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Diagnostic Performance of Hepcidin in Evaluation of Iron Status among Pregnant Women in Rivers State, Nigeria

Authors
Beauty Echonwere[*1], Teddy C. Adias[2]& Evelyn M. Eze[1]
[1]Dept. of Medical Laboratory Science, Rivers State University, Port Harcourt, Rivers Sate, Nigeria
[2]Dept. of Microbiology, Faculty of Science, Federal University Otuoke, Bayelsa State, Nigeria
[*]Corresponding Author
Beauty Echonwere
Email: *beautyechonwere@gmail.com*, Phone: +2348032767471

Abstract
"Iron indices are useful in assessing the state of haemoglobin function and determination of state and degree of anaemia". "In humans, aside iron indices are the role of hepcidin in up and down regulation of iron metabolism in normal state and diverse state including pregnancy". We did evaluate gestational changes in serum hepcidin and iron status among pregnant women". We evaluated serum hepcidin, ferritin, soluble transferrin receptor, serum iron, unsaturated iron binding capacity, total iron binding capacity at 12, 20, and 30 weeks of gestation in a longitudinal study of 428 apparently healthy women who consisted of 328 pregnant women and 100 non-pregnant who served as control in Rivers State of Nigeria". Hepcidin, sTfR, Ferritin, Iron, TIBC and UIBC were assayed using Enzyme Linked Immunosorbent Assay and Photometric methods". Data was analyzed using (Graph Pad Prism version 9.0, and NCSS version 9.0). "Results obtained were expressed mean ± SD, median and 2.5-97.5 percentile. Hepcidin concentration declined throughout the three partitions of pregnancy ($p<.05$) and had greatest sensitivity than other iron parameters at week 20 and 30. The AUC^{ROC} values for hepcidin to detect iron deficiency (ferritin <15ng/ml) were 0.96, $p<.0001$ and 0.97, $p<.0001$ at weeks 20 and 30 respectively. The prevalence of anaemia increased from 39.37%, to 89.33%, and 60.37%, at weeks 12, 20, and 30 respectively. The prevalence of iron deficiency anaemia defined as (sTfR>28nmol/l) was 17.99% at week 12, 17.68% at 20 and 35.98% at week 30. Hepcidin outperformed haemoglobin, ferritin, soluble transferrin receptor in diagnosing iron deficiency. This study found a progressive increase in development of IDA, even with increase iron demand by fetus economic. We had provided data and evidence on changes in serum hepcidin concentration and other iron parameter in pregnancy, and further asserts that hepcidin is a more useful assay in diagnosis of IDA in pregnancy.
Keywords: *Hepcidin, Gestation, Women, Rivers State.*

Introduction

"During pregnancy iron is very important because of the swift cell and tissue development associated with fetal growth"[1]. "Sufficient iron status during pregnancy is vital for healthy pregnancy, normal development of the fetus and maturity of the new born baby[2]. "Deficiency of iron during pregnancy is characterized by increased risks in preterm births, perinatal mortality, low birth weight and intrauterine growth retardation"[3]. Approximately 800mg of iron are required in pregnancy, which is far higher than the "230mg of iron that non-pregnant women needed, and the 150mg that may be lost during" delivery via blood loss[4]. It varies between 14-52% in women who aren't using supplements to 0-25% with women in pregnancy taking usual multivitamins comprising iron and folic acid

formulation[5].

Conventionally, diagnostic markers for iron deficiency comprise total iron binding capacity (TIBC), serum iron, and serum ferritin level. In addition, the diagnostic markers also comprise red blood cell indices and serum transferrin in saturation".[6] "reported that serum iron is not suitable in pregnancy and that low sensitivity of transferrin saturation and daily and even hourly variation of serum iron levels makes it less effective than serum ferritin level for diagnosis of iron deficiency which is the only case linked to reduced serum ferritin concentration"[7]. "Another study also reported that, serum ferritin is beneficial, being a sensitive gauge for iron deficiency, however, since the presence of inflammation increases its concentration, in contrast ferritin was considered to be a non-specific indicator of iron deficiency"[8].

