

Haematological values in pregnant women in Port Harcourt, Nigeria II: Serum iron and transferrin, total and unsaturated iron binding capacity and some red cell and platelet indices

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Summary: Previous studies on the normal values of serum iron, unsaturated iron binding capacity, total iron binding capacity, serum transferrin, percent transferrin saturation, red cell distribution width, and various platelet indices: Platelet count, mean platelet volume, platelet distribution width, plateletcrit and platelet larger cell ratio in pregnant subjects in Nigeria are relatively scanty. Present study aims to determine the values of these parameters in apparently healthy pregnant subjects residing in Port Harcourt south eastern Nigeria; and help establish normal reference ranges of these parameters for the population under reference. Cross sectional prospective study involving 220 female subjects attending for the first time, the ante-natal clinics of a tertiary health care facility in Port Harcourt. Subjects were divided into 73, 75 and 72 subjects in the first, second and third trimester of pregnancy respectively. Serum iron and unsaturated iron binding capacity, red cell distribution width, platelet count and platelet distribution width were determined by automated methods; total iron binding capacity, serum transferrin concentrations, percent transferrin saturation, mean platelet volume and plateletcrit were calculated using appropriate formulas. The values of serum iron, unsaturated iron binding capacity, total iron binding capacity and serum transferrin concentrations were found to show significant variations between the various trimesters of pregnancy. However, while serum iron showed significant decreases during pregnancy; unsaturated iron binding capacity, total iron binding capacity and serum transferrin concentrations were found to show significant increases during pregnancy amongst our subjects ($p < 0.05$). By contrast the values of red cell distribution width, platelet count, mean platelet volume, platelet distribution width, plateletcrit and platelet larger cell ratio did not show any significant differences at the different trimesters of pregnancy in our subjects ($p > 0.05$). The present study reports, for the first time, normative values for these parameters in apparently healthy pregnant subjects in Port Harcourt south eastern Nigeria. Apparently, increases in unsaturated and total iron binding capacity and serum transferrin values seen amongst our subjects with increasing gestation may perhaps be a mechanism to ensure a fetal adequate iron delivery on account of the decreasing serum iron concentration with gestation in our subjects. The study suggests that values of serum transferrin are perhaps a more useful screening tool for iron deficiency anemia during pregnancy amongst our subjects.

Keywords: Serum transferrin; Pregnancy; Serum iron; Total iron binding capacity; Unsaturated iron binding capacity; Mean platelet volume; Plateletcrit; Nigerians

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INTRODUCTION

Several studies have attempted to document normal values for various haematological parameters amongst healthy pregnant Nigerian women at the different trimesters of pregnancy. Reports from various centers across Nigeria including Zaria (Fleming *et al.*, 1985), Jos (Onwukeme and Uguru 1990; Onwukeme 1992), Ibadan (Obisesan *et al.* 1998; Akingbola *et al.*, 2006), and Port Harcourt (Dapper *et al.*, 2006) have attempted to document normative values of a number of haematological

parameters amongst pregnant Nigerian women. These reports have helped establish reference ranges for these haematological parameters for the pregnant Nigerian women population and document the pattern of changes in these parameters in the physiological state of pregnancy in Nigerian women. However, most of these reports have focused essentially on "routine haematological parameters"; data on such other parameters as serum iron and transferrin, total and unsaturated iron binding capacity and some other specific red and platelet cell indices have been relatively scanty. This is despite their diagnostic,

therapeutic, prognostic and physiologic importance especially in pregnant subjects. For instance, serum transferrin has been reported to be an important haematological parameter that can aid the diagnosis of iron deficiency in pregnant women (Carriaga *et al.*, 1991). Further, a low serum transferrin value in early pregnancy appear to be a specific and sensitive marker for iron deficiency anemia; having advantages over both serum ferritin and hemoglobin concentration (Akeson *et al.*, 1998). In a report from India, decreases in hemoglobin concentration and percent transferrin saturation and increases in total iron binding capacity were reported to suggest an increased iron demand, turnover and deficiency especially amongst pregnant subjects of increasing age and gravidae (Kurahde *et al.*, 1994).

