Nature and Science

Websites:
http://www.sciencepub.net/nature
http://www.sciencepub.net

Emails:
naturesciencej@gmail.com
editor@sciencepub.net

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Association between Vitamin D Deficiency and Serum Ferritin and Preterm labor: A Case Control Study

[1] Madiha Mohamed Hanafy, [1]Magdy Olama, and [2]Enshrah Sayed Kamel Ali

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Al-Azhar University, Egypt.
[2]Resident of Obstetrics and Gynecology in El. Hawamdiya Hospital, Egypt.
annoshsayed30@yahoo.com

Abstract: Background: Preterm labor refers to labor from the beginning of fetal viability and the 37 weeks of pregnancy. It is responsible for around 10% of all births and 75% of perinatal mortality and 50% of long-term morbidity. Many studies had been performed discussing the role of vit. D or serum ferritin levels and their association with preterm labor. Objective: To evaluate the relation between vitamin D deficiency and maternal serum Ferritin level and preterm labor. Subjects and Methods: A Case Control Study had been conducted on 60 pregnant women at Department of Obstetrics and Gynecology, El. Hawamdiya hospital. They divided into two groups: Group A: study group (30 cases): Patients presented with established preterm labor within 28 weeks to < 37 weeks gestation i.e. (preterm labor > 20 week to < 37 week, but the cases < 28 week were excluded from the present study). Group B: control group (30 cases): Patients with uncomplicated pregnancies who delivered at early term (37 week and 0 days to 38 week and 6 days) and full-term (39 weeks to 40 week and 6 days). Results: Receiver operating curve (ROC) has been used to determine the cut off value of vitamin D and serum ferritin in prediction of preterm labor. The ROC results revealed that Vitamin D cut off value is lower than 20ng/ml and serum ferritin cut off value is greater than 100ng/ml. The area under the ROC curve was equal to 0.973, and 0.966 respectively indicating that it could be considered as a fair predictor for preterm labor. The sensitivity values of vitamin D and serum ferritin were 93.3%, and 93.3%, respectively. The specificity values of vitamin D and serum ferritin were 90.0% and 86.7% respectively. Conclusion: The results of the present study have been provided further support to the association between maternal low level of vitamin D and risk of preterm delivery. The current study showed that a cut off ≤ 20 ng/ml of serum 25 (OH) D levels for prediction of preterm labor. Also, the results showed that there is an association between elevated concentrations of maternal serum ferritin during pregnancy and the sPTB risk. This implies that an inflammatory process that can be detected in early pregnancy can be a plausible biological mechanism for this association. The current study had helped to evolve a cut off value of ≥ 100 ng/ml of serum ferritin for prediction of preterm labor.

[Madiha Mohamed Hanafy, Magdy Olama, and Enshrah Sayed Kamel Ali. **Association between Vitamin D Deficiency and Serum Ferritin and Preterm labor: A Case Control Study.** *Nat Sci* 2020;18(7):40-47]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 6. doi:10.7537/marsnsj180720.06.

Keywords: Premature rupture of membranes, Vitamin D, Spontaneous preterm birth, Preterm Delivery

1. Introduction

Preterm labor refers to labor from the beginning of fetal viability and the 37 weeks of pregnancy. It is responsible for around 10% of all births and 75% of perinatal mortality and 50% of long-term morbidity **(Movahedi et al., 2016).**

Premature labor pathophysiology is still widely unknown. There is evidence, however, that subclinical infections, intrauterine infections and chronic inflammation are significant risk factors that result in premature labor and premature membrane rupture. **(Koucky et al., 2017).**

Ferritin which has a central role in iron homeostasis, response to acute and chronic infection is also released by infiltration of leukocytes. A number of earlier studies have shown a correlation between high serum ferritin concentration and preterm delivery. The elevated ferritin levels may be an acute phase reaction to subclinical genital tract infection or inflammation. Damage to tissue caused by infection leads to increased levels of serum ferritin acting as an acute phase reactant **(Movahedi et al., 2016).**

Some studies have described the association between vitamin D deficiency during pregnancy and an increased risk of premature birth. **Wagner et al. (2015)** reported that pregnant women with serum vitamin D concentrations below 20 ng / mL had a preterm birth rate of 3.81 times higher than those with serum vitamin D concentrations above 40 ng / mL. **Bodnar et al. (2015)** stated that the risk of preterm birth decreased significantly as the serum concentrations of 25-hydroxyvitamin D increased to approximately 36 ng / mL and then plateaued.