Studies by WHO in2001 and 2008, confirms the fact that, even though there are many deep-rooted tests for iron status, detailed evaluation using a single testis not satisfactory, likewise, to use the conventional marker such as ferritin is still subject to review[9] thus may need adjustment for inflammation levels. However, developing multiple testsis associated with difficulty, expensive, and it demands high levels of interpretation, and is non-achievable in a resource poor setting[10][11]. Therefore, a biomarker that would ensure precise diagnosis of iron status of individual should be developed, and thus hepcidin, a peptide hormone predominately synthesized by hepatocytes of the liver has been discovered as a vital regulator of iron hemostasis in the body[12]. Hepcidin binding to iron exporter ferroportin induces it taking on and subsequent degradation[13]. Iron deficiency suppresses hepcidin concentration to facilitate augmented assimilation and use of iron. Elevation in iron overload coupled with inflammation would hinder the entrance of iron to the plasma. This characteristic of hepcidin positions it to be used as a test for diagnosing iron status[14].

Anaemia with a projected incidence of 35-75% among pregnant women is a key cause of maternal deaths in Nigeria[15]. It is regarded as a difficult public health problem and a most shared pregnancy complication in developing countries, with the emergence of HIV/AIDS pandemic making it more complicated[1]. WHO projects more than half of pregnant women in the developing nations as affected by anaemia. Current projections in the developing countries, not excluding Nigeria, show 60% prevalence in pregnancy and approximate 7% of the women with serious anaemia[16][17][18]. The high prevalence and the aetiology associated with anaemia in pregnancy are complex and their comparative effects differ geographically and by season[19].

Pregnancy-related anaemia could be relative or absolute[20]. Relative anaemia naturally takes place in pregnancy owing increased plasma volume (almost 45% in singleton and 50-60% in twin gestation) than in red cell mass, causing anaemia in pregnancy. Absolute anaemia entails an exact reduction in red cell mass and is accompanied with a rise in red cell destruction [20][21]. Predisposing factors comprises young age, striking multiparity, poverty, ignorance, and short inter-pregnancy periods[22]. The aim of this research focused on evaluating the gestational changes in serum hepcidin and iron status among pregnant women in Rivers State".

Materials and Methods

Study Design

The study was a longitudinal research performed on 428 apparently healthy women of reproductive ages between August 2017 and April 2018. They comprised 328 women with pregnancy and 100 women without pregnancy who served as Control and were all resident in Rivers State, Nigeria.

Study Setting

This study was done in Rivers State, located at the South-South zone of Nigeria with a population of 5,198,716 according to 2006 Census report and is located at Coordinates, 4°55'N,6°50'E.

Study Population

This study was carried out on pregnant women who were attending antenatal clinic at Braithwaite Memorial Specialist Hospital (BMSH), University of Port Harcourt Teaching Hospital (UPTH) and selected Prima1y Health Centres located at each 3 senatorial districts in Rivers State".

Sample Size and Sampling Technique

The sample size was obtained using the formula[23]. Prevalence rate of anaemia in pregnant women receiving regular supplements is" 75%[15].

Laboratory Analysis

Sample Collection

Seven milliliters (7ml) of venous sample was taken from each participant of which 5 ml of the blood was added to a plane sarstedt monovette, for determination of hepcidin, ferritin, serum iron, serum transferrin receptor and TIBC concentration."The blood sample collected in a disposable, non-pyrogenic, and non-endotoxin sarstedt monovette tubes was allowed to clot for 2 hours. "The supernatant was collected, and the assay was conducted at once for the analysis of hepcidin, serum transferrin receptor, ferritin serun iron and total iron-binding capacity

Determination of serum Hepcidin

Sandwich-Enzyme-linked immunosorbent assay (S-ELISA) kit as described by Camaschella and Silvestri, 2011". Catalog No. E-EL-H0077 was used to estimate serum hepcidin.

Quantitative Determinant of Human Soluble Transferrin Receptor (sTfR)

Accu-bind Microplate Enzyme Immunoassay, Colorimetric (ELISA technique as described by[24]. Product code: 8625 -300 which the principle is based on antigen-antibody binding. The method of the assay involves immobilization at the surface of a microplate well by interacting with streptavidi and exogenously added biotinylated monoclonal anti s-TfR antibody". There is direct proportionality between enzyme activity on the well and native free antigen.

