Journal of Hematology & Thromboembolic Diseases
ISSN: 2329-8790

Odiegwu et al., J Hematol Thrombo Dis 2018, 6:2
DOI: 10.4172/2329-8790.1000292

Research Article Open Access

Comparative Studies of Serum Ferritin and Anaemia Status in the Three Stages of Gestation of Some Gravid Women in Nnewi, Anambra State, Nigeria

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Abstract

One Hundred (100), apparently healthy women within the reproductive age at the Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria were investigated to ascertain their Serum Ferritin SF)/ iron and other diagnostic index of anaemia viz, Haemoglobin concentration (HB), Packed Cell Volume (PCV) and Mean Cell Haemoglobin Concentration (MCHC). Out of this number, 20 randomly selected non-pregnant women served as the control. The test groups comprised 19 pregnant women in the first trimester, 33 in the second trimester and 28 in the third trimester of gestation. Statistically significant differences were observed in the assessed parameters with P less than 0.05 ($P<0.05$). Thus, the PCV and HB values of the women in the 1st trimester of gestation were statistically significantly lower with $P<0.001$ when compared with the control group and anaemia without iron deficiency was found to occur in 47.4% of this 1st trimester pregnant women. Similarly, the PCV and HB concentration of the 2nd trimester gravid women were also significantly lower ($P<0.001$) than the control. Here, anaemia, iron deficiency and iron excess were found in 78.8%, 21.2% and 6.1% of these categories of pregnant women. The same were found to be true amongst the 3rd trimester pregnant women. However, the calculated MCHC values in the three stages of gestation, when compared with the control were found not to be statistically significant with $P>0.05$. The blood film report showed varying degrees of Hypochromasia, Macrocytosis, Microcytosis and anisocytosis. This research has succeeded in comparing blood levels and serum ferritin status in the three stages of gestation.

Keywords: Serum ferritin; Anaemia status in gestation periods

Introduction

Blood is a connective tissue albeit in fluid form and is composed of pale yellow fluid called plasma in which are suspended White Blood Cells (WBC) or Leucocytes, Red Blood Cells (RBC) or Erythrocytes and the Platelets (PLT) or Thrombocytes [1,2]. These mature blood cell types are produced by the haematopoietic stem cells in the marrow but in developing embryos, blood formation occurs in aggregates of blood cells in the yolk sac called blood islands [3]. As development progresses, blood formation occurs in the spleen, liver and lymph nodes. When bone marrow develops, it eventually assumes the task of forming most of the blood cells [4,5]. Each of the blood cell types carryout different and specific functions in the body. The Haemoglobin molecule contains iron, an essential mineral found in diet. The Haematocrit is the percentage of the sample that consists of red cells, which contains the Haemoglobin. Ferritin is a ubiquitous 450 kDa protein that stores iron and releases it in a controlled fashion. It provides a reserve of iron readily available for the formation of Haemoglobin (HB) and other haeme proteins (Casiday and Fery). Insufficient iron for HB production leads to Iron Deficiency Anaemia (IDA) which is a major nutritional problem throughout the World (WHO, 2001) [6]. The incidence of anaemia is especially high during pregnancy and lactation due to an increase in the need for iron. The prevalence of iron deficiency and anaemia in pregnant women is high and seems to become higher as pregnancy progresses [7,8]. Anaemia is defined as a Haemoglobin concentration lower than the established cut off defined by the World Health Organization (WHO) [9]. This cut off figure range from 110 g/L for pregnant women to 120 g/L for non-pregnant women. Anaemia during pregnancy is considered as an established risk factor for both the mother and fetus. The diagnostic index for IDA includes red cell indices like Hemoglobin (HB), Packed Cell Volume (PCV), Mean Cell Hemoglobin Concentration (MCHC) etc. and Serum Ferritin (SF) levels. Several studies have proven that serum ferritin is the single, best, non-invasive test and is a very useful and reliable index of iron stores especially during pregnancy with low levels indicating iron deficiency [10]. Pregnancy is the carrying of one or more offspring known as fetus or embryo inside the uterus of a female and it lasts approximately forty (40) weeks, as measured from the 1st day of the last normal cycle [11]. Human pregnancy is divided into three (3) trimester periods which serve as reference to the different stages of prenatal development and changes that take place overtime. One specific term for the state of pregnancy is gravid; hence, a pregnant woman is sometimes referred to as a gravida [12-14]. The first trimester occurs between first one to three months (first 12 weeks). Months four through six of pregnancy are called the second trimester; months seven through nine of pregnancy are called the third trimester [15]. The objective of this research was to assess the extent of effects exerted by the dramatic physiological and hormonal changes of each trimester of pregnancy on iron and blood levels. Specifically, to determine the levels of Serum Ferritin (SF), Haemoglobin (HB), Packed Cell Volume (PCV), Mean

