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Characterization of Asymptomatic Children Infected with the Human Immunodeficiency Virus at Birth

Céline Nguefeu Nkenfou[1,2]*, Edith Saounde Temgoua[1,3], Béatrice Dambaya[1,4], Charlotte Tanguinpundum[1], Vittorio Colizzi[3] and Jacques Theze[5]

[1]*Chantal Biya" International Reference Centre for Research on HIV/AIDS prevention and management- CIRCB, Yaounde, Cameroon*
[2]*Higher Teachers' Training College, University of Yaounde I, Yaounde, Cameroon*
[3]*University of Tor Vergata, Rome, Italy*
[4]*Faculty of Sciences, University of Yaounde I, Yaounde Cameroon*
[5]*Institut Pasteur, Paris, France*

Abstract

Background: Mother-to-child HIV transmission remains very high in Cameroon. Therefore follow-up of numerous HIV-infected infants is a critical issue in the country. Here, we investigated the file of HIV-infected infants remaining asymptomatic in the absence of anti-retroviral therapy (ART). The first goal was to obtain an estimate of the prevalence of infants with an HIV controller like status.

Method: HIV-infected infants, aged 6 months to 17 years presenting at CIRCB for biological examinations were enrolled upon signed a proxy-consent. The enrollment took place from April 2011 to February 2013. From the medical file of 359 HIV vertically-infected infants, 41 were found naive of anti-retroviral therapy and free of clinical symptoms. Diseases related to HIV infection (oral candidosis, zona, chronic diarrhea, pulmonary tuberculosis, dermatitis) were more particularly checked and corresponding infants not included in the study. From the selected infants, CD4 counts and viral load were recorded. Non-exposed children were enrolled as control group.

Results: Of the 359 infants, 41 were ARV-naive and free from HIV clinical symptoms. Five of them (12%) exhibit a viral load < 1200 RNA copies/ml. Their CD4 counts were found not statistically different from those of a control group of HIV negative infants (p=0.33). Furthermore, ten years after contamination, three children did exhibit a viral load < 5500 RNA copies/ml. Altogether, this suggests the existence of pediatric HIV controllers (pHIC) with a frequency much higher (>10%).

Conclusion: Our preliminary cross-sectional study highly suggests the existence of pediatric HIV controllers **like** in Cameroon despite all disfavoring living conditions. However, a longitudinal study would be required to confirm this hypothesis. The development of an HIV vaccine applicable to infants of countries with high incidence of HIV-infected people should benefit from the immunological analysis explaining the HIV controller (HIC) status.

Keywords: Pediatric HIV; Infection Control; Characteristics

Abbreviations: ALT: Asymptomatic Long Term; ART: Antiretroviral Treatment; CIRCB: "Chantal Biya" International Reference Centre; HIC: HIV controller; LTNP: Long Term Non Progressors; pHIC: Pediatric HIV controller; VL: viral load.

Introduction

Mother-to-child transmission of HIV-1 is still very high in Cameroon. The most recent statistics indicate that around 17% of children born to HIV-positive mothers become contaminated [1-3]. Half of these children die before they are two years old [4]. The clinical situation of those who survive is dependent upon a number of factors such as quality of nutrition, interference by other infectious diseases, and unknown factors that remain to be studied [5-9].

The children who survive this HIV contamination beyond two years show different types of progression to AIDS. If no antiretroviral therapy (ART) is given, the standard progressor will develop AIDS in 5 to 10 years [10] while the rapid progressor will develop AIDS in 1 or 2 years after contamination [11]. Long Term Non Progressors (LTNP) or Asymptomatic Long Term (ALT) patients who maintain high CD4 counts for 5 years or more post contamination may also be found among HIV-infected children [12-15]. The latter group includes HIV controllers, or HIC, corresponding to patients with undetectable or low viral loads (VL) after 5 or 10 years of contamination [16-19]. More recently, in efforts to identify the genes involved in controlling HIV replication, patients with low VL one year post infection were also compared to HIC [20].

