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Evaluation of Subclinical Anaemia Using Serum Ferritin Level in Rural Antenatal Mothers

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ABSTRACT
Prevalence of anaemia in all the groups of women is higher in India as compared to other developing countries [1]; leading to maternal mortality, increase in Neonatal Intensive Care Unit (NICU) admission and under nutrition in childhood and adolescence. The aim of this study is to assess serum ferritin levels in non-anaemic (according to Haemoglobin concentration) mothers with Haemoglobin > 10g/dl and to express that Haemoglobin concentration of blood is inadequate to diagnose subclinical iron deficiency anaemia in rural antenatal mothers. Blood samples were collected from 200 antenatal women visiting Government Sivaganga Medical College and Hospital, Sivaganga, Tamil Nadu, India. Among them 56.5 % (n=113) prenatal women were in the 3rd Trimester of pregnancy, 30 % (n=60) in the second Trimester and 13.5 % (n=27) in the first Trimester. Their mean age was 24.5 years, mean height was 153.9 cm. During clinical examination 9 women (4.5 %) were found to be clinically anaemic and the remaining 191 samples were classified according the range of Haemoglobin level. Of the 191 samples 48 samples (24%) containing Hb ≥ 10 g/dl (non anaemic) were taken for study of serum ferritin. The mean level of Ferritin decreases from first trimester to second to third trimester in the decreasing order of 40.8ng/ml, 36.4ng/ml and 27.6 ng/ml respectively. There is a positive correlation between Haemoglobin and Serum Ferritin in three different trimesters of antenatal women. On assessing Haemoglobin alone 76% of antenatal women under study were found to be anaemic. In non-anaemic women 16.7 % were found to be anaemic (iron deficiency anaemia) based on Serum ferritin level (less than 12 ng/ml). Totally 80% of antenatal women are found to be anaemic in this study area. Thus an increase of 4% detection rate and early diagnosis of anaemia is observed with Serum ferritin estimation.
Keywords: *Serum Ferritin, Antenatal Women, Haemoglobin, Subclinical and Anaemia.*

INTRODUCTION

A healthy antenatal mother, during childbirth, would tolerate a blood loss of up to 400 ml during normal delivery and 1000 ml during a Caesarean section But, for an anaemic mother, blood loss as little as 150 ml can be fatal. India contributes about 80 % of the maternal deaths caused by anaemia in South Asia [2]. Maternal anaemia is associated with intrauterine growth retardation, increased risk of preterm births and low birth weight babies which will inevitably lead to poor growth trajectory in infancy. It is commonly

associated with neonatal mortality and morbidity and poses a risk of decreased resistance to infections in mother, postpartum haemorrhage, puerperal venous thrombosis and many other complications in the mother. WHO has estimated that 65-75 % of antenatal mothers in India are anaemic based on serum haemoglobin (Hb) concentration[1]. Normally, iron requirement increases during pregnancy as a result of foetal haemoglobin synthesis, foetal iron reserve formation and increased maternal haemoglobin synthesis in order to counteract the blood loss during parturition. In practice, Government Health care centres use Hb concentration, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Peripheral Blood Smear as the haematological parameters for detecting anaemia. But, Hb levels and PCV are unsatisfactory due to disproportionate increase in plasma volume and red cell mass during pregnancy [3]. MCV, MCH, MCHC and Peripheral Blood Smear, though they are reliable, are a late sign of anaemia [4]. Even serum iron levels may vary from day to day or even from hour to hour.

The best parameter of maternal iron status currently available is serum ferritin concentration. Ferritin is the major iron storage protein found in circulating plasma [5]. Ferritin provides iron for the synthesis of iron containing proteins including haemoglobin and myoglobin and certain enzymes. Serum ferritin is an early and accurate measure of body iron stores particularly in an iron deficient state not severe enough to produce microcytic, hypochromic anaemia or even when haemoglobin or serum iron concentrations are normal [6]. The National Anaemia Control Programme (NACP) envisaged that all pregnant women will be screened for anaemia; Non anaemic women would get iron (100 mg) and folate (500 µg) and those with anaemia would get two tablets daily, which in practice is continued even in the puerperal period through antenatal clinics. Dose of this iron supplementation is increased to meet the requirements only when anaemia is confirmed which is usually done by Haemoglobin (Hb) estimation. But, by the time Hb level decreases it would have been too late for the mother to recover completely from the risks of anaemia and the neonate too wouldn't have received adequate iron stores. If iron depletion is diagnosed at an early stage, preventive measures of iron supplementation can be implemented to improve iron status. Thus Serum Ferritin will let an early and accurate insight on the proneness of antenatal women to become anaemic in the near future.

