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Association of Vitamin B_{12} and Folic Acid with Thyroid Hormones in Pregnant Women with Hypothyroidism.

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ABSTRACT

The present study was aimed to observe the association of Vitamin B12 and folic acid with thyroid profile in pregnant women with overt hypothyroidism. 85 pregnant women with overt hypothyroidism were included in the study after obtaining written informed consent. Assessment of Free T_3, Free T_4 and TSH, folic acid and vitamin B_{12} was performed by standard methods. In first trimester, positive correlation was observed between vitamin B_{12} and FT_3, which is statistically significant (r=0.505) ($P < 0.0001$). In second trimester, positive correlation was observed between vitamin B_{12} and FT_3, which is statistically significant (r=0.510) ($P < 0.0001$). Negative correlation was observed between vitamin B_{12} and TSH, which is statistically significant (-0.229) ($P<0.05$). Positive correlation was observed between folic acid and FT_3, which is statistically significant (r=0.287) ($P<0.01$). In third trimester, positive correlation was observed between vitamin B_{12} and FT_3, which is statistically significant (r=0.486) ($P < 0.0001$). Negative correlation was observed between folic acid and TSH, which is statistically significant (r=-0.228) ($P<0.05$). In cord blood samples, significant positive correlation was observed between vitamin B12 and FT3 (r=0.458) ($P<0.0001$). Negative correlation was observed between folic acid and TSH, which is statistically significant (r=-0.257) ($P<0.05$). We conclude that both Vitamin B12 and FA has significant negative correlation with TSH in all trimester while these have significant positive correlation with FT_3 , hence these values have to be monitored closely throughout the pregnancy for normal development of the fetus.

Keywords: Vitamin B_{12}, folic acid, Thyroid hormones, hypothyroidism

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INTRODUCTION

In recent years, thyroid disorders are common in pregnant women which may cause maternal as well as fetal complications. [1] During pregnancy, thyroid gland undergoes physiological changes such as enlargement and increased vascularity. [2] However, changes in thyroid function in pregnancy is still in debate as some authors reported increase and some as no change or decreased.[3,4] It was reported that, folate supplementation suppressed thyroid hormone function in the periphery and hippocampus and results in motivational and spatial memory deficits.[5] Deficiency of vitamin B12 is highly prevalent among hypothyroid patients.[6] Vitamin B12 deficiency worsens hypothyroidism. Unfortunately, both deficiencies can go unnoticed and they can be difficult to diagnose. [7] The present study was aimed to observe the association of Vitamin B12 and folic acid with thyroid profile in pregnant women with overt hypothyroidism.

MATERIALS AND METHODS

The study was approved by Institutional Human ethical committee. A written informed consent was taken from all the participants.

Patients and controls

85 pregnant women with overt hypothyroidism were included in the study. All pregnancies were dated according to ultrasonographic measurement of the foetal growth - rump length during the first trimester till the time of delivery.The following criteria were followed while selecting the patients.

Inclusion criteria:

- Willing pregnant women attending the antenatal clinic during the first trimester
- Age between 18- 45 years
- FT_4 levels below the range0.86-1.87 ng/dl and TSH levels above 2.5µIU/dl.
- TSH levels above 95th percentile and FT4 levels below 5th percentile
- without any other noted metabolic disorders

Exclusion criteria:

- History of Hypertension, Diabetes Mellitus, Thyroid Disease, Renal disease, Obesity and twins.

Laboratory setting: The current study was conducted at the Apollo general hospital, Department of Obstetrics and Gynecology and Department of Biochemistry, Apollo Institute of Medical Sciences and Research (AIMSR), Jubilee Hills, Hyderabad, Telangana.

Blood Sample collection and handling

5ml each of 12 hours fasting venous blood specimens was collected between 8am to 9am in the morning, from all the subjects in sterile silicon coated glass tube. The blood samples were allowed to stand for complete clot formation at room temperature and subsequently centrifuged for 10 minutes at approximately 3500rpm ensuring no particles or traces of fibrin. Samples were clotted at room temperature, centrifuged and aliquotted for 80ºC storage within 8 hours. Rest of the serum sample is appropriately labelled and stored at minus 80 degree centigrade until batch analysis for FT_3, FT_4, TSH, Folate and Vitamin B_{12} were analyzed.