Aim of the Work

The purpose of this study was to assess the relationship between the deficiency of vitamin D and maternal serum Ferritin level and preterm labor.

2. Patients and Methods

Type of the study: A Case Control Study.

Site of the study: Study had been conducted at Department of Obstetrics and Gynecology, El-Hawamdiya hospital.

This study included sixty patients divided into two groups:

Group A: study group (30 cases): Patients presented with established preterm labor within 28 weeks to < 37 weeks gestation i.e. (preterm labor > 20 weeks to < 37 weeks, but the cases < 28 weeks were excluded from the present study).

Group B: control group (30 cases): Patients with uncomplicated pregnancies who delivered at early term (37 weeks and 0 days to 38 weeks and 6 days) and full-term (39 weeks to 40 weeks and 6 days).

Preterm labor is characterized as frequent uterine contractions that lead to changes in the cervix which include effacement and dilation that start before 37 weeks of pregnancy.

All patients in both groups had been subjected to the following:

Inclusion criteria: Singleton pregnancy. Maternal age:20 -35 years. Parity: primipara or multipara. Patients with hemoglobin concentration: Non anemic patients hemoglobin (≥ 11 gm/dl). Mild anemia patients hemoglobin (≥ 9.5 gm/dl - 11 gm/dl).

Exclusion criteria: Anemia: Moderate anemia (< 9.5gm/dl). Severe anemia (< 7 gm/dl). Iron overload state especially iron transfusion and blood transfusion**.** Congenital uterine abnormalities and incompetent cervix. Multiple pregnancy**.** Polyhydramnios**.** Premature rupture of membranes. Diabetes mellitus**.** Pre-eclampsia and eclampsia**.** The disease of the liver. Renal disease and malignancies**.** Foetal congenital anomalies. Morbid obesity (BMI of ≥ 35 kg/m2).

Methods:

All patients enrolled in this study had the following: Verbal consent: was obtained from the pregnant women on whom the study was performed and they were informed about the objectives of the study. **Complete history taking. Physical examination**: General examination: vital data (pulse, blood pressure and temperature). Abdominal examination: with particular emphasis on fundal height, clinically estimated fetal weight and presence of uterine contractions. Pelvic examination: condition of the membranes, presentation, cervical status (dilatation, length, consistency and position). **Investigations in the form of:** Ultrasonography to ensure fetal life, gestational age, excludes congenital fetal malformation, liquor abnormalities. Laboratory: Complete blood picture. Serum ferritin concentration. Vitamin D assay. Serum creatinine. Randum blood sugar.

Newborn assessment: APGR score, body weight and sex at delivery time.

Measurement of serum ferritin level: Blood sample was taken from all women at 28-36 weeks of gestation. Assay of Ferritin was performed using Enzyme-Linked Fluorescent Assay (ELFA) technique.

Blood sampling and storage: Three millliters of venous blood sample were taken from all women at 28-36 weeks were drawn from the antecubital vein using a disposable plastic syringe and left to clot for 30 minutes. The sample was then centrifuged and serum was separated and kept at - 20°C till assay time. Serum ferritin levels were measured by ELFA technique **(Kimber et al., 1983)**.

Assay procedure: VIDAS Ferritin (Vidas kits of bioMérieux French company) assay is an enzyme-linked fluorescent immunoassay (ELFA) Executed on an automated instrument. Both the test steps and the assay temperature were monitored by the instrument. The Solid Phase Receptacle (SPR ®) is a disposable device which looks like a pipette tip. At the time of manufacture, the SPR was coated with monoclonal anti-ferritin mouse antibodies. The configuration of the VIDAS Ferritin assay prevents non-specific reactions with the SPR. The sample was transferred to a well containing antiferritin conjugated with alkaline phosphatase. The sample / conjugate mixture was cycled inside and outside of the SPR, and the ferritin will bind to SPR-coated antibodies and the conjugate forming a "sandwich". Wash steps to remove the unbound conjugate.

A 4-methylumbelliferyl phosphate, a fluorescent base, was cycled through the SPR.

The remaining enzyme on the SPR wall will catalyze the substrate conversion to 4-methylumbelliferone, a fluorescent product. The fluorescence intensity was determined via the optical scanner of the instrument; it is proportional to the concentration of ferritin in the sample. Upon completion of the VIDAS Ferritin assay, the instrument analyzed the findings automatically and a report was printed for each sample.

