

Anatomopathological characterization of placentas from HIV+ patients associated with p24 expression

Caracterização anatomopatológica da placenta de pacientes HIV+ associada à expressão do p24

Consuelo Lozoya López¹; Andréa Rodrigues Cordovil Pires²; Eliene Carvalho de Fonseca³; Fabiana Resende Rodrigues⁴; Antônio Rodrigues Braga Neto⁵; Gesmar Volga Haddad Herdy⁶; Flávio Augusto Prado Vasques⁷; Vania Gloria Silami Lopes⁸

ABSTRACT

Introduction: The study of placentas from pregnant human immunodeficiency virus (HIV) positive women has become the subject of numerous studies in the literature. Morphological, viral, immune and inflammatory placental aspects have been analyzed in order to grasp the vertical transmission of the virus. **Objective:** To identify the most frequent findings in the placentas by associating them with a viral antigen and correlating them with the infection of newborns. **Material and methods:** Thirty-five placentas from HIV- positive pregnant women were pathologically and immunohistochemically analyzed with the use of p24 antibody in the period from 1992 to 1997 in accordance with the routine laboratory testing from the Anatomopathological Department - *Hospital Universitário Antônio Pedro - Universidade Federal Fluminense* (APD/HUAP/UFF). **Results:** The microscopic alterations detected in all cases, including those with vertical transmission, were arteriopathy in the fetal blood circulation, chorioamnionitis, perivillous fibrin deposition, syncytial knotting, villous edema and villous immaturity. No specific macroscopic or histopathological changes were found in these placentas. The neonatal infection was observed in five cases. Vertical transmission was identified in two out of five placentas that had low weight for the respective stage of pregnancy. Immunohistochemical analysis revealed 14 positive cases, two of which showed vertical transmission. The viral protein was not identified in 10 out of 14 placentas from patients who had been medicated with zidovudine (AZT). **Conclusion:** Our study has contributed to the anatomopathological investigation into placentas from HIV-positive patients, although p24 expression per se did not allow a definite and early diagnosis of the vertical transmission

Key words: placenta; pathologic exam; HIV; immunohistochemistry; p24.

INTRODUCTION

The vertical transmission of the human immunodeficiency virus (HIV) has been widely studied and particular emphasis has been given to the role of the placenta as a selective membrane of the mother-infant pair and prime source of congenital infection.

Transplacental infection has evolved with the adoption of obstetric (cesarean section) and pharmacological (antiretrovirals during gestation, delivery and puerperium) practices, inasmuch as less than a decade ago 14% to 48% of the concepti from infected mothers acquired the infection⁽²⁷⁾, whereas currently this total dropped to 1% to 2%⁽¹⁷⁾.

First submission on 30/11/12; last submission on 13/09/13; accepted for publication on 15/09/13; published on 20/12/13

1. MSc in Pathology by Universidade Federal Fluminense (UFF); adjunct professor at UFF.

2. Doctoral student in Pathology at UFF; adjunct professor at UFF.

3. MSc in Cellular and Molecular Biology by Fundação Oswaldo Cruz; adjunct professor at UFF.

4. PhD in Pathology by UFF; adjunct professor at UFF.

5. PhD in Gynecology, Obstetrics and Mastology by Universidade Estadual Paulista Júlio de Mesquita Filho; Post-doctorate by International Researcher of Harvard Medical School - BWH (USA); adjunct professor at UFF.

6. PhD in Cardiology by Universidade Federal do Rio de Janeiro; professor at the Maternal and Child Health Department - UFF.

7. PhD in Obstetrics by Universidade Federal de São Paulo; associate professor at UFF.

8. PhD in Pathology by UFF; associate professor at UFF.

The use of zidovudine (AZT) has been proven to be unequivocally effective in reducing vertical transmission of HIV. However, in order to yield the best results, these patients should be advised not to breastfeed. Moreover, AZT oral solution should be administered to the newborn for six weeks after delivery. These children must be assessed by specialized units until their diagnosis is defined. Their serology should be performed monthly for the first six months and at least quarterly from the second semester until the 18th week. If there is no evidence of HIV-positive results, the child will be considered uninfected. This process usually causes anguish and uncertainty among relatives, hence the need for tests that can allow an early diagnosis of transplacental transmission of HIV. Nevertheless, the mechanisms of transplacental infection by HIV as well as the details pertaining to the perinatal transmission are still unclear.

The placentas from HIV-infected patients are usually normal and show no specific histological changes that could be attributed to HIV contamination, which differs from other congenital transplacental viral infections⁽⁴⁾. Nonetheless, chorioamnionitis and villous immaturity with hypercellularity of villous stroma have been frequently reported⁽²⁵⁾, hence requiring further investigation into possible histopathological effects of placental infection by HIV. Some morphological findings such as placentitis, mainly chorioamnionitis and funisitis are referred to as risk factors for vertical transmission^(7, 21).

Furthermore, we have striven to identify the presence of HIV in the human placenta through the use of more sophisticated diagnostic procedures. Some authors use a variety of techniques such as electron microscopy⁽¹²⁾, immunohistochemistry⁽¹⁶⁾, in situ hybridization⁽⁷⁾ and polymerase chain reaction⁽¹⁸⁾. They have also identified the virus preference for Hofbauer cells rather than trophoblasts and endothelial cells. One of the targets for the diagnosis of viral infection is p24, which is a protein of 24 kilodaltons present in the HIV nucleocapsid. However, paradoxically, the mere presence of the virus in the placenta did not correlate with the vertical transmission of HIV. Conversely, some cases of congenital infection were observed without the presence of the virus, hence the need for further investigation into the role of the viral presence in the vertical HIV infection⁽⁹⁾.

