Type of HIV influence on iron metabolism in pregnant and non-pregnant women on antiretroviral therapy in Integrated Centre for Bioclinical Research of Abidjan (Côte d'Ivoire)

ABSTRACT

INTRODUCTION: HIV has a significant effect on iron metabolism in women of childbearing age in developing countries. The purpose of this study is to identify in this population layer, the type of HIV truly responsible for changes in iron metabolism.

MATERIALS AND METHODS : Thus, 395 HIV infected women of childbearing age were selected. This population included 120 non-pregnant women and 275 pregnant women in consultation at the Integrated Center for Bioclinical Research Abidjan (ICBRA). Blood samples were collected from each of the subjects for HIV status, hematological, immunological, and various biological indicators of iron status determination.

RESULTS : The results of the study indicated that women infected with HIV-1 and HIV-2 had a high mean values of hemoglobin, serum ferritin and iron stores. In addition, women infected with the two types of HIV had the lowest mean values of total transferrin binding capacity, transferrin saturation factors, and $CD4^+$ levels compared to the other groups of women. However, women infected with HiV-1 showed the highest prevalence of anemia (65.3%). This rate of anemia is higher in pregnant women (45.3%) compared to non-pregnant one (20%). Moreover, 77.5% of women infected with HIV had abnormal iron status with a high prevalence of inflammatory anemia (52.9%). No significant nutritional anemia was observed in the study population.

CONCLUSION : Pregnant women were the most affected by the degradation of iron metabolism. HIV-1 was the type of virus that caused the most impairment of iron status in the subjects of our study.

Keywords: Iron; HIV; Inflammatory anemia; Woman of childbearing age, Cote d'Ivoire.

1-INTRODUCTION

Iron is a trace element important for a good functioning of the body [1]. This micronutrient plays important roles in the formation of hemoglobin and myoglobin, in gas transport, oxidation-reduction reactions, cognitive performance, and resistance to infection [2,3]. The modification of this valuable element for human physiology has dramatic consequences in populations health. The most observed change in iron metabolism is its deficiency, which could lead to anemia [4,5]. There are several fundamental reasons for disrupting iron metabolism in the body. In addition to dietary origin, the confounding factors responsible for infectious and inflammatory syndromes are events that contribute to the degradation of iron in populations [6,7]. This situation has multiple causes among which the syndrome of acquired immunodeficiency (AIDS) caused by a virus HIV is noted. Today, the human immunodeficiency virus (HIV) is the scourge that disrupts all physiological systems in the body [8]. This virus is prevalent among people in developing countries. Despite the decline in HIV prevalence, Côte d'Ivoire has one of the highest HIV prevalence rates in West Africa. This prevalence is higher among women aged 15 to 49 years with 3.7% according to a report of the UN-AIDS-UNAIDS Côte d'Ivoire in 2017 [9]. HIV infection is thus a pathology as much more severe as it attacks the hematopoietic systems. Indeed, during the infection, haematological abnormalities are frequent; Thus, the first observations of pancytopenia

associated with AIDS are described as early as 1983 [10]. In addition, recent studies in Côte d'Ivoire have indicated a significant impact of HIV on iron metabolism in pregnant and non-pregnant women of childbearing age [11,12,13,14,15]. HIV has two serotypes, populations are infected with either HIV-1 or HIV-2 or the combination of the two serotypes [16,17]. What are the characteristic differences of these two types of virus in terms of specificity and sensitivity to iron metabolism? To answer this question, it seemed useful to undertake this study since, to our knowledge, no study has accurately revealed the type of HIV that would be responsible for the reported changes in iron metabolism worldwide. This study therefore aims, in general, to assess the effect of the type of HIV on possible alteration or degradation of iron metabolism in women of childbearing age. Thus, the work will firstly consist in reporting the changes in the biological parameters for evaluation of iron metabolism according to the types of HIV and secondly the physiological state of the study population. Then, the parameters most affected by this degradation will be found and finally find out how the components of iron status evolve according to the progression of HIV.

2-MATERIEL AND METHODS

2-1-Sites and studied population

Our study was undertaken from October 21st, 2009 to December 21st, 2012 in the Integrated Center for Bioclinical Research Abidjan (ICBRA) located in Treichville (Abidjan, Côte d'Ivoire). The general characteristics of the studied population are presented in Table I. The study covered 395 women including 275 pregnant and 120 non-pregnant women. The average age of the studied population is 30.47 ± 0.6; that is 35.9 ± 0.5 for non-pregnant women and 28.1 ± 0.7 for those who are pregnant with extremes of 18 to 45 years. After information and consent, women who did not have any complications of high blood pressure, diabetes, rheumatoid and arthritis were included. In contrast, recently transfused women and those who reported gynecological and digestive diseases were excluded. In the same way, women in

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menstruation were not selected. The types of antiretrovirals commonly used by these patients and prescribed after therapeutic trials are zidovudine, lamivudine and nevirapine. These women were from different areas of Abidjan and the suburbs. Pregnant women included more adolescents (10.2%) than non-pregnant women (1.7%). From the Body Mass Index (BMI), non-pregnant women underweight are 15% against 16% in pregnant women. Overweight women are 30% among non-pregnant women and 30.5% among pregnant women. The normal weight status is 55% and 53.5%, respectively for non-pregnant and pregnant women with an average BMI of 24.4 ± 0.5 and 24.3 ± 0.5. Regarding the number of pregnancies, 32.5% of non-pregnant women were primigest against 67.6% of multigestes. Non-pregnant women presented 32.5% nulliparous against 17.5% primiparous and 50% multiparous. The birth interval is higher than 36 months for 75% of women against 25% of women with an intergenesic space of less than 36 months.

