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## Iron Status and Red Cell Parameters in Pregnant and Non-pregnant Adolescents in Côte d'Ivoire (West Africa)

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### Authors' contributions

Authors MNB, ASNB, MK, DS, PAY contributed equally in the study. They made substantial contributions to the design of the study, the collection of the data as well as the preparation and analysis of the data. They also drafted the manuscript and gave final approval for its submission to the journal for consideration of publication.

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### ABSTRACT

**Aims:** The objective of this study was to evaluate iron metabolism and compare iron stores between pregnant and non-pregnant adolescents in Côte d'Ivoire.

**Place and Duration of Study:** The study was undertaken with 187 volunteers adolescents aged from 15 to 19 years. For this study, adolescents were divided into 2 groups with 75 non-pregnant adolescents and 112 pregnant adolescents. Study population was recruited January 2006 to January 2008 in 4 urban community health centers, of Abidjan. Assays of blood samples were performed in Laboratory of Physiology, Pharmacology and Phytotherapy (Nangui Abrogoua University) and in Laboratory of Medical Biochemistry of University Hospital Centre (Cocody, Félix Houphouët-Boigny University).

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**Methodology:** In each pregnant adolescent a blood sample was collected by venipuncture on a dry and EDTA tubes of 5 ml. With these blood samples, haematological and biochemical parameters were determined.

**Results:** Haematological parameters were decreased in pregnant adolescents at the third trimester of pregnancy compared with control adolescents. Pregnant adolescents were more anaemic during pregnancy (77.7 %) compared with non-pregnant adolescents (42.7 %). Iron stores were greatly decreased in 72.3 %, 83.9 % and 95.6 % of adolescents respectively during the 3 trimesters of pregnancy compared with non-pregnant adolescents (34.7 %). In addition, high prevalence of iron deficiency anaemia was recorded throughout pregnancy in adolescents. Therefore, iron status was more altered in pregnant adolescents (11.6 %, 9.8 % and 0.0 % respectively) compared with non-pregnant adolescents (13.3 %). The results of this study show that the causes of pronounced degradation in iron status are insufficient in size of iron stores in pregnant adolescents and non-pregnant adolescents.

**Conclusion:** Iron metabolism alteration is important in pregnant adolescents in Côte d'Ivoire, causing severe anaemia in this group of population.

*Keywords: Pregnant adolescents, Non-pregnant adolescents, Iron stores, Iron deficiency Anaemia, Côte d'Ivoire.*

## 1. INTRODUCTION

Iron is a key component of hemoglobin and myoglobin which transport gases in organism. This micronutrient is also an essential element of many enzymes that carry out oxidation-reduction reactions necessary to generate energy and produce various metabolic for host defense [1, 2, 3]. Its deficiency is a widespread problem, affecting an estimated two billion people worldwide [4, 5]. Iron deficiency is the most common and prevalent nutritional disorder in the world in both developing and developed countries [6]. It occurs when iron stores, mostly found in the liver, start to become depleted. Thereafter, iron deficiency anaemia arises when the production of red blood cells starts to diminish once the iron stores have been depleted [7]. According to World Health Organization (WHO), the iron deficiency anaemia in pregnancy is a significant problem throughout the world with a prevalence ranging from an average of 14 % of pregnant women in developed countries to 56 % in developing countries [8, 9]. Therefore, anaemia is a public health concern resulting nutritional disorder in the world, affecting mainly women of childbearing age and children under five years of age [10]. It causes developmental delay and cognitive impairment in children and infants, reduced work capacity in adults [11, 12]. Among women, anaemia is primarily prevalent during reproductive age and adversely impacts pregnancy outcomes [13, 14, 15]. In addition, anaemia is more observed in adolescents during pregnancy compared with non-pregnant adolescents in some countries [16, 17, 18].

In Côte d'Ivoire, several studies on iron metabolism were devoted to non-pregnant women, pregnant women, non-pregnant adolescents, adolescents during pregnancy, infants and children [19, 20, 21, 22, 23, 24, 25]. Furthermore, adolescence is a physiological period which recommends a need of iron for organism's growth. Moreover, pregnancy in adolescents is a physiological state that requires a high demand of iron [26, 27].