In Cameroon, very little is known about those children who survive HIV-contamination at birth. Herein, we describe a group of HIV-contaminated children who remained asymptomatic despite the absence of ART. This preliminary study aimed at defining the prevalence of HIV controller like in HIV vertically infected children of Cameroon. This strongly suggests that pediatric HIC (pHIC) can be found in Cameroon, and at a far higher frequency than in Western countries [16,17,19].

***Corresponding author:** Céline Nguefeu nkenfou, "Chantal Biya" International Reference Centre for Research on HIV/AIDS prevention and management-CIRCB, Yaounde, Cameroon,Tel: +237 75 57 35 19; Fax: +237 22 31 54 56; E-mail: nkenfou@yahoo.com

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Patients and Methods

Study design

HIV-infected children presenting at CIRCB for biological examinations were enrolled upon signed consent by their guardian. These children were referred to CIRCB from various hospitals of Yaounde (Centre Mère enfant, University teaching hospital, Efoulan district hospital…). The enrollment took place from April 2011 to February 2013. From these children, 5 ml of blood was collected in EDTA tubes for CD4 count and viral load if ARV naives. The inclusion criterion was been HIV-infected by vertical transmission. The exclusion criteria were clinical signs of HIV related diseases: diarrhea, oral candidosis, skin rash, chronic diarrhea, zona, bronchopneumonia, pulmonary tuberculosis, heavy cough. Upon enrolment, retrospective clinical data were collected from infants' medical record booklet. Retrospective and prospective clinical and biological data were collected and analyzed in order to classify participant either as fast/rapid progressor, slow progressor and LTNP or HIC. This is was a case-control study where participants were age matched.

HIV-positive children

Large numbers (359) of children born to HIV-infected mother were screened. Those who were both ARV-naive and asymptomatic at the time of the medical check-up were further analyzed. The medical records of each child were examined to check that they had not in their lifetime suffered any AIDS-related diseases. In addition, the child's parents or guardians signed an informed consent form and completed a questionnaire that was subsequently used to verify that the child remained asymptomatic up to blood sampling. All children with present or past significant disease conditions, and more particularly any AIDS-related conditions, were excluded (chronic diarrhea, oral candidosis, productive cough…). They were aged 3 years to 17 years old, and represent 12% of the population (45/359); of the 45 (55%); 25 were male and 20 (45%) were female. Blood (5ml on EDTA) was collected from the children included in the study for CD4 counts and VL analysis.

HIV-negative children

Samples were also collected as controls from HIV-negative children consulting for reasons unrelated to HIV/AIDS (suspected malaria, skin disease, persistent cough, etc.). These children were in the same age range as the group of HIV-contaminated children. In all, 23 children aged between 1 and 15 years were enrolled in the HIV-negative control group. Twelve of them were male and 13 were female. Blood (5 ml) was collected from these patients to determine control CD4 counts.

Determination of CD4 counts

CD4 T cells were quantified on a FACSCalibur flow cytometer [Becton Dickinson Immuno-cytometry System (BDIS), San Jose, CA, USA]

Determination of HIV viral load

The HIV-1 viral load was determined from plasma by Abbott Real-Time HIV-1 assay (Abbott Molecular Diagnostics, Wiesbaden, Germany) [21] The detection limit of this method is 40 copies/ml (1.6 log).

Statistics

Analyzis were performed with the GraphPad Prim 5.0 software using non parametric tests in all case. When indicated, the data are reported as mean ± Standard deviation. p values were calculated by the Mann Whitney U test. p values<0.05 are considered to be significant.

Ethics

Our study protocol was approved by the national ethics committee under the n° 103/CNE/SE/2012.Children presenting at CIRCB, fulfilling the inclusion criteria, were enrolled upon signed proxy consent form by their guardian. From these children, 5 ml of blood were collected in EDTA tubes for CD4 count and viral load if ARV naives.