MATERIALS AND METHODS

Antenatal mothers visiting the outpatient department of Government Sivaganga Medical College and Hospital, Sivaganga, Tamil Nadu, India were enrolled in this study. A sample size of 200 patients, irrespective of parity and trimester was selected for the study. Prospective data and retrospective records of the patients were collected using appropriate questionnaire. All women participating in the study gave written informed consent, and the study was approved by the Institutional Ethics Committee. The research work was carried out during the period of August-September, 2015. Patients with pre-existing liver disorders, diabetes mellitus, thyroid disorders or blood or inflammatory disorders or haemoglobinopathies or who might have very high Ferritin concentration due to acute or chronic infections were excluded Clinically, anaemia was assessed by examining the lower palpebral conjunctiva, buccal mucosa and nail beds for pallor. Other findings such as dyspnoea and fatigue were taken into account. Clinically anaemic mothers were excluded from further study. For clinically non-anaemic mothers, irrespective of parity and trimester, blood samples were taken under standard aseptic precautions. On venipuncture, 3ml of blood was collected. It was divided into 2 aliquots. In one tube with EDTA (Ethylene Diamine Tetra acetic Acid) solution, 1ml of blood was added for Haemoglobin estimation. Another 2ml was added in another

tube without EDTA and kept aside for acquiring serum. Haemoglobin was assessed using an automated analyser and values recorded.

The Centre for Disease Control and Prevention defines anaemia as blood haemoglobin level less than 11g/dl during first and third trimester and less than 10.5 g/dl during second trimester [7]. However, in developing countries, like India, a lower level of 10 g/dl is often accepted [8]. Thus, a haemoglobin level of 10 g/dl at any time during pregnancy was used as a cut-off point for evaluating anaemia in this clinical evaluation in consideration of patient with uncertain or inaccurate pregnancy dating. For those, with Haemoglobin > 10 g/dl, the tube containing 2 ml of blood without EDTA was centrifuged and serum collected.

Serum ferritin levels were analysed for those antenatal mothers. Serum ferritin was assayed in a Semi-Automatic RX-50 Biochemistry analyzer based on Latex Turbidimetry method. According to National Academy of Sciences panel on nutrition and pregnancy, subjects with ferritin levels less than 12 ng/ml are considered to be anaemic [9]. The number of patients with serum ferritin < 12 ng/ml was determined and considered anaemic. The percentage of antenatal mothers with Hb< 10g/dl or serum ferritin < 12 ng/ml was calculated which gave the percentage of antenatal mothers with subclinical anaemia. The percentage of total anaemic mothers was calculated and compared with the WHO estimation. The result were analysed by appropriate statistical methods such as Range, Mean, Standard deviation, Student T Test, Pearson Correlation Coefficient and ANOVA. The socioeconomic status of study subjects were assessed based on B.G Prasad's revised (January 2015) socioeconomic status scale, which is applicable to both urban and rural populations and which takes mainly the per capita income per month into consideration [10].

OBSERVATION AND RESULT

In total 200 antenatal women were enrolled in the study. 56.5 % (n=113) prenatal women were in the 3rd Trimester of pregnancy with 30 % (n=60) in the second Trimester and 13.5 % (n=27) in the first Trimester. Their anthropometric characteristics are summarised in the table 1. Their mean age was 24.5 (range: 19 – 35) years, their mean height was 153.9 (140 – 173) cm and their mean weight was 55.5 (35 – 89) kg (Fig.1)