For the red cell indices, red cell distribution width (RDW) has been shown to be a good indicator of anisocytosis, and a good screening index especially for iron deficiency anemia and the thalassaemia trait (Quorton *et al.*, 1989). In non-pregnant subjects, RDW has been shown to be a strong, independent predictor of morbidity and mortality in heart failure (Förhéc *et al.*, 2009). The unexpected increase in the RDW during the last 4-6 weeks of pregnancy leading up to the onset of labor suggests an increase in bone marrow activity (Shehata *et al.*, 1998). Changes in RDW are significant and useful indicators of impending parturition, although the precise mechanism in this regard is uncertain (Shehata *et al.*, 1998). The mean platelet volume (MPV) and platelet distribution width (PDW) are known to increase during platelet activation, and thus these platelet indices are potentially useful markers for the early diagnosis of thrombo-embolic diseases (Vagdatli *et al.*, 2010) and as well as the risk of development of pre-eclampsia (Howarth *et al.* 1999; Dundar *et al.* 2008). There are however, conflicting results on the significance of MPV in pregnancy (Ceyhan *et al.*, 2006) as there are no reliable platelet tests yet to predict the onset of pre-eclampsia (Lazarov *et al.*, 1999). These apparent conflicts have been attributed mainly to the varying methods and equipments used in the determination of automated blood counts by the different studies (Ceyhan *et al.*, 2006). Reports have suggested significant differences in the values of white blood, red blood and platelet cell counts and platelet distribution width in pregnant Caucasian subjects during the trimesters of pregnancy (Howarth *et al.*, 1999; Ceyhan *et al.*, 2006; Förhéc *et al.*, 2009); reports from Nigeria have confirmed such variations in leucocyte counts (Fleming *et al.*, 1985), haematocrit (Akingbola *et al.*, 2006), white blood cell, platelet and red blood cell counts and PDW (Akingbola *et al.*, 2006). However, reports from

Nigeria are still conflicting and would therefore require further investigation (Dapper *et al.*, 2006). Data on the values of platelet large cell ratio in Nigerians, especially in pregnancy, are relatively scanty despite their known diagnostic importance (Laurel 1947).

The aim of the present study therefore is to report the values of some important haematological parameters in apparently healthy pregnant Nigerian subjects residing in Port Harcourt. The parameters assessed include : serum iron, unsaturated and total iron binding capacity, serum transferrin and percent transferrin saturation, red cell distribution width, platelet count, mean platelet volume, platelet distribution width, plateletcrit and platelet large cell ratio. This we hope would further our attempts at establishing normal reference ranges for these haematological parameters for pregnant Nigerian subjects residing in Port Harcourt. In addition, our study could be of value to the Nigerian obstetrician in the ante natal care assessment of the population under reference.

MATERIALS AND METHODS

Subjects

A total number of 220 apparently healthy pregnant subjects aged between 18 and 42 years were recruited into the study. All subjects were attending the ante natal clinics of a tertiary health center in Port Harcourt, Nigeria. Subjects were recruited either at the booking visit and or were attending the clinic for the first time. The subjects were of different socio-economic classes and the ethnic groups in south eastern Nigeria. Subjects were divided into three pregnancy groups, depending on the duration of pregnancy: this consists of 73 subjects in the first trimester, 75 subjects in the second trimester and 72 subjects in the third trimester of pregnancy respectively. For the purpose of the present study the first trimester was considered to end at the thirteenth week, the second to end at the twenty-sixth week and the third to end at forty weeks. Informed consent was sought and obtained from each subjects before recruitment into the study. No subject had a history of haematinic or any other mineral supplementation during the course of the present pregnancy prior to recruitment into the study. No subject had a history of vaginal bleeding during the course of the present pregnancy and none had received any form of blood transfusion within the past eight months.

Methods

Following an overnight fast, 2mls of venous blood was subsequently collected from an ante-cubital vein

with the subject comfortably seated and with minimal stasis into EDTA specimen bottles. The blood samples were properly labeled and stored at room temperature until ready for analysis. All blood samples were collected between 9am and 12noon each day and analyzed within 2hours of collection. Haematological analysis was done at room temperature ($27.5 \pm 0.5^\circ\text{C}$). Serum iron and unsaturated iron binding capacity (UIBC) were both determined by automated methods using the Prestige 24i automated clinical analyzer (Tokyo Boeki Medical System Ltd. Tokyo, Japan) with the appropriate diagnostic kits (PZ Cormay S. A. Lomianki, Poland). Red cell distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) and platelet large cell ratio (P-LCR) were all determined by flow cytometry using the Swelab Alfa Basic model hematological analyzer (Boule Medical AB, Stockholm, Sweden).