Pregnant women were 30.9% primigest against 69.1% of multigeste. They presented 21.5% nulliparous, 32.4% primiparous and 46.2% multiparous. The birth interval is greater than 36 months for 61.5% compared to 38.5% of birth space less than 36 months (Table I).

2-2-Blood sampling and biological parameters assessment

From the elbow of the recruited fasted women, 5 ml of blood samples were collected and put in two different tubes. One containing anticoagulant (EDTA) for the CD4 lymphocyte assay performing using a flow cytometry with Fascalibur ®) and also for the blood count formula using an automaton (Sysmex Xt 2000i). The other one which are dry tubes, blood samples were centrifuged at 3000 rpm for 5 minutes in order to obtain serum. The serum was used for HIV serology and the biochemical status evaluations. Two successive HIV serology determination method were used. Once the first test (Determine) is positive, on discrimination tests (Gen. II HIV-1 / HIV-2) to determine the type of HIV.

Quantitative determination of biochemical parameters (iron, transferrin and ferritin) in human serum is based on a colorimetric technique available on COBAS INTEGRA 400 plus. COBAS INTEGRA Iron Test (IRON), Tina-quant Transferrin Ver.2 Test (TRSF2) TRSF2 Test, Test ID 0-567 and Ferritin Gen.2 Test (FERR2) FERR2, test ID 0-078, containing diagnostic reagents in vitro were used. Each dose of blood from the same sample is duplicated to reduce handling errors. And the average mean of the two is used for the study. The total transferrin binding capacity (CTF) and the transferrin saturation factor (CST) were calculated by the following formulas:

CTF (µmol/l) = transferrin (g/l) x 25 CST (%) = 100 x Iron/CTF [18,19,20].

Table I: General characteristics of the studied population

	Groups of women N = 395	
	Non-pregnant women N = 120	Pregnant women N = 275
Characteristics	n (%)	n (%)
Age (years)	35.9 ± 0.5	28.1 ± 0.7
18 - 20	2 (1.7)	28 (10.2)
21 - 45	118 (98.3)	247 (89.8)
Body mass index ($kg.m^{-2}$)	24.4 ± 0.5	24.3 ± 0.5
< 18.5	18 (15)	44 (16)
18.5 – 26	66 (55)	147 (53.5)
> 26	36 (30)	84 (30.5)
Gravidity	2.02 ± 0.2	2.8 ± 0.2
Primigravidae	39 (32.5)	85 (30.9)
Multigravidae	81 (67.5)	190 (69.1)
Parity	0.7 ± 0.04	1.6 ± 0.2
Nulliparous	39 (32.5)	59 (21.5)
Primiparous	21 (17.5)	89 (32.4)
Multiparous	60 (50)	127 (46.2)
Space between pregnancies (Months)	15.9 ± 1.6	28.8 ± 5.8
< 36	90 (75)	169 (61.5)
> 36	30 (25)	106 (38.5)
Matrimonial status		
Married	35 (29.2)	52 (18.9)
Single	48 (40)	85 (30.9)
Concubinage	37 (30.8)	117 (42.5)

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widowers	0(0)	21 (7.6)
Education attainment		
Uneducated	27 (22.5)	72 (26.2)
Primary school	24 (20)	67 (24.4)
Secondary school	40 (33.3)	94 (34.2)
Higher education	32 (26.7)	42 (15.3)

N: Number of each subject group; n: Number of observed subjects in each group

2-4-Statistical assessment and exploitation of biological parameters

For better appreciation of the parameters of biological analyzes, conventional criteria have been retained. They associated the recommendations of international organizations (WHO), the French Society of Clinical Biology (SFBC/France), the French Society of Hematology (SFH/France-group of cellular hematology), the Society of Nutrition and French Language Dietetics (France), the Center for Disease Control and Prevention (WHO/CDCP) and the Institute of Medicine (IOM/USA) [21,22,23]. The mean values of the biological parameters obtained were subjected to a Student's T-test for independent samples with the *Statsoft Statistica* software version Windows 7.1 [24]. to assess the influence of antiretroviral therapy on iron metabolism. The different observed proportions of the biological indicators of the iron status were compared using the likelihood G-test or test log likehood ratio with the Windows version R.2.0.1 software [25]. The threshold of significance has been defined for a p-value less than 0.05.

