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Interaction of HIV and pregnancy on iron metabolism in Côte d'Ivoire

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Abstract: Background: HIV affects several women of reproductive age in developing countries. In addition, this virus and pregnancy have a real impact on iron metabolism in infected subjects. Objective: An association between HIV and pregnancy could be a factor affecting iron metabolism in women of childbearing age. These investigations are therefore intended to identify and characterize a possible interaction of HIV and pregnancy on iron status in population of Abidjan (Côte d'Ivoire). **Methods:** This work was a prospective, descriptive, analytical, cross-sectional and case-control study. It covered 270 women of reproductive age in all trimesters of pregnancy and in consultation to integrated centre for bioclinical research of Abidjan (ICBRA). These subjects were divided into two groups: 130 pregnant women and 140 apparently healthy pregnant women infected with HIV and on antiretroviral therapy. All biological indicators of metabolism were determined through blood samples from each of pregnant women. The Student t test, factor analysis of variance (ANOVA) with two factors and the G test or log Likelihood ratio test with the software programs Statistica Statsoft Windows version 7.1 and R.2.0.1 Windows version were used for the statistical analyzes. **Results:** Analysis of data showed that pregnant women infected with HIV reported a significant deterioration of their iron status (92 %) against 79.2 % in pregnant control women. Infected women presented overload iron stores during the three trimesters of pregnancy (20 %. 13.3 % and 10 % respectively. Conversely, control women indicated at the end of pregnancy a significant deficit in iron (82 %). in this sense. infected women revealed strong inflammatory anaemia during the three trimesters of pregnancy compared to controls. These controls on the contrary indicated a significant prevalence of iron deficiency anaemia in the last two trimesters and inflammatory anaemia with iron deficiency during the first two trimesters of pregnancy. **Conclusion:** HIV and pregnancy degrade iron metabolism in women of childbearing age. This could be explained by the diet of women and more specifically by antiretroviral therapy.

Key words: Iron metabolism; Interaction HIV-Pregnancy; Iron deficiency anaemia; Inflammatory anaemia; Côte d'Ivoire.

INTRODUCTION

Iron is a micronutrient that contributes to the smooth process of blood cell synthesis. Its metabolism in the body is required to perform many functions biological chemical and carried out by all blood cells. Its quantity and quality are more important in the body to conduct at best its metabolism (Cook, 1990). For some populations of developing countries including infants, children, adolescents and women of childbearing age, iron metabolism is slowing (Dallman, 1986; Allen. 2000). During pregnancy, iron requirements are greatly increased and are estimated at 1290 mg to resolve difficulties of increased plasma volume, red cell mass, synthesis of new red blood cells, formation of the placenta, fetal growth and blood loss during delivery (Beaton, 2000; Dreyfuss *et al*., 2001). Numerous studies have shown that metabolism of iron in women of childbearing age is largely degraded during pregnancy for several reasons. The main reason for alteration of iron metabolism is attributed to the physiology of pregnancy itself through hemodilution (Yapo *et al*., 2008). Results of investigations revealed that hemodilution is not a risk factor for degradation of the iron status of pregnant women that early in pregnancy when the iron stores are considerably reduced. Consequently, food is related to the quality and quantity of iron in the body.

Other factors are also known today namely infectious and inflammatory syndromes such as malaria, infections bacterial and parasitic gastrointestinal (Achidi *et al*., 2005; Cottrell *et al*., 2007). Among these factors,

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there is the human immunodeficiency virus (HIV) which decimated the populations of developing countries (Antelman *et al*., 2000). This virus modifies metabolic pathways of iron during pregnancy (Dallman, 1987). A recent study in Côte d'Ivoire by our team (submitted for publication) showed that the evaluation parameters of iron metabolism is severely degraded in women infected with HIV during the three trimesters of pregnancy. We would like in this present study to determine the real impact of HIV on biological indicators of iron status assessment during pregnancy. In other words, the association between HIV and physiological state of pregnancy do vary changes of iron metabolism in women of childbearing age in Côte d'Ivoire? Is a possible interaction can cause an overload of the iron reserves among women since they are already supplemented in this micronutriment? Could this overload cause in its turn a proliferation of the VIH within the women organism, and that lead to an attack of the physical integrity of the women?

In this context, a case-control study was conducted with pregnant women infected with HIV and on antiretroviral therapy in pregnant women and healthy pregnant women of Abidjan. It aims to identify and characterize a possible interaction of HIV and pregnancy in women of childbearing age in Côte d'Ivoire. This investigation will indicate as shown in the previous study. a possible modification of biological parameters of iron metabolism during the three trimesters of pregnancy in control women to make a comparison with other studies in Côte d'Ivoire (Bléyéré *et al*., 2007). In addition, another comparison of different biological parameters of iron metabolism obtained between the two groups of pregnant women is performed. Moreover, a comparison of various components prevalences of iron status is reported.

MATERIALS AND METHODS

Study population:

The investigations were cross-sectional and descriptive study which took place from 21 October 2009 to 21 December 2012 in the Integrated Centre for Bioclinical Research of Abidjan (ICBRA). The study involved 270 women aged from 18 to 45 years during pregnancy.

This sample of pregnant women consists of 130 control subjects and 140 HIV-infected women receiving antiretroviral therapy. These pregnant women came for consultation in Integrated Centre for Bioclinical Research of Abidjan (ICBRA) for prenatal examinations and biological monitoring of their HIV infection for some. Control pregnant women were composed of 40 subjects in the first trimester, 40 in the second trimester and 50 subjects in the last trimester (Figure1). Women infected with HIV and on antiretroviral therapy from at least one year, are composed of 40 subjects in the first trimester, 45 subjects in the second trimester and 55 women in the last trimester. In addition, this group of women included 99 % of HIV-1 against 1 % HIV-2. Tritherapy most widely prescribed to pregnant women is characterized by Zidovudine (AZT), Lamivudine (3TC) and Nevirapine (NVP). This set of women from different municipalities and suburbs of Abidjan (Côte d'Ivoire) was selected after informed consent of each woman on the objectives of the investigation. The 270 selected pregnant women have not presented major complications of hypertension, diabetes, rheumatoid arthritis. In contrast, those with recently reported major health concerns that is transfused, indicating digestive and gynecological diseases were excluded.

