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RESEARCH ARTICLE

CAN VITAMIN D SUPPLEMENTAL THERAPY IMPROVE PREGNANCY-ASSOCIATED ANEMIA? AN INTERVENTIONAL PROSPECTIVE CLINICAL TRIAL.

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Abstract

Objectives: To evaluate impact of vitamin D supplemental therapy (VD-ST) and iron ST (IST) on hemoglobin concentration (HC) in pregnant women.

Patients & Methods: 187 women pregnant were clinically evaluated and gave blood samples at 1st trimester for estimation of HC and serum 25OH-VD, ferritin concentration (FC), hepcidin (HPC) and interleukin-6 (IL-6). Women were categorized according to the levels of estimated parameters into control and study groups A-D. Women of groups A and C received VD-ST alone, group B received IST alone and group D received both ST. At delivery, HC, extent of change and frequency of pregnancy- associated anemia (PAA) were determined.

Results: At 1st trimester, 71.7% women were anemic and 38.5% had hypoferremia, 77% had VD deficiency (VDD) and 59.4% had both VDD and PAA. Serum HPC and IL-6 levels in anemic women especially those had VDD were significantly higher than control women. At delivery, 35% of control women developed PAA and mean HC was significantly lower than 1st trimester HC with a median deficit of 4.72 gm/dl (IQR: 2.8-6.4) among studied population. Frequency of anemic women in groups B-D was decreased at delivery by 21.7%, 30.8% and 30.6%, respectively. Statistical analyses defined high serum HPC and IL-6 levels at 1st trimester as specific early predictors for PAA development, but early institution of ST especially VD-ST is one of the best significant predictors for improved HC at time of delivery.

Conclusion: VDD, hypoferremia, higher HPC and IL-6 levels constitute a vicious circle entrapping pregnant women and inducing iron deficiency anemia at delivery. Early institution of VD-ST and IST can burst this circle and induce improvement of HC up to normal range in about 30% of women presented by PAA. Thus, early estimation of HC and serum VD can be used as a guide for early institution of ST.

Introduction:-

Vitamin D deficiency (VDD) is very common and prescriptions of both assay and supplementation are increasing [(1)]. Serum 25-hydroxy vitamin D (25OH-VD) levels in women are variable as it tends to be higher during luteal phase than follicular phase in non-pregnant and were lower in pregnant than in non-pregnant women [(2)].

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Vitamin D is a significant regulator of both innate and adaptive immunity by regulating cell proliferation, differentiation and apoptosis [3]. During the first weeks of pregnancy, calcitriol is increasing by >2-3 fold to correspond for fetal demands [4]. During pregnancy, vitamin D receptor and regulatory metabolic enzymes are expressed in the placenta and deciduas [3], indicating a potential critical point in the immunomodulation at the maternal-fetal interface [5], so maternal VDD was associated with pregnancy-related disorders [6].

Anemia affects upwards of 50% of pregnant women in developing countries and is associated with adverse outcomes for mother and child [7]. Iron is an essential micronutrient. During pregnancy, the placenta adapts by up-regulating its transfer systems to maintain fetal requirements of iron at the expense of the mother's stores [8]. Iron deficiency (ID) constitutes the main cause of anemia and hemoglobin concentration (HC) below 10.5 g/dl could be considered as true anemia [9].

Trans-placental iron transfer involves binding transferrin-bound iron to its receptor, uptake into an endosome, acidification, release of iron through divalent metal transporter 1, efflux across the basolateral membrane through ferroportin (FPN) and oxidation to ferrous ion [8]. Acute and chronic inflammation has a major impact on iron homeostasis as it affects iron trafficking and its availability to the host [10]. Hepcidin (HPC) is a mediator of innate immunity and the master regulator of systemic iron bioavailability in humans [11] as it regulates its intestinal absorption, tissue distribution, and macrophage iron release [12]. Hepcidin regulates extracellular iron concentration by binding to the iron exporter FPN leading to functional hypoferremia through intracellular iron sequestration [13]. Thus, elevated HPC levels especially during inflammatory conditions lead to iron-release restriction [14] and so may interfere with trans-placental iron transfer [15].