3-Results

3-1-States of hematological parameters between the different groups of women

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The hematological parameters revealed and susceptible to establish a comparison between groups of women are hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCHT) and mean hemoglobin concentration (MCHC). Results didn't show any significant difference. However, women infected with HIV-1 and 2 had a high value of hematocrit and MCHT. HIV-2 infected women had a high level of MCHC, with little difference from the other two groups of women. However, on the MCV level, the HIV1 and 2 infected women's group stood out with 106.1fL compared to HIV-1 women with around 90 fL and HIV-2 women around 85fL (Figure 1).

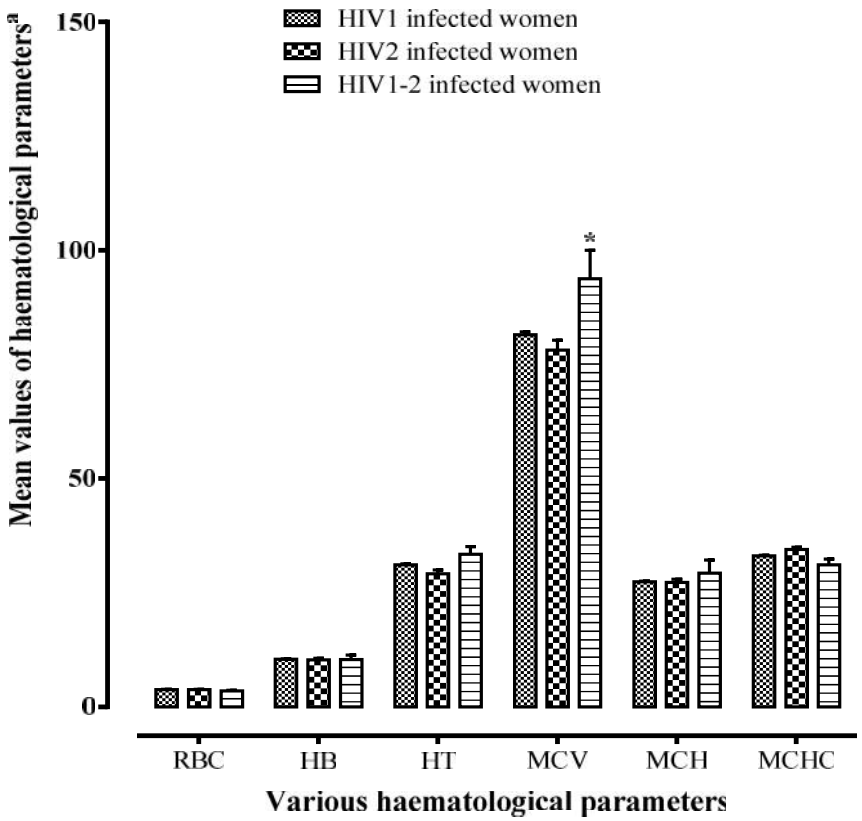

Figure 1: Changes of haematological parameters in women of reproductive age

a: Units of measure of the various haematological parameters; RBC: Red blood cells (10^9/l); HB: Hemoglobin (g/dl); HT: Hematocrit (%); MCV: Mean corpuscular volume (fl); MCH: Mean corpuscular hemoglobin (pg); MCHC: Mean corpuscular hemoglobin concentration (g/dl); *: Difference statistically significant for $p < 0.05$

3-2-Variations of biochemical and immunological parameters among different Groups of women

Significant differences ($p<0.01$) were observed between the three groups of women concerning parameters such as proportions of ferritin, iron stores, $CD4^+$ level, and total iron binding capacities below normal and saturation factors of transferrin with values between 15% and 35%. As for transferrin, results did not show any significant difference ($p>0.05$) between these three groups of women for (Figure 2).

Overall, seropositive women of childbearing age had the highest pathological proportions except normal values of transferrin saturation. CTF, CST and $CD4^+$ levels are higher in Women infected with HIV1 plasma than the other women's groups. In fact, the CTF of HIV1 infected women had a value close to 300 µmol/l while those infected with HIV2 and HIV1-2 were 200 µmol/l and less than 100 µmol/ respectively. Although the values of CST and CTF are not the same, we found similar variation of these parameters women's groups.

Thus, HIV1 women revealed a higher value, which averaged around 50% followed by HIV-infected women2 (about 30%) and HIV-2 women (less than 15%). At the $CD4^+$ level, HIV1 women averaged 400 Cells/mm^3, HIV2 women slightly more than 350 Cells/mm^3 and those with both types of virus had a CD4 count approaching 25 Cells/mm^3.