Fig. 1 Age and Anthropometric Characteristics of Pregnant Women

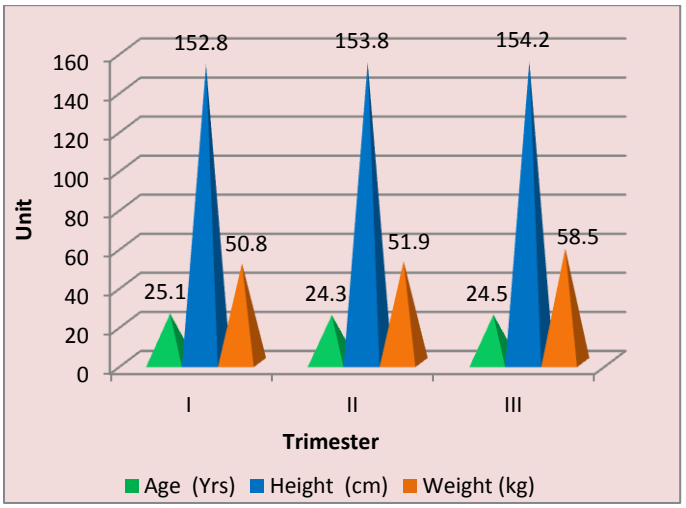

Table 1 Level of Haemoglobin Concentration in Prenatal Women

Range Hb (g / dl)	No. of Sample	% sample
Greater than 10	48	24.00
9 to 9.9	95	47.50
8 to 8.9	31	15.50
less than 8	17	8.50
Clinically Anemic	9	4.50
Total	200	100.00

During clinical examination 9 women (4.5 %) were found to be anaemic and the remaining 191 samples were classified according the range of Haemoglobin level. Of the 191 samples 48 samples (24%) containing Hb ≥ 10 g/dl (non-anaemic) were taken for study of serum ferritin. Serum ferritin value was found by Semi-Automatic Bio analyser based on Latex Turbidimetry method.

Table 2 Level of Haemoglobin Concentration in Different Trimester

Trimester	No. of Sample	Haemoglobin (g/dl)	
		Range	Mean
I	24	6.0 - 12.5	9.20
II	57	7.7 - 12.2	9.50
III	110	6.5 - 13.0	9.40
Total	191	6.0 - 13.0	9.60

In 191 antenatal women the average content of haemoglobin was 9.60 g/dl with a range of 6 – 13 g/dl. The Hb level is high in II Trimester (9.50) followed by III (9.40) and I trimester (9.20) (Table 4).

The 48 samples were classified according to the ferritin concentration level among different Trimester of the pregnancy (Table 4).

Of the 48 samples analysed the average serum ferritin level was 31.4ng/ml and the corresponding Hb level was 10.9g/dl. 5 (10.4%) of antenatal women in first Trimester had an average ferritin concentration of 40.8 ng/ml (mean Hb = 11.7 g/dl), 15 women (31.3 %) in second Trimester had 35.4 ng/ml (mean Hb = 10.6 g/dl) and 28 (58.3 %) antenatal mothers in third Trimester had 27.6 ng/ml (mean Hb = 10.8 g/dl) (Fig. 3). In total 8 pregnant women (16.7 %) had ferritin level below 12 ng/ml which implies iron deficient and of these, 6 with 10.3 ng/ml ferritin in third Trimester and the remaining two in the second Trimester with S.F. = 9.4 ng/ml.

The level of Ferritin decreases from first trimester to second and third trimester in the decreasing order 40.8 ng/ml, 36.4 ng/ml and 27.6 ng/ml respectively.

In the case of 48 antenatal women who are classified as non anaemic, 8 people were found to be anaemic by serum ferritin evaluation (Iron deficiency anaemia).

Totally 152 anemic women are there by Hb evaluation. In Hb greater than 10, 8 were anaemic by serum ferritin. So totally 160 women are anaemic which amounts to 80%. Thus totally 80% of antenatal women in this study are truly anaemic.

Table 3 Association between Haemoglobin and Serum Ferritin Level in Different Trimesters of Prenatal Women

Variables	Haemoglobin (g/dl)		Ferritin (ng/ml)		T - Calculated	Degree of Freedom	T - Table value	P	r
	Mean	Std Deviation	Mean	Std Deviation					
I Trimester (n=5)	11.70	0.57	40.80	22.80	2.85	8	2.306	0.0220	0.445
II Trimester (n=15)	10.60	0.70	35.40	17.80	5.39	28	2.048	0.0001	0.231
III Trimester (n=28)	10.70	0.60	27.60	14.10	6.34	54	2.005	0.0001	0.304

*The values are significant at $p \leq 0.05$ ** The values are positively correlated

Table 3 shows the result of relationship between Haemoglobin and Serum Ferritin in three different Trimesters of antenatal women and are expressed as the mean, standard deviation, Student't' test and Pearson Correlation coefficient. The calculations were done using online Biostatistics Calculator (http://www.socscistatistics.com/tests/studentttest/Default2.aspx) [11]

The result of t- test revealed that the calculated values of `t` was higher than that of the table value of `t`. Hence, it is significant at $p \leq 0.05$. The result of correlation coefficient analysis indicated that the value of `r` is 0.445, 0.231, and 0.304 for the first, second and third trimester respectively. This shows the positive correlation between Haemoglobin and Serum Ferritin. However, such a low value indicates that the relationship is weak one.