The mean age of enrolled women in the study was 25.2 ± 0.4 years and 30.8 ± 0.9 years respectively in control subjects and HIV-infected women. Control subjects included more adolescents (8.5 %) compared to women receiving antiretroviral therapy (2 %). These had generally a body mass index (BMI) of 24.6 ± 0.6 $kg.m^{-2}$ 54 % of women had normal weight status against 46 % for abnormal weight status with underweight (14 %) and 32 % overweight. In controls, mean BMI was 23.5 ± 0.3 $kg.m^{-2}$ 67.7 % indicated a normal weight status against 32.3 % for abnormal weight status with underweight (10 %) and 22.3 % overweight (Table 1). The gravidity and parity had mean values of 1.76 ± 0.16 and 1.8 ± 0.2 in women infected and 2.7 ± 0.1 and 1.1 ± 0.1 for control subjects. Several study subjects (100 and 84 respectively) had indicated a pregnancy for their obstetric histories. In this same context, respectively 52.3 % and 94 % of women selected had at least one childbirth. In terms of space between births, 63.8 % of control women against 56 % of infected women have observed less than 3 years between pregnancies. Study population also included married, Widows, single and subjects living in concubinage (Table 1).

Blood Sampling And Assays Of Biological Parameters:

For each of the recruited women, a blood sample collected in dry tubes and tubes with 5 ml anticoagulant each were performed fasting bend of the elbow in the morning. Whole blood collected in tubes with anticoagulant (EDTA) achieved the CD4 counts by flow cytometry with Fascalibur® and the blood count by the Sysmex PLC Xt 2000i. The collected blood in dry tubes was centrifuged at 3000 tours for 5 minutes to obtain the serum. The obtained serum allowed to determine HIV status and biochemical data assessment of iron status. For HIV serology, the method most used in the care centers, is the use of two successive tests. Once the first test (Determine) is positive, we proceed to discrimination test (Genie II HIV-1/HIV-2) to determine the type of HIV. The quantitative determination of biochemical parameters (serum iron. serum transferrin and serum ferritin) in

human serum is based on a colorimetric technique available on most automated COBAS INTEGRA 400. For this determination, the COBAS INTEGRA kits Iron (IRON), Tina-quant Transferrin ver.2 (TRSF2) TRSF2 test and Ferritin Gen.2 ID 0-567 test (FERR2), ID 0-567 test containing *in vitro* diagnostic reagents were used. The total iron binding capacity (TIBC) and the saturation coefficient of transferrin (SCT) were obtained by calculations.

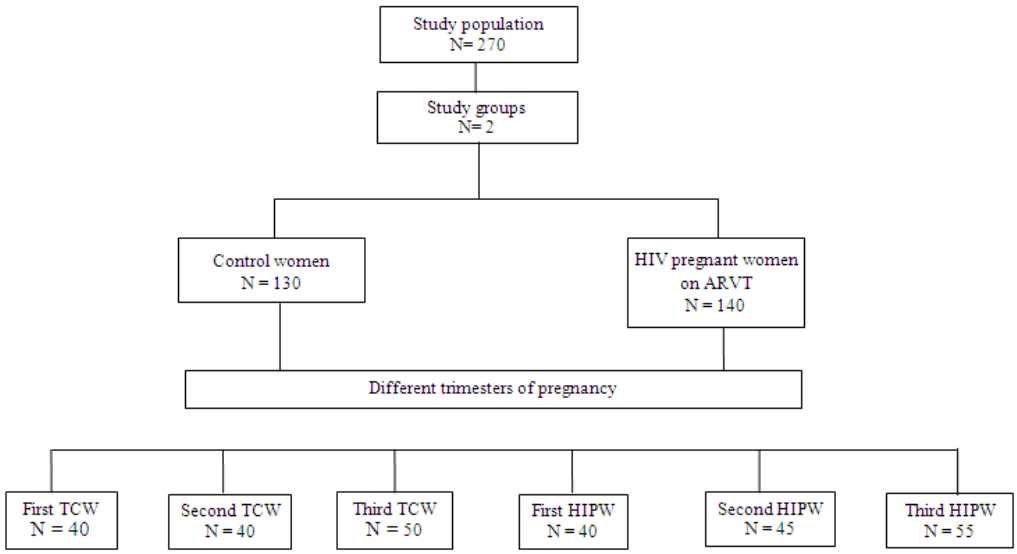

Fig. 1: Sampling of study population according to selected sites and different trimesters of pregnancy.
N: Total number of each subject group; TCW: Trimester control women; HIPW: HIV pregnant women

Assessment And Statistical Analysis Of Biological Parameters:

To better appreciate the parameters of biological assays, conventional criteria were selected. They associated the recommendations of international organizations (WHO), French Society of Clinical Biology (SFBC/France), French Society of Hematology (SFH/France-Group of Cellular Haematology), Society of Nutrition and Diet of the French Language (France), Centre for Disease Control and Prevention (WHO/CDCP) and Institute of Medicine (Vernet *et al*., 2001; IOM/USA, 1990; UNICEF/UNU/WHO, 2001; SNDLF, 2001).

The Student t test for independent samples was used to estimate possible changes of biological indicators of iron status in two groups of pregnant women. The obtained values of the biological parameters of iron status were subjected to a factorial analysis of variance (ANOVA) with two factors (the three trimesters of pregnancy and HIV) in order to evaluate one hand, their evolution during different trimesters in control pregnant women. And secondly, these tests aimed to reveal an eventual influence of HIV on biological indicators of iron metabolism in two groups of selected pregnant women. These statistics treatments with Statistica Statsoft program Windows version 7.1 were associated NEWMAN-KEULS as multivariate test post hoc to specify the probable groups of women significantly different (Statsoft, 2005). Different observed proportions of biological indicators of iron status were compared by the G test or log Likelihood ratio test with the software R.2.0.1 Windows version (Ihaka and Gentleman, 1996). The level of significance was defined for a p value < 0.05.

Ethics:

Experimental procedures and protocols used in this study were approved by ethical committee of Health Sciences in Nangui Abrogoua University. 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 and the Ministry of Health and Public Hygiene in the Republic of Côte d'Ivoire.