Results

Characterization of asymptomatic HIV-infected children

The study involved 359 children who were HIV-infected at birth. They were between 6 months and 17 years old; 152 (42%) were male and 177 (58%) were female. In all, 97 participants were aged from 0.5 to 2 years, 56 from 2.1 to 5 years, and the majority (206) was between 5.1 and 17 years old. At the time of the study, 89 children were ARV-naive and 41 of these were found to be asymptomatic as described in Patients and Methods. Several reasons could justify why these children were not yet on treatment: the recommendations of WHO were not yet in vigor, some of these children do not have access to health service and their status has been known late or the health personnel were not trained to provide the appropriate services.

The results of the CD4 counts and VL analyses for these 41 children are given in Table 1 and show that all these children except two (one with minor cough and the other with dermatitis) did not exhibit any significant clinical symptoms. CD4 counts in these patients were very heterogeneous, ranging from 36 to 3168/mm^3. CD4 counts were between 500 and 1000/mm^3 in 35% of the children, between 300 and 500/mm^3 in 21% and >1000/mm^3 in 17%. The threshold for CD4 count used for WHO stage classification is 15% 25% [22] According to CDC 1994 classification, in children CD4 percentage of more than 25% depicts no immunodeficiency, a percentage of 15-25% denotes a moderate immunodeficiency and less than 15% describes a severe immunodeficiency. Currently treatment initiation of infants infected at birth does take into consideration neither the CD4 count nor the viral load count. Their VLs were also found to be very heterogeneous, and it is noteworthy that no correlation was found between their VL and CD4 counts. Correlations were analyzed with the Spearman's coefficient R.

We also notice that among the 41 asymptomatic children, 13 were male (31%) and 28 were female (68%), showing the higher percentage of controllers among female infants. This has been shown in other studies. [20,23-29]

Preliminary evidence of HIV Controllers like status

Figure 1 part A shows VL for all 41 asymptomatic patients together with their classification into groups based on VL value. Two groups of patients emerged. The first includes 5 patients (12%) with viral load <1200 RNA copies/ml. The second group includes 36 patients (87%) with viral load >1200 RNA copies/ml. The graph shows a clear VL discontinuity between these two groups and is therefore suggestive of a pHIC group. However, none of the children in this group showed total control of HIV replication, only a very clear reduction in detectable VL. Two of these children were 7 and 8 years old, further supporting the notion of persistent HIV control. The VL in the three other younger children (1, 2 and 3 years old) in this group was very low compared to far younger children in the other group exhibiting very high VL (Table 1).

ID	Age (year)	Sex	CD4 Mean (Cells/mm³)	Average CD4 (%)	VL mean (copies/ml)	Clinic
19212	0.75	F	1340 (2)	31	68952	
19236	0.75	M	1342	18	124317	
19098	0.75	F	386	10	339088	
19178	0.75	F	870	9	247274	
19111	1	F	670	24	409820	
22116	1	F	810	15	201745	
19082	1	F	3168 (2)	53,5	278	
19142	2	F	863 (2)	11,5	267116	
19459	2	F	857 (2)	18,5	14624	
19467	2	M	800	13	52077	
19208	2	F	2255 (2)	32	222	
21796	2	F	1036	35	72205	
19335	3	F	688 (2)	27,5	294689	
21843	3	M	558 (2)	15	28283	
19140	4	M	958	25	1045	
20284	4	M	653,5 (2)	25,5	19363	Cough
21841	5	F	356	11	147119	
22074	6	F	475	24	9532	
19601	6	F	450 (2)		119192	
20731	7	F	663		113 (2)	
4901	7	M	1094 (2)	28	121031 (2)	
12968	8	F	1140 (2)	32,5	98423	
2757	8	M	438 (3)	18,5	88355	
18897	8	F	996 (2)	31,5	1115	
2223	9	F	389 (3)	14	23833	
3597	9	M	605	25	505132	
19087	9	F	389 (2)	31	11931	
19189	9	F	242 (2)	39,5	17674	
19038	9	F	559	22	3258	
21772	10	F	289,5 (2)	2	152372	
21341	11	M	610	27	3016	
12522	12	F	401	14	44928	
7949	12	F	687 (2)	23	55533 (2)	
10907	13	F	846 (2)	34,5	8986 (4)	
19188	13	M	424 (2)	31	19093	
21840	13	M	36	2	45898	
21811	13	F	187 (2)	20	5250	Dermatitis
19190	14	M	351 (2)	20,5	2313,5 (2)	
21753	14	F	167		14021	
22078	15	F	294	12	957926	
4957	17	M	189	11	1346449	
( ) number of measurements and average						
	VL<1500 RNA copies/ml					
	VL>1500 RNA copies/ml and <5500 RNA copies/ml					