Thus, the objective of the present study is to correlate the morphology and p24 immunoreactivity in placentas from HIV-positive pregnant women with the vertical transmission in patients that had been treated or not with prophylactic AZT during pregnancy or delivery.

MATERIAL AND METHODS

We conducted a prospective study with pregnant patients followed at Antonio Pedro University Hospital, *Universidade Federal Fluminense* (APUH/UFF), in the period of 1992 to 1997. The pregnant women included in this investigation met the following criteria: two results for enzyme-linked immunosorbent assay (ELISA) and one positive result for HIV Western blot, delivery at APUH/UFF with more than 32 weeks of gestation, pediatric follow-up with ELISA anti-HIV in the first six months of life monthly and quarterly until the 18th week of life⁽¹⁹⁾. Patients younger than 18 years of age, those who had been in treatment for acquired immunodeficiency syndrome (AIDS) and those who did not fulfill the complete follow-up criteria were excluded from this research protocol.

We analyzed 35 HIV-infected pregnant women without prior antiretroviral treatment who were medicated with AZT during pregnancy and intrapartum. After delivery, their placentas were placed in vials with 10% buffered formalin and sent to the Pathology Service (PS). The anatomopathological analysis was conducted by the same pathologist. The macroscopic study of the placentas was performed according to the protocol proposed by Benirschke & Kaufmann⁽⁵⁾. The tissue samples were fixed in 10% buffered formalin for subsequent paraffin embedding and standard histological processing. 4 µm histological sections were obtained from the paraffin block and stained with hematoxylin and eosin (HE), which were examined and the histopathological diagnoses were recorded. In the immunohistochemical study, HIV p24 antigen was used as primary antibody (clone Kal-1, Dako, code M857, Batch 062), adopting the Avidin - Biotin - Peroxidase method⁽¹³⁾.

The following clinical variables were analyzed: patient's age, gestational age at delivery, associated obstetric complications, type of delivery, indication for cesarean delivery and vertical transmission of HIV (assessed up to 18 months after delivery). We also described the morphological variables of the placenta (weight in grams, chorionic plate diameter, thickness, umbilical cord length and color of the maternal and fetal sides as well as Wharton's jelly) and p24 immunoreactivity in different cells from the placental parenchyma. Statistical analysis was carried out and mean/median, ranges, standard deviation (SD) and percentage were calculated according to the variable type. We employed a nonparametric corrected X^2 (chi-square) test. The level of significance was set at $p < 0.05$.

This study was approved by the Ethics in Research from APUH/UFF.

RESULTS

The median age of patients was 25 years (ranges: 19-37; SD = 4.3 years). The mean gestational age at delivery was 38 weeks (ranges: 33-40; SD = 1.4 weeks).

Obstetric complications occurred in 22/35 (62.8%) patients: urinary tract infection and prematurity in seven patients each (7/35-20%); genital condylomata (6/35-17%); syphilis, tuberculosis, genital candidiasis and premature amniorrhexis in three patients each (3/35-8.5%); pulmonary pneumocystosis, hepatitis B, genital trichomoniasis, placental abruption, cord prolapse and prolonged amniorrhexis in one patient each (1/35-2.8%).

Cesarean section was performed in 80% of patients (28/35) with the following indications: elective (18/28-64.2%), prematurity (7/28-25%), prolapsed umbilical cord (1/28-3.4%), chorioamnionitis by prolonged amniorrhexis (1/28-3.4%) and acute fetal distress - placental abruption with live fetus (1/28-3.4%). Vaginal delivery occurred in 20% of patients (7/35), all admitted to the maternity hospital in advanced labor (active phase, with more than 7 cm dilation, fetal head fixed in the pelvis). 2 out of 5 patients who had their children infected by HIV had preterm delivery, one with placental abruption and acute fetal distress. All presented ascending infection (chorioamnionitis) and two of them had other associated infections.

Regarding placental morphology, the macroscopic study revealed that the weight of the 28 placentas from full term pregnancies averaged 466.6 g (ranges: 200-862; SD = 65 g), whereas the weight of the seven preterm placentas averaged 372 g (extremes: 215-530; SD = 32g). 78.5% (22/28) of the placentas from full term pregnancies had suitable weight for the gestational age (between 10th and 90th percentile), whereas 14.2% (4/28) of them were considered small for the gestational age (below the 10th percentile) and 7.1% (2/28) were large for the gestational age (above the 90th percentile). 57% of the preterm placentas (4/7) had suitable weight for the gestational age (between the 10th and 90th percentile), whereas 33% (3/7) were small for the gestational age (below the 10th percentile), with no cases of large placenta for the gestational age in this group. The diameter of the placental chorionic plate measured 18.2 cm on average (ranges: 13.5-27.5; SD = 0.4 cm). The thickness of the placentas was on average 2 cm (ranges: 3.5-1.0; SD = 0.4 cm). The length of the umbilical cord measured 31.6 cm on average (ranges: 18-68; SD = 8 cm).

Vertical transmission of HIV was observed in two neonates (2/5-40%) whose placentas had low weight for the gestational age among the seven placentas with low weight from a total of 35 cases (7/35-20%).