Results:

Evolution Of Biological Parameters Of Iron Status According Different Group Of Women During Pregnancy

The study results in Table 2 showed that all blood counts parameters were abnormal compared to reference values in both groups of women. In the same way, mean corpuscular volume (MCV) in control women (76 ± 0.8 fl) and mean corpuscular hemoglobin concentration (MCHC) in infected women (31.4 ± 0.2 g/dl) presented abnormal values against standards. Conversely, other red cell indices indicated normal values compared to references in all pregnant women (Table 2). Determined values of biochemical indicators in all were normal

compared to standards with the exception of saturation coefficient of transferrin (10.5 ± 0.5 % and 10.9 ± 0.7 % respectively).

Comparison between the two groups of women showed statistically significant differences for hematocrit ($p = 0.02$), mean corpuscular volume ($p = 0.04$), mean corpuscular hemoglobin concentration ($p = 0.04$), serum iron ($p = 0.04$) and serum ferritin ($p = 0.0001$). However, other biological parameters of iron status showed no significant difference ($p > 0.05$) between the two groups of pregnant women. Iron stores in pregnant infected women are more increased than 4 times higher than control women. The same observation was made for hematocrit, MCV, MCHC and serum iron (Table 2).

The general and significant ($p < 0.05$) evolution of blood cells count parameters (red blood cell count. hemoglobin and hematocrit) of the pregnant control women during pregnancy is decreased. In the same way, mean corpuscular volume initially increased in the second trimester of pregnancy before fall significantly ($p < 0.05$) in the last trimester. In addition, biochemical indicators in general, have significantly ($p < 0.05$) decreased during pregnancy in controls of study (Table 3).

Analyzes revealed a significant interaction ($p < 0.05$) between HIV and pregnancy in three haematological parameters (hemoglobin, hematocrit and MCV) and in all biochemical indicators of iron metabolism to exception of serum transferrin. Thus, the hemoglobin was low in the first trimester of pregnancy in infected women compared with control women. However, at the beginning and at the end of pregnancy, infected pregnant women reported values of hematocrit and MCV significantly ($p < 0.05$) higher compared to control women (Table 3). In the same way, MCHC showed a mean value significantly ($p < 0.05$) decreased in infected women to first trimester of pregnancy compared with control women without any significant interaction ($p > 0.05$).

For biochemical parameters, a significant interaction ($p < 0.05$) between HIV and pregnancy has been observed in the last trimester of pregnancy on serum iron, total iron binding capacity and saturation coefficient of transferrin. Thus, infected women indicated values significantly ($p < 0.01$) greater than control women except total iron binding capacity (Table 3). Another interaction between HIV and the three trimesters of pregnancy was revealed regarding serum ferritin throughout pregnancy. Therefore, the iron stores of infected pregnant women were significantly ($p < 0.001$) higher compared to control women in all trimesters of pregnancy (Table 3).

Proportions' Distribution Of Iron Status Main Parameters In Different Pregnant Women Group:

During pregnancy, high prevalences of anaemia were observed in control women with 86 % last trimester. A significant hemodilution has been reported in all these subjects throughout pregnancy with high proportions of hematocrit abnormal values (< 33 % or < 32 %). The prevalences of microcytosis and hypochromia were higher in control subjects during the three trimesters of pregnancy (Table 4). For the three observed types of anaemia (HMA, NNA and HNA), according to erythrocyte indices, normochromic normocytic anaemia (NNA) only significantly increased in the second trimester before declining in the last trimester of pregnancy. However, hypochromic microcytic anaemia (HMA) has observed in control pregnant women with high prevalence compared to other types of anaemia (Table 4).

The results of the study showed that anaemia was influenced by HIV and pregnancy in women of reproductive age. This impact was significant ($p < 0.01$) in early pregnancy in infected women with 90 %. In contrast, hemodilution has been more impacted by HIV and pregnancy in control pregnant women in the first and last trimesters of pregnancy (85 % and 94 % respectively) compared to infected women. The same observation was made for the microcytosis (85 % and 86 % respectively). However, the prevalence of macrocytosis was only impacted significantly ($p < 0.001$) in infected pregnant women (56 %) compared with control women.

Regarding hypochromia, infected pregnant women revealed a significant lower prevalence (40 %) in early pregnancy ($p < 0.05$). This prevalence has twice increased in the second trimester (80 %) before falling towards the end of pregnancy (68 %) compared to control women. The interaction between HIV and pregnancy has impacted different types of anaemia according to erythrocyte indices. In this case, to the first trimester of pregnancy, hypochromic microcytic anaemia (HMA) was observed in all control women (100 %). whereas it was reported 25 % in infected women. In this same period, three 75 % of infected women were indicated normochromic normocytic anaemia (NNA) against no control. In the second trimester, hypochromic microcytic anaemia (HMA) was more prevalent among control women (90 %) against 60 % of infected women. In contrast, to this trimester infected women reported more hypochromic normocytic anaemia (20 %) versus controls (0 %). In the last trimester of pregnancy the interaction between HIV and pregnancy strongly influenced ($p < 0.001$) different observed types of anaemia in our study. At this stage of pregnancy, infected women showed high rates of normocytic normochromic anaemia and hypochromic normocytic anaemia (20 % and 25 % respectively) compared to control women (0.3 % and 0 % respectively). However, infected pregnant women reported a low rate of hypochromic microcytic anaemia (25 %) versus controls (Table 4).

The proportion of pregnant control women with low serum iron increased significantly during the three trimesters of pregnancy (10 %, 37.5 % and 86 %). In contrast, no control women revealed high concentrations of serum iron. Control women with low values of serum transferrin, total iron binding capacity (TIBC) and saturation coefficient of transferrin decreased significantly at different stages of pregnancy. However, number of these women has increased to high values of serum transferrin and TIBC. It was opposite for saturation coefficient of transferrin for which no control woman revealed strong values above 35 %. In the same way, no control woman also indicated an overload of iron stores. However, a deficit of iron stores has been reported during pregnancy in control women (Table 5).