Supplemental therapy (ST) during pregnancy is still a matter of discrepancy; despite of improved maternal hematological indexes on routine iron supplemental therapy (IST) during pregnancy, its clinical significance for both pregnant women and infants remains unclear [16]. Moreover, high intake of heme iron is associated with increased risk of gestational diabetes [17] and glucose intolerance-related outcome was similar for selective or routine IST [18]. Concerning VD-ST, the current evidence base could not allow definite conclusions regarding the optimal maternal circulating concentration of 25OH-VD during pregnancy, and how this might best be achieved [19] and VD-ST during pregnancy was associated with increased its circulating levels, birth weight, and length, but was not associated with other outcomes [20].

Hypothesis:-
Pathogenesis of pregnancy-associated anemia (PAA) is multi-factorial and the response to IST is either unpredicted or inefficient. The current study hypothesizes that VDD may underlay the development of PAA or the weak response to IST.

Objectives:-
The current study targets to evaluate the impact of vitamin D and iron supplemental therapy on hemoglobin concentration in pregnant women at time of delivery

Design:-
Prospective observational clinical study

Setting:-
Benha University Hospitals

Patients & Methods:-
The study protocol was approved by the Local Ethical Committee and all enrolled women signed a written fully-informed consent to participate in the study. The study was conducted at departments of Obstetrics & Gynecology and Clinical Pathology, Benha University Hospitals. To exclude the seasonal variations of VD levels, all women attended the antenatal care unit at Benha University Hospital during their 1st trimester since March 21st till June 21st each year for three years starting at 2015 were eligible to clinical evaluation and were asked to attend the clinic fasting on the next day to give blood samples for investigations. At time of delivery, another blood sample was obtained for re-determination of HC. Exclusion criteria included presence of multiple pregnancy, fetal congenital anomalies, current DM or essential hypertension, obesity-inducing endocrinopathy, evident manifestations of hypo-

parathyroidism, thyrotocixosis, renal or hepatic diseases and women gave birth outside the hospital were also excluded from the study.

Vitamin D sufficiency status was defined according to 25-OHD concentration as follows: ≥75 nmol/L sufficient level, 50-75 nmol/L insufficient level and <50 nmol/L deficient level. Vitamin D deficiency was categorized as mild, moderate and severe if 25-OHD concentration was 25-50 nmol/L, 12.5-25 nmol/L and <12.5 nmol/L, respectively [21]. PAA was defined as Hb conc. of <11 gm/dl either at 1st trimester (T1) or at delivery (T2). Iron deficiency (ID) was diagnosed if serum ferritin was <15 ng/ml [22].

Investigations:-
Under complete aseptic conditions, 5 ml blood samples were withdrawn from the antecubital vein during 1st trimester for:
- Routine investigations included estimation of HC and fasting blood glucose.
- A part of blood sample was collected in a plane tube, till clotted and then was centrifuged at 3000 rpm for 10 min. Serum was removed put in pyrogen-free Eppendorf tubes and stored at -70°C until ELISA assayed for serum levels of 25OH-VD, using an ELISA kit from Cayman Chemical, Ann Arbor, MI, USA [23], ferritin concentration (FC), using an ELISA kit from Eurogenetics UK [24], hepcidin using ELISA kit from Quantikine r&d systems Inc. Minneapolis MN USA [25] and IL-6 using ELISA kit from Pelikine™ Inc., Concord, USA [26].