The chorion laeve was transparent in most placentas (26/35-74%) and opalescent in 9/35 (26%). The coloring of the Wharton's jelly was predominantly white (28/35-80%), although it also presented other hues: yellow (3/35-8.8%), violet (2/35-5.6%), green (1/35-2.8) and pink (1/35-2.8%). The fetal side was described as predominantly bluish (32/35-91.5%) and in a few cases greenish (3/35-7.5%). The fetal side of the amnion was transparent in 23/35 (65.5%) and opalescent in 12/35 (34.5%) placentas. On the maternal side, the lobes were well defined in 25/35 (71.5%) and poorly defined in 10/35 (28.5%) placentas. Sectioning revealed that the tissue was spongy, wine-red (29/35-82.8%) and light red (6/35-17.2%), with calcifications (11/35-31.5%), infarcts (10/35-28.5%) and retroplacental hematoma (1/35-2.8%), whereas 13 sections revealed no morphological changes (13/35-37.2%). As far as microscopy is concerned, those five placentas with vertical transmission presented slightly opalescent chorion indicating chorioamnionitis. Subsequently, the diagnosis was confirmed by microscopy.

The incidence of placental microscopic lesions observed in 35 placentas and in the five placentas from children who had acquired HIV infection by vertical transmission are shown in

Figure 1.

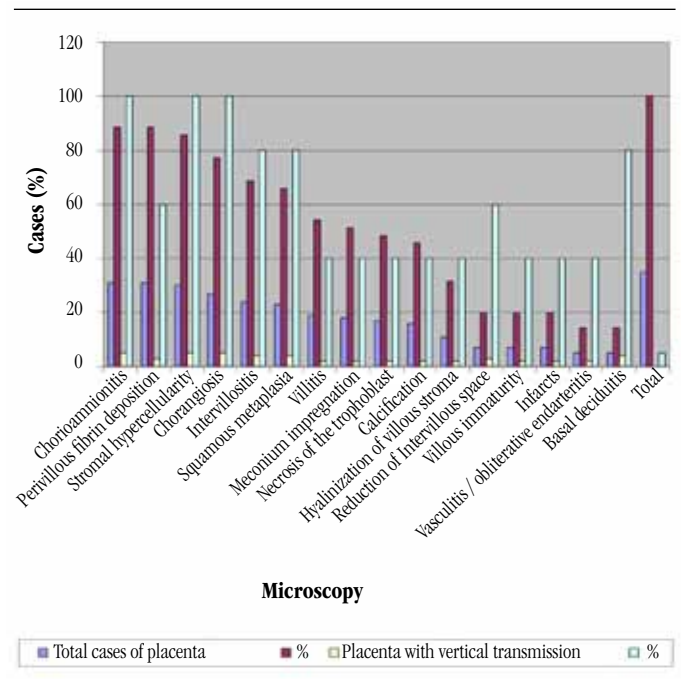


FIGURE 1 – The growing incidence of microscopic findings of 35 placentas from HIV-positive patients and the incidence of findings in the group of five children with concomitant HIV infection

HIV: human immunodeficiency virus.

The microscopic study indicated that all placentas were from the third trimester of pregnancy, including the premature ones. The fetoplacental vasculopathy was the most common histopathological finding, which was observed in 33/35 (94.2%) placentas. This change was characterized by thickening of the vascular walls due to an endothelial edema extending from the subendothelial layer to the middle and adventitial layers as well as hyperplasia of the middle layer and occasionally perivascular fibrosis with consequent reduction of the vascular lumen (**Figure 2**). Vasculopathy was present in all five cases of fetal infection. Chorioamnionitis was the second most frequent 31/35 (88.6%), present in all cases in which there was fetal infection. Villous immaturity (**Figure 3**) characterized by stromal villous hypercellularity was the third most frequent infection observed in 30/35 (85.7%) HIV + placentas, which was present in all cases of vertical transmission.

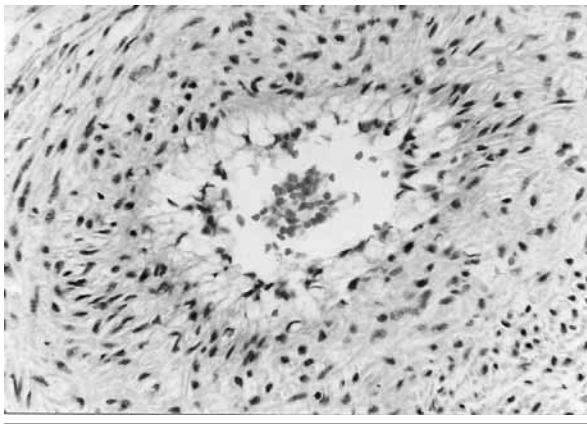


FIGURE 2 – Microscopy (HE): arteriopathy – wall thickening with edema, hyperplasia of the middle layer and lumen reduction . 40 × / 0.65
HE: hematoxylin and eosin.

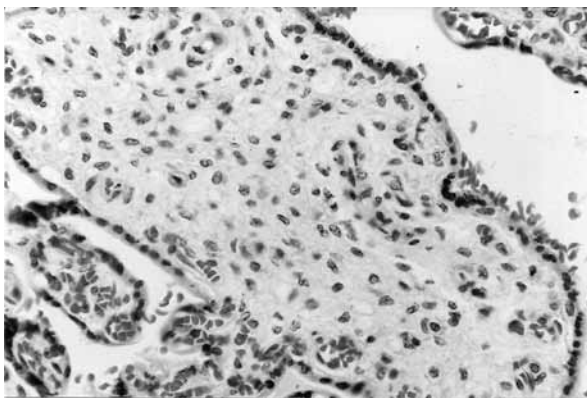


FIGURE 3 – Microscopy (HE): immaturity - villous stromal hypercellularity. 40 × / 0.65
HE: hematoxylin and eosin.