However, no investigation was concerned with comparison of iron metabolism between adolescents according to their physiological state in Côte d'Ivoire. In view of this, the aim of this study was to determine the group of adolescents in Côte d'Ivoire which is exposed to nutritional anaemia by comparing changes of iron metabolism. In this same way, non-

pregnant and pregnant subjects aged 15 to 19 years from Abidjan were selected to evaluate and characterize the iron stores through their haematological and iron metabolism parameters.

## 2. MATERIALS AND METHODS

### 2.1 Study Population

The investigation was a longitudinal, analytic and descriptive study in pregnant adolescents. In addition, study design was a cross sectional analytic study for non-pregnant adolescents. Subjects in this study aged 15 to 19 years, were recruited January 2006 to January 2008 in 4 urban community health centers, of Abidjan (Côte d'Ivoire). These are urban and health centers of Abobo south, south Cocody, urban community health centers "Les Hortensias" Port-Bouet and Municipal Hospital of Port-Bouet. A total of 187 adolescents in consultation in these 4 urban community health centers were selected based on clinical data to exclude those with complications of health like blood transfusion, hypertension, diabetes rheumatism and other infections. These adolescents were divided into 2 groups with 75 non-pregnant adolescents regarded as a control group and 112 pregnant adolescents followed from first trimester to third trimester of pregnancy. The characteristics of study population were summarized in Table 1.

**Table 1. General characteristics of study population**

Characteristics	Non-pregnant adolescents N=75	adolescents during pregnancy (N=112)
	n (%)	n (%)
Age (years)	17.04 ± 0.2	17.6 ± 0.1
15 - 17	46 (61.3)	48 (42.9)
18 - 19	29 (38.7)	64 (57.1)
Body mass index (kg.m <sup>-2</sup> ) <sup>a</sup>	20.6 ± 0.9	21.5 ± 0.4/24.2 ± 0.4/26.1 ± 0.4
< 18.5	20 (26.7)	26 (23.2)/30 (26.8)/17 (15.2)
18.5 – 26	54 (72)	72 (64.3)/60 (53.6)/87 (77.7)
> 26		14 (12.5)/22 (19.6)/8 (7.1)
Gravidity	1.7 ± 0.1	3 ± 0.2
Primigravidae	60 (80)	27 (24.1)
Multigravidae	15 (20)	85 (75.9)
Parity	1 ± 0.1	0.6 ± 0.1
Nulliparous	47 (62.7)	80 (71.4)
Primiparous	13 (17.3)	19 (17)
Multiparous	15 (20)	13 (11.6)
Space between pregnancy (Months)	52.6 ± 1.9	17.8 ± 2
< 36	10 (13.3)	100 (89.3)
> 36	65 (86.7)	12 (10.7)
Matrimonial status		
Married	1 (1.3)	2 (1.8)
Single	73 (97.3)	105 (93.8)
Concubinage	1 (1.3)	5 (4.4)
Education attainment		
Uneducated	2 (2.7)	39 (34.8)
Primary school	3 (4)	19 (17)
Secondary school	70 (93.3)	54 (48.2)

*n*: Number of observed subjects in each group; *a*: Mean values and proportions of Body Mass Index's variation during the three trimesters of pregnancy