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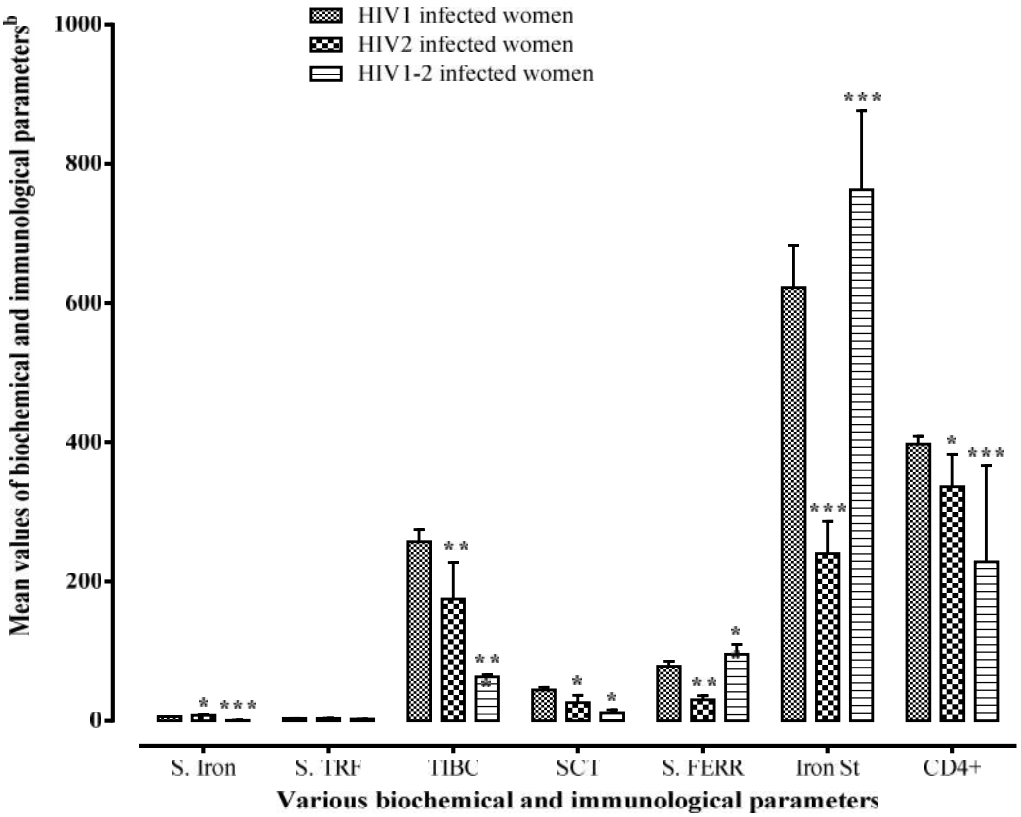

Figure 2: Variation of biochemical and immunological parameters in women of reproductive age

b: Units of measure of the various biochemical and immunological parameters; S. Iron: Serum iron (µmol/l); S.TRF: Serum transferrin (g/l); TIBC: Total iron binding capacity (µmol/l); SCT: Saturation coefficient of transferrin (%);S. Ferr: Serum ferritin (µg/l); Iron St (mg): Iron stores; CD4+ (Cells/mm^2); *: Difference statistically significant for $p < 0.05$; **: Difference statistically significant for $p < 0.01$; ***: Difference statistically significant for $p < 0.001$

3-3-Proportions of anemia and major hematological parameters

The proportions of anemia and hypochromia among the different groups of women as a whole showed no significant differences. However, infected pregnant women reported a high rate of hypochromic (50%) microcytic anemia compared with other groups of non-pregnant women (Table II). This made it possible to compare the various erythrocyte parameters between the groups of women according to the type of HIV. The recorded results indicated high rates of anemia (67.2% for HIV1, 75% for HIV2 and 100% for co-infection), hypochromia (65.1% for HIV1, 62, 5% for HIV2 and 66.7% for co-infection) among the three groups of women. Women with both types of virus reported the highest proportions except for microcytosis. In

addition, the combination of several haematological parameters (hemoglobin level, mean corpuscular volume and mean hemoglobin content) indicated in selected women the prevalence of normochromic necrotic anemia, hypochromic normocytic anemia and microcytic anemia. hypochromia and macrocytic hypochromic anemia (Table II).

In Table III, the types of anemia observed in the subjects in our study were hypochromic microcytic anemia (AMH), normochromic normocytic anemia (ANN), hypochromic normocytic anemia (ANH), anemia normochromic microcytic (AMN) and macrocytic hypochromic anemia. No group presents the five types of anemia; however pregnant women have the first four types of anemia and non-pregnant women also have four types but instead of hypochromic macrocytic anemia one has AMN. The prevalence of macrocytosis (53%), normochromic normocytic anemia (21.4%) and hypochromic normocytic anemia are higher in non-pregnant women compared with pregnant women. Hypochromic microcytic anemia was observed in all infected women, except for HIV-1-2 co-infection. In addition, no non-pregnant women developed normochromic microcytic anemia and no pregnant women developed hypochromic macrocytic anemia (Table III).