Study Grouping:-
- Control group included women with HC>11 gm/dl, 25OH-VD ≥75 nmol/L and FC ≥15 ng/ml.
- Study groups:
  - Group A: included women with HC>11 gm/dl, 25OH-VD <75 nmol/L and FC ≥15 ng/ml.
  - Group B: included women with HC<11 gm/dl, 25OH-VD ≥75 nmol/L, irrespective of FC.
  - Group C: included women with HC<11 gm/dl, 25OH-VD <75 nmol/L and FC ≥15 ng/ml.
  - Group D: included women with HC<11 gm/dl, 25OH-VD<75 nmol/L and FC <15 ng/ml.

Study Design & Medications:-
- Women of groups A and C received only vitamin D3 ST according to **Grant et al.** [27] as a daily oral dose of 400 IU to be taken as 4 drops daily with mail (Vidrop, Oral solution, Medical Union Pharmaceuticals, Abu Sultan, Ismailia, Egypt, provided as 100 IU/drop). Vitamin D supplementation started since 1st antenatal visit after giving blood sample and assuring insufficieny-deficiency till delivery.
- Women of group B received IST as a daily oral ferrous fumarate 350 mg caps (HAEMOTON cap; containing ferrous fumarate 350 mg, vitamin B_{12} 7.5 µg, folic Acid 2 mg, ascorbic acid 50 mg and other vitamins; Glaxo Smith Kline Co., Egypt).
- Women of group D received both IST and VD-ST, while control women received no supplemental therapy

Study outcome:-
- Determination of frequencies of PAA, ID, ID anemia (IDA) and VDD.
- Evaluation of the effect of VD-ST on the severity of PAA and if this effect can be modulated by combination with IST or not.
- Trying to determine the association between studied parameters and early prediction of the oncoming HC at time of delivery.

Statistical analysis:-
Obtained data were presented as mean±SD, numbers and percentages. Results were analyzed using Student and paired t-test and Chi-square test (X^2 test). Data were analyzed using Regression analysis (Stepwise Method) to define the significant predictors for HC and its extent of change at time of delivery in relation to 1st trimester HC and for development of PAA at time of delivery in pregnant women. ROC curve analysis for laboratory parameters estimated at 1st trimester for prediction of development of PAA at time of delivery and for the appropriate supplemental therapy for PAA minimization or prevention. Statistical analysis was conducted using the IBM SPSS (Version 23, 2015) for Windows statistical package. P value <0.05 was considered statistically significant.

Results:-
The study included 217 pregnant women eligible for evaluation; 30 were excluded and 187 were enrolled in the study. According to estimated serum 25OH-VD levels, 43 women had sufficient, 49 women had insufficient, 44

women had mild VDD and 51 women had moderate VDD. As regards PAA, 53 women had no anemia, while 134 women had PAA. For grouping, 20 women were considered as control women, and 33, 23, 39 and 72 women were included in study groups A-D, respectively. Enrolment data of studied women as shown in table 1 showed non-significant difference between groups.

Table 1:-Patients' enrolment data

Data		Control group	Study groups			
			A	B	C	D
Number		20 (10.7%)	33 (17.7%)	23 (12.3%)	39 (20.9%)	72 (38.4%)
Age (years)		27.7±2.6	27.6±2.7	27.9±2.3	27.4±2.5	28.2±2.7
Body mass index data	Weight (kg)	77.3±4.8	79.8±3.4	74.8±3.9	76.2±4.4	79.4±7.9
	Height (cm)	170.4±3.3	169.8±2.7	167.1±3.1	169.8±2.7	168.3±3.2
	BMI (kg/m^2)	26.5±3.4	27.2±4.1	27.5±3.2	27.3±2.3	28±2.7
Gravidity	Primi	6 (30%)	12 (36.4%)	6 (26.1%)	11 (28.2%)	23 (31.9%)
	Multi	14 (70%)	21 (63.6%)	17 (73.9%)	28 (71.8%)	39 (68.1%)
Blood pressure (mmHg)	Systolic	113.5±3.4	114.8±3.1	114.5±3.9	114.7±3.2	114.3±9.8
	Diastolic	72.3±3.5	72.5±3.4	72.2±2.9	71.8±3.2	71.9±4.5
Fasting blood glucose (mg/dl)		97.1±3.7	96.4±3.9	98.2±3.3	96.6±3.8	99.4±5

Data are presented as mean±SD & numbers; BMI: Body mass index

Mean initial HC was significantly lower in groups B-D compared to groups A and B. Mean estimated FC levels were significantly lower in women of group D compared to women of all other groups and in women of group C compared to those of group A. Estimated 25OH-VD levels were significantly lower in women of groups D compared to women of other groups and in women of groups A and C compared to women of control group and group B, (Table 2).