## 2.2 Blood Samples and Assays of Biological Parameters

In each study adolescent, a blood sample was collected by venipuncture on a dry and EDTA tubes of 5 ml. Blood sampling was performed on fasting in elbow in morning and during each trimester of pregnancy (between 8 and 15 weeks of pregnancy in the first trimester, between 16 and 28 weeks of pregnancy for the second trimester and between 28 and 36 weeks for the last trimester of pregnancy). Haematological parameters were immediately measured on samples collected in EDTA tubes by a haematological analyzer "Sysmex automatic Poch-100i" (1-5-1 Wakino-hama-Kaigandori, Chuo-Ku, Kobe 651-0073, Japan). Samples collected in dry tubes were centrifuged at 3000 tours/min during 5 minutes and the serum was used for the determination of biochemical parameters. Iron concentrations were determined by the colorimetric method from kit "Iron FerroZine. The rates of transferrin and ferritin were estimated by immunoturbidimetric method according respectively to kits "Transferrin Immunoturbidimetric" and "Ferritin Turbilatex." The reagents of such analyzes have been provided by Spinreact SA company (Ctra-Santa Coloma, Spain). LisaBio 300 (Hycl group, Pouilly en Auxois, France) allowed the reading values serum iron, transferrin and ferritin. Each assay of blood sample from same sample was performed twice to minimize potential manipulation errors. The average of these obtained 2 values was used.

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

Total iron binding capacity (TIBC), saturation coefficients of transferrin (SCT) and iron stores have been obtained by calculations as follows:

TIBC ( $\mu\text{mol/l}$ ) = 25. Serum transferrin (g/l) [28].

SCT (%) =  $[100 \cdot \text{Serum iron } (\mu\text{mol/l}) / \text{TIBC } (\mu\text{mol/l})]$  [28].

For Iron stores (mg): 1  $\mu\text{g/l}$  of serum ferritin = 8 mg of iron stores [29]

## 2.3 Statistical Design

The results of study are expressed as averages associated with standard errors of mean (SEM). The determination of iron status components indicated in Table 2, was performed according to recommendations of WHO, French Society of Clinical Biology, French Society of Haematology (Group of Cellular Haematology), Society nutrition and dietetics French (France) and Center of disease control and prevention [10, 28, 30, 31]. Possible changes of haematological and biochemical parameters between various groups of adolescents were evaluated by STUDENT test. The analysis of variance (ANOVA) with repeated measures multivariate tests involving as post hoc Newman-Keuls test were used to compare the means of biological parameters between the different trimesters in pregnant adolescents.

These statistical analyses were performed by computer program Statistica Statsoft Windows version 7.1 [32]. For comparisons of different obtained proportions, Loglikelihood ratio test (Test "G") was conducted by statistical software "R" Windows version 2.0.1 [33]. A probability level (p) of less than 0.05 was chosen for significance in all statistical analyzes.

**Table 2. Assessment of iron status in population [10, 28, 30, 31]**

<b>Haematological and biochemical parameters</b>	<b>Iron deficiency</b>	<b>Iron deficiency anaemia</b>	<b>Inflammatory anaemia</b>	<b>Iron deficiency + Inflammatory anaemia</b>
Hemoglobin	Normal	Low	Low	Low
MCV	Normal	Low	Normal	Normal or Low
MCH	Normal	Low	Normal or Low	Normal or Low
Serum iron	Normal or low	Low	Normal or Low	Low
Transferrin	Normal or high	High	Low	Normal or Low
SCT	Normal	Low	Normal or Low	Low
Serum Ferritin	Low	Very high	Normal or high	Normal

*SCT: Saturation coefficient of transferrin*

### 3. RESULTS

#### 3.1 Changes in Biological Parameters of Iron Status Assessment and Iron Stores

After analyses of data with STUDENT test, all haematological parameters were changed between non-pregnant and adolescents during pregnancy. Indeed, red blood cells, hemoglobin, hematocrit, MCV, MCH were increased in non-pregnant adolescents compared with pregnant adolescents. In addition, the results with ANAVO test indicated that these haematological parameters were decreased in pregnant adolescents at the third trimester of pregnancy compared with control adolescents. However, Mean corpuscular hemoglobin concentration (MCHC) was higher in adolescents throughout pregnancy than in control non-pregnant adolescents (Fig. 1). For biochemical parameters, serum iron, saturation coefficient of transferrin (SCT) and serum ferritin values were higher in control non-pregnant adolescents compared with values in pregnant adolescents. These different biochemical indicators were decreased in third trimester pregnant adolescents. Conversely, serum transferrin and total iron binding capacity were significantly increased in pregnant adolescents during all trimesters of pregnancy beside control non-pregnant adolescents (Fig. 2).