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Table II: Distribution of anaemia, microcytosis, macrocytosis and hypochromia in different groups of women according to type of HIV

	Groups of women according to type of VIH N = 395			p values
	HIV1 N = 384	HIV2 N = 8	HIV1-2 N = 3	
Haematological parameters	n (%)	n (%)	n (%)	
Hemoglobin (g/dl)				
Anaemia	258 (67.2)	06 (75)	3 (100)	$2.2\ 10^{-16}$ (S)
Normal	126 (32.8)	02 (25)	00 (0)	$2.2\ 10^{-15}$ (S)
Types of anaemia				
Crude	128 (33.3)	05 (62.5)	01 (33.3)	$2.9\ 10^{-13}$ (S)
Moderate	78 (20.3)	03 (37.5)	02 (66.7)	$2.5\ 10^{-7}$ (S)
Severe	52 (13.5)	00 (0)	00 (0)	$3.2\ 10^{-7}$ (S)
Hypochromic Microcytic Anaemia	184 (47.9)	04 (50)	00 (0)	$2.2\ 10^{-16}$ (S)
Hypochromic Normocytic Anaemia	28 (7.3)	01 (12.5)	01 (33.3)	0.006 (S)
Normochromic Normocytic Anaemia	45 (11.7)	01 (12.5)	02 (66.7)	0.0002 (S)
Normochromic Microcytic Anaemia	05 (01.3)	02 (25)	00 (0)	0.4 (NS)
Hypochromic macrocytic Anaemia	16 (04.2)	00 (0)	00 (0)	0.01 (S)
Hematocrit (%)				
Low	263 (68.5)	07 (87.5)	03 (100)	$2.2\ 10^{-16}$ (S)
Normal	221 (57.6)	01 (12.5)	00 (0)	$2.2\ 10^{-16}$ (S)
Mean corpuscular volume (fl)				
Microcytosis	133 (34.6)	06 (75)	00 (0)	$7.9\ 10^{-15}$ (S)
Normal	147 (38.3)	02 (25)	02 (66.7)	$9.9\ 10^{-16}$ (S)
Macrocytosis	104 (27.1)	00 (0)	01 (33.3)	$1.4\ 10^{-12}$ (S)
Mean corpuscular hemoglobin (pg)				
Hypochromia	250 (65.1)	05 (62.5)	02 (66.7)	$2.2\ 10^{-16}$ (S)
Normal	127 (33.1)	03 (37.5)	01 (33.3)	$8.2\ 10^{-14}$ (S)

N: Total number of each subject group; n: Number of observed subjects in each group; S: Difference statistically significant for $p<0.05$; NS: Difference not statistically significant for $p>0.05$.

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Table III: Proportions of main hematological parameters in groups of women in relation to physiological state and type of HIV

Hematological parameters	Groups of women depending on physiological state					
	Non-pregnant women, N = 120			Pregnant women, N = 275		
	HIV1, N = 117	HIV2, N = 0	HIV1-2, N 3	HIV1, N = 267	HIV2, N = 8	HIV1-2, N = 0
	n (%)$^{\alpha}$	n (%)	n (%)	n (%)$^{\alpha}$	n (%)	n (%)
Hemoglobin (g/dl)						
Anaemia	79 (67.5)^{a***}	ND	03 (100)	179 (67)	06 (75)$^{+++}$	ND
Normal	38 (32.5)***	ND	00 (0)	88 (33)	02 (25)$^{+++}$	ND
Types of anaemia						
Crude	55 (47)^{a}	ND	01 (33.3)	73 (27.3)	05 (62.5)$^{+++}$	ND
Moderate	18 (15.4)^{a**}	ND	02 (66.7)	60 (22.5)	03 (37.5)$^{+++}$	ND
Severe	06 (05.1)^{a***}	ND	00 (0)	46 (17.2)	00 (0)$^{+++}$	ND
Hypochromic Microcytic Anaemia	38 (32.5)^{a***}	ND	00 (0)	146 (54.7)	04 (50)$^{+++}$	ND
Hypochromic Normocytic Anaemia	20 (17.1)^{a*}	ND	01 (33.3)	08 (03)	01 (12.5)	ND
Normochromic Normocytic Anaemia	25 (21.4)^{a}	ND	02 (66.7)	20 (07.5)	01 (12.5)$^{++}$	ND
Normochromic Microcytic Anaemia	00 (0)	ND	00 (0)	05 (01.9)	02 (25)	ND
Hypochromic macrocytic anaemia	16 (13.7)^{a*}	ND	00 (0)	00 (0)	00 (0)	
Hematocrit (%)						
Low	106 (90.6)^{a**}	ND	03 (100)	157 (58.8)	07 (87.5)$^{+++}$	ND
Normal	11 (09.4)^{a***}	ND	00 (0)	110 (41.2)	01 (12.5)$^{+++}$	ND
Mean corpuscular volume (fl)						
Microcytosis	17 (14.5)^{a***}	ND	00 (0)	116 (43.4)	06 (75)$^{+++}$	ND
Normal	38 (32.5)***	ND	02 (66.7)	109 (40.8)	02 (25)$^{+++}$	ND
Macrocytosis	62 (53)^{a*}	ND	01 (33.3)	42 (15.7)	00 (0)$^{+++}$	ND
Mean corpuscular hemoglobin (pg)						
Hypochromia	73 (62.4)***	ND	02 (66.7)	177 (66.3)	05 (62.5)$^{+++}$	ND
Normal	37 (31.6)***	ND	01 (33.3)	90 (33.7)	03 (37.5)$^{+++}$	ND

