Analysis Comparison of Viral Load Measurement with Quantitative Polymerase Chain Reaction (qPCR) and of p24 antigen by ELISA on HIV-1 virus after Hyperbaric Oxygen exposure

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Background: In making a diagnosis or monitoring therapy, an examination is needed to detect the presence of pathogens. In the monitoring of therapy, there will be a decrease in the number of viruses or protein components as well as the amount of enzymes and components of viral nucleic acids that will determine whether a therapy successfully eradicates the virus infection or not. In this regard, a method of examination that is high in sensitivity and specificity is needed, in order to suppressing the number of false positive or false negative that can make the diagnosis and evaluation of therapy easy, precise and efficient.

Objective: This study emphasizes the sensitivity of qPCR method that detects viral nucleic acid compared with the enzyme linked immunosorbent assay (ELISA) method that detects p24 HIV-1 antigen, after hyperbaric oxygen therapy at therapeutic doses

Method: After isolation of PBMCs (Peripheral Blood Mononuclear Cells) from healthy whole blood, inoculation of HIV-1 / MT4 virus in PBMC cell cultures was performed. Then exposure with hyperbaric oxygen at 2.4 ATA O2 100% for 3x30 'for 5 days. And, the P24 HIV-1 antigen was measured by ELISA and quantitatively measured viral nucleic acid by qPCR. Analyze the results of the influence of hyperbaric oxygen on both groups was performed, as well as the correlation between the two methods of examination.

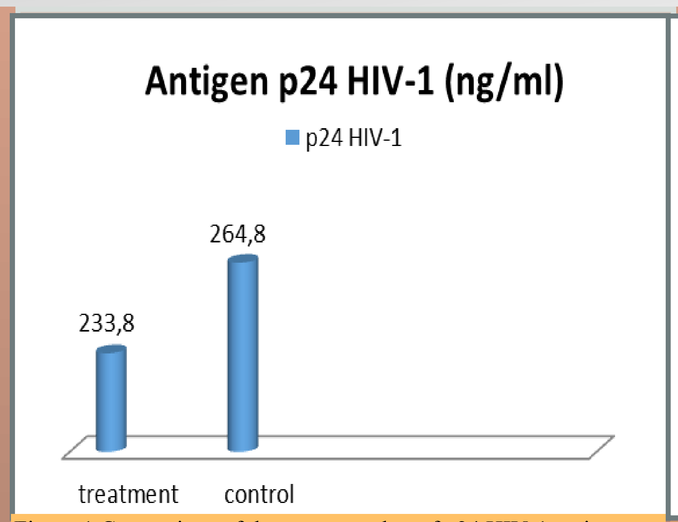

Figure 1 Comparison of the mean number of p24 HIV-1 antigens (ELISA)

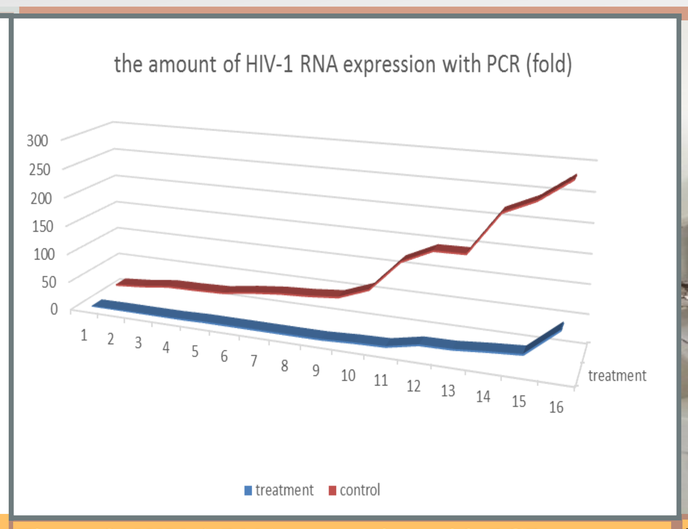

Figure 2 HIV-1 RNA expression with PCR

Figure 3 Animal Hiperbarik Chmber

Paired t test showed p = 0.017 (p <α), there was a significant difference the amount of p24 HIV-1 between the treatment group and control group. Whereas examination of HIV-1 mRNA with RT PCR using Paired t test showed the results of p = 0.001 (p <α), there were also differences in the expression of absolute HIV-1 RNA between treatment and control groups.

The results of p24 HIV-1 different test (ELISA) and HIV-1 RNA expression (PCR) in the treatment group showed a correlation coefficient of 0.2 (α> 0.05) which means there is no correlation between the two types of methods. While the paired t test test shows α = 0,000 (α <0,05) which shows that there are significant differences between the two methods of examination.

Conclusion: Examination of HIV-1 with qPCR which detected the amount of HIV-1 RNA was more sensitive than ELISA which detected the p24 protein that found in the capsid of each virion. Both of these examination methods have benefits depending on whether the goal is to establish, to diagnosis in the acute or chronic phase, to monitor the results of antiretroviral therapy or to evaluate the progression of the disease.

Key word : HIV-1,qPCR, ELISA, p24 antigen, Hyperbaric Oxygen

Refference :

Barletta, J.M., Edelman, D.C., Constantine, N.T., 2004. Lowering the detection limits of HIV-1 viral load using real-time immuno-PCR for HIV-1 p24 antigen. Am.J.Clin.Pathol. 122, 20–27. doi:10.1309/529T2WDNEB6X8VUN

Dheda, K., Huggett, J.F., Bustin, S.A., Johnson, M.A., Rook, G., Zumla, A., 2004. Validation of housekeeping genes for normalizating RNA expression in real-time PCR. Biotechniques 37.

Kozera, B., Rapacz, M., 2013. Reference genes in real-time PCR. J. Appl. Genet. 54, 391–406. doi:10.1007/s13353-013-0173-x

Reillo, M.R., Altieri, R.J., 1996. HIV antiviral effects of hyperbaric oxygen therapy. J. Assoc. Nurses AIDS Care 7, 43–45. doi:http://dx.doi.org/10.1016/S1055-3290(96)80037-9

Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. 3, 1101–1108. doi:10.1038/nprot.2008.73