Measurement of vitamin D level: All samples were screened to measure 25-OH vitamin D (vitamin D2 and vitamin D3) in serum with 25-Hydroxy (25-OH) vitamin D ELISA. The kit name: (The Calbiotech, Inc.25-hydroxy (25-OH) Vitamin D ELISA was used as manufacturer`s instructions.

Sample collection and preparation: Blood samples (5 ml) were collected by venipuncture into test tube with no additives, clotting was allowed then centrifuged for 10 minutes at 5.000 g to separate the serum at laboratory of obstetrics and gynecology department. Serum was transported to be frozen at -20° C.

Statistical analysis:

Based on Microsoft Excel software, data collected over the course of the history, basic clinical assessment, laboratory testing and outcome measures have been coded, entered and analyzed. SPSS (Statistical Package for Social Science), version 25 (Armonk, NY: IBM Corp) has tabulated and analyzed data collected from on an IBM compatible computer.

There were two statistical types:

Descriptive statistics: Depending on the type of qualitative data, the quantitative continuous group represents a mean ± SD as a number and percentage.

Analytic statistics: Chi-square test ($\chi 2$): used to compare and associate two qualitative variables with each other. **Independent samples Student t-test (T):** is the significance test used for comparison between two groups of normal distribution quantitative variables A P-value of ≤ 0.05 was considered statistically important. The collected and submitted information for statistical analysis. The following parameters and statistical tests were used.

3. Results

Table (1): Comparison of the two groups studied according to maternal age and Gestational age

Parameter		Groups		T-test of sig.	P-value
		Study Group	Control Group		
Maternal age (years)	Range	20 - 30	20 - 32	-0.573	0.569
	Mean ±SD	25.10 ± 3.155	25.57 ± 3.159		
Gestational age (weeks)	Range	29 - 36	37 - 40	-14.83	0.001**
	Mean ±SD	32.83 ± 1.895	38.27 ± 0.740		

The means of maternal age (years) were nearly equal between both groups with no statistically significant difference (p>0.05), while the means of the gestational age were significantly lower in the study group in comparison to that of the control group (p-value=0.001) (Table 1).

Table (2): Comparison of the two groups studied according to parity and abortion:

Parameter		Groups		X^2 -test of sig.	P-value
		Study Group	Control Group		
Parity No (%)	0	7 (23.3%)	12 (20%)	3.393	0.494
	1	14 (46.7%)	10 (33.3%)		
	2	7 (23.3%)	6 (20%)		
	3	2 (6.7%)	1 (3.3%)		
	4	0 (0.0%)	1 (3.3%)		
Abortion No (%)	0	16 (53.3%)	20 (66.7%)	1.130	0.568
	1	8 (26.7%)	6 (20%)		
	2	6 (20%)	4 (13.3%)		

This table showed that there was no statistically significant difference between the two patients' groups as regards parity and abortion (Table 2).

Table (3): Comparison of the two groups studied according to Laboratory data.

Parameter		Groups		T-test of sig.	P-value
		Study Group	Control Group		
Vit D	Range	5.0 - 30.7	10.0 - 50.0	-11.298	0.001**
	Mean ±SD	11.067 ± 5.4794	33.880 ± 9.6071		
Ferritin	Range	75 - 615	20 - 158	8.416	0.001**
	Mean ±SD	283.67 ± 136.00	68.40 ± 33.666		
HB	Range	10 - 12.2	10.2 - 12.3	0.110	0.913
	Mean ±SD	11.12 ± 0.591	11.103 ± 0.5857		
HB (9.5-11) (N=32)	Range	10 - 11.00		-0.056	0.955
	Mean ±SD	10.66 ± 0.33	10.66 ± 0.29		
HB >11 (N=28)	Range	11.2 - 12.3		0.310	0.759
	Mean ±SD	11.64 ± 0.31	11.60 ± 0.41		

The mean of Vit D was significantly lower in the study group than the control group (p-value=0.001), while the mean of the serum ferritin in study group was significantly higher than the control group (p-value=0.001).

As regards HB levels there were no statistically significant differences between two groups (p-value>0.05) (Table 3).

Table (4): Comparison of the two groups studied according to newborn sex.

Parameter		Groups		Test of sig.	P-value
		Study Group	Control Group		
Newborn Sex No (%)	Male	21 (70%)	14 (46.7%)	X^2 =3.36	0.067
	Female	9 (30%)	16 (53.3%)		

This table showed no statistically significant difference between two patients' groups (p- value>0.05) (Table 4).

Table (5): Correlation between gestational age with maternal age, Vit. D, maternal serum ferritin and HB.