Table 1: Characteristics of the 41 asymptomatic infants HIV-infected at birth.

The 5 patients identified as possible HIC were further analyzed and the results, as expected and as illustrated in Figure 1B, show that their VL was significantly lower than in the 36 other patients. Even more interestingly, Figure 1C shows that CD4 counts in the putative HIC patients were not statistically different from those in the HIV-negative control group defined in Patients and Methods (p=0.33). By contrast, CD4 counts in the group of 36 viremic patients were significantly lower than those in the control group.

The case of three children with viral load <5500 RNA copies/ml also warrants special comment. These children were 9, 11 and 15 years old, also asymptomatic, and to some extent were partially controlling their VL. It is noteworthy that the first two had CD4 counts >500 / mm³. This group may also represent another form of control over HIV replication, sufficient to avoid the onset of clinical symptoms.

Discussion

Several points noted during this study of 41 children who were HIV-infected at birth, had never received ARV therapy, but were nevertheless asymptomatic, warrant further discussion.

Of the 359 infants surveyed, only 41 (11%) of those not receiving

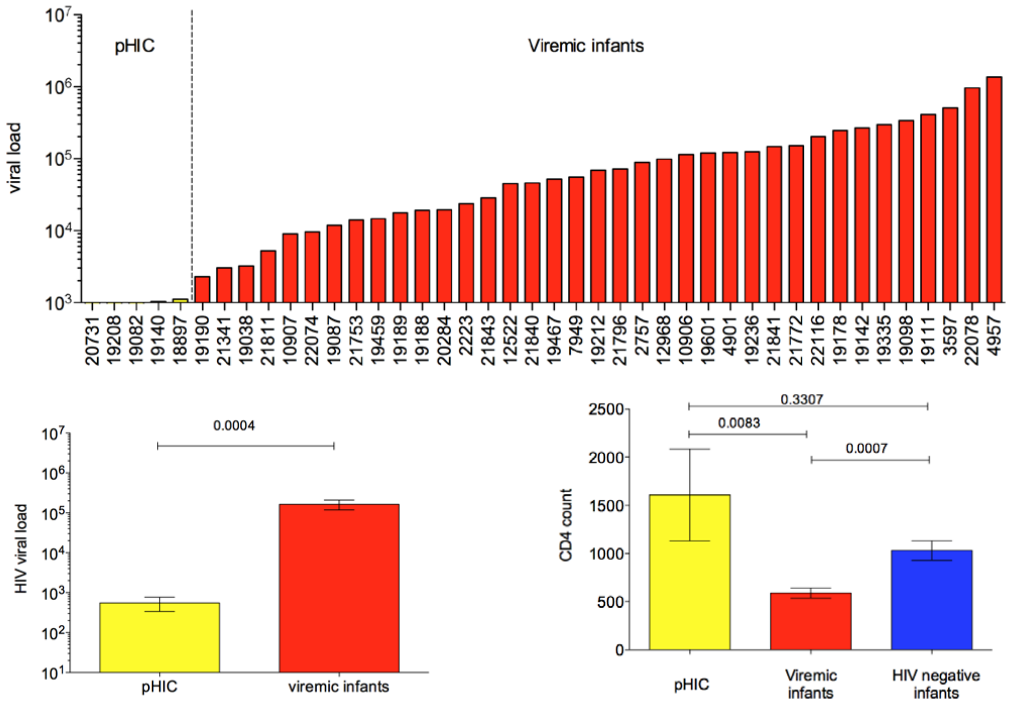

Figure 1: Analysis of the viral load and CD4 counts of HIV-infected infants.

A – HIV RNA copies/ml. 5 children have RNA copies/ml<1200 and could be considered as pediatric HIV controllers (pHIC).

B – HIV/RNA copies/ml (mean ± SD) for the 5 pHIC (see above) and for the 36 viremic infants. The statistical difference between the two groups is significant (p=0.0004).

C- CD4 counts (mean ± SD) for the 5 pHIC and 36 viremic children. The difference is statistically significant (p=0.0085). By contrast, the difference between the CD4 counts in the pHIC infants and control group (HIV-negative infants) is not statistically significant (p=0.33).

therapy were asymptomatic. This group included very young children < 2 years old with very high VL and high CD4 counts, and also children >15 years with very high VL (around 10^6 RNA copies/ml) and low CD4 counts (<300 CD4/mm^3). Since no correlation was found in our study between the results of the VL analysis and CD4 counts, it may be concluded that disease progression to clinical symptoms in these Cameroonian HIV-contaminated children was not strictly dependent upon conventional parameters such as CD4 counts and VL.