N: Total number of each subject group; n: Number of observed subjects in each group; α: Proportions according to comparison in the same group (HIV1 non-pregnant women vs HIV1-2 non-pregnant women)and between various groups of infected women (HIV1 non-pregnant women vs HIV1 pregnant women); **: Difference statistically significant for $p<0.01$ in the same group of non-pregnant women; ***: Difference statistically significant for $p<0.001$ in the same group of non-pregnant women; ++: Difference statistically significant for $p<0.01$ in the same group of pregnant women; +++: Difference statistically significant for $p<0.001$ in the same group of pregnant women; a: Group significantly

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3-4-Distribution of proportions of biochemical parameters

At the level of the plasma compartment, highly significant differences ($p<0.01$) were observed between the different groups of women for proportions of serum iron, transferrin, and total iron binding capacities at below normal values. and transferrin saturation coefficients with values between 15% and 35%. No other significant difference ($p> 0.05$) was shown for iron stores (serum ferritin). Overall, females had high pathological proportions except at the normal values for the saturation coefficient. transferrin (Table IV). Taking into account the physiological status of women, we noted the following observations: in non-pregnant women HIV1, 83.1% of normal cases of serum iron, 78.3% of normal transferrin status, same for CTF. Normal values are also in the majority for iron stores (44.2%) and ferritin (58.9%). Only the saturation coefficient shows more subjects with lower than normal values (79.1%). HIV-1 pregnant women experienced a similar situation to non-HIV-1 pregnant women; indeed, all parameters are mostly normal except the saturation coefficient (79.6%) which is low (Table V).

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Table IV: Repartition of main biochemical parameters proportions in different groups of women depending on type of HIV

	Groups of women according to type of VIH N = 395			p values
	HIV1 N = 384	HIV2 N = 8	HIV1-2 N = 3	
Biochemical parameters	n (%)	n (%)	n (%)	
Serum iron (µmol/l)				
Low	53 (13.8)	03 (37.5)	03 (100)	0.0003 (S)
Normal	323 (84.1)	05 (62.5)	00 (0)	$2.2\ 10^{-16}$ (S)
High	08 (02.1)	00 (0)	00 (0)	0.1 (NS)
Serum transferrin (g/l)				
Low	29 (07.6)	01 (12.5)	00 (0)	0.0007 (S)
Normal	305 (79.4)	02 (25)	03 (100)	$2.2\ 10^{-16}$ (S)
High	46 (12)	05 (62.5)	00 (0)	$4.7\ 10^{-5}$ (S)
Total iron binding capacity (µmol/l)				
Low	29 (07.6)	01 (12.5)	00 (0)	0.0007 (S)
Normal	293 (76.3)	01 (12.5)	03 (100)	$2.2\ 10^{-16}$ (S)
High	58 (15.1)	05 (62.5)	00 (0)	$3.4\ 10^{-6}$ (S)
Saturation coefficient of transferrin (%)				
Low	314 (81.8)	05 (62.5)	02 (66.7)	$2.2\ 10^{-16}$ (S)
Normal	70 (18.2)	01 (12.5)	01 (33.3)	$8.8\ 10^{-8}$ (S)
High	00 (0)	02 (25)	00 (0)	0.5 (NS)
Serum ferritin (µg/l)				
Low	25 (06.5)	02 (25)	00 (0)	0.005 (S)
Normal	302 (78.6)	06 (75)	03 (100)	$2.2\ 10^{-16}$ (S)
High	57 (14.8)	0 (0)	00 (0)	$7.5\ 10^{-8}$ (S)
Iron stores (mg)				
Low	126 (32.8)	03 (37.5)	00 (0)	$1.6\ 10^{-14}$ (S)
Normal	213 (55.5)	05 (62.5)	02 (66.7)	$2.2\ 10^{-16}$ (S)
High	45 (11.7)	00 (0)	01 (33.3)	$2.6\ 10^{-5}$ (S)

N: Total number of each subject group; n: Number of observed subjects in each group; S: Difference statistically significant for $p<0.05$; NS: Difference not statistically significant for $p > 0.05$.