Table 1: General characteristics of the study population

Anthropometric and sociodemographic parameters	Control women N = 130	HIV pregnant women/ARVT N = 140
	n (%)	n (%)
Age (years)	25.2 ± 0.4	30.8±0.9
18 – 19	11 (8.5)	3 (2)
20 – 45	119 (91.5)	137 (98)
BMC ($kg.m^{-2}$)	23.5 ± 0.3	24.6 ± 0.6
< 19.8	13 (10)	20 (14)
19.8 – 26	88 (67.7)	76 (54)
> 26	29 (22.3)	44 (32)
Gravidity	2.7 ± 0.1	1.8± 0.2
Primigravidae	30 (23.1)	56 (40)
Multigravidae	100 (76.9)	84 (60)
Parity	1.1 ± 0.1	1.8 ± 0.2
Nulliparous	62 (46.7)	8 (6)
Primiparous	32 (24.6)	56 (40)
Multiparous	36 (27.7)	76 (54)
Space between births (Months)	27.6 ± 3	30.8 ± 8.9
< 36	83 (63.8)	78 (56)
> 36	47 (36.2)	62 (44)
Matrimonial status		
Married	11 (8.5)	39 (28)
Widowed	12 (9.2)	8 (6)
Single	46 (35.4)	37 (26)
Concubinage	61 (46.9)	56 (40)
Education attainment		
Uneducated	35 (26.9)	25 (18)
Primary school	36 (27.7)	31 (22)
Secondary school	59 (45.4)	42 (30)
Superior	0 (0)	42 (30)

N: Total number of each subject group; n: Number of observed subjects in each group;
ARVT: Antiretroviral therapy

Table 2: Mean values of iron status biological parameters

Biological parameters of iron status	Control women N = 130	HIV Pregnant women ARVT N = 140	p-values	Reference values[α]
Red blood cell counts				
Red blood cells (10^{12}/l)	3.8 ± 0.4	3.7 ± 0.1	0.3 (NS)	4-5.4
Hemoglobin (g/dl)	10.3 ± 0.1	10 ± 0.2	0.3 (NS)	10.5-14
Hematocrit (%)	28.8 ± 0.4	31.9 ± 0.6	0.02 (S)	32-45
Erythrocyte indices				
MCV (fl)	76 ± 0.8	87 ± 1.7	0.04 (S)	80-100
MCH (pg)	27 ± 0.2	27.5 ± 0.6	0.3 (NS)	27-31
MCHC (g/dl)	34.4 ± 0.1	31.4 ± 0.2	0.04 (S)	32-36
Plasma compartment				
Serum iron (µmol/l)	7.1 ± 0.2	13.1 ± 0.7	0.04 (S)	6.6-26
Serum transferrin (g/l)	3.3 ± 0.1	3 ± 0.1	0.1 (NS)	2-3.6
Total iron binding capacity (µmol/l)	82.6 ± 2.7	74.8 ± 3.5	0.1 (NS)	50-90
Saturation coefficient (%)	10.5 ± 0.5	10.9 ± 0.7	0.6 (NS)	15-35
Iron stores compartment				
Serum ferritin (µg/l)	21 ± 0.8	99.4 ± 15.3	0.0001 (S)	15-150

N: Total number of each subject group; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular concentration of hemoglobin concentration; ARVT: Antiretroviral therapy; α: Reference values of the assessment biological parameters of iron status in women during pregnancy (Vernet *et al*.. 2001; IOM/US. 1990; UNICEF/UNU/WHO. 2001; SNDLF. 2001; WHO/CDCP. 2004); S: Difference statistically significative for $p < 0.05$; NS: Difference not statistically significative for $p < 0.05$

Table 3: Changes of iron status parameters according to different women groups during pregnancy

Iron status biological parameters	Different trimesters of pregnancy					
	First		Second		Third	
	Control women N = 40 n (%)	HIV Pregnant women ARVT N = 40 n (%)	Control women N = 40 n (%)	HIV Pregnant women ARVT N = 45 n (%)	Control women N = 50 n (%)	HIV Pregnant women ARVT N = 55 n (%)
Red blood cell counts						
Red blood cells (10^{12}/l)	4 ± 0.1ab	3.8 ± 0.2	3.8 ± 0.05ac	3.8 ± 0.1	3.6± 0.04cd	3.6 ± 0.1
Hemoglobin (g/dl)	10.9 ± 0.2a	10.3 ± 0.5*	10.4 ± 0.2b	10.2 ± 0.4	9.7 ± 0.2c	10 ± 0.2
Hematocrit (%)	30.2 ± 0.5a	33.3 ± 1.7*	31 ± 0.7a	32.2 ± 1.1	26.1 ± 0.5c	31.7 ± 0.7*
Erythrocyte indices						
MCV (fl)	74.5 ± 0.8b	87.6 ± 1.4*	82.3 ± 1.4a	85.2±3.1	72.3 ± 1.4b	89.1 ± 2.4*
MCH (pg)	26.6 ± 0.3	27.2 ± 0.4	27.8 ± 0.5	26.9±1.3	26.6 ± 0.4	28.5 ± 0.8
MCHC (g/dl)	35.2 ± 0.1	31 ± 0.4*	33.8 ± 0.3	31.5± 0.5	34.3 ± 0.3	31.7 ± 0.2
Plasma compartment						
Serum iron (µmol/l)	8.8 ± 0.4a	11.1 ± 1.4	7.8 ± 0.3a	11.8±1.4	5.2 ± 0.2b	14.5 ± 1.3**
Serum transferrin (g/l)	2.7 ± 0.2b	2.3 ± 0.1	2.8 ± 0.2b	3 ± 0.3	4.2 ± 0.1a	3.1 ± 0.2
Total iron binding capacity (µmol/l)	68.2 ± 4.1b	57.9 ± 3	69.8 ± 4.1b	74.6 ± 6.6	104.5 ± 3.5a	78.2 ± 4.6**
Saturation coefficient (%)	14.2 ± 0.8a	10.4 ± 1	12.6 ± 0.7a	9.8 ± 1.2	5.9 ± 0.6b	11.8 ± 1.2**
Iron stores compartment						
Serum ferritin (µg/l)	28.3 ± 1.5a	122.1±47.8***	22.9 ± 1.3a	92.8 ± 29.4***	13.6 ± 0.4b	90.8±17.4***

N: Total number of each women group; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular concentration of hemoglobin concentration; ARVT: Antiretroviral therapy; *: Difference statistically significative for $p < 0.05$; a. b. c and d: Women groups statistically different for $p < 0.05$; **: Difference statistically significative for $p < 0.01$; ***: Difference statistically significative for $p < 0.001$

Table 4: Compared proportions of erythrocyte parameters depending on different trimesters of pregnancy