Immunohistochemistry showed positivity for p24 antigen in 14/35 (40%) cases. The following results were yielded: five positive cases in the cells and endothelium of the decidua reflexa vessels; four cases in decidua basalis cells; three cases in lymphocytes from the intervillous space; one case in trophoblasts; four cases in Hofbauer cells from the villous stroma; one case in lymphocytes from the lumen of a blood vessel in the villous branch. The immunopositivity occurred in six patients in different locations of the same placenta (**Figures 4 and 5**).

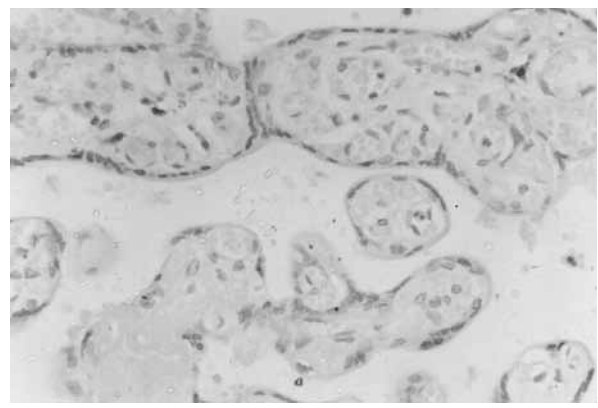


FIGURE 4 – p24 IHC : Positive immunostaining in Hofbauer cells from the villous stroma and in trophoblasts . 40 × / 0.65
IHC: immunohistochemistry.

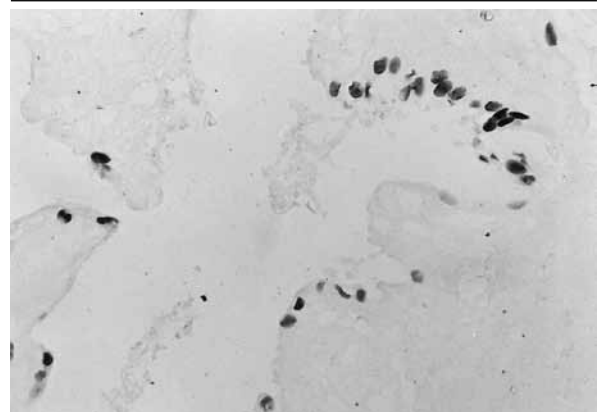


FIGURE 5 – p24 IHC: positive immunostaining in the decidua and trophoblasts. 40 × / 0.65
IHC: immunohistochemistry.

From a total of 14 placentas that showed immunopositivity for p24 antigen, seven newborns were not infected, five had no clinical follow-up and two were infected presenting immunostaining for p24 in trophoblasts and Hofbauer cells. From the total of 21 cases with negative immunohistochemistry, three newborns acquired infection, eight were not infected and ten had no follow-up (**Table 1**).

From a total of 14 placentas from patients treated with AZT, the immunohistochemistry confirmed positivity for the viral protein

in 4 cases (28.5%), involving basal decidua cells and lymphocytes from the intervillous space. In the other 21 placentas from patients not treated with AZT, p24 antigen was detected in 10 cases (52.3%) (Table 2).

From a total of 14 patients treated with AZT, the prophylaxis was efficient in five neonates, two children were infected and seven of them had no follow-up records. From a total of 21 patients who were not treated with AZT, ten were not infected, three were infected and eight of them had no clinical follow-up (Table 3).

TABLE 1 – Correlation between p24 viral rotein and vertical transmission

p24	Infected	Non-infected
Positive	2 (14%)	7 (50%)
Negative	3 (12%)	8 (38%)
Total	5 (14%)	15 (43%)

$\chi^2 = 0.49$; $p > 0.05$ non-significant. The remaining 15 children from our sample had no outpatient follow-up.

TABLE 2 – Correlation between the use of AZT in pregnant women and p24 viral protein in placentas.

Use of AZT	p24		Number of cases
	Presence	Absence	
Yes	4 (28%)	10 (72%)	14 (100%)
No	10 (50%)	11 (50%)	21 (100%)
Total	14 (40%)	21 (60%)	35 (100%)

AZT: zidovudine.

$\chi^2 = 1.26$; $p > 0.005$ non-significant. It was not possible to verify infection prophylaxis in 15 children who had no outpatient follow-up.

TABLE 3 – Correlation between the use of AZT in pregnant women and vertical transmission

Use of AZT	Vertical transmission	
	Infected	Non-infected
Yes	2 (14%)	5 (36%)
No	3 (14%)	10 (50%)

AZT: zidovudine.

$\chi^2 = 0.49$; $p > 0.005$ non-significant. It was not possible to verify infection prophylaxis in 15 children who had no outpatient follow-up.

DISCUSSION

Burton *et al.*⁽⁹⁾ studied 17 HIV-positive pregnant women and demonstrated that fetal infection is not correlated with placental infection when the immunohistochemistry with p24 antigen is applied, that is to say that there were cases in which the viral antigen was detected in the placenta and the child was not infected with

HIV and vice versa, insofar as the child was infected and the viral antigen was not detected in placental tissue. Baurakiades *et al.*⁽⁴⁾ demonstrated by histomorphometry that in placentas from HIV-positive pregnant women the villi were smaller in comparison with seronegative patients. Furthermore, the immunohistochemistry revealed that anti-p24 antibody presented lower immunostaining (2 / 57) to detect the viral protein, but they observed CD8 T cells and the intercellular adhesion molecule (ICAM-1) as biological markers of inflammatory placental changes associated with HIV-1 infection. The positivity for the viral protein was detected in all 57 cases by polymerase chain reaction (PCR), a genomic amplification method.