The samples were collected thrice from each individual-once in first trimester, second trimester and in third trimester or at the time of delivery respectively. The same procedure of sample collection and handling was followed and all the samples were processed for thyroid assay and vitamin B_{12} and Folic acid estimation. Cord blood collected after delivery was immediately processed for folic acid and vitamin B_{12} parameters.

Cord blood: 3ml of cord blood was drawn into disposable plain polystyrene tubes. Cord blood is collected at birth. The samples were collected, handled and transported to the lab according to the guidelines given by clinical and laboratory standards institute/ NCCLS (National Clinical Chemistry Laboratory Standards). The

blood samples were centrifuged at 3500 rpm for 10 minutes and the serum is immediately analyzed for folate and vitamin B_{12}.

Assessment of Free T_3, Free T_4 and TSH: It was assessed by Immulite 1000, automated immunoassay analyser, continuous random access instrument based on chemiluminescent method, (IMMULITE/IMMULITE 1000 FreeT3).[8,9,10,11]

Assessment of Folate: It was assessed by Immulite 1000, automated immunoassay analyser, continuous random access instrument based on chemiluminescentmethod, competitive liquid-phase ligand-labeled protein binding chemiluminescent assay. [12]

Vitamin B12: by Elecsys 2010 and **cobase** immunoassay analyzers based on electrochemiluminescence immunoassay. Solid-phase electrode chemiluminescent emission. [13]

Data analysis: Data was analyzed by SPSS 20.0 Pearson correlation was used to determine the association of the parameters. P<0.05 was considered as significant.

RESULTS

Mean values of Free T_3, Free T_4, TSH, vitamin B12 and folic acid was presented in table no1. In first trimester, positive correlation was observed between vitamin B_{12} and FT_3, which is statistically significant (r=0.505) (P < 0.0001). Correlation between folic acid and FT_3, folic acid and TSH, vitamin B_{12} and TSH, vitamin B_{12} and FT_4, folic acid and FT_4 was not statistically significant (table 2, figure1).

In second trimester, positive correlation was observed between vitamin B_{12} and FT_3, which is statistically significant (r=0.510) (P < 0.0001). Negative correlation was observed between vitamin B_{12} and TSH, which is statistically significant (-0.229) (P<0.05). Correlation between vitamin B_{12} and FT_4 was not significant. Positive correlation was observed between folic acid and FT_3, which is statistically significant (r=0.287) (P<0.01). Correlation between folic acid and TSH and folic acid and FT_4 was not statistically significant (table 3, figure 2). In third trimester, positive correlation was observed between vitamin B_{12} and FT_3, which is statistically significant (r=0.486) (P < 0.0001). Correlation between vitamin B_{12} and TSH, vitamin B_{12} and FT_4 was not statistically significant. Correlation between folic acid and FT_3 and folic acid and FT_4 was not statistically significant. Negative correlation was observed between folic acid and TSH, which is statistically significant (r=-0.228) (P<0.05) (table 4, figure 3).

In cord blood samples, significant positive correlation was observed between vitamin B12 and FT3 (r=0.458) (P<0.0001). Correlation between vitamin B_{12} and TSH, vitamin B_{12} and FT_4 was not statistically significant. Correlation between folic acid and FT_3 and folic acid and FT_4 was not statistically significant. Negative correlation was observed between folic acid and TSH, which is statistically significant (r=-0.257) (P<0.05) (table 5, figure 4).