Studied Parameters	Gestational age (week)	
	Spearman's Correlation	p-value
Maternal age (years)	0.146	0.460
Vit D	0.660	0.001**
Maternal serum ferritin	-0.742	0.001**
HB	-0.044	0.824

P value>0.05 insignificant, * P-value≤0.05 significant, **P- value ≤0.001 highly significant

The Table showed according to Spearman's rank correlation coefficient analysis, gestational age (weeks) was positively correlated with: Vit D where (r = 0.660, P = 0.001). And the Gestational age (weeks) was negatively correlated with: Ferritin where (r = -0.742, P = 0.001). And there was non-significant correlation with maternal age (years) and HB (Table 5).

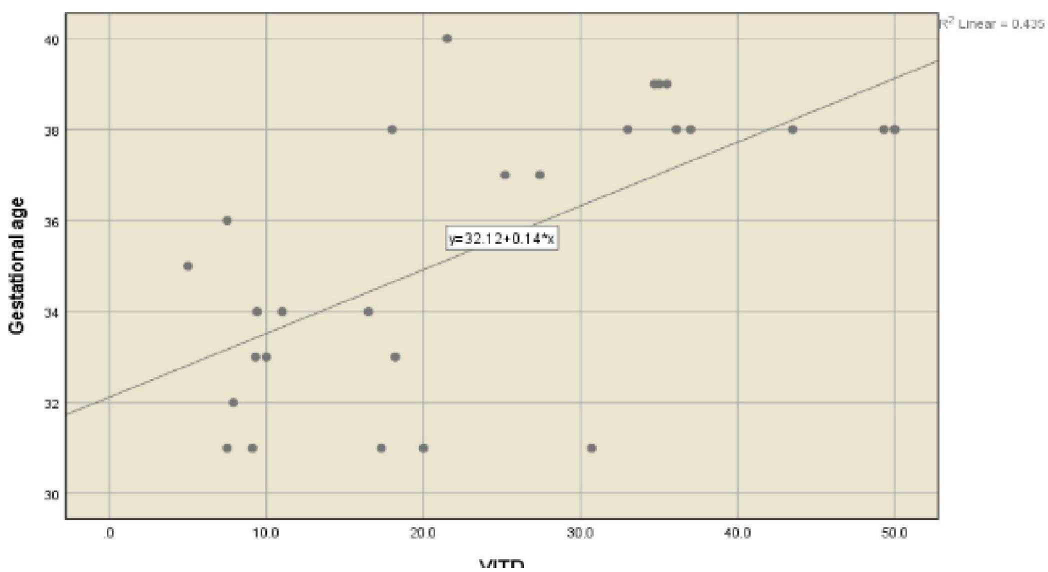

Figure (1): Correlation between gestational age (weeks) with Vit.D.

Table (6): Vitamin D Deficiency and Serum Ferritin as a predictor of preterm labor.

Test Result Variable (s)	Cut off*	AUC	P-value	95% C.I.		Sensitivity	Specificity
Vitamin D	≤ 20	0.973	0.000	0.938	1.00	93.3%	90.0%
Serum Ferritin	≥ 100	0.966	0.000	0.927	1.00	93.3%	86.7%

AUC: Area Under a Curve p value: Probability value CI: Confidence Intervals

*: Statistically significant at p ≤ 0.05; #Cut off was choose according to Youden index

(Figure 1, Table 6)