Although the exact mechanisms of vertical transmission remain unclear, some authors such as Moussa *et al.*⁽¹⁸⁾ applied PCR to detect placental cytokines such as macrophage inflammatory proteins (MIP) 1 α , MIP-1 β , interleukin (IL) -8 and IL-6 in HIV + patients. They observed a reduction of these substances in their sample, thus the newborns were not infected. The study by Vidricaire, Tardif and Tremblay⁽³⁰⁾ revealed an increase in the tumor necrosis factor alpha (TNF- α) and IL-1 in placentas of HIV + patients and correlated the inflammation of placental tissue, namely chorioamnionitis and chronic villitis, with HIV vertical transmission. Bento *et al.*⁽⁶⁾ and Pornprasert *et al.*⁽²²⁾ developed comparative studies with pregnant women who had a controlled or uncontrolled viral load and showed a rise in the secretion of IL-10 cytokine by T cells in the first group of patients, which caused a decrease in viral replication, hence influencing the reduction of vertical transmission. Scarlatti⁽²⁴⁾ studied the advances and disputable aspects of HIV-1 vertical transmission and reported that research into the role of viral characteristics, immune response and genomic polymorphisms of the host have not always produced final results. Even viral strains that are involved in the vertical transmission, namely those that use CCR5 receptor, may undergo mutations that may not be transferred, especially during the perinatal transmission. Derrien *et al.*⁽¹¹⁾ and Faye *et al.*⁽¹²⁾ investigated the role of the placenta in vertical transmission and suggested that placental cells such as trophoblasts, Hofbauer cells and the villous capillary endothelial cells have CD4 positive receptors, which makes them susceptible to HIV infection. Moreover, they revealed that placental cytokines (TNF- α and IL-8) and chemokines (regulated on activation, normal T cell expressed and secreted [RANTES] and MIP-1 β) are modulators through their receptors in order to prevent viral replication of HIV and to induce it. In the same study, from the genetic analysis of the HIV-1 sequencing, the interaction of certain genotypic viral characteristics with placental tissue was observed. Therefore, vertical transmission of

HIV-1 was characterized by the selection of the genotypic variant of the virus that is escaping the mother's immune system. Kumar *et al.*⁽¹⁴⁾ indicated in their research into the genetic sequencing of HIV-1 subtype C that the genomic differences in certain hydrophobic and "Env" particle regions are associated with tropism for certain placental cells and, therefore, these gene mutations may be involved in vertical transmission.

Soilleux & Coleman⁽²⁷⁾, attempting to describe vertical transmission, studied binding lectins dendritic cell-specific ICAM-grabbing non- integrin (DC-SIGN), a receptor for HIV binding lectin that is expressed on the dendritic cell-specific ICAM-grabbing non- integrin. Boily-Larouche *et al.*⁽⁸⁾ also investigated these viral DC-SIGN strains that underwent mutations and were linked with intrauterine infection.

The vertical transmission is correlated with viral load, symptomatology, co-infections and obstetric complications in HIV-positive pregnant women, according to data in the literature⁽¹⁵⁾ and similarly corroborated by Zorrilla⁽³²⁾, who reported a higher incidence of coinfections that interfere in the vertical transmission. In our sample, we also observed co-infections and obstetric complications in 22 patients (22/35-62.9%), including in this total five patients who had transmitted the virus to their children.

As similarly reported by Baurakiades *et al.*⁽⁴⁾, the analysis of our study did not allow us to identify any specific or abnormal macroscopic features of the placentas from HIV-positive pregnant women, inasmuch as the macroscopic changes were related to obstetric complications and maternal diseases.

According to Anderson⁽²⁾, placentas exposed to HIV infection exhibited the following microscopic features: edema, villous immaturity, villous necrosis, focal necrosis of trophoblasts, numerous Hofbauer cells, excessive intervillous fibrin deposition and chorangiomas. However, these characteristics are not specific due to the fact that the patients were smokers, drug addicts and had coinfections, which may influence morphology of the placenta. We described the arteriopathy in the fetal circulation as the most frequent lesion in our cases (94%). Some authors also detected fetal vasculopathy in placentas from HIV-positive pregnant women^(3, 23). We cannot assert that the observed vasculopathy is HIV specific, insofar as the patients were affected by other coinfections and diseases of different etiologies. Nevertheless, we may claim that HI, as other etiological agents, may be accountable for this lesion. The fact that immunopositivity for p24 antigen was evidenced in the villous capillary endothelium of one placenta from a mother who had no coinfection reinforces the idea that the virus is somehow

responsible for this lesion, since the arteriopathy was observed in all five placentas from newborns who were HIV infected.