Furthermore, iron stores were greatly decreased throughout the three trimesters of pregnancy compared with non-pregnant adolescents (Fig. 2).

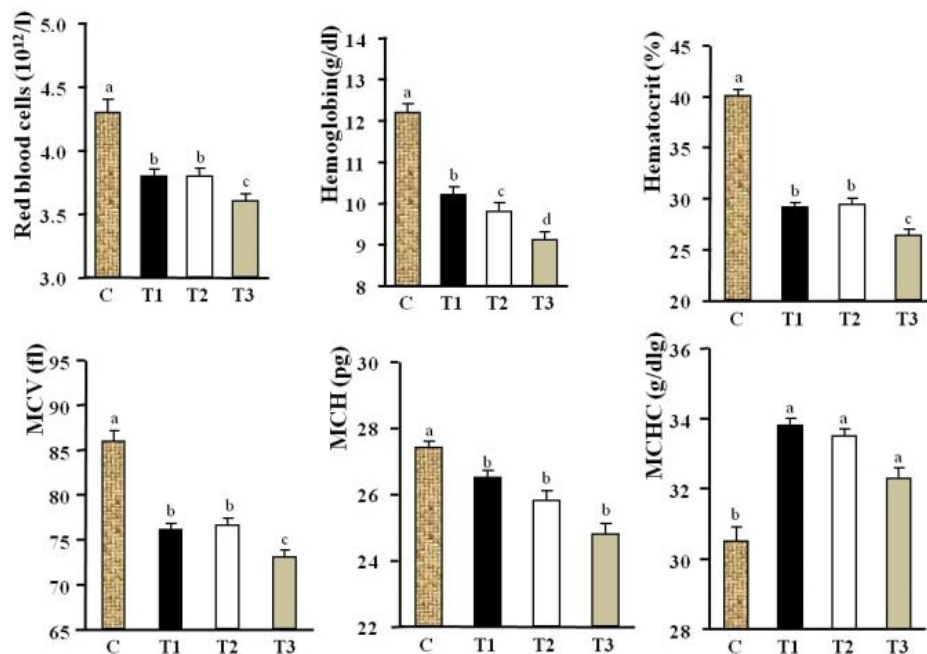


Figure 1. Changes in Haematological parameters of two adolescent groups

C: Control Non-Pregnant Adolescents 
  T1: First Trimester 
  T2: Second Trimester 
  T3: Third Trimester  
 MCV: Mean Corpuscular Volume MCH: Mean Corpuscular Hemoglobin  
 MCHC: Mean Corpuscular Hemoglobin Concentration a, b, c, d: Group statistically significant for  $p < 0.05$

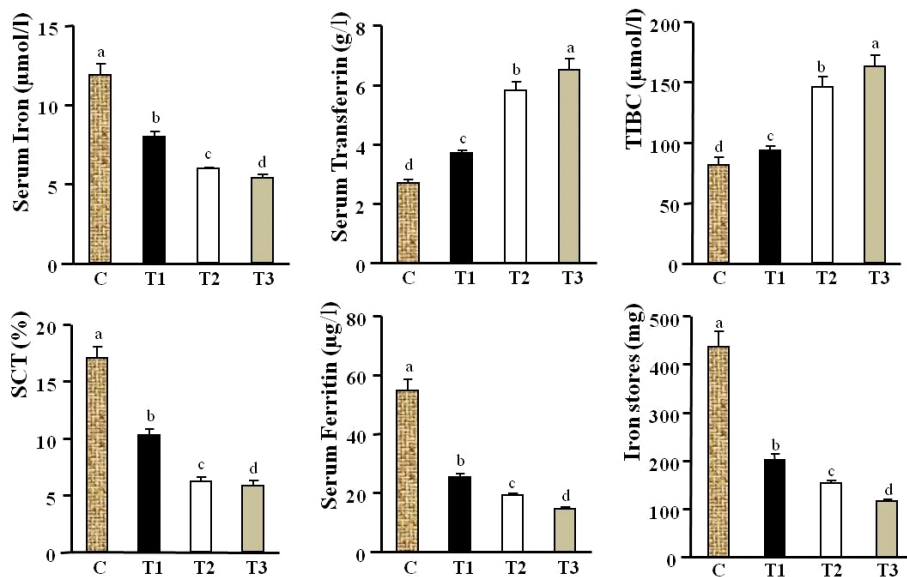


Figure 2. Evaluation of Biochemical Parameters and Iron Stores in Adolescents groups