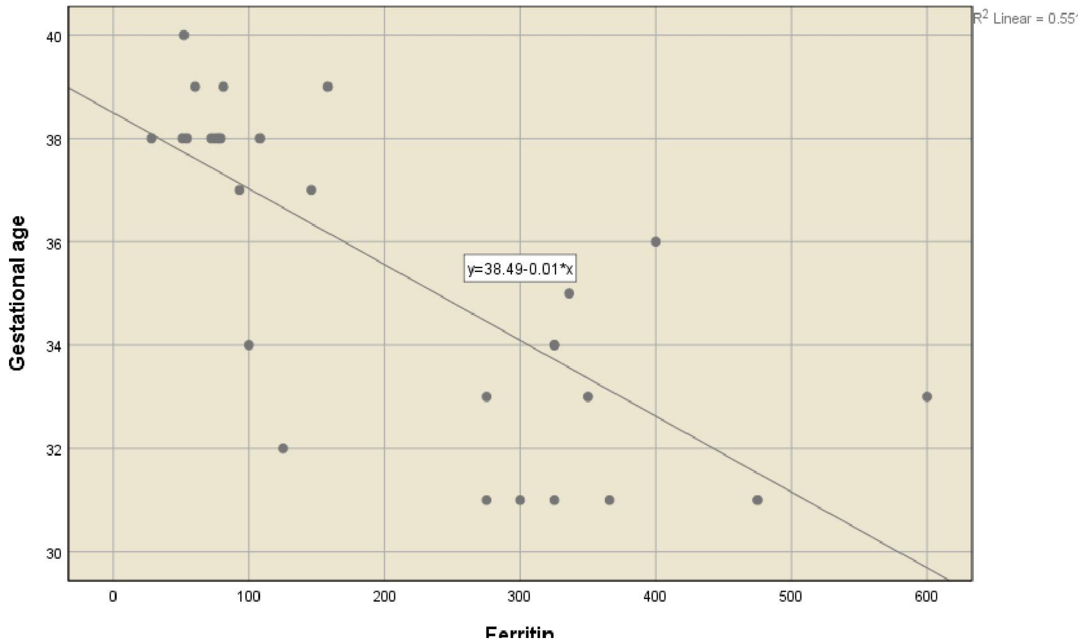

Figure (2): Correlation between gestational age (weeks) with maternal serum ferritin.

Receiver operating curve (ROC) was used to determine the cut off value of vitamin D and serum ferritin in prediction of preterm labor. Our ROC results revealed that vitamin D cut off value is lower than 20nmol/L and serum ferritin cut off value is greater than 100ng/ml and the area under the ROC curve is equal to 0.973, and 0.966 respectively indicating that they are fair predictors for preterm labor. The sensitivity values of vitamin D and serum ferritin were 93.3%, and 93.3% respectively and the specificity values of vitamin D and serum ferritin were 90.0% and 86.7% respectively (Figures 2, 3, 4).

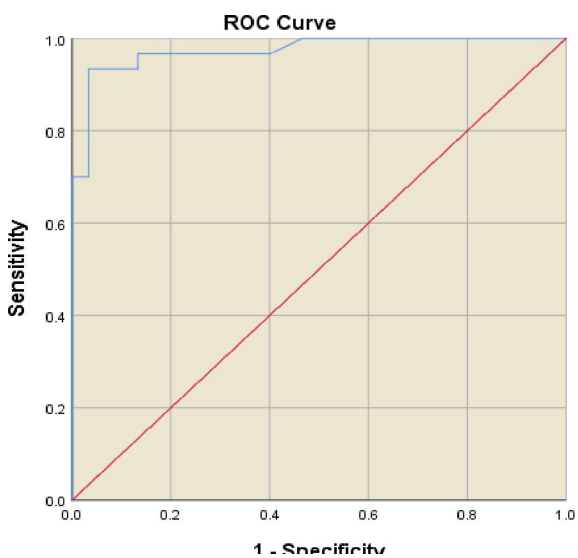

Figure (3): ROC curve of Vitamin D.

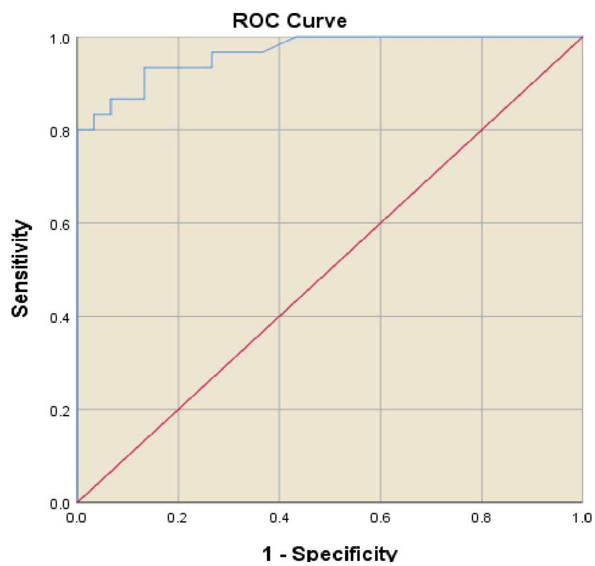

Figure (4): ROC curve of serum ferritin.