All automated analysis was done after proper bar-coding to ease identification. Values of total iron binding capacity, serum transferrin and percentage transferrin saturation were subsequently calculated from the values of the serum iron concentration and unsaturated iron binding capacity using the appropriate formula. Total Iron Binding Capacity (TIBC) was calculated as the arithmetic sum of serum iron concentration and Unsaturated Iron Binding Capacity (UIBC); percent transferrin saturation was calculated as the ratio of serum iron and TIBC expressed as a percentage (Dacie and Lewis 1991). Serum transferrin was calculated using the formula of the Miami Valley Burn Unit: Serum transferrin = $0.7 \text{ TIBC} + 24$ (Douglass 1981).

Statistical Analysis

Table 1:

Values of serum iron, unsaturated and total iron binding capacity, serum transferrin, percent transferrin saturation, red cell distribution width, platelet count, mean platelet volume, platelet distribution width, plateletcrit and platelet large cell ratio in the 3 trimesters of pregnancy

Parameters	1st trimester (n=73)	2 nd trimester (n=75)	3 rd trimester (n=72)
Iron ($\mu\text{g/dl}$)	$98.62 \pm 31.41^*$ (15.79-151.02)	$90.45 \pm 36.50^*$ (15.25-221.79)	$80.10 \pm 53.28^*$ (25.73-316.32)
UIBC ($\mu\text{g/dl}$)	$260.10 \pm 53.70^*$ (134.38-405.72)	$325.92 \pm 75.58^*$ (116.30-654.92)	$334.78 \pm 88.04^*$ (43.15-625.00)
TIBC ($\mu\text{g/dl}$)	$340.20 \pm 53.79^*$ (221.75-449.77)	$416.38 \pm 64.2^*$ (258.38-702.96)	$433.40 \pm 71.17^*$ (287.72-699.68)
Transferrin mg/dl)	$238.89 \pm 36.80^*$ (155.00-315.00)	$296.07 \pm 48.26^*$ (180.00-492.00)	$303.04 \pm 49.81^*$ (201.00-489.00)
PTS (%)	23.72 ± 9.09 (6.15-43.05)	22.27 ± 9.93 (3.80-65.60)	23.19 ± 13.35 (6.04-87.10)
RDW-SD (fl)	43.04 ± 14.23 (29.7-78.1)	41.15 ± 14.53 (26.00-95.70)	40.43 ± 11.87 (29.70-81.30)
RDW-CV (%)	13.91 ± 0.84 (11.7-16.5)	13.76 ± 0.90 (12.00-16.90)	13.90 ± 1.15 (11.30-18.20)
PLT ($10^3/\mu\text{l}$)	289.40 ± 21.68 (87.00-594.00)	259.93 ± 98.96 (71.00-558.00)	279.63 ± 107.97 (117.00-693.00)
MPV (fl)	8.45 ± 0.79 (6.60-10.90)	8.65 ± 0.76 (7.00-10.20)	8.69 ± 0.75 (7.00-10.70)
PDW (%)	9.93 ± 1.40 (6.90-14.4)	10.10 ± 1.46 (6.90-13.40)	9.86 ± 1.37 (7.70-14.90)
PCT (%)	0.26 ± 0.14 (0.09-0.90)	0.24 ± 0.18 (0.10-0.90)	0.25 ± 0.11 (0.08-0.80)
P-LCR (%)	12.18 ± 5.79 (3.00-36.1)	12.17 ± 5.56 (14.00-29.00)	12.61 ± 5.52 (4.20-34.00)

*Significant difference at $P < 0.05$, UIBC=Unsaturated Iron Binding Capacity, TIBC= Total Iron Binding Capacity, PTS= Percent transferrin saturation, RDW= Red Cell Distribution Width, PLT= Platelet count, MPV= Mean Platelet Volume, PDW= Platelet Distribution Width, PCT= Plateletcrit, P-LCR= Platelet large cell ratio

The results obtained were presented in Tables I and II as mean \pm standard deviation. Statistical differences in the values of each hematological parameter at the different trimesters of pregnancy were determined using the one-way analysis of variance; a p value less than 0.05 were considered significant.