The results were calculated by exploring a dose response curve to guarantee the concentration of sTfR in unknown specimens. We recorded the absorbance gotten from the print out of the micro plate reader, the absorbance for all duplicate serum references were plotted against the corresponding sTfR concentration in nmol/L on linear graph paper and linked points with a best-fit curve.

Quantitative Determination of Serum Ferritin

Accu-Bind Microplate Immunoenzymometric Assay. ELISA technique as described by Tietz (1999). Product code: 2825-300. Necessary reagents involved in immunoenzymometric assay have high affinity and specific antibodies of various excess epitope recognition**,** and native antigen. These specific antibodies are enzyme and immobilized. The method of the assay involves immobilization at the surface of a microplate well by interacting with streptavidi and exogenously added biotinylated monoclonal anti-ferritin antibody.

At the end of incubation, separation of the antibody-antigen bound fraction from unbound antigen was done by decanting or aspirating, then followed by adding another antibody labelled with an enzyme. There is direct proportionality between enzyme activity on the well and native free antigen.

Quantitative Determination of Iron, Total Iron Binding Capacity (TIBC)

Photometric method as described by Henry 1984

The dissociation of iron in serum from its Fe (III) - transferrin complex is initiated by the adding an acidic buffer containing hydroxylamine. Consequently, Fe (III) ions are reduced to Fe (II). Immensely colored Fe (II)-complex that is measured photometrically at 560nm is produced by the chromogenic agent ferene. UIBC is evaluated by addition of Fe (II) iron to serum to initiate binding to the unsaturated sites on the transferring.

Statistical Analysis

Graph Prism 6.1 and National Council of Social Science (NCSS 9.0) were used to analyze the data generated. An error probability p ($\leq$ 0.05) was considered significant.

Results

Demographic Characteristics of Enrolled Subjects

A total of 466 subjects were initially enrolled into our longitudinal study. Of this number, 428 were finally enrolled, while 18 were excluded. Details are cited in Table 4.1.

Table 4.1: Some Demographic Characteristics of Enrolled Subjects

Parameter	Frequency	%	Mean ± SD
Total No. of Enrolled Subjects	428	95.96	
Pregnant Subjects			
• **Total**	328	100.00	
• **Nulliparous**	161	49.08	
• **Primiparous**	77	23.48	
• **Multiparous**	90	27.44	
• **Married**	280	85.37	
• **Single**	48	14.63	
• **Age (y)**			31.12 ± 4.59
Control (Non-pregnant)			
• **Total**	100	100.00	
• **Nulliparous**	36	36.00	
• **Primiparous**	25	25.00	
• **Multiparous**	39	39.00	
• **Married**	75	75.00	
• **Single**	25	25.00	
• **Age (y)**			29.54 ± 5.41
BMI (kg/m^2)			
Control			27.64 ± 3.65
1st Trimester (Wk. 12)			28.38 ± 8.76
2nd Trimester (Wk. 20)			29.32 ± 5.05
3rd Trimester (Wk. 30)			30.17 ± 4.58

Key: y = years, NA = not applicable, % = percentage, SD = standard deviation, BMI = body mass index, Wk. = week.

Comparison of Hepcidin and Iron Parameters between Trimesters of Pregnancy and Control

Table 4.2 shows the comparison of serum hepcidin and other iron parameters between each trimesters and control. A list for normality indicated that these values were non-parametric, hence percentile distribution (2.5th and 97.5th) with median values were determined.

Table 4.2: Comparison of Hepcidin and Iron Parameters between Trimesters of Pregnancy and Control