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Table V: Proportions of main biochemical parameters in groups of women in relation to their physiological state and type of HIV

Biochemical parameters	Groups of women depending on physiological state					
	Non-pregnant women, N = 120			Pregnant women, N = 275		
	HIV1, N = 117	HIV2, N = 0	HIV1-2, N = 3	HIV1, N = 267	HIV2, N = 8	HIV1-2, N = 0
	n (%)$^{\alpha}$	n (%)	n (%)	n (%)$^{\alpha}$	n (%)	n (%)
Serum iron (µmol/l)						
Low	15 (12.6/3.8)^{a**}	ND	3 (2.5)	36 (13.1/9.1)	3 (1.1)$^{++}$	ND
Normal	99 (83.3/25.1)^{a***}	ND	0 (0)	229 (83.3/58)	5 (1.8)$^{+++}$	ND
High	3 (2.5/0.8)^{a}	ND	0 (0)	2 (0.7/0.5)	0 (0)	ND
Serum transferrin (g/l)						
Low	19 (16/4.8)^{a}	ND	0 (0)	14 (5.1/3.5)	1 (0.4)$^{+}$	ND
Normal	93 (78.3/23.5)^{a***}	ND	3 (2.5)	212 (77.1/53.7)	2 (0.7)$^{+++}$	ND
High	5 (4.2/1.3)^{a**}	ND	0 (0)	41 (14.9/10.4)	5 (1.8)$^{++}$	ND
Total iron binding capacity (µmol/l)						
Low	19 (16/4.8)^{a***}	ND	0 (0)	14 (5.1/3.5)	1 (0.4)$^{+}$	ND
Normal	93 (78.3/23.5)^{a***}	ND	3 (2.5)	200 (72.7/50.6)	1 (0.4)$^{+++}$	ND
High	5 (4.2/1.3)^{a**}	ND	0 (0)	53 (19.3/13.4)	6 (2.2)$^{+++}$	ND
Saturation coefficient of transferrin (%)						
Low	94 (79.1/23.8)***	ND	2 (1.7)	219 (79.6/55.4)	5 (1.8)$^{+++}$	ND
Normal	23 (19.4/5.1)***	ND	1 (0.8)	47 (17/11.9)	1 (0.4)$^{+++}$	ND
High	0 (0/0)	ND	0 (0)	0 (0/0)	2 (0.7)	ND
Serum ferritin (µg/l)						
Low	15 (12.6/3.8)^{a***}	ND	0 (0)	10 (3.6/2.5)	2 (0.7)	ND
Normal	70 (58.9/17.7)^{a***}	ND	3 (2.5)	232 (84.4/58.7)	6 (2.2)$^{+++}$	ND
High	32 (26.9/8.1)^{a}	ND	0 (0)	25 (9.1/6.3)	0 (0)$^{++}$	ND
Iron stores (mg)						
Low	22 (18.3/5.6)^{a***}	ND	0 (0)	104 (37.8/26.3)	3 (1.1)$^{+++}$	ND
Normal	53 (44.2/13.4)***	ND	2 (1.7)	160 (58.2/40.5)	5 (1.8)$^{+++}$	ND
High	42 (35/10.6)^{a***}	ND	1 (0.8)	3 (1.1/0.8)	0 (0)	ND

N: Total number of each subject group; n: Number of observed subjects in each group; α: Proportions according to comparison in the same group (HIV1 non-pregnant women vs HIV1-2 non-pregnant women) and between various groups of infected women (HIV1 non-pregnant women vs HIV1 pregnant women); ; *: Difference statistically significant for $p < 0.05$ in the same group; **: Difference statistically significant for $p < 0.01$ in the same group; ***: Difference statistically significant for $p < 0.001$ in the same group; +: Difference statistically significant for $p < 0.01$ in the same group of pregnant women; ++: Difference statistically significant for $p < 0.01$ in the same group of pregnant women; +++: Difference statistically significant for $p < 0.001$ in the same group of pregnant women; a: Group significantly different for $p < 0.05$ in various groups with the same type of HIV; ND: Not determined

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4-Discussion

The results of this study revealed an impact of the types of HIV on biological indicators for the evaluation of body iron metabolism. During the HIV infection the producing of erythropoietin and red blood cells were affected. HIV can also directly affect the bone marrow and limit the production of red blood cells. Thus, an alteration of erythropoiesis accounts for anemia in most HIV-infected individuals. The effects of HIV on erythropoiesis due to HIV infection itself may be related to the release of inhibitors and/or abnormal production of trophic cytokines [26]. Among the types of HIV, HIV-1 has had a significant impact on women of childbearing age and pregnant women. This type is the most widespread in the world; it is also the type most frequently encountered in Côte d'Ivoire. The results of our study showed that 97.2% of women had HIV-1, 2.03% of type 2, and 0.76% of the two types. These results were similar to those of [27] who found a prevalence of 90.97% of people infected with HIV-1 followed by 2, 5.3% with HIV-2 and finally 3.7% of people infected with the two type of HIV. In this work, we observed an impact of HIV types on mean corpuscular of hemoglobin cell, CD4, and all mean values of the iron status evaluation endpoints apart from transferrin. This impact led to changes in the hematological parameters observed in the subjects of this study. This has also been reported by investigations in India undertook by Thankachan *et al.* [28] This survey revealed a significant reduction in hemoglobin in Indian with a deficiency of iron stores. This observation was made in women infected with HIV-1 and HIV-2. Our founding may be justified by the fact that the clinical latency period is significantly longer and the rate of progression to the AIDS stage was significantly lower in HIV-2 than in HIV-1. Viral load is also much lower in VIH type 2 than the one of type-1. Similarly, the configuration and composition may also promote the impact of HIV-1 on the degradation of iron metabolism.