TIBC: Total Iron Binding capacity SCT: Saturation coefficient of Transferrin  
 a, b, c, d: Group statistically significant for  $p < 0.05$   C: Control Non-Pregnant Adolescents  
 T1: First Trimester 
  T2: Second Trimester 
  T3: Third Trimester

### 3.2 Frequency of Abnormal Parameters of Iron Status

The findings in Table 3 were reported that pregnant adolescents were more anaemic during pregnancy compared with non-pregnant adolescents. In the same way, severe anaemia was not observed in non-pregnant adolescents. However, this type of anaemia was revealed throughout the three stages of pregnancy in these study adolescents. In addition, in these same subjects, our study was recorded, hypochromic microcytic anaemia, hypochromic normocytic anaemia, normochromic normocytic anaemia and normochromic microcytic anaemia. But, in non-pregnant adolescents only hypochromic microcytic anaemia and hypochromic normocytic anaemia were indicated (Table 3). For low values of hematocrit, microcytosis and hypochromia, different prevalence rates were significantly more high in adolescents during pregnancy beside control non-pregnant adolescents. Moreover, no macrocytosis was observed among pregnant adolescents in contrast to non-pregnant adolescents (8 %). The findings of study were shown that non-pregnant adolescents were indicated proportions of low values of serum iron, saturation coefficient of transferrin and serum ferritin less large relative to those observed in adolescents during pregnancy (Table 4). Conversely, adolescents throughout pregnancy were revealed decreased proportions of low values of serum transferrin and total iron binding capacity compared with control non-pregnant adolescents. However, for high values of serum transferrin and total iron binding capacity, the same pregnant adolescents were recorded increased rates beside non-pregnant adolescents (Table 4). An analysis involving all biological parameters of iron metabolism showed in Table 5 that iron status of all study adolescents was altered. This iron status was more degraded in pregnant adolescents compared with non-pregnant adolescents. In addition, non pregnant adolescent was reported a normal iron status in the third trimester of pregnancy. In the same way, the abnormal iron status was composed of iron deficiency, iron deficiency anaemia, inflammatory anaemia and inflammatory anaemia associated with iron deficiency. Furthermore, pregnant adolescents throughout pregnancy were concerned by high prevalence rates of iron deficiency anaemia. But, non-pregnant adolescents were observed inflammatory anaemia (10.7 %) and inflammatory anaemia associated with iron deficiency compared with pregnant adolescents (Table 5).

Table 3. Compared proportions of main haematological parameters between two groups of adolescents