RESULTS

From table 1, only serum iron, unsaturated iron binding capacity, total iron binding capacity and serum transferrin values were shown by Analysis of Variance to be significantly different in the subjects of the three pregnancy groups. For instance, values of serum iron concentration in the three pregnancy groups were $98.62 \pm 31.41 \mu\text{g/dl}$, $90.45 \pm 36.50 \mu\text{g/dl}$ and $80.10 \pm 53.28 \mu\text{g/dl}$ respectively for the first, second and third trimester; apparently suggesting a significant reduction in the values of serum iron with pregnancy progression ($p < 0.05$). By contrast, though the values of unsaturated iron binding capacity, total iron binding capacity and serum transferrin concentrations all showed significant differences amongst our subject groups ($p < 0.05$); however, unlike the values of serum iron the mean values of each of these three haematological parameters were lowest amongst subjects in the first trimester and highest amongst subjects in the third trimester of pregnancy; apparently suggesting an increase with progression of pregnancy. For instance, values for unsaturated iron binding capacity were $260.10 \pm 53.70 \mu\text{g/dl}$ for subjects in the first semester, $325.92 \pm 75.58 \mu\text{g/dl}$ for subjects in the second semester and $334.78 \pm 88.04 \mu\text{g/dl}$ for subjects in the third semester of pregnancy respectively, while values for total iron

Table 2:

Values of red cell distribution width, platelet count, mean platelet volume, platelet distribution width, plateletcrit and platelet large cell ratio amongst the subjects.

PTS (%)	RDW-SD (fl)	RDW-CV (%)	PLT ($10^3/\mu\text{l}$)	MPV (fl)	PDW (%)	PCT (%)	P-LCR (%)
23.05±10.91 (3.80-87.10)	41.57±13.67 (26-95.7)	13.85±0.97 (11.3-18.2)	276.2±110.11 (71-693)	8.60±0.77 (6.6-10.9)	9.97±1.41 (6.9-14.9)	0.24±0.11 (0.08-0.9)	12.33±5.57 (2.4-36.1)

PTS= Percent transferrin saturation, RDW= Red Cell Distribution Width, PLT= Platelet count, MPV= Mean Platelet Volume, PDW= Platelet Distribution Width, PCT= Plateletcrit, P-LCR= Platelet large cell ratio

binding capacity and serum transferrin were 340.20 ±53.79 µg/dl and 238.89±36.80 mg/dl for subjects in the first trimester, 416.38 ±64.20 µg/dl and 296.07±48.26 mg/dl for subjects in the second trimester and 433.40 ±71.17 µg/dl and 303.04±49.81 mg/dl for subjects in the third trimester of pregnancy respectively. Noteworthy are the values of percent transferrin saturation that did not show any significant differences amongst our three subject groups ($p>0.05$) despite its close association with the haematological parameters described above.

Furthermore, the values of both red cell distribution width parameters did not show any significant differences amongst our three subject groups ($p>0.05$); additionally, none of the platelet indices determined in the present study: platelet count, mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) and platelet large cell ratio (P-LCR) were found to show any significant differences amongst our three subject groups ($p>0.05$). Therefore, on account of lack of significant differences in the values of both the red cell distribution width parameters and the various platelet cell indices studied amongst our subjects, results obtained for these haematological parameters were pooled irrespective of the duration of pregnancy of our subjects. The results obtained are thus presented in Table 2.

DISCUSSION

Statistically significant differences were observed in the values of serum iron concentration amongst the three groups of pregnant subjects involved in the present study: highest value being observed amongst subjects in the first trimester and lowest value amongst subjects in the third trimester of pregnancy. This is consistent with reports of changes in serum iron concentration during pregnancy in both Filipino (Africa *et al.*, 1997) and Caucasian subjects (Kanishegi 1981) and have been attributed to the increased iron demand during the first and second trimester of pregnancy likely due to an increased fetal and placental demand (Kanishegi 1981). The values obtained for serum iron concentration in the present study are in the same range for pregnant Filipino subjects (Africa *et al.*, 1997) though lower than

values obtained in pregnant Caucasian subjects (Kanishegi 1981).

