus and fluid velocity ($P<0.05$). In this work, the highest intensity of fluid leakage found in the cell culture infected by DENV-3, followed by DENV-2, DENV-4 and DENV-1. It is also found that the higher the flow rate, the higher the intensity of fluid leakage. Smaller height of the channel resulted in higher intensity of fluid leakage. In general, the higher the fluid viscosity, the lower intensity of the fluid leakage found in the cell culture. The value of the wall shear stress (WSS) in the simulation varying from 0.06 – 2.33 dynes/cm^2. Those value of computed WSS are relatively agreed with the result of the in vitro experiment of the recent work that have value 0.32 – 3.20 dynes/cm^2 , and the value of the real WSS in the postcapillary venules (1-5 dynes/cm^2). In the simulation, it was also shown that the velocity contour of the Newtonian and Non Newtonian fluids were slightly different. At the

same fluid velocity, the peak value of WSS of the Newtonian fluid was about 12.5% lower than the WSS of the Non Newtonian fluid.

Recent work concludes that in static condition, the intensity of the leakage flow through the dengue virus infected-vero cells line are affected by the serotype and the titer of dengue virus. In dynamic condition, the intensity of the leakage flow through the dengue virus infected-vero cells line are affected by dengue serotype, fluid velocity, height of the channel and fluid viscosity. The results of simulation can explain a more comprehensive visualization about the velocity contour of fluid leakage and the value of the WSS on the dengue virus infected-vero cells.

Keywords: wall shear stress, permeability, dengue virus, computational fluid dynamics

INTISARI

Sebagaimana dijumpai di negara lain, penyakit infeksi dengue di Indonesia masih merupakan permasalahan yang belum sepenuhnya terpecahkan. Patologi paling utama penyakit dengue akut ditandai dengan peningkatan permeabilitas vascular yang berakibat pada kebocoran plasma. Perubahan permeabilitas vascular akan berpengaruh pada hemodinamik, terutama kecepatan darah terakit dengan perubahan tegangan gesr dan viskositas darah. Penelitian ini mengkarakterisasi permeabilitas pada sel terinfeksi virus dengue, pada kondisi statis dan dinamis mempergunakan empat serotype virus dengue. Pendekatan CFD juga dilakukan untuk mensimulasi aliran fluida di atas permukaan sel dan kebocoran fluida akibat peningkatan permeabiltas sel.

Pada uji in vitro kondisi statis, sel vero (CCL-81, ATCC) dikultur pada membrane permeable polyester 24 mm dengan ukuran pori 3 micrometer. Kultur sel dibagi menjadi lima kelompok dan masing-masing diinfeksi dengan DENV-1, -2, -3, -4 serta control. Fluida yang bocor diambil pada jam ke 4, 24, 48, 72 dan 80 setelah diinfeksi. Protein pada sample dianalisa mempergunakan teknik ELISA, sementara keberadaan virus dianalisa mempergunakan metode Yong - one step RT-PCR. Metode ini diaplikasikan pada virus titer rendah dan titer tinggi. Pada pengujian kebocoran fluida pada kondisi dinamis, analisa dilakukan dengan variasi kecepatan fluida, tinggi channel serta viskositas fluida. Variasi kecepatan fluida yang diaplikasikan pada kultur adalah setara kapasitas fluida sebesar 1, 2, 5, 7.5 dan 10 ml/menit. Kapasitas tersebut bersesuaian dengan tegangan geser sebesar 1.38, 2.56, 6.39, 9.59, and 12.78 dynes/cm^2 (dengan tinggi channel 100 micrometer). Kapasitas tersebut juga bersesuaian dengan WSS sebesar 0.32, 0.64, 1.60, 2.40, and 3.20 dynes/cm^2(channel height 200 micrometer). Pengaruh viskositas fluida pada permeabilitas dilakukan dengan mempergunakan 3 jenis media kultur, yakni RPMI, F12K dan M199. Adapun simulasi dilakukan dengan pemodelan sel tunggal serta empat sel yang dikultur pada membrane 3 micrometer.

Pada kondisi statis, hasil penelitian menunjukkan bahwa dengan analisa ANOVA 2 arah, serotype virus dengue mempengaruhi intensitas kebocoran pada sel yang terinfeksi virus dengue. Pada pengamatan selama 80 jam, urutan kultur dengan kebocoran tertinggi adalah yang diinfeksi oleh DENV-2, DENV-3, DENV-4 dan DENV-1, masing-masing dengan nilai 6895, 6880, 6720 and 6395 microliter. Pada pengujian kondisi statis, diperoleh pula bahwa tingkat kebocoran dipengaruki oleh titer virus, yakni makin tinggi titer, makin tinggi pula tingkat kebocoran kultur sel. Selain itu dipengaruhi serotype dan titer, diperoleh pula bahwa tingkat kebocoran dipengaruhi oleh interaksi antar serotype dan titer. Pada pengujian dengan kondisi dinamis, diperoleh bahwa tingkat kebocoran dipengaruhi oleh serotype, kecepatan serta interaksi antar keduanya. Pada kondisi dinamis, diperoleh bahwa tingkat kebocoran tertinggi terjadi pada sel uyang dikultur dengan DENV-3, diikuti oleh DENV-2, DENV-4 dan DENV-1. Pada kondisi dinamis diperoleh hasil bahwa semakin tinggi kapasitas fluida yang mengalir di atas permukaan sel, semakin tinggi pula kebocoran fluida yang terjadi. Hasil lain yang diperoleh adalah semakin kecil tinggi channel, semakin besar kebocoran, serta semakin tinggi viskositas fluida, semakin rendah pula kebocoran fluida yang terjadi pada kultur sel.

Kata kunci : *wall shear stress*, permeabilitas, virus dengue, *computational fluid dynamics*