Parameter		1st Trim (Wk. 12)	Control	*P*	2nd Trim (Wk. 20)	Control	*p*	3rd Trim (Wk. 30)	Control	*p*
Hepcidin (ng/ml)	*Mdn*	45.20	43.81	.01*	14.00	43.81	.00**	13.30	43.81	.00**
	P2.5 – P97.5	22.80 – 55.12	11.76 – 53.50		9.80 – 18.40	11.76 – 53.50		10.30–16.30	11.76 – 53.50	
STfR (nmol/l)	*Mdn*	14.30	15.91	.00**	18.74	15.91	.03*	23.56	15.91	.00**
	P2.5 – P97.5	6.09 - 23.51	10.54 – 39.52		9.23 – 35.40	10.54 – 39.52		10.73–32.07	10.54 – 39.52	
Ferritin (ng/ml)	*Mdn*	30.58	25.15	.03*	23.04	25.15	.59	18.29	25.15	.01*
	P2.5 – P97.5	5.10 – 123.12	3.55 – 130.06		6.70 – 75.84	3.55 – 130.06		6.02 – 48.71	3.55 – 130.06	
SI (µg/dl)	*Mdn*	111.00	173.00	.00**	185.00	173.00	.74	187.00	173.00	.96
	P2.5 – P97.5	19.00 – 312.00	65.60 – 383.55		32.00-298.00	65.60 – 383.55		100.00-287.00	65.60 – 383.55	
TIBC (µg/dl)	*Mdn*	471.00	519.50	.06	529.00	519.50	.06	512.00	519.50	.23
	P2.5 – P97.5	142.00-677.00	23.63 –769.08		239.00-744.00	23.63 –769.08		268.00-704.00	23.63 –769.08	
UIBC (µg/dl)	*Mdn*	341.00	353.50	.04*	375.00	353.50	.01*	328.00	353.50	.21
	P2.5 – P97.5	68.00 – 455.00	24.00 – 452.00		41.00–486.00	24.00–452.00		166.00-458.00	24.00 – 452.00	

Key: *Mdn* = median, P2.5 = 2.5th percentile, P97.5 = 97.5th percentile, 95%CI = 95% Confidence Interval of the difference, Wk. = week, t = t-test statistic, *p* = error probability, Trim = trimester, STfR = soluble transferrin receptor, SI = serum iron, TIBC = total iron binding capacity, UIBC = unsaturated iron binding capacity, **Significant difference observed, $p < .01$, *Significant difference observed $p \leq .05$ using the paired t-test.

Comparison of Hepcidin and Iron Parameters between Various Trimesters of Pregnancy

Comparisons were made between the hepcidin and iron parameters of various trimesters of pregnancy. As shown in Table 4.3, strongly significant differences were observed for all pair wise comparisons of parameters between the various trimesters ($p < .01$), with the exception of 1st and 2nd, and 1st and 3rd unsaturated iron binding capacity (UIBC) values ($p > .05$).

Table 4.3: Comparison of Hepcidin and Iron Parameters between Various Trimesters of Pregnancy

Parameter		1st Trim (Wk. 12)	2nd Trim (Wk. 20)	*p*	2nd Trim (Wk. 20)	3rd Trim (Wk. 30)	*p*	1st Trim (Wk. 12)	3rd Trim (Wk. 30)	*p*
Hepcidin (ng/ml)	*Mdn*	45.20	14.00	.00**	14.00	13.30	.00**	45.20	13.30	.00**
	P2.5 – P97.5	22.80 – 55.12	9.80 – 18.40		9.80 – 18.40	10.30–16.30		22.80 – 55.12	10.30–16.30	
STfR (nmol/l)	*Mdn*	14.30	18.74	.00**	18.74	23.56	.00**	14.30	23.56	.00**
	P2.5 – P97.5	6.09 - 23.51	9.23 – 35.40		9.23 – 35.40	10.73–32.07		6.09 - 23.51	10.73–32.07	
Ferritin (ng/ml)	*Mdn*	30.58	23.04	.00**	23.04	18.29	.00**	30.58	18.29	.00**
	P2.5 – P97.5	5.10 – 123.12	6.70 – 75.84		6.70 – 75.84	6.02 – 48.71		5.10 – 123.12	6.02 – 48.71	
SI (µg/dl)	*Mdn*	111.00	185.00	.00**	185.00	187.00	.19	111.00	187.00	.00**
	P2.5 – P97.5	19.00 – 312.00	32.00-298.00		32.00-298.00	100.00-287.00		19.00 – 312.00	100.00-287.00	
TIBC (µg/dl)	*Mdn*	471.00	529.00	.00**	529.00	512.00	.28	471.00	512.00	.00**
	P2.5 – P97.5	142.00-677.00	239.00-744.00		239.00-744.00	268.00-704.00		142.00-677.00	268.00-704.00	
UIBC (µg/dl)	*Mdn*	341.00	375.00	.49	375.00	328.00	.04*	341.00	328.00	.13
	P2.5 – P97.5	68.00 – 455.00	41.00–486.00		41.00–486.00	166.00-458.00		68.00 – 455.00	166.00-458.00	

Sensitivity, Specificity, and Youden Index of Putative Hepcidin Cut off Values across Trimesters of Pregnancy

Table 4.4 shows the sensitivity, specificity, and Youden Index of putative hepcidin cut off values across trimesters of pregnancy.