According to Bhoopat *et al.*⁽⁷⁾, the histological evidence of HIV replication provided by p24 is found in approximately one quarter of the placentas obtained from full term pregnancies. Chorioamnionitis was also reported as a major risk factor for vertical transmission in 31 (86%) placentas. In our investigation, chorioamnionitis was identified in 31 (88.6%) placentas. The newborns were not infected in 15 of them and the mother transmitted the virus to their children in 5 cases. Chandwani *et al.*⁽¹⁰⁾ did not observe villitis, nor did they prove that chorioamnionitis may have been caused by HIV. However, in our material the immunopositivity for p24 antigen on the membranes was present in nine cases of chorioamnionitis, suggesting that the HIV was the cause of the inflammatory process. The decidua basalis, the intervillous space, the chorion of the chorionic plate and the umbilical cord may be involved in cases of chorioamnionitis depending on the severity and extent of the inflammatory process. Inflammation involving these structures in severe chorioamnionitis was found in our sample. We suggest the probable correlation of the HIV inflammatory process with these structures, which are detected in viral proteins by the immunohistochemistry method. The viral protein was identified in 14 cases of chorioamnionitis and 5 placentas of newborns who had acquired HIV infection. We were not able to associate villitis exclusively with HIV, since infections such as syphilis, tuberculosis, infection by human papillomavirus (HPV), toxoplasmosis and urinary tract infection can also cause it. In four placentas from patients who had no coinfection during pregnancy, immunopositivity for p24 antigen associated with villitis was evidenced in Hofbauer cells from the villous stroma, in trophoblasts, in the intervillous space and in the endothelium of villous vessels, hence suggesting that villitis, intervillitis and vasculitis were caused by HIV. Therefore, there was vertical transmission in these cases. Shearer *et al.*⁽²⁶⁾ reported the presence of villitis in four cases out of 30 HIV-positive patients. Moreover, HIV in trophoblasts was detected by in situ PCR, thus corroborating the hypothesis of villitis by HIV.

Mwanyumba *et al.*⁽²¹⁾ described nonspecific placental changes related to chronic hypoxia in HIV-positive patients. Herein these changes were observed in all placentas from infected children, who presented villous stromal edema, excess in syncytial knots and chorangiomas. These features were also attributed to hypertensive disorders of pregnancy, smoking, sickle cell disease and various infections. The stromal hypercellularity (immaturity) was detected in 30 cases (85.7%) from the total sample, and in all five cases of vertical transmission (Figure 4). Chandwani *et al.*⁽¹⁰⁾ also reported the same incidence of villous immaturity in all cases of

vertical transmission of HIV-1. The hyalinization of villous stroma observed in 11 (31.4%) cases may be indicative of villitis repair process, since in these cases there was no evidence of fetal artery thrombosis or other causes of intrauterine hypoxia. This change was observed in two out of five placentas from children who had been infected. The villous immaturity identified in seven (20%) patients was correlated with prematurity and was evident in two out of five cases of vertical transmission, which demonstrates that this obstetric complication is an aggravating factor in this transmission. Landesman *et al.*⁽¹⁵⁾ reported that children born before 32 weeks have twice the risk of being contaminated by the virus through vertical transmission.

Table 1 shows that the detection of viral antigen in the placenta did not always correspond with fetal infection in our sample, inasmuch as seven out of twelve cases in which the viral protein was detected were not infected with HIV. Vidricaire & Tremblay⁽³¹⁾ showed that there are placental protective factors such as trophoblasts, in which the p24 viral particle is predominantly concentrated in their endosomal vesicles after the entry of HIV-1, thus determining low productive infection of various strains. According to Muñoz *et al.*⁽²⁰⁾, another placental protective factor is progesterone since it inhibits HIV-1 replication in placental cells by reducing TNF- α levels, which are required for replication. Al-Husaini⁽¹⁾ also demonstrated that placental infection does not imply fetal infection and established that IL-10 produced by placental macrophages (Hofbauer cells) is a potent inhibitor of HIV-1 viral replication in these cells. Moreover, they act as reservoirs of the virus by blocking transmission to the fetus.

Baurakiades *et al.*⁽⁴⁾ reported that the genetic structure of the virus strain is crucial for vertical transmission, showing that there is genotypic selection and less heterogeneity in its genetic makeup. Tripathi *et al.*⁽²⁹⁾ studied chemokine co-receptors CCR51 and CXCR7, indicating that certain features of the viral antigen can determine fetal infection. The authors claimed, therefore, that the detection of the placental viral protein is not enough. The presence of these molecular viral features is required to predict neonatal infection.

Martin *et al.*⁽¹⁶⁾ applied immunohistochemistry and yielded positive results in only four out of nine placentas from HIV-infected children. Herein, immunohistochemistry did not provide similar correlation between the detection of viral protein in placental tissue and neonatal infection, since this factor was negative in 21 placentas and three from this total corresponded to children that had been infected. The probable causes of this negativity may account for the fact that the infection occurred in the perinatal period and antiretroviral medication had been applied.

Placentas from HIV-infected newborns showed viral protein near the vascular-fetal circulation. Accordingly, immunohistochemistry associated with histopathological study may aid the diagnosis of probable fetal infection. Nonetheless, the presence of the placental viral antigen is not a determinant predictive factor of fetal infection, as reported by Baurakiades *et al.*⁽⁴⁾, who deployed in situ PCR to detect the location of viral antigen.

Sturt *et al.*⁽²⁸⁾ showed that the neonatal infection rates dropped to 1% to 2% due to vertical transmission prevention, mainly when the diagnosis is established during prenatal care. In our investigation, in the group of 21 women who were not medicated with AZT, immunohistochemistry revealed the viral protein in more cases (14/21, 52.3%), though there was no significant difference between the two groups according to the applied statistical test. The effectiveness of AZT prophylaxis in newborns was not confirmed in our study (Table 2).