4. Discussion

In the early 20th century, vitamin D was classified as a vitamin, and as a prohormone in the second half of the 20th century ("conditional" vitamin). "It is a rare nutrient since it can be endogenously synthesized (skin) and act as a hormone. Impaired vitamin D status during pregnancy is associated with adverse outcomes of pregnancy such as preterm birth and poor neonatal outcome **(Bener et al., 2013).**

Preterm birth is the most daunting problem in obstetrics worldwide, but it was difficult and ineffective to avoid premature birth due to its multifactorial and partially still unknown etiology **(Bodnar et al., 2015).**

However, infections alone can be associated with up to 40% of spontaneous preterm births, particularly those occurring at an early gestational age. In the last two decades, the relationship between maternal genital tract infections and increasing choriodecidual interface infections leading to premature birth has been of particular interest **(Weinert & Silveiro, 2015).**

Maternal 1, 25-(OH)2D in normal pregnancy gradually increases from the first trimester to a rate of twice the point of non-pregnancy in the third trimester. Pregnancy doesn't alter the 1,25-(OH)2D clearance. The rise in maternal serum levels is attributed to increased production and a rise in the protein binding vitamin D **(Shibata et al., 2011).**

Potential mechanisms that result in high levels of ferritin associated with the risk of sPTB include: intrauterine infection; failure to expand maternal plasma volume; infection and inflammation. As part of the acute response, ferritin production increases with infection and inflammation, so interpretation of these studies is challenging. **(Scholl, 2005).**

This is why this study was selected to be conducted to evaluate the relation between vitamin D deficiency and maternal serum Ferritin level and preterm labor.

In the present study (Table-1) showed that the mean age in the study group was 25.10±31.155 and in the control group it was 25.57± 31.59 years. Comparison between the two groups showed that means of age were nearly equal between both groups with no significant differences (p>0.05), while the mean value of the gestational age in study group (32.83 ± 1.895) weeks was lower than that of control group (38.27 ± 0.740) weeks with a significantly high difference (p- value=0.001).

The findings of the this study were in agreement with study of **Khambalia et al. (2015)** as they reported that age of majority of the studied groups was between 25-34 years with no significant difference between both groups p= 0.36. Furthermore, **Bodnar et al. (2015)** found that there was no significant difference among their studied groups as regard age of mothers who had preterm labour. Most of them had age between 20-34 years.

In the present study (Table-2) showed that there was no statistically significant difference between the two groups as regards parity and abortion.

In the current study (Table-3) showed that the mean of Vit D in study group was 11.067 ± 5.4794ng/ml and in the control group it was 33.880 ± 9.6071 ng/ml. Comparison between the two groups showed that, the mean of Vit D in the study group was significantly lower than in the control group (p-value=0.001). In (Table-5), gestational age (weeks) was positively correlated with Vit D where (r = 0.660, P = 0.001). In (Table-6), ROC results revealed that vitamin D cut off value was lower than 20ng/ml and the area under the ROC curve was equal to 0.973, indicating that it could be considered as a fair predictor for preterm labor. The sensitivity values of Vitamin D were 93.3%, and the specificity values of Vitamin D were 90.0%.

In a meta-analysis of observational studies done by **Qin et al. (2016)** there were total eleven studies out of 237 studies included in this meta-analysis study; six studies conducted in the United States of America, two in Spain, and one in Australia, China, and Canada. In this meta-analysis, they found pregnant women with low levels of vitamin D have an increased risk of premature delivery, and the Odds ratio was 1.29; 95% confidence interval was 1.16, 1.45. Cochrane Review showed that supplementation with vitamin D during pregnancy reduces the incidence of low birth weight (<2.5 kg) by 52%.

In the same way, in a meta-analysis of 24 observational studies by **Wei et al. (2013)** they found association between maternal serum level of vitamin D less than 50 nmol/l and preterm delivery.

The results of the present study were supported by the study of **Abd El Hameed et al. (2018)** as they reported that preterm group mothers had a significantly lower serum vitamin D level with a mean of 4.48± 2.5ng/ml compared to 24.9 ±13.7ng/ml among full term mothers.

Mehrdad et al. (2013) stated that sufficient vitamin D would protect against preterm birth from a preliminary study of a large cohort of pregnant women complemented with vitamin D in South Carolina, and their initial findings showed that preterm birth risk among women taking vitamin D was reduced at 37 and 32 weeks. They also reported that mean gestational age was 38.8 ± 1.8 weeks in women with 2000 IU vitamin D supplementation.

On the other hand **Flood-Nichols et al. (2015)** found no association between low maternal serum vitamin D and preterm delivery and spontaneous abortion, odds ratio was1.01 (p value =0.738, 95% Confidence Interval was 0.947–1.164).