However, values of unsaturated and total iron binding capacity and the serum transferrin concentrations showed a pattern opposite that of serum iron concentration: the lowest value for each parameter was observed amongst subjects in the first trimester and the highest value amongst subjects in the third trimester of pregnancy. Total iron binding capacity is known to be increased in pregnancy and during iron overload (Dacie and Lewis 1991). The reported increases in total iron binding capacity with pregnancy in our subjects is therefore expected considering previous reports from our centre of possible pre-existing iron deficiency anemia amongst pregnant subjects (Dapper *et al.*, 2006); therefore, the values of total and unsaturated iron binding capacity and serum transferrin concentration obtained in the present study were generally lower than reported values in non-pregnant Caucasian females (Jacob *et al.*, 1969). Though unsaturated iron binding capacity has diagnostic use in genetic haemochromatosis (Dacie and Lewis 1991) the determination of the reference values of this parameter remains relevant.

The pattern of differences in the mean values of serum transferrin amongst our subject groups is consistent with established pattern earlier described amongst Caucasian women (Lazarov *et al.*, 1999) and also amongst Filipino women (Africa *et al.*, 1997). However, the present study reports lower values of serum transferrin concentration amongst our pregnant subject groups compared to values previously reported amongst Caucasian and Filipino women (Africa *et al.*, 1997; Lazarov *et al.*, 1999). Differences in the values of most haematological parameters have been reported between Nigerian and Caucasian pregnant subjects with Nigerian subjects having lower values (Dapper *et al.*, 2006). Racial differences have been established in the values of WBC counts between Africans and Caucasians; with a leuko-neutropenia seen in the African (O'Brien and Horton 1983; Adewuyi *et al.*, 1994). The lower values of serum transferrin seen amongst our subjects compared to Caucasians may be a corollary to the lower values of serum iron concentration also observed amongst our subjects.

The pattern of a significant increase in serum transferrin concentration and decrease in serum iron concentration with increasing gestation seen amongst our subjects is consistent with previous reports in Caucasians (Laurel *et al.*, 1947). It has been fairly established that there is a marked increase in the need for iron at the third trimester of pregnancy due mainly to the increased rate of fetal growth with an enhanced deposition of iron in fetal and placental tissues and an accompanying increase in red cell mass (Laurel *et al.*, 1947). Further to the aforementioned factors, the reduced percent transferrin seen amongst our subjects has been proposed to improve iron transfer to the fetus due to increase in the intestinal absorption and mobilization of iron from maternal storage sites (Laurel *et al.*, 1947). The increase in serum transferrin concentration with increasing gestation seen amongst our subjects may therefore be a protective mechanism ensuring fairly adequate iron delivery to the developing fetus. The present study suggests that since iron deficiency may affect pregnancy outcome (Akesson *et al.*, 1998), the iron status of pregnant Nigerian subjects be determined routinely.

The mean values of other parameters assessed in the present study; red cell distribution width, platelet count, mean platelet volume, platelet distribution width, plateletcrit and platelet large cell ratio were not found to show any significant differences amongst the three groups of subjects. However, values of all parameters determined for each trimester fell within the reference ranges for pregnant Caucasians (Dacie and Lewis 1991). This finding is consistent with previous reports from our centre describing none statistically significant differences in the values of most haematological parameters amongst our female pregnant population (Dapper *et al.*, 2006). However, significant increases in platelet distribution width have been described in pregnant Greek (Howarth *et al.*, 1999) and American (Tygart *et al.*, 1986) subjects. Amongst Chinese women, a gradual decrease in platelet count with no change in mean platelet volume has been reported (Liu *et al.*, 2009). Platelet counts were found to decrease slightly as pregnancy progresses in Pakistani women (Karim *et al.*, 1992) though no changes have been reported in American women (Tygart *et al.*, 1986). On account of this lack of significant differences in the values of these particular haematological parameters under reference amongst our subjects; values obtained for subjects at the different trimesters of pregnancy were pooled together and are thus proposed as reference values for this population, along with data presented in Table 1.

In conclusion, the present study reports, for the first time, values of some important haematological

parameters for pregnant subjects in Port Harcourt, Nigeria. Significant differences were found only in the values of serum iron, unsaturated and total iron binding capacity amongst our subjects. No significant differences were found in the values of red cell distribution width and various platelet cell indices amongst our pregnant subject groups. Our study recommends that the iron status of pregnant Nigerian subjects be determined routinely during gestation by determination of serum iron and iron binding capacity.

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