Haematological parameters <sup>a</sup>	Non-pregnant adolescents N=75 n (%)	Pregnant adolescents (N=112)			P values
		First trimester n (%)	Second trimester n (%)	Third trimester n (%)	
Hemoglobin (g/dl) <sup>b</sup>					
Anaemia (< 10.5 or 11 or 12)	32 (42.7)	76 (67.9)	60 (53.6)	87 (77.7)	< 0.001
Normal (10.5-14 or 12-16)	43 (57.3)	36 (32.1)	52 (46.4)	25 (22.3)	< 0.001
Types of anaemia					
Mild (10.5 or 11 or 12-9)	16 (21.3)	10 (8.9)	33 (29.5)	64 (57.1)	< 0.001
Moderate (8-9)	16 (21.3)	40 (35.7)	10 (8.9)	24 (21.4)	< 0.001
Severe (< 8)	0 (0)	26 (23.2)	17 (15.2)	9 (8)	< 0.001
HMA	29 (38.7)	40 (35.7)	38 (33.9)	59 (52.7)	< 0.001
HNA	3 (4)	9 (8)	7 (6.3)	4 (3.6)	> 0.05
NNA	0 (0)	21 (18.8)	12 (10.7)	21 (18.8)	< 0.001
NMA	0 (0)	6 (5.4)	3 (2.7)	3 (2.7)	> 0.05
Hematocrit (%) <sup>b</sup>					
Low (< 32 or 33)	36 (48)	88 (78.6)	65 (58)	87 (77.7)	< 0.01
Normal (32-42 or 36-47)	39 (52)	24 (21.4)	47 (42)	25 (22.3)	< 0.01
MCV (fl)					
Microcytosis (< 80)	29 (38.7)	85 (75.9)	76 (67.9)	90 (80.4)	< 0.001
Normal (80-100)	40 (53.3)	30 (24.1)	36 (32.1)	22 (19.6)	< 0.01
Macrocytosis (> 100)	6 (8)	0 (0)	0 (0)	0 (0)	< 0.001
MCH (pg)					
Hypochromia (< 27 and >31)	35 (46.7)	69 (61.6)	74 (66.1)	94 (83.9)	< 0.01
Normal (27-31)	40 (53.3)	43 (38.4)	38 (33.9)	18 (16.1)	< 0.001

*n*: Number of observed subjects in each group; *a*: Haematological reference parameters according to French Society of haematology [21], [52]; *b*: for these two haematological parameters, the reference values were defined according to the stage of pregnancy to obtain prevalences of types' anaemia and hemodilution; hemoglobin levels are similar in the first and third trimesters of pregnancy, it is the same for hematocrit. MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; HMA: Hypochromic Microcytic Anaemia; HNA: Hypochromic Normocytic Anaemia; NNA: Normochromic Normocytic Anaemia; NMA: Normochromic Microcytic Anaemia.



Table 4. Iron stores parameters in non-pregnant and pregnant subjects

Biochemical indicators <sup>a</sup>	Non-pregnant adolescents N=75 n (%)	Pregnant adolescents (N=112)			P values
		First trimester n (%)	Second trimester n (%)	Third trimester n (%)	
Serum iron (µmol/l)					
Low (< 7.16)	29 (38.7)	57 (50.9)	112 (100)	79 (70.5)	< 0.001
Normal (7.16-26.85)	46 (61.3)	55 (49.1)	0 (0)	33 (29.5)	< 0.001
Serum transferrin (g/l)					
Low (< 2)	23 (30.7)	14 (12.5)	9 (8)	2 (1.8)	< 0.001
Normal (2-3.6)	40 (53.3)	44 (39.3)	35 (31.3)	31 (27.7)	< 0.05
High (> 3.6)	12 (16)	54 (48.2)	68 (60.7)	79 (70.5)	< 0.001
TIBC (µmol/l)					
Low (< 50)	23 (30.7)	14 (12.5)	9 (8)	1 (0.9)	< 0.001
Normal (50-90)	40 (53.3)	44 (39.3)	35 (31.3)	32 (28.6)	< 0.05
High (> 90)	12 (16)	54 (48.2)	68 (60.7)	79 (70.5)	< 0.001
SCT (%)					
Low (< 15)	27 (36)	84 (75)	111 (99.1)	91 (81.6)	< 0.001
Normal (15-35)	48 (64)	28 (25)	1 (0.9)	21 (18.4)	< 0.001
Serum ferritin (µg/l)					
Low (< 20)	26 (34.7)	67 (59.8)	72 (64.3)	97 (88.6)	< 0.001
Normal (20-110)	49 (65.3)	45 (40.2)	40 (35.7)	15 (13.4)	< 0.001
Iron stores (mg)					
Low (< 200)	26 (34.7)	81 (72.3)	94 (83.9)	107 (95.6)	< 0.001
Normal (200-900)	49 (65.3)	31 (27.7)	18 (16.1)	05 (4.4)	< 0.001

*n*: Number of observed subjects in each group; TIBC: Total iron binding capacity; SCT: Saturation coefficient of transferrin, *a*: the proportions of various biochemical parameters were defined according to established references [21], [23], [24], [53].