Erythrocyte parameters	Different trimesters of pregnancy					
	First		Second		Third	
	Control women N = 40 n (%)	HIV Pregnant women ARVT N = 40 n (%)	Control women N = 40 n (%)	HIV Pregnant women ARVT N = 45 n (%)	Control women N = 50 n (%)	HIV Pregnant women ARVT N = 55 n (%)
Red blood cell counts						
Hemoglobin (g/dl)						
< 11 ou < 10.5	22 (55)b	36 (90)**	20 (50)b	18 (40)	43 (86)a	46 (84)
> 11 ou > 10.5	18 (45)a	4 (10)**	20 (50)a	27 (60)	7 (14)b	9 (16)
Hematocrit (%)						
< 33 ou < 32	34 (85)a	16 (40)**	26 (65)b	24 (53.3)	47 (94)a	33 (60)**
> 33 ou > 32	6 (15)b	24 (60)**	14 (35)a	21 (46.7)	3 (6)b	22 (40)***
Erythrocyte indices						
MCV (fl)						
< 80	34 (85)a	8 (20)***	18 (45)b	15 (33.3)	43 (86)a	11 (20)***
80-100	6 (15)b	32 (80)***	21 (52.5)a	27 (60)	7 (14)b	13 (24)
> 100	0 (0)	0 (0)	1 (2.5)	3 (6.7)	0 (0)	31 (56)***
MCH (pg)						
< 27 ou > 31	25 (62.5)b	16 (40)*	23 (57.5)b	36 (80)*	47 (94)a	37 (68)*
27-31	15 (37.5)b	24 (60)*	17 (42.5)b	9 (20)*	3 (6)a	18 (32)**
Types of anaemia						
HMA	22 (100)	9 (25)***	18 (90)	10 (60)*	42 (97.7)	11 (25)***
NNA	0 (0)b	27 (75)**	2 (10)a	4 (20)	1 (0.3)a	10 (20)***
HNA	0 (0)	0 (0)	0 (0)	4 (20)**	0 (0)	25 (55)***

N: Total number of each women group; n: Subjects' number observed in each group; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular concentration of hemoglobin concentration; ARVT: Antiretroviral therapy; HMA: Hypochromic Microcytic Anaemia; NNA: Normochromic Normocytic Anaemia; HNA: Hypochromic Normocytic Anaemia; *: Difference statistically significative for $p < 0.05$; a and b: Women groups statistically different for $p < 0.05$; **: Difference statistically significative for $p < 0.01$; ***: Difference statistically significative for $p < 0.001$

The association between HIV and pregnancy had a significant effect ($p < 0.001$) on the proportion of women with high and low rates of iron at the end of pregnancy. Infected women indicated a low prevalence (4 % against 86 %) of decreased serum iron compared with control women.

For other biochemical parameters, the proportions of women during pregnancy showed a significant influence ($p < 0.05$) of the association between HIV and pregnancy. At low values of serum transferrin and TIBC and high levels, infected women showed low proportions compared to control women (Table 5).

However, infected women reported high proportions for low values of saturation coefficients of transferrin (SCT) below 15 % during the first two trimesters of pregnancy compared with control women. No woman in the study reported values of SCT above 35 % except for 4 % of infected pregnant women in the third trimester.

During pregnancy no infected women revealed any significant depletion of iron stores than control women who showed a 82 % in this regard at the end of pregnancy ($p < 0.05$). In the same way, no control woman indicated overload of iron stores contrary to infected women who reported throughout pregnancy significant proportions in this context (Table 5).

Table 5: Distribution of proportions of biochemical iron status parameters depending on different trimesters of pregnancy

Biochemical iron status parameters	Different trimesters of pregnancy					
	First		Second		Third	
	Control women N = 40 n (%)	HIV Pregnant women ARVT N = 40 n (%)	Control women N = 40 n (%)	HIV Pregnant women ARVT N = 45 n (%)	Control women N = 50 n (%)	HIV Pregnant women ARVT N = 55 n (%)
Serum iron (µmol/l)						
< 6.6	4 (10)c	4 (10)	15 (37.5)a	9 (20)	43 (86)b	2 (4)***
6.6-26	36 (90)a	36 (90)	25 (62.5)b	36 (80)*	7 (14)c	51 (92)***
> 26	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)*
Serum transferrin (g/l)						
< 2	15 (37.5)b	4 (10)*	9 (22.5)a	3 (6.7)**	1 (2)c	2 (4)
2-3.6	15 (37.5)c	36 (90)**	20 (50)a	42 (93.3)**	8 (16)b	47 (76)***
> 3.6	10 (25)b	0 (0)**	11 (27.5)b	0 (0)***	41 (82)a	6 (10)***
TIBC (µmol/l)						
< 50	15 (37.5)a	4 (10)*	9 (22.5)b	3 (6.7)**	1 (2)c	2 (4)
50-90	15 (37.5)c	36 (90)**	20 (50)a	36 (80)**	8 (16)b	40 (72)***
> 90	10 (25)b	0 (0)**	11 (27.5)b	6 (13.3)*	41 (82)a	13 (24)***
SCT (%)						
< 15	23 (57.5)b	40 (100)***	21 (52.5)b	39 (86.7)**	43 (86)a	40 (72)
15-35	17 (42.5)a	0 (0)***	19 (47.5)a	6 (13.3)**	7 (14)b	13 (24)
> 35	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)*
Serum ferrintin (µg/l)						
< 15	0 (0)b	0 (0)	7 (17.5)c	12 (26.7)	41 (82)a	0 (0)***
15-150	40 (100)a	32 (80)*	33 (82.5)b	27 (60)	9 (18)c	44 (80)***
> 150	0 (0)	8 (20)**	0 (0)	6 (13.3)*	0 (0)	11 (10)**

N: Total number of each women group; n: Subjects' number observed in each group; TIBC: Total iron binding capacity; SCT: Saturation coefficient of transferrin; ARVT: Antiretroviral therapy; *: Difference statistically significative for $p < 0.05$; a. b and c: Women groups statistically different for $p < 0.05$; **: Difference statistically significative for $p < 0.01$; ***: Difference statistically significative for $p < 0.001$

Evolution Of Different Iron Status Components During Pregnancy:

Observed components of iron metabolism in all women of study were iron deficiency. iron deficiency anaemia, inflammatory anaemia and inflammatory anaemia with iron deficiency (Table 6a). However, inflammatory anaemia with iron deficiency has not been reported in infected pregnant women. Very few infected pregnant women indicated a normal iron status (8 %) compared to control women (20.8 %). No significant difference ($p > 0.05$) between the two groups of women for the prevalence of iron deficiency was observed. Iron deficiency anaemia was significantly ($p = 1.3.\ 10^{-8}$) more common in women compared to control infected women. In contrast, 82 % of infected women reported inflammatory anaemia against 11.5 % in control women. In addition, infected pregnant women have presented inflammatory anaemia with iron deficiency unlike control women who indicated 16.2 % of this type of anaemia (Table 6a).