Our anatomopathological and immunohistochemical analysis of the p24 virus particle contributed to the identification of the most common placental alterations in HIV + pregnant women caused by transplacental viral infection, though it was not sufficient to determine fetal infection. The correlation with the aforementioned studies on the advances in molecular biology have enabled to verify that there is a higher sensitivity and specificity in molecular methods in terms of the host immune system, placental cells and the viral strain. Thus, complementary exams, namely molecular pathology methods such as PCR and viral gene sequencing of the viral genome, are required for a better understanding and diagnosis of fetal infection.

CONCLUSION

The most frequent placental lesions observed in our sample and in vertical transmission are arteriopathy in the fetal vascular circulation, chorioamnionitis, villous stromal hypercellularity, excessive syncytial knots, villous stromal edema and chorangiomas. These lesions are not specific to HIV or vertical transmission. The viral protein in the placenta was 67% lower in the cases under AZT treatment, but it was not possible to verify its effectiveness in the prevention of vertical transmission. The viral p24 antigen in placental tissue was not correlated with fetal infection. Our research revealed that it is possible to establish a definitive and early diagnosis of neonatal infection by histopathological examination of the placenta combined with immunohistochemistry for p24.

RESUMO

Introdução: A importância do estudo da placenta de gestantes com o vírus da imunodeficiência humana (HIV) soropositivas tornou-se alvo de inúmeros trabalhos na literatura. Aspectos morfológicos, virais, imunes e inflamatórios intrínsecos ao tecido placentário foram analisados para o entendimento da transmissão vertical do vírus. **Objetivo:** Identificar as lesões mais frequentes nas placentas, associando-as ao antígeno viral e correlacionando-as com a infecção dos recém-nascidos. **Material e métodos:** Trinta e cinco placentas de gestantes HIV soropositivas foram analisadas por estudo anatomopatológico e imuno-histoquímico, utilizando o anticorpo p24, no período de 1992 a 1997, segundo a rotina do laboratório do Serviço Anatomia Patológica/Hospital Universitário Antônio Pedro/Universidade Federal Fluminense (SAP/HUAP/UFF). **Resultados:** As alterações microscópicas registradas em todos os casos, inclusive nos de transmissão vertical, foram arteriopatia no circuito vascular fetal, corionamnionite, depósito fibrinoide perivilositário, excesso de nós sinciciais, edema do estroma viloso e dismaturidade vilosa. Nenhuma alteração microscópica ou macroscópica específica do HIV foi encontrada nas placentas. A infecção neonatal pôde ser constatada em cinco casos. A transmissão vertical foi identificada em duas placentas entre cinco que tinham baixo peso para a idade gestacional. Análise da imuno-histoquímica do p24 mostrou 14 casos positivos, dois dos quais apresentaram transmissão vertical. A proteína viral não foi identificada em 10 das 14 placentas cujas pacientes foram medicadas com zidovudina (AZT). **Conclusão:** Nosso estudo contribuiu para o estudo anatomopatológico da placenta de pacientes soropositivas para o HIV, porém a expressão do p24 por si só não permitiu um diagnóstico definitivo e precoce da transmissão vertical.

Unitermos: placenta; exame anatomopatológico; HIV; imuno-histoquímica; p24.

REFERENCES

- AL-HUSAINI, A. M. Role of placenta in the vertical transmission of human immunodeficiency virus. *J Perinatal*, v. 29, n. 5, p. 331-6, 2009.
- ANDERSON, V. M. The placental barrier to maternal HIV infection. *Obstet Gynecol Clin North Am*, v. 24, n. 4, p. 797-820, 1997.
- BACKÉ, E. *et al.* Vertical human immunodeficiency virus transmission: a study of placental pathology in relation to maternal risk factors. *Am J Perinatol*, v. 11, n. 5, p. 326-30, 1994.
- BAURAKIADES, E. *et al.* Histomorphometric and immunohistochemical analysis of infectious agents, T-cell subpopulations and inflammatory adhesion molecules in placentas from HIV-seropositive pregnant women. *Diagn Pathol*, v. 6, p.101-7, 2011.
- BENIRSCHKE, K.; KAUFMANN, K. B. P. *The pathology of the human placenta*. 5. ed. New York: Springer Verlag, 2006.
- BENTO, C. A. IL-10-secreting T cells from HIV-infected pregnant women downregulate HIV-1 replication: effect enhanced by antiretroviral treatment. *Aids*, v. 23, n. 1, p. 9-18, 2009.
- BHOOPAT, L. *et al.* Chorioamnionitis is associated with placental transmission of human immunodeficiency virus-1 subtype E in the early gestational period. *Mod Pathol*, v. 18, n. 10, p. 1357-64, 2005.
- BOILY-LAROCHE, G. *et al.* Naturally-occurring genetic variants in human DC-SIGN increase HIV-1 capture, cell-transfer and risk of mother-to-child transmission. *PLoS One*, v. 7, n. 7, p. 6-17, 2012.
- BURTON, G. J. *et al.* Significance of placental damage in vertical transmission of human immunodeficiency virus. *J Med Virol*, v. 50, n. 3, p. 237-43, 1996.
- CHANDWANI, S. *et al.* Pathology of the placenta in HIV-1 infection. *Prog AIDS Pathol*, v. 3, n. 1, p. 65-81, 1992.
- DERRIEN, M. *et al.* Impact of the placental cytokine-chemokine balance on regulation of cell-cell contact-induced human immunodeficiency virus type 1 translocation across a trophoblastic barrier in vitro. *J Virol*, v. 79, n. 19, p. 12304-10, 2005.
- FAYE, A. *et al.* Characterization of the main placental cytokine profiles from HIV-1-infected pregnant women treated with antiretroviral drugs in Milev, France. *Clin Exp Immunol*, v. 149, n. 3, p. 430-9, 2007.
- HSU, S. M.; RAINE, L.; FANGER, H. A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am J Clin Path*, v. 75, p. 734-6, 1981.
- KUMAR, S. B. *et al.* Different regions of HIV-1 subtype C env are associated with placental localization and in utero mother-to-child transmission. *J Virol*, v. 85, n. 14, p. 7142-52, 2011.
- LANDESMAN, S. H. *et al.* Obstetrical factors and the transmission of human immunodeficiency virus type 1 from mother to child. The Women and Infants Transmission Study. *N Engl J Med*, v. 334, n. 25, p. 1617-23, 1996.
- MARTIN, A. W. *et al.* Immunohistochemical localization of human immunodeficiency virus p24 antigen in placental tissue. *Hum Pathol*, v. 23, n. 4, p. 411-4, 1992.
- MCINTYRE, J. Use of antiretrovirals during pregnancy and breastfeeding in low-income and middle-income countries. *Curr Opin HIV AIDS*, v. 5, n. 1, p. 48-53, 2010.