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Received June 01, 2018; **Accepted** June 30, 2018; **Published** July 08, 2018

Citation: Odiegwu CNC, Ogbuakanne JC, Onwuasoanya UF, Onwurah OW, Odiegwu UO, et al. (2018) Comparative Studies of Serum Ferritin and Anaemia Status in the Three Stages of Gestation of Some Gravid Women in Nnewi, Anambra State, Nigeria. J Hematol Thrombo Dis 6: 292. doi: 10.4172/2329-8790.1000292

Cell Haemoglobin Concentration (MCHC) and the red blood cell morphology examinations of well-made and stained blood films.

Materials and Methods

Ethical clearance and the provisions of informed consent were duly followed before the antenatal women's blood samples were collected. One hundred (100) apparently healthy women attending antenatal clinic at the Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria randomly selected were studied. Twenty (20) of this number were the randomly selected non pregnant women that served as the control group [16]. The remaining eighty (80) subjects were the Nineteen (19) gravid women in the first (1st) trimester of gestation; Thirty-three (33) in the second (2nd) trimester and the remaining Twenty-eight (28) in the third (3rd) trimester stage. Five (5) ml of blood were collected from the cubital veins of each of these pregnant women. Two and half (2.5) ml of these blood samples were delivered into the appropriate Ethylene diamine tetra acetic acid (EDTA) containing tubes, mixed thoroughly and used within 1 hour of collection for the assay of Haemoglobin (HB) in g/dl, Packed Cell Volume (PCV) in %, Mean Cell Haemoglobin Concentration (MCHC) in g/dl, blood film preparation, staining and examination. The HB, PCV and MCHC were assayed using Sysmex Kx-21N automation while the blood film preparation, staining and examination were performed using standard haematological techniques. The remaining two and half (2.5) ml were delivered into dry plain test tubes, allowed to clot, retract and then centrifuged. The sera obtained were separated into micro tubes, stored frozen and assayed for ferritin levels in ng/ml within two (2) weeks of collection using standard solid phase direct sandwich ELISA (Enzyme-linked immuno sorbent assay) Biosafety kits method (Cheesbrough, Ochei and Kolhatkar) [17]. The value limits for defining anaemia, iron deficiency and iron excess are as follows: HB less than 12.0 g/dl, SF less than 15 ng/ml and SF greater than 120 ng/ml respectively.

Results

Table 1 shows the mean values of HB, PCV, MCHC and Serum Ferritin (SF) of the control and the pregnant women (test groups) in the three (3) stages of gestation. Table 2 shows the percentage (%) distribution of anaemia and iron status in the three (3) trimester periods. Table 3 is the statistical comparison of the mean values of HB, PCV, MCHC and SF between the pregnant women in the three trimesters of gestation with the control group. Table 4 is the percentage presence or absence of abnormal Red Cell morphologies in the three stages of gestation. The results obtained in this research are as shown in the tables below.

Parameters investigated	Control group n=20	Test groups		
		1st trimester N=19	2nd trimester N=33	3rd trimester N=28
HB (g/dl)	12.09 ± 0.36	10.88 ± 0.84	10.39 ± 0.91	10.24 ± 1.16
PCV (%)	35.56 ± 1.09	32.16 ± 2.46	30.58 ± 2.70	29.79 ± 3.25
MCHC (g/dl)	34.03 ± 0.045	34.01 ± 0.023	34.01 ± 0.043	34.00 ± 0.05
SF (ng/ml)	38.06 ± 16.38	49.23 ± 29.07	39.17 ± 34.86	48.04 ± 63.85