Table 2:-PAI and VDD data of studied patients

Parameters	Data		Control (n=20)	Study groups			
				A (n=33)	B (n=23)	C (n=39)	D (n=72)
HC (mg/dl)	PAA frequency	>11	20(100%)	33 (100%)	0	0	0
		<11	0	0	23 (100%)	39 (100%)	72 (100%)
	Mean (±SD)		12.3±0.6	12.1±0.5	10.7±0.2*†	10.8±0.14*†	10.7±0.2*†
FC (ng/ml)	IDA frequency	>15	20(100%)	33 (100%)	23 (100%)	39 (100%)	0
		<15	0	0	0	0	72 (100%)
	Mean (±SD)		17±1.2	17±1.33	16.9±1.27	16.2±0.8†	13.1±1.2*†‡ٱ
Serum 25OH-VD (nmol/l)	Frequency according to sufficiency	Sufficient	20(100%)	0	23 (100%)	0	0
		Insufficient	0	14 (42.4%)	0	26 (66.7%)	9 (12.5%)
		Mild VDD	0	15 (45.5%)	0	8 (20.5%)	21 (29.2%)
		Moderate VDD	0	4 (12.1%)	0	5 (12.8%)	42 (58.3%)
	Mean (±SD)		77.9±1.2	53.8±18.4*‡	76.7±1	54±18.5*‡	31.8±13.1*†‡ٱ

HC: Hemoglobin concentration; FC: Ferritin concentration; 25OH-VD: 25 hydroxy vitamin D; PAA: Pregnancy-associated anemia; IDA: Iron deficieny anemia; VDD: Vitamin D deficiency; *: significant difference versus control group; †: significant difference versus Group A; ‡: significant difference versus Group B; ٱ: significant difference versus Group C

Women of groups C and D had significantly ($p<0.05$) higher serum HPC levels compared to women of control and study groups A and B. Women of group A had significantly ($p<0.05$) higher, while women of group B had non-significantly ($p>0.05$) higher serum HPC levels compared to control women. Serum IL-6 levels were significantly ($p<0.05$) higher in women of groups A, C and D, and non-significantly ($p>0.05$) higher in women of group B compared control women. Moreover, estimated serum IL-6 levels were significantly ($p<0.05$) higher in women of study groups A-D compared to group B and in women of group D than those of group A (Table 3).

Table 3:-Serum Hepcidin and IL-6 levels estimated in studied patients at the 1st trimester

Parameters	Control (n=20)	Study groups			
		A (n=33)	B (n=23)	C (n=39)	D (n=72)
HPC (ng/ml)	17.5±3.7	25.5±8.6*	20.5±4.5	32.8±10*†‡	34.7±13.5*†‡
IL-6 (ng/ml)	22.5±10.1	39.9±14.9*	26.6±10.6†	47.4±12.9*‡	48.7±13.5*†‡

Data are presented as mean±SD; HPC: hepcidin; IL-6: Interleukin-6; *: significant difference versus control group; †: significant difference versus Group A; ‡: significant difference versus Group B

All women showed decreased HC at time of delivery that was significantly ($p<0.05$) lower than at 1st trimester HC in women of control group and study groups A and B, but was non-significant ($p>0.05$) in women of groups C and D. Moreover, the frequency of PAA increased in control and study group A by 35% and 27.3% with non-significantly ($p>0.05$) higher frequency and extent of deficit among women of control group than women of study group A. On contrary, the frequency of PAA was decreased by 21.7%, 30.8% and 30.6% among women of group B-D, respectively with non-significantly ($p>0.05$) higher frequency and extent of deficit among women of group B than women of groups C and D (Table 4).