Table 4.4: Sensitivity, Specificity, and Youden Index at Putative Hepcidin Cut off Values across Trimesters of Pregnancy

Hepcidin Cut off (ng/ml)	1st Trimester (12 weeks)			2nd Trimester (20 weeks)			3rd Trimester (30 weeks)		
	Sensitivity	Specificity	Youden Index	Sensitivity	Specificity	Youden Index	Sensitivity	Specificity	Youden Index
15.00	100.00	4.00	0.040	75.30	96.00	0.713	86.28	96.00	0.823
17.80	100.00	10.00	0.100	96.34	90.00	0.863	100.00	90.00	0.900*
20.65	98.78	11.00	0.098	100.00	89.00	0.890*	100.00	89.00	0.890
27.30	93.90	17.00	0.109	100.00	83.00	0.830	100.00	83.00	0.830
33.21	92.38	23.00	0.154*	100.00	77.00	0.770	100.00	77.00	0.770

Key: Sensitivity = true positive detection rate, specificity = true negative detection rate, Youden Index = (sensitivity/100 + specificity/100)-1, *Hepcidin cut off with the highest Youden Index for each Trimester

Correlation between Log Hepcidin and Other Iron Parameters among Pregnant Subjects

Strong significant correlations with moderate effect sizes were observed between Log Hepcidin and other iron parameters among the studied pregnant subjects ($r > .10$, $p < .01$).

Table 4.5: Correlation between Log Hepcidin and Some Iron Parameters among Pregnant Subjects

		LogHep	LogSTfR	LogFerr	SI	TIBC	UIBC
LogHep	*r*	1.00	-.41**	.28**	-.43**	-.22**	.03
	p		.00	.00	.00	.00	.36
	n	984	984	984	984	984	984
LogSTfR	*r*	-.41**	1.00	-.29**	-.04	-.09**	-.08**
	p	.00		.00	.19	.00	.01
	n	984	984	984	984	984	984
LogFerr	*r*	.28**	-.29**	1.00	-.06	-.08*	-.06
	p	.00	.00		.07	.01	.06
	n	984	984	984	984	984	984
SI	*r*	-.43**	-.04	-.06	1.00	.57**	.03
	p	.00	.19	.07		.00	.31
	n	984	984	984	984	984	984
TIBC	*r*	-.22**	-.09**	-.08*	.57**	1.00	.79**
	p	.00	.00	.01	.00		.00
	n	984	984	984	984	984	984
UIBC	*r*	.03	-.08**	-.06	.03	.79**	1.00
	p	.36	.01	.06	.31	.00	
	n	984	984	984	984	984	984

Key: LogHep = Log hepcidin, Log STfR = Log soluble transferrin receptor, LogFerr = Log ferritin, SI = serum iron, TIBC = total iron binding capacity, UIBC = unsaturated iron binding capacity, *r* = Pearson correlation coefficient, *p* = error probability, n = total number of samples, **Correlation is significant at the .01 level (2-tailed), *Correlation is significant at the .05 level (2-tailed).

Receiver Operator Curve (ROC) of Hepcidin at 1st, 2nd, and 3rdTrimester of Pregnancy showing the Mean Area under the Curve (AUC).

Figure 4.1 shows the Receiver Operating Characteristic (ROC) Curves of hepcidin for determination of iron status at 1st, 2nd, and 3rd trimesters of pregnancy. The area under the curve (AUC) increased from 0.55 although not significant in the 1st trimester (p = .41), to 0.96 in the 2nd trimester (p< .0001) and peaking at 0.97 in the 3rd (p< .0001).