KEY: N=Number of subjects
HB=Hemoglobin
PCV=Packed Cell Volume
MCHC=Mean Cell Hemoglobin Concentration
SF=Serum Ferritin

Table 1: Mean values of the investigated parameters for the control and the test groups in the three stages of gestation

Parameters investigated	1st trimester N=19	2nd trimester N=33	3rd trimester N=28
Anaemia (%)	47.4	78.8	82.1
Iron deficiency (%)	0	21.2	28.6
Iron excess (%)	0	6.1	10.7

Table 2: Percentage (%) distribution of anaemia and iron status in the three trimester stages of pregnancy

Assessed parameters	1st trimester		2nd trimester		3rd trimester	
	DF	P-VALUE	DF	P-VALUE	DF	P-VALUE
HB (g/dl)	33	P>0.05	47	P<0.05	42	P<0.05
PCV (%)	33	P<0.05	47	P<0.05	42	P<0.05
MCHC (g/dl)	33	P>0.05	47	P>0.05	42	P>0.05
SF (ng/ml)	33	P>0.05	47	P>0.05	42	P>0.05

KEY: P<0.05=Significant
P>0.05=Not significant
DF=Degree of freedom

Table 3: Statistical comparison of the mean values of Haemoglobin (HB), Packed Cell Volume (PCV), Mean Cell Haemoglobin Concentration (MCHC) and Serum Ferritin (SF) between the pregnant women in the three trimesters of gestation with the control group

Red cell morphologies seen in blood film	1st trimester N=19	2nd trimester N=33	3rd trimester N=28
Hypochromasia (%)	50	76	65
Microcytosis (%)	12.5	16	13
Macrocytosis (%)	0	12	17
Anisocytosis (%)	25	28	30
Ovalocytosis (%)	0	4	0
Burr cells (%)	0	0	8.5
Tear drop cells (%)	0	0	8.5

Table 4: Percentage (%) presence/absence of abnormal red cell morphologies in the three trimesters of gestation

Discussion

Iron deficiency is a global health problem including developing countries like Nigeria. It remains undetected in a person until clinical features sets in. Various factors like unavailability of food, unawareness, illiteracy, poverty, deficiency in diet, dietary inhibitors are responsible due to which it remain a major health problem in the society. The Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria is tertiary care institute serving health care facility to a large population of South-East Geopolitical Zone of Nigeria being a Government run institute it is easily accessed by those who cannot afford the expenses of private run facilities. At the antenatal clinic of Obstetrics and Gynecology Department, routine hematological tests including complete blood indices are advised. Estimation of serum ferritin and transferrin are not advised to the pregnant women during their antenatal visits on routine basis. The study was conducted with the aim to assess the levels of serum ferritin during pregnancy. Documented evidences have shown that results obtained on this topic vary in some parts of the world. This therefore prompted this research so as to deduce what are obtainable in our local environment. In this study, the values determined in each trimester period were compared with those of the control group (apparently healthy non pregnant women within the reproductive age). Thus our findings show that the results of the haematological indices relating to anaemia viz, HB, PCV and MCHC in the first trimester of gestation were lower than those of the control. However, only the PVC values were of statistical significant variation. Also, out of the 19 gravid women in the first trimester group studied, 9 or 47.4%, of them were anaemic. This is in contrast to the