Parameter	First trimester	Second trimester	Third trimester	Cord blood
$FT_{3\ (pg/ml)}$	2.48±0.76	2.01±0.34	1.78±0.24	
$FT_{4(ng/dl)}$	0.60±0.19	1.03±0.20	2.10±8.33	
TSH(µlU/ml)	6.65±2.77	1.69±0.72	1.47±0.41	
Vitamin B_{12}	666.10±604.66	732.11±604.53	784.35±584.78	1061.53±618.80
Folic acid	14.49±12.02	14.65±5.11	15.44±4.55	18.61±2.36

Table 1: FT_3, FT_4, TSH, folic acid and vitamin B12 values in y pregnant women with hypothyroidism. (Data presented are mean ± SD)

Parameter	Correlation of Vitamin B12 with (r)	P value	Correlation of folic acid (r) with	P value
FT_3	0.505	<0.0001***	0.068	0.534
TSH	-0.13	0.235	0.205	0.059
FT_4	-0.021	0.846	-0.0028	0.979

Table 2: Association between Vitamin B_{12} and folic acid with thyroid hormones in first trimesters in pregnant women with hypothyroidism. (*P<0.05 is significant, **P<0.01 is significant, ***P<0.001 is significant)

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Parameter	Correlation of Vitamin B12 with (r)	P value	Correlation of folic acid (r) with	P value
FT_3	0.510	<0.0001***	0.287	0.007**
TSH	-0.229	0.034*	-0.057	0.602
FT_4	0.209	0.053	0.154	0.157

Table 3: Association between Vitamin B_{12} and folic acid with thyroid hormones in second trimesters in pregnant women with hypothyroidism. (*P<0.05 is significant, **P<0.01 is significant, ***P<0.001 is significant)

Parameter	Correlation of Vitamin B12 with (r)	P value	Correlation of folic acid (r) with	P value
FT_3	0.486	<0.0001***	0.126	0.247
TSH	0.057	0.603	-0.228	0.035*
FT_4	-0.062	0.570	0.109	0.3188

Table 4: Association between Vitamin B_{12} and folic acid with thyroid hormones in third trimesters in pregnant women with hypothyroidism. (*P<0.05 is significant, **P<0.01 is significant, ***P<0.001 is significant)

Parameter	Correlation of Vitamin B12 with (r)	P value	Correlation of folic acid (r) with	P value
FT_3	0.458	<0.0001***	0.069	0.526
FT_4	0.078	0.475	0.0613	0.577
TSH	0.027	0.804	-0.257	0.017*

Table 5: Association of cord blood Vitamin B_{12} and folic acid with thyroid hormones of third trimester. (*P<0.05 is significant, **P<0.01 is significant, ***P<0.001 is significant)