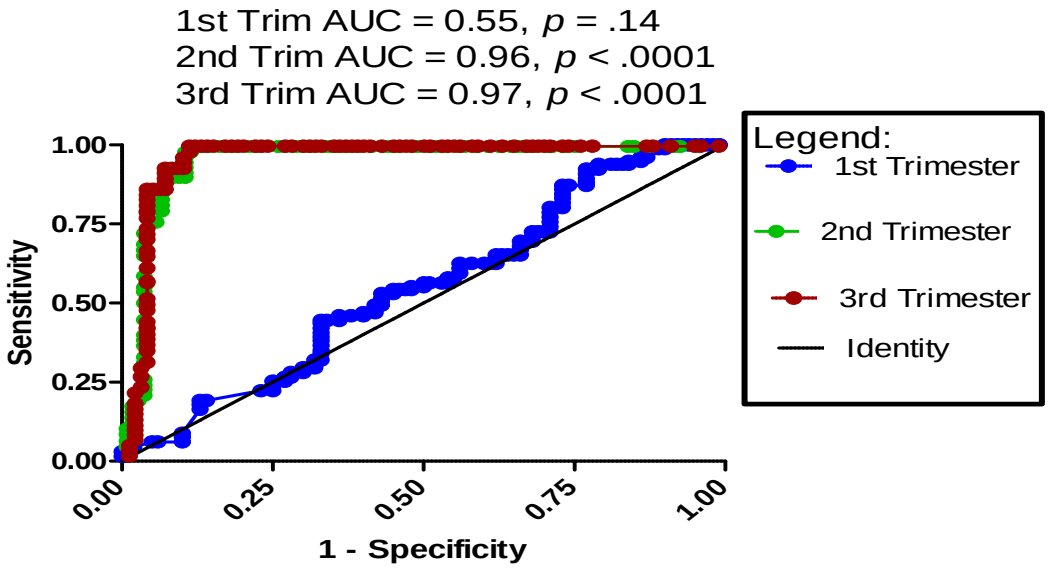

Figure 4.1: Receiver Operator Curve (ROC) of Hepcidin for 1st, 2nd, and 3rdTrimester of Pregnancy showing the Mean Area under the Curve (AUC).
Key: Trim = Trimester, *p* = error probability.

Receiver Operator Curve of Hepcidin, Haemoglobin, Ferritin, and Soluble Transferrin Receptor at 1st Trimester (12 Weeks of Gestation).

Figure 4.2.below shows receiver operator curves of hepcidin, haemoglobin, ferritin, and soluble transferrin receptor (STfR) were compared at week 12 of gestation.

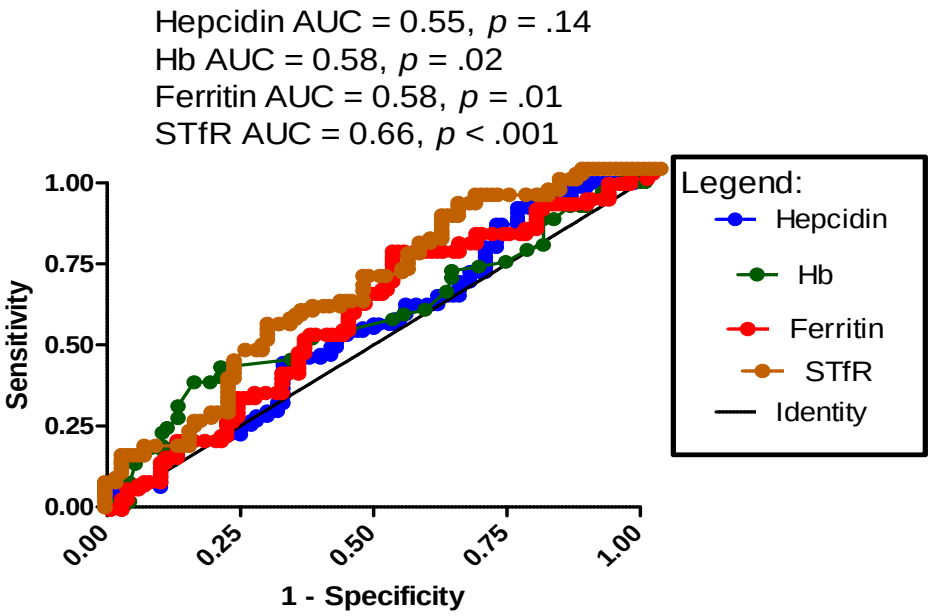

Figure 4.2: Receiver Operator Curve of Hepcidin, Haemoglobin, Ferritin, and Soluble Transferrin Receptor at 1st Trimester (12 Weeks of Gestation).
Key: AUC = Mean Area Under the Curve, *p* = error probability, Hb = haemoglobin, STfR = soluble transferrin receptor