works of Mashaael, where 1 or 5.89% of the 17 pregnant women studied had anaemia [18]. The status of iron stores in these first trimester group were determined by the assay of their Serum Ferritin (SF) levels. The results obtained show that their SF levels were within the normal range of 15-120 ng/ml. This is also not in line with the works of Mashaael, who reported a lower than normal serum ferritin levels in 7 (41.2%) out of the 17 women in the first trimester of gestation studied. However, the ferritin results obtained in this work show association with the works of Kaneshige, and Hou et al. [19] which state that during pregnancy serum ferritin level remain at higher level during first trimester and tends to decrease as pregnancy progresses. The importance of this study area have prompted Kumar et al. following their findings from the work they conducted in India to suggest that serum ferritin levels measurement could be a better parameter for predicting pregnancy out come and can be advised along with other routine haematological investigations. The findings in the second trimester subjects show that their HB, PCV and MCHC values were lower than those of the non-pregnant control group and unlike in the first trimester group, their HB and PCV values were statistically significant ($P<0.05$). It was discovered that 26% or 78.8% out of the 33 antenatal women in this stage of pregnancy had lower PCV and HB values and thus 78.8% of the second trimester group were anaemic. This prevalence is higher than that reported by Mashaael, where 20 (36%) of the 56 second trimester gravid women studied were anaemic. This higher prevalence could be due to various factors like unavailability of food, unawareness, illiteracy, poverty, deficiency in diet, dietary inhibitors to which it remain a major health problem in the society. On the other hand, the mean serum ferritin levels of these antenatal women were higher than in control group but this increase in variation was not statistically significant ($P>0.05$). This finding is not in line with the works of since there is increase instead of decrease in serum ferritin levels of the antenatal women as the pregnancy progressed. Break down of the figures in the second trimester period show that only 7 (21.2%) out of the 33 pregnant women had lower serum ferritin levels, 24 (72.7%) had higher serum ferritin levels, while 2 (6.1%) had values higher than the upper limits of the normal range which is 15-120 ng/ml. The numbers of gravid women in the third trimester stage studied were 28. Their HB, PCV and MCHC values were found to be lower than the control. As obtained in the second trimester group, variations in their HB and PCV but not MCHC values were deduced to be statistically significant ($P<0.05$). The serum ferritin levels of 8 of these antenatal women were lower than normal, while 3 were higher than normal. Thus, 82.1%, 26.6% and 10.7% of these third trimester women had anaemia, iron deficiency and iron excess respectively while the remaining 17 (60.7%) had normal iron levels. These relatively rise or prevalent normalcy in serum ferritin levels observed in the second and third trimester periods as compared to the first trimester are in support of the works and does not hold true with the findings of and the blood film reports show that in the 1st trimester stage, 25% of the antenatal women had anisocytosis, 50% hypochromasia, and 12.5% microcytosis. In the second trimester group, Hypochromasia were found in the 76% of the pregnant women, anisocytosis in 28%, 16% had microcytosis, 12% of them with macrocytosis and ovalocytosis in 4%. The blood film appearance of the gravid women in the third trimester period revealed that hypochromasia, anisocytosis, microcytosis, macrocytosis, teardrop cells and burr cells were found in 65%, 30%, 13%, 17%, 8.5% and 8.5% of these category of women respectively. The comparative examinations of the blood film of the test (pregnant women) and the control (non-pregnant 20 women) show that the appearances of the control group were in most cases normocytic- normochromic although there were few cases of poikilocytosis. The characteristic variations in anaemia status between the control and the test groups including the differences in each of the three trimesters of gestation associated with this research may be attributed to the haemo-dilution which results in an increase in plasma volume exceeding the increase in the red cell mass. On the other hand, iron deficiency in the pregnant women can be attributed to the expansion of maternal red cells which is maximal at weeks 20-25 of gestation and the deposition of substantial amount of iron in the fetus and placenta. Also, the iron excess found in some of the 2nd and 3rd trimester antenatal women can be explained as due to iron supplementation administered. There are various investigations available nowadays which can diagnose and describe anemia accurately although there could not be a single parameter for solving this problem. The findings of the present study suggest that serum ferritin level measurements could be a better parameter which can be advised along with other routine haematological investigations. At tertiary level health institution there is still more possibilities available for research to be done in this field. The drop out of subjects during follow up antenatal visits and at the time of delivery limits the number of subjects included in the study. Considering the cost for serum ferritin estimation during entire duration of pregnancy effort should be undertaken to reduce cost of tests so that benefits of medical advancement can be afforded by individuals of all income groups.

Recommendations

As a result of the findings obtained in this research including those deduced from the literature, it is hereby recommended as follows:

1. It is imperative that all pregnant women attend antenatal clinic and undergo periodic and regular laboratory investigations.
2. Anaemia and iron status of all antenatal women should be monitored in the three stages of gestation using sensitive indices like HB, PCV, and Serum Ferritin levels.
3. Iron supplements should be given to gravid women so as to avoid iron deficiency anaemia in both mother and foetus.
4. However, administration of iron supplements should be controlled and monitored with a view to avoiding iron overdose or excesses and its attendant adverse effects in mother and foetus.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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