Table 5. Compared components of iron status between two groups of adolescents

Components of iron status <sup>β</sup>	Non-pregnant adolescents N=75 n (%)	Pregnant adolescents (N=112)			P values
		First trimester n (%)	Second trimester n (%)	Third trimester n (%)	
Normal iron status	10 (13.3)	13 (11.6)	11 (9.8)	0 (0.0)	< 0.001
Abnormal iron status	65 (86.7)	99 (88.4)	101 (90.2)	112 (100)	> 0.05
Iron deficiency	20 (26.7)	27 (24.1)	40 (35.7)	25 (22.3)	> 0.05
Iron deficiency anaemia	12 (16)	65 (58)	61 (54.5)	86 (76.8)	< 0.001
Inflammatory anaemia	8 (10.7)	6 (5)	0 (0.0)	1 (0.9)	< 0.001
Inflammatory anaemia + Iron deficiency	25 (33.3)	1 (1.3)	0 (0.0)	0 (0.0)	< 0.001

*n*: Number of observed subjects in each group; *β*: The prevalences of the various components of iron status were defined depending on established references [6], [21], [23], [25], [26], [53]

#### **4. DISCUSSION**

The results of this study indicate that haematological and biochemical parameters were decreased in pregnant adolescents at the third trimester of pregnancy compared with control adolescents. The findings show that iron stores were greatly decreased throughout pregnancy compared with non-pregnant adolescents. These results are similar to those of some authors who reveal that women of reproductive age require more iron. These demands are indispensable for the growth, menstruation and pregnancy [29, 34]. According to some authors, the low iron status early in pregnant women was found to be inversely related to placental size [35]. The total iron requirement is 1040 mg during pregnancy with 840 mg to the foetus [36]. Other studies have shown that for a adolescent who has not finished growing, competition takes place between fetal growth and maternal growth [37, 38]. This phenomenon and the dilution of plasma (normal pregnancy) cause the depletion of maternal iron throughout the pregnancy [34, 39, 40]. According to Ivorian study, women were presented iron deficiency during pregnancy [20]. In addition, the same authors were reported that, adolescents are exposed to an alteration of iron metabolism in the third trimester of pregnancy. Furthermore, non-pregnant adolescents in this same country were more observed an abnormal iron status (86.7 %) [24]. This comparative study shows more clearly the degradation of iron metabolism through iron stores in adolescents than other previous studies. Iron deficiency anaemia is still a major health problem. WHO estimates that 10.3 % of women in industrialized countries are anaemic, as compared with 42.3 % in non-industrialized countries [10]. Pregnancy is considered an important risk factor for iron deficiency and iron deficiency anaemia [41, 42, 43]. In addition, our study indicated that iron deficiency anaemia was higher in pregnant (76.8 %) than non-pregnant women (10.7 %). These results are similar to those which estimated the prevalence of anaemia in pregnant and non-pregnant women in Latin America [44]. These authors showed that anaemia was 38.5 % in pregnant versus 17.3 % in non-pregnant women. In developing countries, the prevalence of iron deficiency anaemia most often is attributed to nutritional deficiencies worsened by chronic blood loss due to parasitic infections and malaria [34, 45, 46, 47]. This could also explain the collapse of the iron stores of all adolescents in our study.

#### **5. CONCLUSION**

Our study reports the main changes of haematological parameters in adolescents (pregnant and non-pregnant). In addition, biochemical indicators are modified in the same study population. In this way, iron status of the two adolescent groups is altered with high prevalence of abnormal iron status. This abnormal iron status includes iron deficiency, iron deficiency anaemia, inflammatory anaemia and inflammatory anaemia associated with iron deficiency. However, iron status is more degraded in pregnant adolescent throughout the pregnancy.

This investigation reveals that iron metabolism of pregnant adolescents must be particularly followed during all stages of pregnancy.

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## **CONSENT**

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this research paper.

## **ETHICAL APPROVAL**

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

## **COMPETING INTERESTS**

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

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