18. MOUSSA, M. *et al.* Placental cytokine and chemokine production in HIV-1-infected women: trophoblast cells show a different pattern compared to cells from HIV-negative women. *Clin Exp Immunol*, v. 125, n. 3, p. 455-64, 2001.
19. Ministério da saúde. Secretaria de Vigilância em Saúde. Departamento de DST, Aids e Hepatites Virais. Recomendações para profilaxia da transmissão vertical do HIV e terapia antirretroviral em gestantes. Série manuais nº 46. Brasília, 2010. Disponível em: http://bvsm.s.saude.gov.br/bvs/publicacoes/recomendacoes_profilaxia_transmissao_vertical_hiv_5ed.pdf. Acesso em: 10 out. 2012.
20. MUÑOZ, L. D. *et al.* Progesterone inhibits HIV-1 replication in human trophoblast cells through inhibition of autocrine tumor necrosis factor secretion. *J Infect Dis*, v. 195, n. 9, p. 1294-1302, 2007.
21. MWANYUMBA, F. *et al.* Placental inflammation and perinatal transmission of HIV-1. *J Acquir Immune Defic Syndr*, v. 29, n. 3, p. 262-9, 2002.
22. PORNPRASERT, S. *et al.* Higher placental anti-inflammatory IL-10 cytokine expression in HIV-1 infected women receiving longer zidovudine prophylaxis associated with nevirapine. *Curr HIV Res*, v. 7, n. 2, p. 211-7, 2009.
23. REDLINE R. W. *et al.* Fetal vascular obstructive lesions: nosology and reproducibility of placental reaction patterns. *Pediatr Dev Pathol*, v. 7, n. 5, p. 443-52, 2004.
24. SCARLATTI, G. Mother-to-child transmission of HIV-1: advances and controversies of the twentieth centuries. *AIDS Rev*, v. 6, n. 2, p. 67-8, 2004.
25. SCHWARTZ, D. A. *et al.* Placental abnormalities associated with human immunodeficiency virus type 1 infection and perinatal transmission in Bangkok, Thailand. *J Infect Dis*, v. 182, n. 6, p. 1652-7, 2000.
26. SHEARER, W. T. *et al.* Role of placental cytokines and inflammation in vertical transmission of HIV infection. *Acta Paediatr*, Suppl. v. 421, p. 33-8, 1997.
27. SOILLEUX, E. J.; COLEMAN, N. Transplacental transmission of HIV: a potential role for HIV binding lectins. *Int J Biochem Cell Biol*, v. 35, n. 3, p. 283-7, 2003.
28. STURT, A. S.; DOKUBO, E. K.; SINT, T. T. Antiretroviral therapy (ART) for treating HIV infection in ART-eligible pregnant women. *Sao Paulo Med J*, v. 128, n. 4, p. 247-8, 2010.
29. TRIPATHI, V. *et al.* Differential expression of RDC1/CXCR7 in the human placenta. *J Clin Immunol*, v. 29, n. 3, p. 379-86, 2009.
30. VIDRICAIRE, G.; TARDIF, M. R.; TREMBLAY, M. J. The low viral production in trophoblastic cells is due to a high endocytic internalization of the human immunodeficiency virus type 1 and can be overcome by the pro-inflammatory cytokines tumor necrosis factor-alpha and interleukin-1. *J Biol Chem*, v. 278, n. 18, p. 15832-41, 2003.
31. VIDRICAIRE, G.; TREMBLAY, M. J. For a better understanding of the mechanisms involved in vertical transmission of HIV. *Med Sci*, v. 20, n. 8-9, p. 784-7, 2004.
32. ZORRILLA, C. D. Obstetric factors and mother-to-infant transmission of HIV-1. *Infect Dis Clin North Am*, v. 11, n. 1, p. 109-18, 1997.

MAILING ADDRESS

Consuelo Lozoya Lopez

Hospital Universitário Antonio Pedro; Rua Marques de Paraná, 303; Departamento de Patologia; 4º andar; Centro; CEP: 24033-900; Niterói-RJ, Brazil; Tel.: (21) 2629-9033/2629-9108; e-mail: consueloheizy@hotmial.com.