The women who had normal delivery had higher Hb level 9.28 g/dl (8.7 to 12g/dl) when compared to those who had caesarean (9.28 g/dl), with a

range of 6.5 – 11.5g/dl. But in case of Serum ferritin, caesarean antenatal women had higher ferritin content 34.29 ng/ml; (9 – 103ng/ml) than the who had normal delivery during the previous pregnancy (28.95 ng/ml) with a range of 11.0 – 50ng/ml.

As per previous research [12] applying a cut-off value of 30 ng/ml for iron-deficiency in this study, Out of 48 ferritin tested women 23 people had ferritin level less than 30 (47.92 %) and 3 people (6.3 %) had greater than 60 ng/ml.

Table 4 Level of Haemoglobin and Ferritin for Primi and Multigravida Antenatal Women

No. of Child	Hb (g/dl)		Ferritin (ng/ml)	
	Sample size	Mean Hb	Sample size	Mean Ferritin
Primigravida	106	9.8	24	34.1
Multigravida	85	9.8	24	29.75

Though the mean Hb level of primigravida and multigravida women were assessed to be same (9.8mg/dl), Serum Ferritin level is decreased in multigravida women (Table 4).

Table 5 Association of Haemoglobin and Serum Ferritin status against Inter conception period

Time gap between child	Mean Haemoglobin (g/dl)		Mean Ferritin (ng/ml)	
	1 Child	2 Child	1 Child	2 Child
less than 2 years	9.5	9.2	25.2	27.0
2 - 5 years	9.6	10.1	26.7	23.0
Greater than 5 years	9.9	10.4	46.0	43.5

If the interconception period is high – greater than 5 years in our case, the level of both Hb as well as Ferritin level was at the maximum for those having 1 as well as 2 child (Table 5).

DISCUSSION

Among the non-anaemic women i.e. whose Hb > 10 g/dl, 1st trimester women had the least and 2nd trimester women had the highest Hb levels followed by 3rd trimester women. The possible explanation would be since the mean age of women under study is low, which predisposes these study individuals to have very low Hb at the start. As the pregnancy progresses there is increased iron absorption and also due to additional iron supplementation Hb increases in 2nd trimester which again faces a fall as and when the maternal iron are prioritised for the foetal iron reserves. Their corresponding Serum ferritin levels however were higher in the 1st trimester, decreased during 2nd trimester, but its level was found to be lowest during the 3rd trimester. This is in accordance with the earlier research [13] wherein serum ferritin levels were found to lowest in the 3rd trimester. A woman at her early stages of pregnancy has comparatively higher iron reserve. This is in accord with the research by various authors [3], [14] and [15] during pregnancy, serum ferritin concentration was maximum at 12-16 weeks gestation. But probably as a result of iron utilization for expansion of the maternal red blood cell mass, Serum ferritin usually falls markedly between 12 and 25 week of gestation [16], when haemoglobin status tends to rise. Most iron transfer to the foetus occurs after 30 week of gestation thereby depleting the iron reserve when serum ferritin falls to the lowest and the efficiency of maternal iron absorption rises to its peak [17]. The iron needs of the foetus thus seem to take priority over maternal requirements with respect to iron absorbed from the gut [18].

Among the 48 women (Hb >10g/dl) whose serum ferritin was analysed 16.7% had serum ferritin < 12ng/ml. According to National Academy of Sciences panel on nutrition and pregnancy, subjects with ferritin levels less than 12 ng/ml are considered to be anaemic [9]. Hence according to both Hb and serum ferritin concentration totally 80% (n=160) of women are found to be anaemic in my place of study. This is in contrast to the WHO's estimation of 65 – 75 % anaemic Indian mothers. The remaining 83.3% of the 48 antenatal mothers had comparatively good reserve of ferritin and were non- anaemic.

In a similar research for assessing anaemia using serum ferritin (S.F.) levels, ferritin value of 30 ng/ml or less was stated to be the best indicator of

iron deficiency in pregnant women [12]. Applying a cut-off value of 30 ng/ml for iron-deficiency in this study 47.9 % (n=23) of pregnant women with Hb> 10g/dl were deficient in iron, of which 31.25%, (n=15), due to deficient iron stores (12ng/ml >S.F. < 30ng/ml) are highly prone to become anaemic (S.F < 12ng/ml). Hence iron supplementation instead of, as blanket therapy, if given in appropriate therapeutic doses can prevent these women from becoming anaemic in the near future.