Receiver Operator Curve of Hepcidin, Haemoglobin, Ferritin, and Soluble Transferrin Receptor at 2nd Trimester (20 Weeks of Gestation).

Figure 4.3 shows the receiver operator curve of hepcidin, haemoglobin, ferritin, and soluble transferrin receptor (STfR) for the evaluation of iron status at week 20 of gestation.

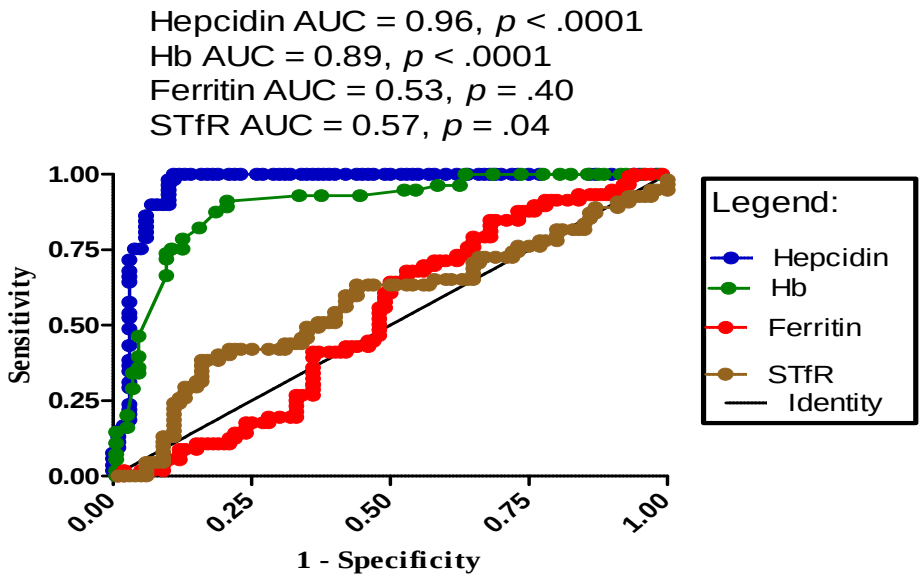

Figure 4.3: Receiver Operator Curve of Hepcidin, Haemoglobin, Ferritin, and Soluble Transferrin Receptor at 2nd Trimester (20 Weeks of Gestation).
Key: AUC= Mean Area Under the Curve, *p* = error probability, Hb = haemoglobin, STfR = soluble transferrin receptor

Discussion

Iron indices are useful in assessing the state of haemoglobin function and in the determination of state and degree of anaemia. In humans, aside these iron indices, are the role of hepcidin as the regulator of iron haemostasis. These roles result in up and down regulation of iron metabolism in normal state and other diverse state including pregnancy. Hepcidin is a peptide hormone predominantly synthesized in the liver.

Few studies had documented longitudinal changes in serum hepcidin in pregnancy[25][12], we had observed significant decrease in serum hepcidin concentration between the pregnant and non-pregnant control (p=.00) (Table 4.2). The decrease in serum hepcidin concentration was seen throughout the three partitions of pregnancy (p<.05) (Table 4.2). This is in harmony with a cohort study in Gambia by[25] where it was documented that hepcidin concentrations decline by week 20 of pregnancy, before the onset of biochemical evidence of changes in iron store. Our study also re-affirmed van Santen and colleagues, (2013) findings while investigating serum hepcidin changes in cohort of Danish pregnant women. However, our findings are in contrast with the study by[26] were no decline in hepcidin concentration was observed across pregnancy in Turkish women. The decline of serum hepcidin across the trimesters of pregnancy was a result of physiologic response to iron need to the mother and fetus.

