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Quantification and importance of broad neutralizing antibodies in vertical transmission of HIV in Cameroon

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General Objective

In the present study, we aimed to analyze the association between neutralizing antibody titers and MTCT taking in to consideration the great genetic diversity of HIV strains and the socio-economic environment in Cameroon; and also determine the role of neutralizing antibodies in HIV/AIDS disease protection in HIV exposed but non infected infants.

Specific Objectives

The objectives were as follows:

1) Viral strain (serotyping)
2) Viral load
3) CD4 count
4) Level and nature of neutralizing antibodies in the couple "Mother-Baby"

Which of these factors' combination best defined vertical transmission of HIV in our context?

Main Activities Conducted

Patient Recruitment

The major activity that we have conducted since the beginning of the project has been patient enrollment. We have enrolled so far **81** couples of mother-child samples (HIV infected mothers with their new born). Samples from mothers were collected as fresh blood, while those from their new born were collected as dried blood spot (DBS).

Samples Analysis

Diagnosis

HIV testing of infants was performed by Roche Amplicor HIV-1 DNA PCR protocol using DBS (as described in the National EID HIV Programme). Among the **81** HIV-exposed infants we have already enrolled, early infant diagnosis of HIV infection was carried on **66** of them among which we had 65 HIV negative new born and only **1** HIV positive infant.

HIV Serotyping

The first step we did so far was to develop the protocol to be used on fresh blood as well as on DBS. In fact here we used known HIV infected samples other than enrolled ones. HIV serotyping was then performed by an immunoenzymatic assay (a discriminant indirect) ELISA which used specific peptidefor each type of virus (HIV-1&HIV-2), and for each group of HIV-1 (M, O, N & P if possible).These peptides came from the V3 loop (variable domain) of the glycoprotein gp 120 of the virus envelop.

At this level we encountered a major difficulty: lack of the positive controls of HIV-2 and HIV-1 group M, N and O. We have contacted several collaborators for this and we are still waiting for their feedback.

Viral Load (VL) determination

HIV-1 viral load was performed on specimens using Abbott HIV-1 RNA Realtime PCR protocol. From our **81** HIV infected patients, we have so far determined the viral load of **58** HIV infected mothers participating in this study (**14** had non detectable viral load, **8** had VL‹ 40 copies/ml and the **59** remaining had VL› 40copies/ml that is between **43** and **248154** copies/ml).

CD4 Count

We used the flow cytometry (Facs Calibur) to determine the level of CD4 in our patients. Among the 81 HIV infected mothers we enrolled, 42 of them had their CD4 count determined.

Level and nature of neutralizing antibodies in the couple "Mother-Baby"

The first step at this level was the development of a protocol that can permit us to identify antibodies on DBS. We have so far identified an optimum buffer to be used to elute antibodies from DBS. Secondly, we tried to obtain monoclonal antibodies that can serve as positive controls during for our testing.

Difficulties

The main difficulty we have resides in sample collection. We wanted to collect more of them before starting the analysis. The first step that was the obtention of ethical clearance took about 4 months. Also, we had some delay due to the slowness of administrative procedures for purchasing the needed reagents. Our last difficulty is still to acquire control antibodies.