In Table 6b, control women revealed prevalence of iron deficiency anaemia significantly ($p < 0.05$) increased during pregnancy. In contrast, they reported rates significantly decreased during the three trimesters of pregnancy in inflammatory anaemia and inflammatory anaemia with iron deficiency.

Multiple analyzes showed that the association between the two factors namely HIV and pregnancy interfered significantly ($p < 0.05$) with components of iron metabolism. Thus, the abnormal iron status in infected women was highly ($p < 0.01$) observed during the first two trimesters of pregnancy (100 % and 93.3 % respectively) compared to control women (77.5 % and 62. 5 %). Infected women showed high rates of

inflammatory anaemia during the three trimesters of pregnancy (90 %. 80 % and 80 % respectively) relative to control women (20 %. 17.5 % and 0 % respectively). Furthermore, no infected woman revealed inflammatory anaemia with iron deficiency during pregnancy. In contrast, the control women showed high prevalence of iron deficiency anaemia (15 %, 22.5 % and 84 % respectively) versus infected women (10 %, 0 % and 8 % respectively).

Table 6: Repartition of iron status components in study pregnant women
Table 6a: Distribution of iron status components according to two pregnant women group

Iron status components	Pregnant women group		p-values
	Control women N = 130	HIV pregnant women/ARVT N = 140	
	n (%)	n (%)	
Normal iron status	27 (20.8)	10 (8)	0.01 (S)
Abnormal iron status	103 (79.2)	130 (92)	0.01 (S)
Iron deficienc	10 (7.7)	6 (4)	0.3 (NS)
Iron deficiency anaemia	57 (43.8)	8 (6)	$1.3. 10^{-8}$ (S)
Inflammatory anaemia	15 (11.5)	116 (82)	10^{-14} (S)
Inflammatory anaemia + Iron deficiency	21 (16.2)	0 (0)	$2.2 10.^{-6}$ (S)

Table 6b: Changes of iron status components during pregnancy in women group

Iron status components of pregnant women	Different trimesters of pregnancy					
	First		Second		Third	
	Control women N = 40	HIV Pregnant women ARVT N = 40	Control women N = 40	HIV Pregnant women ARVT N = 45	Control women N = 50	HIV Pregnant women ARVT N = 55
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Normal iron status	9 (22.5)a	0 (0)**	15 (37.5)a	3 (6.7)**	3 (6)b	7 (12)
Abnormal iron status	31 (77.5)b	40 (100)**	25 (62.5)b	42 (93.3)**	47 (94)a	48 (88)
Iron deficienc	3 (7.5)	0 (0)*	3 (7.5)	6 (13.3)	4 (8)	0 (0)**
Iron deficiency anaemia	6 (15)b	4 (10)	9 (22.5)b	0 (0)**	42 (84)a	4 (8)***
Inflammatory anaemia	8 (20)a	36 (90)***	7 (17.5)a	36 (80)**	0 (0)b	44 (80)***
Inflammatory anaemia + Iron deficiency	14 (35)a	0 (0)***	6 (15)b	0 (0)**	1 (2)c	0 (0)

N: Total number of each women group; n: Subjects' number observed in each group; a. b and c: Women groups statistically different for $p < 0.05$; **: Difference statistically significative for $p < 0.01$; ***: Difference statistically significative for $p < 0.001$

Discussion:

The results of the study that we conducted revealed an interaction of HIV and pregnancy on overall biological indicators of iron metabolism assessment. The combination of these factors had a significant impact in women of reproductive age during pregnancy. But the women most affected during pregnancy are those infected with HIV. Control pregnant women indicated a significant decrease of four haematological parameters. Biochemical indicators of iron status in these women were all degraded during pregnancy. These findings are similar to the work among pregnant women in Abidjan (Bléyéré *et al*., 2007). But in contrast to this study, investigations reported a significant deterioration in all biological parameters of iron status assessment. In the present study, we observed upstream, an interaction of HIV and pregnancy on hemoglobin and MCHC in the first trimester, hematocrit and MCV in the first and third trimesters of pregnancy. Serum iron, TIBC and the SCT have been modified in the last trimester of pregnancy. The association between HIV and pregnancy altered serum ferritin during the three trimesters of pregnancy.

This impact has led to high rates of prevalence of overload's iron stores in infected pregnant women in all trimesters of pregnancy and iron deficiency in control pregnant women in the last trimester. The observed change in haematological parameters in study subjects was also reported by investigations carried out in India. This work in Indians revealed a significant reduction in hemoglobin when they indicate an insufficiency of iron stores (Thankachan *et al*., 2010).

Downstream in our study, no pregnant woman infected with HIV has presented a sufficiently appropriate and adapted in early pregnancy compared to control women to conduct the proper functioning of iron metabolic pathways. The consequence downstream, leads to upstream high prevalence of abnormal iron status among all subjects in the first two trimesters of pregnancy. This type of status involved the inflammatory anaemia observed in most infected women and iron deficiency anaemia in control women was significant more. Another component of abnormal iron status is iron deficiency which is not influenced by the association between HIV and pregnancy in study subjects. In contrast, inflammatory anaemia associated iron deficiency is affected by the interaction to the first two trimesters of pregnancy in control women compared infected women. The results of

this study in infected pregnant women are contrary to those reported in Ethiopia in subjects infected with HIV and tuberculosis. Conversely, they are similar to those reported in control women (Kassu *et al*., 2006).

The main reasons for the interaction of HIV and pregnancy on biological indicators of iron metabolism evaluation are diet, physiological state of pregnancy, HIV and even antiretroviral treatment (Sullivan *et al*., 1998; Bléyéré *et al*., 2007; Obirikorang *et al*., 2009, Johannessen *et al*., 2011; Oladeinde *et al*., 2011). In developing countries, the diet of populations is strong base of plants. The food of these populations is therefore very low in animal protein (Dillon, 2000; Ogotona *et al*., 2003). Iron from plant is abundant in the populations of these countries. And yet, this type of iron is in non heminic form so is very little bioavailable. It therefore does not participate in strengthening of iron stores in women of reproductive age especially in women during pregnancy (Backstrand *et al*., 2002). In this way, other food in the diet of pregnant women would inhibit the intestinal absorption of iron (Mennen *et al*., 2007).