In our findings, we observed that hepcidin out performed haemoglobin, ferritin, soluble transferrin receptor in the various trimesters (Table 4.2 and 4.4). This is on the basis that hepcidin occurs the largest area under the curve (AUC) on the receiver operator characteristics (ROC) plot, which is with equally highest Youden Index (Table 4.4; Figure 4.1- 4.4). This is in conformity with the study by[25] were it was documented that hepcidin outperformed other indices predictor for definition of iron deficiency.

In the comparison made in Table 4.3, between hepcidin and various trimesters of pregnancy, we observed significant differences for all pairwise comparison of parameters between the various trimesters (p< .01), with exception of first and second, and first and third UIBC values (p>.05). However, our findings were in line with the studies in Southern Malawi by[19], on the increase observed in TIBC values but disagrees with the increase in UIBC. These authors reported that both TIBC and UIBC values increased throughout pregnancy with more pronounced UIBC as pregnancy advanced to term. The decrease in UIBC in our study in third trimester could be a result of malnutrition, inflammation, liver disease or nephrotic syndrome.

Results from Table 4.5 shows a strong correlation between log hepcidin and other iron parameters among the pregnant women studied (r>.10,p<.01), an inverse correlation was observed between log hepcidin and (log sTfR r = -.41, p=.00; serum iron r = -.43, p=.00; and TIBC r = -.22, p=.00), while log hepcidin had a direct relationship with only log ferritin (r =.28, p=.00). No significant correlation was found between log hepcidin values and UIBC (P>.05). The correlation between log hepcidin and other iron biomarkers is an indication that hepcidin is implicated in increasing bioavailability of iron to both mother and fetus.

Out of a total of 328 pregnant subjects studied, 129 (39.33%) had anaemia in the first 12 weeks of gestation, 293 (89.33%) at week 20, and 198 (60.37%) at week 30 (p = .00). The prevalence of anaemia in this study was higher than that obtained by[25] who obtained prevalence of 34.62% at week 14.50% at week 20, and 54.62% at week 30 of gestation, in Gambia. In addition, the prevalence of anaemia in this study far exceeds that obtained by[27] across the various trimesters of pregnant women in Abeokuta (9.8% in 1st trimester, 63.5% in the 2nd, and 26.6% in the 3rd). Nonetheless, a similar trend was observed in comparison with these two studies: anaemia peaked at the 2nd trimester of gestation. The increased anaemia in the 2nd trimester of pregnancy may be due to rapid growth of the fetus, as there seems to be an inverse relationship between the maternal Hb and the fetal birth weight[28]. Poor diet, with especially inadequate bioavailable iron, among the women could be another likely factor[29].

Iron Deficiency Anaemia (IDA) was 17.99% in the 1st trimester, 17.68% in the 2nd trimester, and 35.98% in the 3rd (Table 4.7). The trends in our results are in agreement with those previously reported by[30] who obtained the highest prevalence of IDA in the 3rd trimester of pregnancy (20.00%) among women in Calabar, Nigeria. Transferrin receptors (TfRs) are proteins that comprise the putative pathway for cellular iron absorption in lieu of physiological needs. In hypoferraemia, these proteins are elevated on the surface of bone marrow (BM) erythroid precursors, this results in sequestration of iron in the cells[31]. These results from our study seem to show that a large proportion of the pregnant women were iron deficient, this could possibly because of parity, poor dietary habits and socio-economic status [32][33].

Despite our reported findings, we were unable to provide reasons for the large proportion of iron deficiency in subject studied and the decrease observed in the value of Unsaturated Iron Binding Capacity of the third trimester (Table 4.2). We therefore, recommend further studies on UIBC across the three partitions of pregnancy and at term to ascertain the underlining cause of this decrease.

Conclusion

The evaluation of serum hepcidin concentration is a novelty and useful biomarker for early detection of bioavailability which is crucial to both mother and developing fetus throughout pregnancy. The understanding of the role of hepcidin in the regulation of iron haemostasis has given insight to

The decline in hepcidin concentration in all trimester of pregnancy suggest a window for optimal timing for antenatal iron intervention to ensure enough increase in iron absorption, thus enhancing adequate supply of iron to the fetus through the placenta.

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