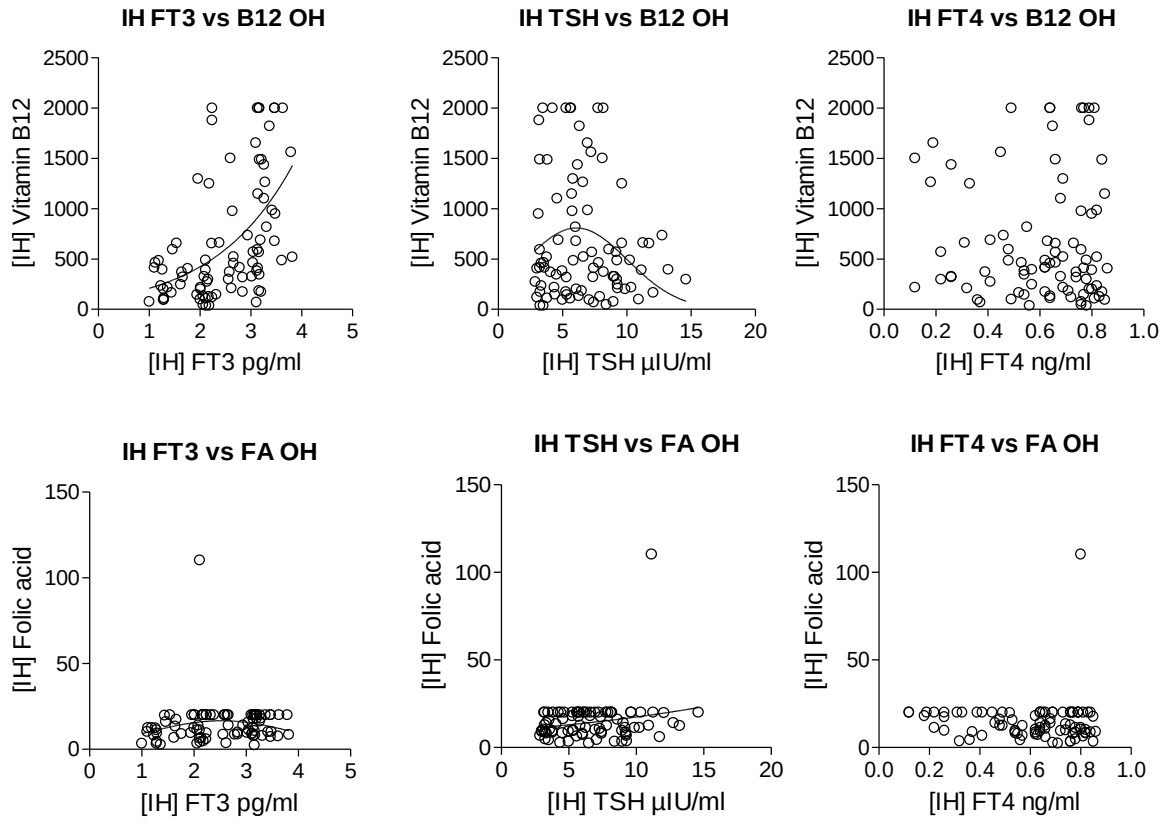

Figure1: Association between Vitamin B_{12} and folic acid with thyroid hormones in first trimesters.

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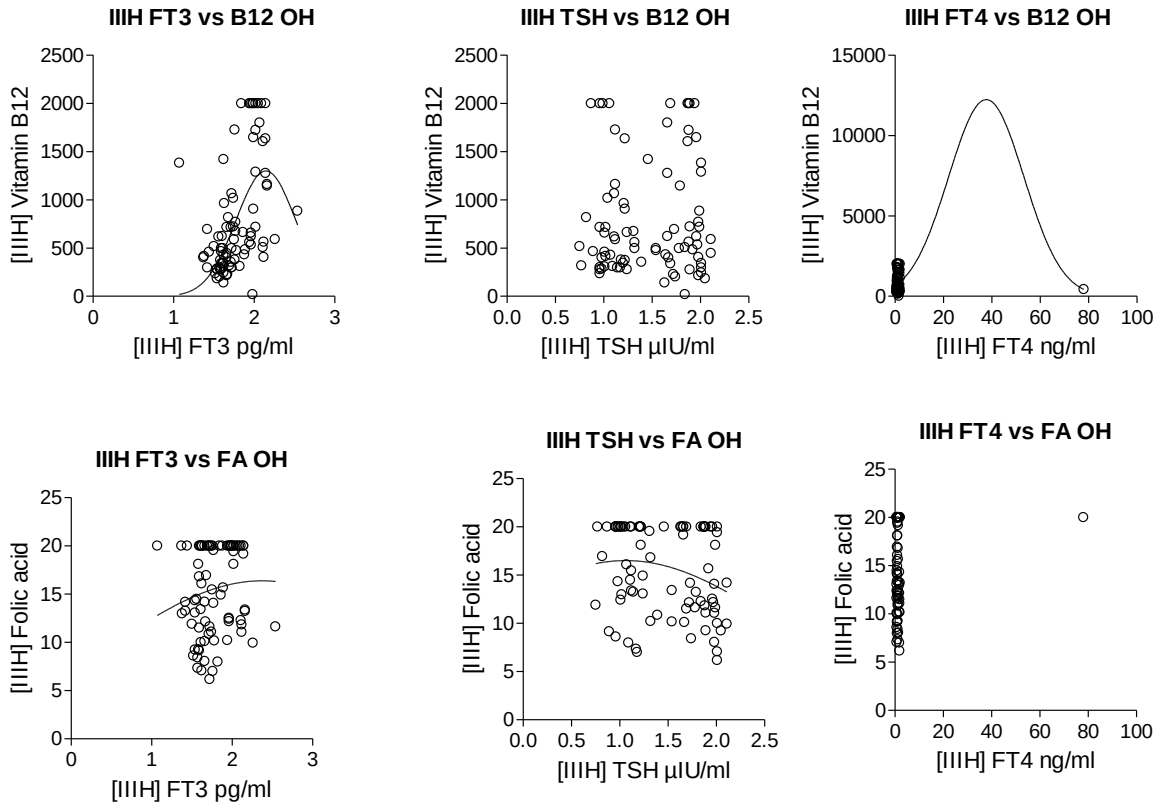

Figure 2: Association between Vitamin B_{12} and folic acid with thyroid hormones in second trimesters.