Table 4:-HC estimated at time of delivery and its extent of change in relation to concentration estimated at 1st trimester

Parameter		HC				Frequency of PAA		
Group	Time	1st trimester	Delivery	P value	Deficit	1st trimester	Delivery	Change
Control (n=20)		12.3±0.6	11.5±0.8	0.001	6.9±3.7	0	35%	↑ by 35%
Study	A (n=33)	12.1±0.5	11.5±0.6	0.011	4.54±2.2	0	27.3%	↑ by 27.3%
	B (n=23)	10.7±0.2	10.4±0.6	0.026	2.78±5.6	100%	78.3%	↓ by 21.7%
	C (n=39)	10.8±0.1	10.6±0.7	0.218	1.23±6.1	100%	69.2%	↓ by 30.8%
	D (n=72)	10.7±0.2	10.6±0.8	0.094	1.34±6.6	100%	69.4%	↓ by 30.6%

Regression analysis defined presence of VDD manifested as low serum 25OH-VD level and high HPC serum, estimated at 1st trimester, as the significant negative predictor for low HC at time of delivery. Moreover, Regression analysis defined the early initiation of VD-RT alone or in combination with IST as positive predictors for maintained or improved HC at time of delivery. For prediction of HC deficit at time of delivery, Regression analysis defined high serum 25OH-VD as positive and high serum IL-6 as negative significant predictors for small HC deficit and early initiation of VD-ST as a positive significant predictor for small HC deficit. Also, regression analysis defined high serum IL-6 estimated at the 1st trimester as a positive, while high serum 25OH-VD and early initiation of VD-ST alone or in combination with IST as negative significant predictors of persistence or development of PAA at time of delivery, (Table 5).

Table 5:-Regression analysis for predictors of HC status at time of delivery in studied population

	At booking parameters	Standardized coefficient	p
HC	HPC	-3.699	0.0009
	VDD	-7.988	0.0003
	Combined VD-ST & IST	5.554	0.0007
	VD-ST	4.081	0.0005
HC deficit	25OH-VD	0.621	0.0004
	VD-ST	0.453	0.0007
	IL-6	-0.199	0.011
Persistence or development of PAA	25OH-VD	-0.804	0.0001
	VD-ST	-0.531	0.0005
	IL-6	0.200	0.008
	Combined VD-ST & IST	-0.216	0.009

VDD: vitamin D deficiency; VD-ST: VD supplemental therapy; IST: Iron supplemental therapy; 25OH-VD: 25-hydroxy vitamin D; IL-6: Interleukin 6

The ROC curve analysis defined low serum 25OH-VD and FC as sensitive and high serum HPC and IL-6 levels as specific early predictor for development of PAA at time of delivery (Fig. 1). Moreover, ROC curve analysis defined

early initiation of VD-ST as the best specific predictor for correction or avoidance of development of PAA (Table 6, Fig. 2).

Table 6:-ROC curve analysis for predictors of PAA at time of delivery in studied population

Parameters estimated at 1st trimester					Supplemental therapy				
Parameter	AUC	SE	P	95% CI	Line	AUC	SE	P	95% CI
25OH-VD	0.267	0.036	0.0002	0.196-0.339	VD	0.629	0.042	0.003	0.547-0.71
FC	0.327	0.041	0.0006	0.246-0.407	Iron	0.506	0.043	>0.05	0.421-0.59
HPC	0.635	0.043	0.002	0.550-0.719	Both	0.580	0.042	>0.05	0.498-0.663
IL-6	0.614	0.043	0.009	0.528-0.698					