However, **Wetta et al. (2014)** found after adjusting for cofounders (age, race, parity, weight, prior preterm birth and season of specimen collection), there was no association between deficiency (less than 15ng/ml) or insufficiency (less than 30 ng/ml) of vitamin D during mid-trimester and preterm delivery (prior to 35week gestation).

In the present study (Table-3) showed that the mean value of the serum ferritin in study group (283.67 ± 136.00 ng/ml) was significantly higher than that of the control group which was (68.40 ± 33.666 ng/ml) (p-value=0.001). In (Table-5) gestational age (weeks) had a strong association with serum ferritin where (r = - 0.742, P = 0.001). In (Table-6), ROC results revealed that serum ferritin cut off value was greater than 100ng/ml and the area under the ROC curve was equal 0.966 indicating that it could be considered as a fair predictor for preterm labor. The sensitivity values of serum ferritin were 93.3% and the specificity values of serum ferritin were 86.7% respectively.

The results of the present study were supported by the study of **Khambalia et al. (2015)** as who suggested Serum ferritin levels were increased as part of the acute phase response and the sPTB-related inflammatory process was evident from the first trimester of pregnancy. The results of previous studies that examined the association between high levels of serum ferritin and preterm birth were inconsistent **(Wen et al., 2004, Weintraub et al., 2005).**

In the study of **Khambalia et al. (2015)**, Serum ferritin levels 75th percentile (≥ 43 mg / l) were found to be correlated with increased sPTB (37 weeks) and moderate to late sPTB (34–36 weeks) subcategories. Nevertheless, only the higher (90th percentile) serum ferritin threshold (≥68 mg / l) was significantly associated in the study with early sPTB.

Weintraub et al. (2005) published similar findings showing that serum ferritin levels below 30 mg / l were linked to with preterm birth. Inconsistent results across studies may be correlated with variations in sample populations and the severity of sPTB, decreased numbers of women in certain categories of exposure and/or outcome and the types of confounders used in the adjusted analyses. Past studies were mainly cross-sectional, and later in pregnancy or at birth, limited to serum ferritin tests **(Xiao et al., 2002).**

In the present study (Table-3) showed that there were no significant differences regarding Hb levels and its relation to preterm delivery (p-value>0.05). In (Table-5), there was non-significant correlation with gestational age (weeks) & Hb levels.

In the study of **Zhang et al. (2018**), maternal Hb concentrations weren't associated with the first or second trimester risk of premature birth. Nevertheless, in the first trimester, women with low Hb concentration along with high Hb concentration in the second trimester had an increased risk of premature birth relative to women with higher Hb concentration in the first trimester who remained comparable in the second trimester. As observed in (Table-4) in the present study the newborn sex in the study and control groups were males [21(70%) – 14 (46.7%)] and females [9(30%) – 16 (53.3%)], respectively. There were no significant differences between both groups.

However, **McGregor et al. (1992)** reported that, at 33 to 36 weeks of gestation, males were more likely to deliver than females (OR = 1.21; 95% CI: 1.02-1.42). This rise in PTB among males was not followed by an increased number of males with low birth weight; rather, between 2000 and 2499 gm (OR = 0.71; 95 % CI: 0.60-0.84) were less likely than females to weigh. The gender imbalance in PTB could not be clarified by the growing occurrence of PROM, chorioamnionitis, endometritis or other infection-related processes. Their results indicate that shorter gestation may be related to our comparatively increased size and birth weight in males in this population. During pregnancy, male gender-associated factors predisposing to infection-mediated preterm birth play a greater role in populations at higher risk of infection of the reproductive tract.

Likewise, in most populations, including IVF births (odds ratio: 1.09–1.24), **Zeitlin et al. (2002)** found that, both preterm and early preterm births there were more males than between preterm births. Induced early births in the general population with two cohorts of black births, and spontaneous onset after IVF.

Conclusion

The results of this study further assisted an association between low maternal vitamin D levels and risk of premature delivery. The current study showed that a cut off ≤ 20 ng/ml of serum 25 (OH) D levels for prediction of preterm labor. Also, the result showed that, there's a correlation between high maternal serum ferritin levels during pregnancy and the risk of sPTB. Importantly, this indicates that a possible biological mechanism could be used for this association with an inflammatory process that is detectable in early pregnancy. The current study had helped to evolve a cut off value of ≥ 100 ng/ml of serum ferritin for prediction of preterm labor.