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Indeed, several studies have shown that HIV mainly affects the prevalence of anemia in pregnant and infected women. In India, Nigeria and Côte d'Ivoire, investigations made by Sinha *et al.* [29], Obrikorang and Yeboah (2011) [30] and Kamagaté *et al.* [31] reported high rates of anemia (38.7%, 49.3% and 44.4% respectively). These rates were lower than those observed in our study, which were 67.2 for women infected with HIV-1, 75% for women infected with HIV-2 and 100% for women infected with the both types of HIV. In addition, these high prevalences of anemia were comparable to those obtained by Kamagaté *et al.* [11] and Bléyéré *et al.* [13] who observed respectively 67.5% of cases in HIV-positive non-pregnant women and 72% in HIV-positive pregnant women with antiretroviral treatment.

In this study, the exploitation of the different biological parameters of evaluation of iron status revealed that the influence of HIV types led to a high prevalence of abnormal iron status in both women's groups. This prevalence was significantly elevated, especially in non-pregnant women infected with HIV-1. In addition, this type of status included inflammatory anemia, which was more observed in these HIV-1 infected women.

These high prevalences of inflammatory anemia indicate that there was a deviation of the iron metabolic pathways in the women of our study by the secretion of cytokine mediators of inflammation. These have changed the exchanges at the level of different compartments of the body. Iron has been sequestered by HIV in seropositive women causing an apparent increase in the reserves to result in its alteration. In fact, inflammatory anemia occurs in situations of activation of the immune and inflammatory system. In these situations, mediators of inflammation such as interleukin IL1 and IL6, Tumor Necrosis Factor (TNFα), α1 antitrypsin, etc., can:

- inhibit the precursors of erythropoiesis, shorten the life of red blood cells and disrupt the synthesis and action of erythropoietin;

- alter the metabolism of iron released by haemolysis in the reticuloendothelial system. Reserves (appreciated by ferritin) are therefore normal or increased, but serum iron and iron available directly for erythropoietin are decreased [32]. This mechanism involves a recently identified protein, hepcidin, which prevents the export of iron from duodenal cells and the reticuloendothelial system [33]. Likewise, the synthesis of ferritin is directly increased by inflammation or remains normal and this, independently and beyond the level of iron reserve. Ferritin therefore no longer strictly reflects the iron stores of the body in this situation [34].

Other main reasons for the alteration of iron metabolism in addition to HIV would be:

- the physiological state of pregnancy resulting in increased plasma volume, erythrocyte mass and fetal growth;
- antiretroviral treatments, some of which have anemic effects and gastrointestinal manifestations [35,36,37,38,39].

In our study, the impact of HIV on iron status assessment parameters led to low prevalence rates of iron depletion. This is also reflected by reduced levels of microcytosis and lower-than-normal proportions of women with serum iron, serum transferrin, total binding capacity, and serum ferritin. This observation is even more plausible for women infected with HIV-1 (7.5% and 2.2% iron deficiency respectively in non-pregnant women and pregnant women with HIV-1). These low prevalence of iron deficiency (3.3% and 4%) were reported respectively by Kamagaté *et al.* [12] and Bléyéré *et al*. [14,15] in non-pregnant and HIV-positive pregnant women in Côte d'Ivoire.

5-Conclusion

Our work reveals that iron metabolism is impaired by HIV in women of childbearing age in Abidjan (Côte d'Ivoire). All hematological and biochemical parameters are collapsed in the study population. In addition, the degradation of iron metabolism highly depends on the type of HIV in women of childbearing age. In this context, HIV-1 indicates a significant impact on

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the biological markers of iron metabolism in the subjects of our investigation compared to HIV-2 and the HIV1-2 combination. In the same vein, 77.5% of women of childbearing age infected with HIV-1 have abnormal iron status. This abnormal status has an increased prevalence of inflammatory anemia. A low prevalence of nutritional anemia (iron deficiency anemia) is observed. Also, both groups of women of childbearing age are affected by the alteration of iron metabolism. However, pregnant women infected with HIV-1, are more vulnerable with 76% of abnormal iron status. Pregnancy, diet, syndromes and inflammatory drugs, antiretroviral therapy and some confounding factors may justify the vulnerability of pregnant women in our study. Since some of these women of childbearing age were on ARVs and others without antiretroviral therapy, we plan to conduct a comparative study of the impact of HIV type on non-ARV women. pregnant treatment and those without treatment and secondly between pregnant women under treatment and those without treatment.

ETHICS

Experimental procedures and protocols used in this study were approved by ethical committee of Health Sciences and the University of Nangui Abrogoua (Abidjan/Côte d'Ivoire). These guide line were in accordance with the internationally accepted principles for laboratory use and care. Then, this study was approved by the Ministry of Higher Education and Scientific Research, the Ministry of Education and the Ministry of Health and Public Hygiene in the Republic of Côte d'Ivoire.

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