Serum ferritin <12 ng/ml was considered indicative of depleted iron stores, ferritin level between 12 ng/ml and 60ng/ml was considered "normal," and ferritin above 60ng/ml was considered elevated [19].

In our current study 6.3% of women had comparatively higher S.F. levels (>60 ng/ml). Serum ferritin determination has the advantage that its results are not significantly affected by other types of anaemia or oral iron therapy [20] and only a small amount (0.2m1), of serum is required for the serum ferritin assay. One limitation of our study was that it relied solely on serum ferritin as the only biomarker of iron status. Because serum ferritin is an acute phase protein and its level increases in response to inflammation normal or elevated levels may be falsely interpreted as iron sufficiency, if the subject is in an inflammatory state [21]. Although we can be confident that women with serum ferritin <12 µg/L were in fact iron deficient anaemic (16.7% of our study group), we cannot be certain that women with ferritin concentrations >12 µg/L were necessarily iron replete. This can be overcome if their (hs- CRP) High sensitivity C - reactive protein (hs- CRP), another acute phase protein which is independent of iron status is measured. If both S.F. and hs- CRP are elevated, it can be confirmed that the rise of S.F was due to inflammation and not due to iron sufficiency.

The results of the study meet our objectives and it establishes that Haemoglobin status alone cannot evaluate the prevalence of anaemia in antenatal women. Haemoglobin estimation fails to identify lesser 'extent of iron deficiency when iron stores are depleted but Hb synthesis is not impaired.

Primigravida women are found to have significantly better iron status than multigravida women. Among the multigravida women, women who have given birth to 2 children had higher Hb status and ferritin status than women with 1 child in our study. This is because the antenatal women in our study had better or greater interconception period between the last and current pregnancy, due to which there was considerable amount of time for the iron stores to get replenished.

Also Hb status among women with Hb < 10 g/dl and the ferritin status in mothers with Hb >10g/dl was compared respectively with the previous pregnancy whether it was a normal or caesarean. There was significant decrease in Hb alone in women in whom previous LSCS was done than in women who underwent normal delivery. However serum ferritin levels were found to be more in women who had previous Caesarean section .This may be due to Greater interconception period in these women and better post-operative care.

In the present study, among the women taking iron supplementation, their compliance in consumption was assessed and any patient with complaints of nausea and vomiting soon after taking iron tablets for a particular duration were enquired and that particular duration was not included in the duration of iron tablets taken. There was no significance between iron supplementation and Serum Ferritin status, however there is little significance between Haemoglobin status and iron supplementation. This questions the efficiency of blanket therapy in antenatal women. A therapeutic approach to iron supplementation, rather than a public health–based approach, needs to be implemented in India in order to counteract this maternal anaemia in a large scale and real, or perceived, problems of compliance can be overcome by means of better awareness programmes and health education. Prenatal mineral and vitamin supplement and therapeutic iron supplementation after the assessment of S.F given from the first trimester

would maintain serum ferritin and Haemoglobin levels at a higher concentration [14] and [15].

The advisability of routine iron supplementation during pregnancy, regardless of whether the mother is anaemic, has been heavily debated in the United States [22] and routine supplementation is not universally practiced in all industrialized countries [23]. This is because higher than the required iron levels in serum is associated with increased oxidative product formation and the initiation of various pathogenic processes such as cardiovascular disease, neuropathologies, and cancer [24]. But in the author's view, as far as India is considered, unless antenatal women have concomitant inflammation or infection or other disorders which will raise serum ferritin levels, there are very less percentage of people with higher than normal iron stores to warrant such a causal action. But by the analyses of serum ferritin in early pregnancy , subclinical infections or inflammations or high iron content might be detected which may be subjected to further investigations .And in case of high iron status, need for iron supplementation be evaluated.

CONCLUSION

From this study it is evident that Serum Ferritin estimation will enable to find higher number of anaemic antenatal women (80 %) than Haemoglobin estimation (76%) alone. Serum ferritin estimation provides an early and accurate method of identifying those patients with depleted iron stores who require active treatment and for assessing the adequacy of such treatment. Serum Ferritin, in addition to being a deciding factor for starting therapeutic iron supplementation, its increase may warrant suspicion of underlying subclinical infections or disorders that can be further evaluated. As far as further research is concerned, an extensive research needs to be carried out in Indian antenatal women taking into consideration the parameters discussed above, also including dietary iron intake, C - reactive protein level, type of contraceptive if any used menstrual loss evaluation, effects of calcium tablets and the compliance of the rural antenatal women.

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