ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Association of Vitamin B_{12} and folic acid with thyroid hormones in healthy pregnant women and pregnant women with Subclinical hypothyroidism.

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Abstract

The study aimed to observe the association of Vitamin B_{12} and folic acid with thyroid hormones in healthy pregnant women and pregnant women with Subclinical hypothyroidism. 68 healthy, age matched pregnant women (controls) and 92 pregnant women with Subclinical hypothyroidism (cases) were included in the study. We have observed significant positive correlation was observed between vitamin B_{12} and FT3 and vitamin B_{12} and FT4 in first trimester. Significant positive correlation was observed between vitamin B_{12} and FT3 and significant negative correlation was observed between vitamin B_{12} and FT4 in second trimester. Significant positive correlation was observed between vitamin B_{12} and FT3 in third trimester. Significant positive correlation was observed between cord blood vitamin B_{12} and FT3 of third trimester, significant negative correlation was observed between cord blood vitamin B_{12} and TSH of third trimester in pregnant women with subclinical hypothyroidism.

KEY WORDS: Pregnant women, Subclinical hypothyroidism, Vitamin B_{12}, folic acid.

1. INTRODUCTION

Folic acid supplementation is recommended during pregnancy to prevent incidence of neural tube disorders in offspring (Hannah Blencowe, 2010). It was reported that, folate prevents degeneration of neurons in adults (Zhuom & Pratico, 2010) and also improves cognitive functions and decreases depression (Beydown, 2010). Deficiency of vitamin B_{12} may cause infertility or spontaneous miscarriage or causes birth defects (Molloy, 2008). Animal studies have reported that the animals were functionally hypothyroid after excessive folate administration (L. J. Sittig, 2012). Thyroid disorders were one of the common clinical problems observed during pregnancy. Supplementation of Folic acid is essential to the body to produce healthy new cells, and plays a role in mental and emotional health (Bailey, 2009; Goh, 2008). Supplementation of folic acid was prescribed in the first trimester to prevent neural tube defects (NTDs) (Laurence KM, 1981). As humans cannot produce folate, it must be supplied by dietary sources like fresh and frozen green leafy vegetables, citrus fruits and juices, liver, wheat bread and legumes, such as beans. It was reported that the optimal daily intake of folate/FA in the periconception period is about 0.66 mg per day to prevent NTDs (McPartlin, 1993; Daly, 1995). Two forms of vitamin B_{12} available in the body are methylcobalamin or 5-deoxyadenosyl cobalamin, plays major role in cell growth and development of the human body (Ulrich, 2006). Most of the patients of Hypothyroidism were reported to have low levels of vitamin B_{12}. However, very little literature exists on the same in humans. Hence, the present study was undertaken in the interest of public health, assuming that if the same effect implies in humans with increased folate supplementation during pregnancy i.e. high folate intake may lead to suppression of maternal plasma thyroid hormonal levels, would have alarming implication for the health of the fetus. The study aimed to observe the association of Vitamin B_{12} and folic acid with thyroid hormones in healthy pregnant women and pregnant women with Subclinical hypothyroidism.

2. 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: 68 healthy, aged 24.77±3.55 years of pregnant women (controls) and 92 pregnant women with Subclinical hypothyroidism (cases) aged 24.13±3.38 years were included in the study. All pregnancies were dated according to ultrasonography measurement of the fetal growth - rump length during the first trimester till the time of delivery. The following criteria were followed while selecting the cases.