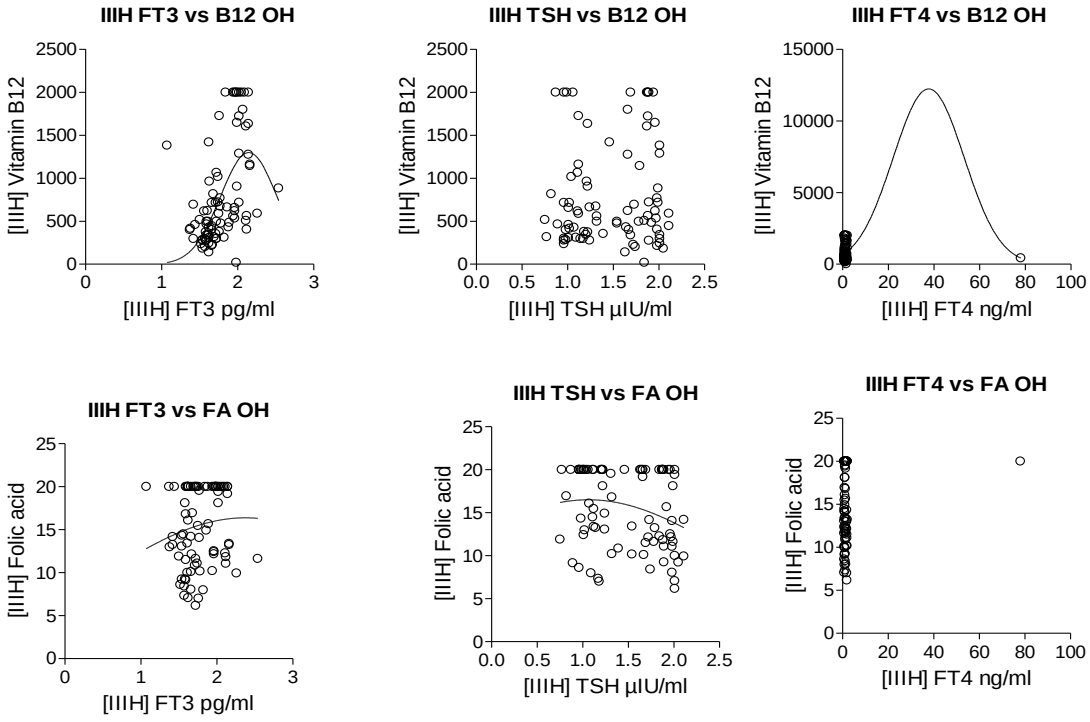

Figure 3: Association between Vitamin B_{12} and folic acid with thyroid hormones in third trimesters.