References

1. Abd El Hameed SM, Mansour DY, Zaghla HMAA, Rishi SH (2018): Association between Vitamin D Deficiency and Preterm: A Case Control Study. The Egyptian Journal of Hospital Medicine, 73(3), 6198-6205.
2. Bener A, Al-Hamaq AO, Saleh NM (2013): Association between vitamin D insufficiency and adverse pregnancy outcome: global comparisons. International journal of women's health, 5: 523.
3. Bodnar L, Platt R, Simhan H (2015): Early pregnancy vitamin D deficiency and risk of preterm birth subtype: Obstet. Gynecol., 125:439– 447.
4. Flood-Nichols SK, Tinnemore D, Huang RR et al. (2015): Vitamin D deficiency in early pregnancy. Plos One, 10(4): e0123763.
5. Khambalia AZ, Collins CE, Roberts CL, Morris

JM, Powell KL, Tasevski V, Nassar N (2015): High maternal serum ferritin in early pregnancy and risk of spontaneous preterm birth. British Journal of Nutrition, 114(3): 455-461.
6. Kimber R, Rusaki Z, Blunden R (1983): Immunodeficiency and iron over load: Serum ferritin and serum iron in clinical medicine. Pathology;15:497-503.
7. Koucky M, Germanova A, Hajek Z, et al. (2017): Pathophysiology of preterm labour: Prague Medical Report 110:13–24.
8. McGregor JA, Leff M, Orleans M, Baron A (1992): Fetal gender differences in preterm birth: findings in a North American cohort. American journal of perinatology. 9(01):43-8.
9. Mehrdad S, Mohamad R, Iranmanesh Y (2013): Vitamin D requirement in pregnancy to prevent deficiency in neonates: a randomised trial. Singapore Med J.; 54(5):285-8.
10. Movahedi M, Saiedi M, Gharipour M, et al. (2016): Diagnostic performance and discriminative value of the serum ferritin level for predicting preterm labor. Journal of Research in Medical Sciences: the Official Journal of Isfahan University of Medical Sciences 17:164–166.
11. Qin LL, Lu FG, Hui S, et al. (2016): Does Maternal Vitamin D Deficiency Increase the Risk of Preterm Birth: AMeta-Analysis of Observational Studies. Nutrients, 8(5):301.
12. Scholl T (2005): Iron status during pregnancy: setting the stage for mother and infant. Am J Clin Nutr 81: 1218–1222.
13. Shibata M, Suzuki A, Sekiya T, Sekiguchi S, Asano S, Udagawa Y, Itoh M (2011): High prevalence of hypovitaminosis D in pregnant Japanese women with threatened premature delivery. Journal of bone and mineral metabolism, 29(5): 615-620.
14. Wagner C, Baggerly C, McDonnell S, et al. (2015): Post-hoc comparison of vitamin D status at three time points during pregnancy demonstrates lower risk of preterm birth with higher vitamin D closer to delivery. J. Steroid Biochem. Mol. Biol., 148:256–260.
15. Wei SQ, Qi HP, Luo ZC, Fraser WD (2013): Maternal vitamin D status and adverse pregnancy outcomes: a systematic review and meta-analysis. The Journal of Maternal-Fetal & Neonatal Medicine, 26(9): 889-899.
16. Weinert LS and Silveiro SP (2015): Maternal–fetal impact of vitamin D deficiency: a critical review. Maternal and Child Health Journal, 19(1): 94-101.
17. Weintraub A, Sheiner E, Mazor M, et al. (2005): Maternal serum ferritin concentration in patients with preterm labor and intact membranes. J Matern Fetal Neonatal Med 18: 163– 166.
18. Wen S, Smith G, Yang Q, et al. (2004): Epidemiology of preterm birth and neonatal outcome.
19. Semin Fetal Neonatal Med 9: 429–435.
20. Wetta LA, Biggio JR, Cliver S, et al. (2014): Is Midtrimester Vitamin D Status Associated with Spontaneous Preterm Birth and Preeclampsia? Am J Perinatol., 31(6):541–546.
21. Xiao R, Sorensen T, Frederick I, et al. (2002): Maternal second-trimester serum ferritin concentrations and subsequent risk of preterm delivery. Paediatr Perinat Epidemiol; 16(4):297-304.
22. Zeitlin J, Saurel-Cubizolles MJ, de Mouzon J, Rivera L, Ancel PY, Blondel B, Kaminski M (2002): Fetal sex and preterm birth: are males at greater risk?. Human Reproduction. 17(10):2762-8.
23. Zhang Q, Ananth C, Li Z, Smulian J (2018): Maternal anaemia and preterm birth: a prospective cohort study. International Journal of Epidemiology; 38:1380–1389.

7/13/2020