AUC: Area under curve; SE: Standard error; CI: confidence interval; 25OH-VD: 25 hydroxy vitamin D; FC: Ferritin concentration; HPC: Hepcidin; IL-6: Interleukin-6; PAA: Pregnancy-associated anemia; IDA: Iron deficiency anemia

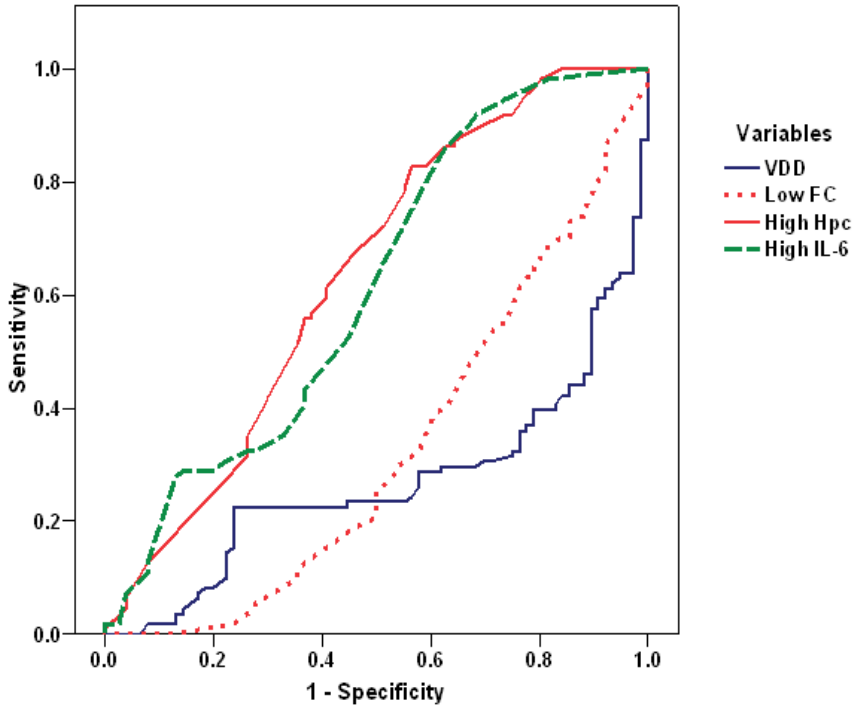

Fig. 1:-ROC curve analysis of laboratory parameters estimated at the 1st trimester for prediction of PAA at time of delivery