This also contributes to the depletion of iron stores in pregnant women. A recent study in Tanzania in pregnant women infected with HIV has shown that these women are identified as subjects with a high risk of poor nutritional status of micronutrients (Mehta *et al*., 2010). Worse nowadays, the food in developing countries is influenced by modern food from industrialized countries. These foods unsuitable to us and consumed by the high social class still create nutritional imbalances among populations in developing countries (Sodjinou *et al*., 2007). This is the story of the nutritional burden.

Several studies have shown that HIV has an impact on the prevalence of anaemia among pregnant women and infected. India and Nigeria investigations have reported high rates of anaemia (38.7 % and 49.3 % respectively), but lower than those observed in our study (Sinha *et al*., 2007; Oladeinde *et al*., 2011). Besides iron, HIV affects other micronutrients such as copper and zinc. A study in Kenya showed a significant deficit in zinc in subjects infected with HIV. And this, because of the inflammation caused by the virus (Mburu *et al*., 2010). Indeed, the inflammatory process caused by HIV leads to a series of reactions with cytokines that entails the sequestration of iron by macrophages. This whole action of HIV among women of reproductive age leads to a particular type of anaemia, inflammatory anaemia (Semba *et al*., 2002; Handelman and Levin, 2008). This justifies the rates of hypochromic microcytic anaemia and normochromic normocytic anaemia in infected pregnant women of study. In addition, antiretroviral therapy is associated with numerous complications including anaemia. It was reported in Côte d'Ivoire (Moh *et al*., 2005; Drain *et al*., 2007; Islam *et al*., 2012).

Conclusion:

Biological parameters of iron status assessment in women of reproductive age in Abidjan are altered by the association of HIV and pregnancy. This interaction leads to abnormal iron status more important in infected pregnant women. These subjects have shown high prevalence of inflammatory anaemia in different trimesters of pregnancy compared with control women. These women had a high prevalence of iron deficiency anaemia in the last two quarters and inflammatory anaemia associated with iron deficiency in the first two trimesters of pregnancy. The main reasons for this altered of iron metabolism would be the diet of women, their physiological state of pregnancy, HIV and even antiretroviral therapy. To more understand and interpret this association between HIV and pregnancy on the metabolism of iron. It will be necessary to decrypt the phenomenon of inflammation, the role of hepcidin and diet in pregnant women infected with HIV. In addition, another factor must be understood in pregnant women infected with HIV. It concerns their antiretroviral therapy on iron metabolism. Moreover, an evaluation study of other micronutrients such as zinc, copper and selenium should be performed in infected pregnant women by HIV. All these future studies will allow us to provide micronutrient supplementation in pregnant women infected with HIV in Côte d'Ivoire.

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Abbreviations:

TIBC: Total Iron Binding Capacity; SCT: Saturation Coefficient of Transferrin; NIS: Normal Iron Status; ID: Iron Deficiency; IDA: Iron Deficiency Anaemia; IA: Inflammatory Anaemia; IA+ID: Inflammatory Anaemia associated with Iron Deficiency; S: Statistically different for p value < 0.05; NS: Not statistically significant for p value < 0.05; N: Total number of each subject group; SEM: Standard error of mean;

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Declaration Of Interest:

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

REFERENCES

Achidi, E.A., A.J. Kuoh, J.T. Minang, B. Ngum, B.M. Achimbom, S.C. Motaze, M.J. Ahmadou and M. Troye-Blomberg, 2005. Malaria infection in pregnancy and its effects on haemoglobin levels in women from a malaria endemic area of Fako Division, South West Province, Cameroon. Journal of Obstetric and Gynaecology, 25(3): 235-40.

Allen, L.H., 2000. Anemia and iron deficiency: effects on pregnancy outcome. American Journal of Clinical Nutrition, 17(5): 1280s-1284s.

Antelman, G., G.I. Msamanga and D. Spiegelman, 2000. Nutritional factors and infectious disease contribute to anemia among pregnant women with human immunodeficiency virus in Tanzania. Journal of Nutrition, 130: 1950-7.

Backstrand, J.R., L.H. Allen, A.K. Black, M.D. Mata and G.H. Pelto, 2002. Diet and iron status of nonpregnant women in rural Central Mexico. American Journal of Clinical Nutrition, 76: 156-64.

Beaton, H.G., 2000. Iron needs during pregnancy: do we need to rethink our target? American Journal of Clinical Nutrition, 72: 265S-71S.

Bleyere, N.M., D. Ekaza- Joulia, A.P. Yapo, B.B. N'guessan., C.A.M. Neil, M.O. Vanga, M. Koné and E.E. Ehilé, 2007. Hétérogénéité du statut en fer chez la femme au cours de la grossesse en Cote d'Ivoire. Annales de Biologie Clinique, 65(5): 525-532.

Dallman, P.R., 1986. Iron deficiency in the Weanling: a nutritionnal problem on the way to resolution. Acta Paediatrica Scandinavica, 323: 59-67.

Dallman, P.R., 1987. Iron deficiency and the immune response. American Journal of Clinical Nutrition, 46: 329-314.

Cook, M., 1990. Adaptation in iron metabolism. American Journal of Clinical Nutrition, 51: 301-25.

Cottrell, G., J.Y. Mary, D. Barro and M. Cot, 2007. The Importance of the Period Of Malarial Infection During Pregnancy On Birth Weight In Tropical Africa. American Journal of Tropical Medicine and Hygiene, 76(5): 849-854.

Dillon, J.C., 2000. Prévention de la carence en fer et des anémies ferriprives en milieu tropical. Medecine Tropicale, 60: 83-91.

Dreyfuss, M.L., G.I. Msamanaga, D. Splegelman, D.J. Hunter, E.J. Urassa, K.E. Hertzark and W.W. Fawzi, 2001. Determinant of low birth weight among HIV infected pregnant women in Tanzania. American Journal of Clinical Nutrition, 74(6): 814-826.