Surprisingly, 8 of the 41 children were exerting at least some degree of control over HIV replication. The greatest control was observed in a 7-year-old child where viral load was 113 RNA copies/mm^3 and CD4 count 663/ml. Two other children also showed marked control of HIV replication (278 and 222 RNA copies/ml; respectively for CD4 count of 3168 and 2255 cells/mm^3). Despite the fact that these latter children were very young (1 and 2 years old), they may nonetheless considered to be HIC (consistent with the study by Fellay et al. [20]. Furthermore, if we consider that significant control of viral replication corresponds to VL < 5500 RNA copies/ml, three more children may also be considered to be HIC. Altogether, our preliminary analysis suggests that 5 or 8 children of the 41 young patients analyzed were pHIC. This is a very high percentage (12 or 19%) compared to that seen in Western countries (< 1%) [16,17,19]

How can this percentage of HIC infants be far higher in Cameroon than in Western countries? Since most HIV-contaminated infants die within two years of birth, it may be considered that the asymptomatic children studied had been naturally selected thanks to some form of viral resistance or viral control. Of the many parameters that may be involved in this control, it could be speculated that the high prevalence of infectious disease in Cameroon may promote a more efficient immune response against HIV [6,30] Obviously, the genetic background of the Cameroon population may also play a role [31] Probably other genes like CCR2-64 I may be involved, as shown by the study carried out in a population of Cameroon where this gene was detected at an allelic frequency of 17.6%; meanwhile, CCR5 delta 32 could not account for protection as it was not found in this population [32] (nkenfou et al. BMC paper) and given the overall low frequency of this gene in African population [33-34]. It is as well evident that the virus types will be investigated as other study have shown progression-virus strain relationship [34].

At this stage, it is important to recall that our data are preliminary as generated by a horizontal study. A longitudinal study in a large group of children would be required to draw more definitive conclusions. The data presented herein are nevertheless very challenging and encourage us to further study this group of children in order to test our hypothesis.

We prospect then in the future to look at the infecting virus, new genes through the GWAS, AIDS related known genes, the microbiome of infants and the nutritional status.

If HIC or different forms of HIV control are definitively characterized, then investigations into the mechanism that results in immunological control of the virus could pave the way to a very significant step toward developing new vaccination strategies for children before their first sexual experience.

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