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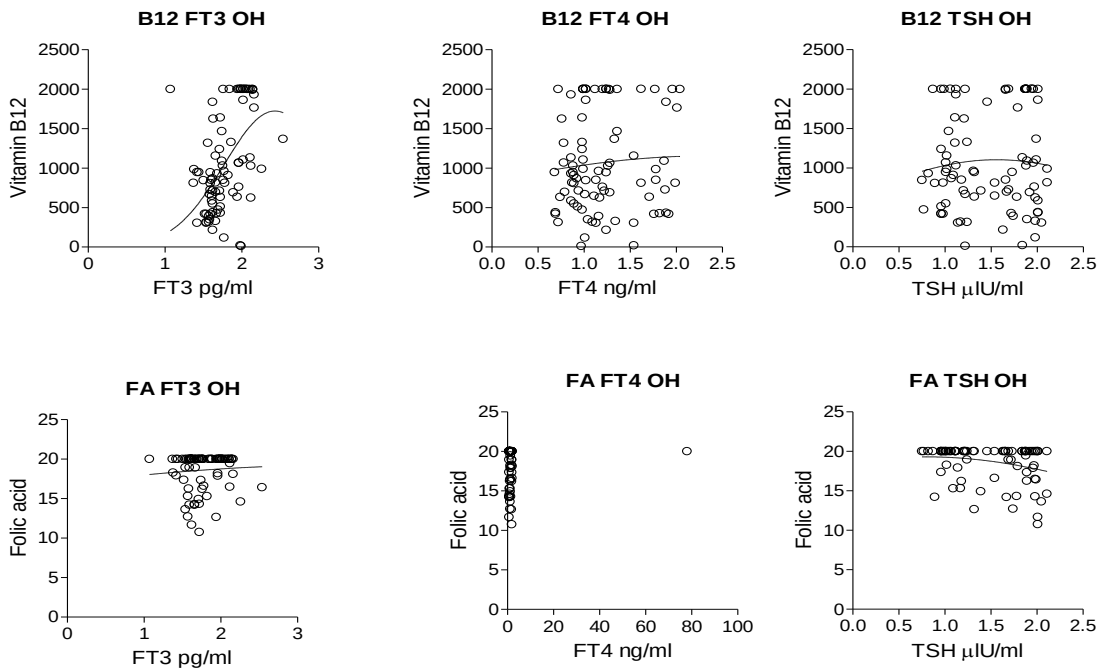

Figure 4: Association of cord blood Vitamin B_{12} and folic acid with thyroid hormones of third trimester.

DISCUSSION

The aim of this study was to determine the relationship between Vitamin B_{12}, folic acid levels and thyroid hormones in pregnant women with overt hypothyroidism. Vitamin B_{12} is very essential in all stages of life and its demand increases during pregnancy, fetal development and infant growth (in lactation) states the dependency of the fetus on maternal Vitamin B_{12} for proper development and growth.[17] Cobalamin status in mother is a strong predictor of vitamin B_{12} in breastfed infants up to at least 6 months of age.[18] It was reported that a strong correlation between Vitamin B12 deficiency and hypothyroidism. [18, 19] In the current study, we have observed positive correlation of vitamin B12 with FT_3 ($P<0.0001$) in three trimesters and in cord blood. No correlation was observed between vitamin B_{12} and FT_4 in all trimesters. Interestingly, significant negative correlation was observed between vitamin B_{12} and TSH in second trimester. Supplementation of folate to the women during pregnancy is common all-around the world.[20] Increased demand of folic acid is observed in pregnancy, may exceed more than 5 times of normal and such demand may not be met by the usual diet. Hence, folic acid supplementation is prescribed in the first trimester of pregnancy. This supplementation helps to prevent neural tube defects (NTDs). [21] Sittig et al; reported that, prolonged effects of folate supplementation in adolescence. Has suppressive effect on thyroid hormones (T3 and T4).[8] In contrast, it was reported that there is controversial information on the correlation between thyroid stimulating hormone (TSH), folic acid and B12.[22] In the present study, we have not observed correlation between folate and FT_3, FT_4 and TSH in first trimester, however significant positive correlation was observed between folate and FT_3 in second trimester, which may be due to decrease in the FT_3 levels as folic acid was are not varied significantly. Significant negative correlation was observed between folic acid and TSH in third trimester, which may be due to decrease in TSH levels.

Limitations

Generalization of the results may not be possible as our study was conducted at one centre.

CONCLUSION

In all three trimesters and in cord blood samples, vitamin B_{12} has positive correlation with FT_3. Significant negative correlation was observed between vitamin B_{12} and TSH in second trimester. Significant

positive correlation was observed between folate and FT_3 in second trimester and significant negative correlation was observed between folic acid and TSH in third trimester and cord blood. We recommend further detailed, multi centre studies to understand the association between vitamin B_{12}, folate and thyroid profile for the benefit of female population in general.

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