Drain, P.K., R. Kupka, F. Mugusi and W.W. Fawzi, 2007. Micronutrients in HIV-positive persons receiving highly active antiretroviral therapy. American Journal of Clinical Nutrition, 85: 333-45.

Handelman, G.J. and N.W. Levin, 2008. Iron and anemia in human biology: a review of mechanisms. Heart Failure Reviews, 13(4): 393-404.

Ihaka, R., and R. Gentleman, 1996. R: a language for data analysis and graphics. Journal of Computation Graphic and Statistic, 5: 299-314.

Institute of Medecine (IOM/USA), 1990. Comittte on nutritional status during pregnancy and lactation. Nutrition during pregnancy: weight gain and nutrient supplements. Washington DC. National Academy Press.

Islam, F.M., J. Wu and J.D.P. Jansson, 2012. Wilson. Relative risk of renal disease among people living with HIV: a systematic review and meta-analysis. BMC Public Health, 12:234 doi:10.1186/1471-2458-12-234.

Johannessen, A., E. Naman, S.G. Gundersen and J.N. Bruun, 2011. Antiretroviral treatment reverses HIV-associated anemia in rural Tanzania. BMC Infectious Diseases, 11:190http://www.biomedcentral.com/1471-2334/11/190.

Kassu, A., T. Yabutani, Z.H. Mahmud, A. Mohammad, N. Nguyen, B.T. Huong, G. Hailemariam, E. Diro, B. Ayele, Y. Wondmikun, J. Motonaka and F. Ota, 2006. Alterations in serum levels of trace elements in tuberculosis and HIV infections. European Journal of Clinical Nutrition, 60(5): 580-6.

Mburu, A.S.W., D.I. Thurnham, D.L. Mwaniki, E.M. Muniu and F.M. Alumasa, 2010. The influence of inflammation on plasma zinc concentration in apparently healthy, HIV+ Kenyan adults and zinc responses after a multi-micronutrient supplement. European Journal of Clinical Nutrition, 64: 510-517 doi:10.1038/ejcn.2010.33.

Mehta, S., D. Spiegelman, S. Aboud, E.L. Giovannucci, G.I. Msamanga, E. Hertzmark, F.M. Mugusi, D.J. Hunter and W.W. Fawzi, 2010. Lipid-soluble vitamins A, D, and E in HIV-infected pregnant women in Tanzania. European Journal of Clinical Nutrition 64: 808-817 (August 2010) doi:10.1038/ejcn.2010.76

Mennen, L., T. Hirvonen, N. Arnault, S. Bertrais, P. Galan and S. Hercberg, 2007. Consumption of black, green and herbal tea and iron status in French adults. European Journal of Clinical Nutrition., 61: 1174-1179.

Moh, R., C. Danel, S. Sorho, D. Sauvageot, A. Anzian, A. Minga, O.B. Gomis, C. Konga, A. Inwoley and D. Gabillard, 2005. Haematological changes in adults receiving a zidovudine-containing HAART regimen in combination with cotrimoxazole in Cote d'Ivoire. Antiviral Therapy, 10: 615-624.

Obirikorang, C., F.A. Yeboah, 2009. Blood haemoglobin measurement as a predictive indicator for the progression of HIV/AIDS in resource-limited setting. Journal of Biomedical Science, 16: 102.

Oguntona, C.R.B. and I.O. Akinyele, 2002. Food and nutrient intakes by pregnant Nigerian adolescents during the third trimester. Nutrition, 18: 673-679.

Oladeinde, B.H., R.M. Phil, M. Olley and J.A. Anunibe, 2011. Prevalence of HIV and anemia among pregnant women. North American Journal of Medical Science, 3: 548-51.

Semba, R.D., N. Shah, S.A. Strathdee and D. Vlahov, 2002. High prevalence of iron deficiency and anemia among female injection drug users with and without HIV infection. Journal of Acquired Immune Deficiency Syndromes, 29: 142-144.

Sinha, G., T.J. Choi, U. Nayak, A. Gupta, S. Nair, N. Gupte, P.M. Bulakh, J. Sastry, S.D. Deshmukh, M.M. Khandekar, V. Kulkarni, R.A. Bhosale, K.E. Bharucha, M.A. Phadke, A.S. Kshirsagar and R.C. Bollinger, 2007. Clinically significant anemia in HIV-infected pregnant women in India is not a major barrier to zidovudine use for prevention of maternal-to-child transmission. Journal of Acquired Immune Deficiency Syndromes, 1; 45(2): 210-7.

Société de Nutrition et de Diététique de Langue Française, 2001. Anémies nutritionnelles (SNDLF). Cah Nutr Diét 36 (Hors série): 76-81.

Sodjinou, R., V. Agueh, B. Fayomi and H. Delisle, 2007. Dietary patterns of urban adults in Benin: relationship with overall diet quality and socio-demographic characteristics. European Journal of Clinical Nutrition, 1–7 doi:10.1038/sj.ejcn.1602906.

Statsoft, 2005. Statistica (Data Analysis Software System) Version 7.1. Available at www. Statsoft.com. Accessed 11/04/2006

Sullivan, P.S., D.L. Hanson, S.Y. Chu, J.L. Jones and J.W. Ward, 1998. Epidemiology of anemia in human immunodeficiency virus (HIV)-infected persons: results from the multistate adult and adolescent spectrum of HIV disease surveillance project. Blood, 91: 301-308.

Thankachan, P., S. Kalasuramath, A.L. Hill, T. Thomas, K. Bhat and A.V. Kurpad, 2011. A mathematical model for the hemoglobin response to iron intake, based on iron absorption measurements from habitually consumed Indian meals. European Journal of Clinical Nutrition, (5 October 2011) | doi:10.1038/ejcn.2011.169

UNICEF/UNU/WHO, 2001. Iron deficiency anemia: assessment, prevention, and control. WHO/NHD/01.3 Geneva, Switzerland: WHO.

Vernet, M., J. Corberand and V. David, 2001. Algorithmes de prescription recommandés pour le diagnostic d'un déficit et d'une surcharge en fer. Annale de Biologie Clinique (Paris), 59: 149-55.

Yapo, P.A., N.M. Bleyere, D. Joulia-Ekaza, D.J. Yao, B.B. N'guessan and E.E. Ehile, 2008. Prévalence de carences martiales et d'anémies chez des femmes en âge de procréer, non enceintes et des femmes enceintes. Annale de Biologie Clinique Québec, 45(3): 24-28.