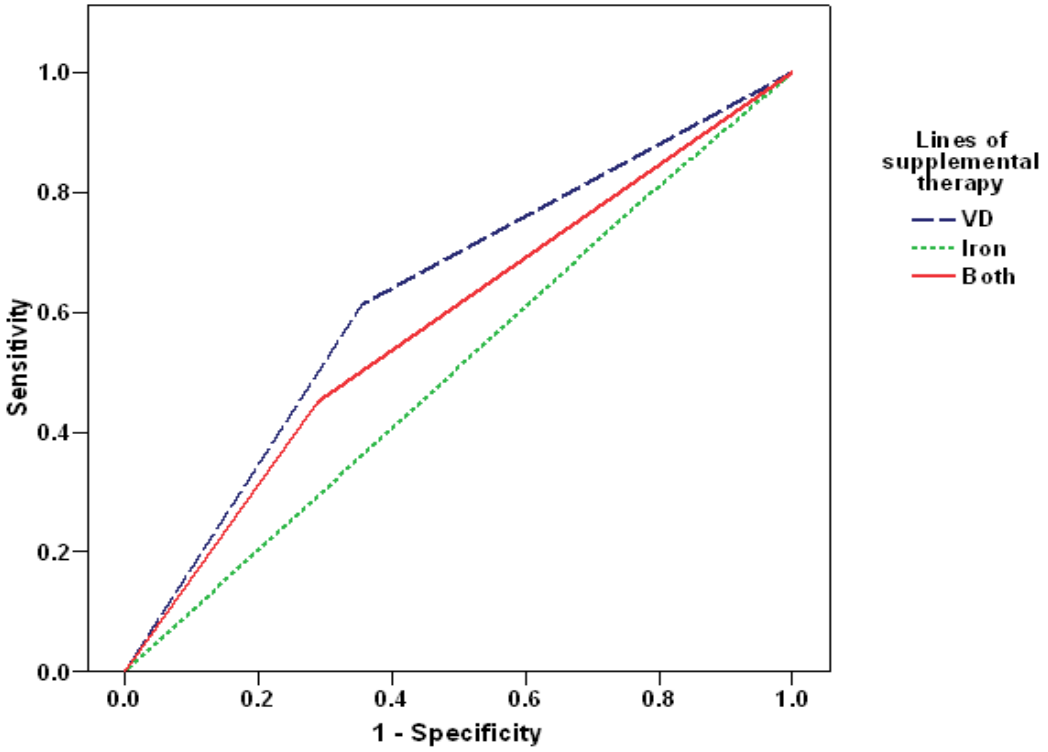

Fig. 2:-ROC curve analysis of lines of supplemental therapies for prevention or minimization of development of PAA at time of delivery

Discussion:-

At the 1st trimester, 71.7% women were anemic and 38.5% had hypoferremia indicating decreased iron stores, such frequency is in line with **Breymann** [9] who documented that worldwide prevalence of IDA ranged between 20-80% and the majority is females. Also, **Abbas et al.** [28] detected frequencies of anemia, hypoferremia and IDA of 57.7%, 21.3% and 12.1% among Sudanese pregnant women. Moreover, **Milman et al.** [29] reported that about 20-35% of European women of reproductive age had sufficient iron stores to complete a pregnancy without developing ID or IDA without receiving iron supplements

The obtained results illustrated the deleterious effect of pregnancy on maternal iron homeostasis as manifested by the reported frequency of anemic women at time of delivery among control women who were not anemic and had sufficient VD and FC at the 1st trimester and the mean HC estimated at time of delivery was significantly lower than that estimated at 1st trimester with a median deficit of 4.72 gm/dl (IQR: 2.8-6.4) among studied population.

These results go in hand with **Milman et al.** [29] who suggested that between 32-39 wk GA, prevalence of ID and IDA was 28-85% and 21-35%, respectively, among European women and **Bah et al.** [30] who found the prevalence of anemia increased from 34.6% at 14 wk to 50% at 20 wk GA. Recently, **Ahmed et al.** [31] reported an overall frequency of anemia, ID and IDA of 34.7%, 27% and 13.4% of their series of pregnant women.

At the 1st trimester, 77% of the studied population had VDD, 111 women had both VDD and PAA, 33 women had VDD with normal HC and 23 were anemic without VDD. Thus, a relationship between both VDD and PAA was evident in 59.4% of studied population.

Multiple clinical studies detected a similar association where **Michalski et al.** [32] found low vitamin D status may be linked to reduced HC in pregnant women. Also, **Yuan et al.** [33] reported that maternal serum 25OH-VD < 50.0 nmol/L may be a risk factor for gestational anemia, and it should be monitored for the high-risk pregnant women. Recently, **Al-Ajlan et al.** [34] detected VDD (< 50 nmol/l) in about 82.5% of 515 pregnant Saudi women during the

1st trimester. Also, **Krieger et al.** [35] detected 53.4% prevalence of VDD in their sample of pregnant women in Switzerland and **Wheeler et al.** [36] documented that VDD in women in New Zealand is very common during pregnancy and lactation and accounted for a prevalence of 65%.

The results of the current study showed significantly higher serum levels of HPC and IL-6 in anemic women especially those had VDD. In support of the association between VDD and inflammatory processes manifested as high serum levels of pro-inflammatory cytokines, **Akoh et al.** [37] found maternal serum 1,25-$(OH)_2$VD was significantly inversely associated with serum TNF-α especially at delivery and suggested that maternal VDD may increase risk of infection across gestation.

Statistical analyses defined high serum HPC and IL-6 levels at 1st trimester as specific early predictors for development of PAA at time of delivery. These findings are in accordance with **Bah et al.** [30] who found HPC was superior to hemoglobin and sTfR as an indicator of ID with high AUC^{ROC} values for HPC to detect ID since the 14th wk GA. **Cutone et al.** [38]**, (2017)** explored the relationship between anemia and inflammation and found increased IL-6 and IL-1β induced up-regulation of cytosolic ferritin and down-regulation of FPN leading to intracellular iron overload that induces higher host susceptibility to infections and ID with subsequent anemia. Also, **Rosa et al.** [39] attributed the curative effect of bovine lactoferrin on anemia of inflammation to its anti-inflammatory activity against IL-6 with subsequent up-regulation of FPN and transferrin receptor 1, while down-regulation of ferritin leading to inhibition of intracellular iron overload and so increasing iron availability for erythropoithesis.

Despite of the still active discrepancy concerning VD-ST and/or IST during pregnancy, supplemental therapy in the form of VD-ST alone, IST alone or combination fructified the outcome of pregnant women of groups B-D, respectively as manifested by the frequency of women who showed increased HC at time of delivery, despite being anemic at 1st trimester. Furthermore, statistical analyses defined early institution of ST especially concerning VD-ST is one of the best significant predictors for improved HC at time of delivery.

In support of the role of ST, the frequency of PAA reported at time of delivery in control women who had within normal range HC and serum VD and FC at 1st trimester and in support of the combined ST, the frequency of PAA at time of delivery in women of group A and the decreased frequency in group D.

In line with the reported outcome, **Krieger et al.** [35] documented that VD supplement intake was the most actionable determinant of VD status at the 3rd trimester and in neonatal cord blood, suggesting that VD-ST during pregnancy should receive more attention in clinical practice. Also, **Wheeler et al.** [36] recommended 1st trimester maternal VD screening and targeted supplementation for those "at risk".

The reported beneficial effect of VD-ST could be attributed to multiple mechanisms illustrating how VD can control iron homeostasis; where it was supposed that VDD induces local calcitriol production by bone marrow with concomitant increased membrane calcium permeability with susbsequent decline of erythropoiesis [40]. Later on these theoretical suggestions were approved experimentally to occur at mRNA and protein levels [41, 42]. Another supposition was that VDD induces hyperparathyroidism with subsequent increased proliferation of erythroid progenitor cells [43]. Clinically, multiple suggestions were provided for the association between VDD and anemia as **Thomas et al.** [44] attributed the effect of VDD on HC in pregnant women to both a direct effect and an indirect effect mediated by erythropoietin. Interestingly, **Azizi-Soleiman et al.** [45] detected a positive relationship between iron status and VD and attributed it a possible reciprocal relation where VDD is associated with higher HPC level causing reduction of iron release which in turn induced reduction of activation of hydroxylases that yield calcitriol, so constituting a vicious circle. Recently, **Liu et al.** [46] experimentally in antenatal lipopolysaccharide-treated rats found VD increased the methylation percentage of the VD-response element in IFN-γ-promoter region and suppressed its LPS-induced and reported clinically an association between maternal VD exposure during pregnancy and neonatal IFN-γ levels, so suggested that supplementation of VD could suppress IFN-γ production with its hazardous maternal, fetal and neonatal effects.

Conclusion:-

Vitamin D deficiency, hypoferremia, higher HPC and IL-6 constitute a vicious circle entrapping the pregnant women and inducing iron deficiency anemia at time of delivery. Early institution of supplemental therapy of vitamin D and iron can burst this circle and induce improvement of HC up to the normal range in about 30% of women presented by PAA. Thus, early estimation of HC and serum VD can be used as a guide for early institution of ST.

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