Inclusion criteria:

- Pregnant women attending the antenatal clinic during the first trimester
- Age between 18- 45 years
- FT_4 levels between 0.86 - 1.87ng/dl and TSH levels between 0.2-3.5μIU/ml (Control-TSH and FT4 levels are between 5th and 95th percentile) and (Subclinical group- TSH levels above 95th percentile and FT4 levels are 5th and 95th percentile (Ross Douglas S, 2016)
- 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 3500 rpm ensuring no particles or traces of fibrin. Samples were clotted at room temperature, centrifuged and aliquoted for 80ºC storage within 8 hours. Rest of the serum sample is appropriately labelled and stored at minus 80^0C until batch analysis for FT3, FT4, 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 T3, Free T4 and TSH: It was assessed by Immulite 1000, automated immunoassay analyser, continuous random access instrument based on chemi luminescent method, (IMMULITE/IMMULITE 1000 FreeT3) (Beck-Peccoz, 1982; Wosila, 1977; Nicoloff, 1990; Tietz NW, 1995).

Assessment of Folate: It was assessed by Immulite 1000, automated immunoassay analyser, continuous random access instrument based on chemiluminescent method, competitive liquid-phase ligand-labeled protein binding chemiluminescent assay (Rothenberg, 1972).

Vitamin B_{12}: It was assessed by Elecsys 2010 and cobase immunoassay analyzers based on electro chemi luminescence immunoassay. Solid-phase electrode chemiluminescent emission (Gutcho, 1977).

Data analysis: Data was analyzed by SPSS 20.0. After checking the normality, analysis was performed. Pearson correlation coefficient was used to determine the association of the parameters. $P<0.05$ was considered as significant.

3. RESULTS

In healthy pregnant women, no correlation was observed between vitamin B_{12} and thyroid profile and folic acid and thyroid profile in first trimester (table.1, figure.1). In second trimester, significant positive correlation was observed between vitamin B_{12} and FT3 ($r=0.2564$), ($P<0.05$) and vitamin B_{12} and FT4 ($r=0.3076$), ($P<0.05$), folic acid and TSH ($r=0.3576$), ($P<0.01$) and no correlation was observed between vitamin B_{12} and TSH, folic acid and FT3, FT4 in healthy pregnant (table.2, figure.2). In third trimester, significant positive correlation was observed between vitamin B_{12} and FT3 ($r=0.4047$), ($P<0.001$) and vitamin B_{12} and FT4 ($r=0.36$), ($P<0.01$), folic acid and TSH ($r=0.3646$), ($P<0.01$) and no correlation was observed between vitamin B_{12} and TSH, folic acid and FT3, FT4 in healthy pregnant women (table.3, figure.3). In cord blood, significant positive correlation was observed between vitamin B_{12} and FT3 ($r=0.4142$), ($P<0.001$), folic acid and TSH ($r=0.3279$), ($P<0.01$) and no correlation was observed between vitamin B_{12} and TSH, vitamin B_{12} and FT4, folic acid and FT3, FT4 (table.4, figure.4). Mean values of FT3, FT4, TSH, vitamin B_{12}, folic acid are presented in table.10.

In pregnant women with subclinical hypothyroidism, significant positive correlation was observed between vitamin B_{12} and FT_3 ($r=0.3664$), ($P<0.001$) and vitamin B_{12} and FT_4 ($r=0.2342$), ($P<0.05$) and no correlation was observed between vitamin B12 and TSH, folic acid and FT3, FT4 and TSH in first trimester (table.5, figure.5). In second trimester, significant positive correlation was observed between vitamin B_{12} and FT3 ($r=0.2134$), ($P<0.05$) and significant negative correlation was observed between vitamin B_{12} and FT4 ($r=-0.2123$), ($P<0.05$) and no correlation was observed between vitamin B_{12} and TSH, folic acid and FT3, FT4 and TSH (table.6, figure.6). In third trimester, significant positive correlation was observed between vitamin B_{12} and FT3 ($r=0.2976$), ($P<0.01$) and no correlation was observed between vitamin B_{12} and TSH, vitamin B_{12} and FT4, folic acid and FT3, FT4 and TSH (table.7, figure.7). In cord blood, significant positive correlation was observed between vitamin B_{12} and FT3 ($r=0.3289$), ($P<0.01$), significant negative correlation was observed between vitamin B_{12} and TSH ($r=-0.2641$), ($P<0.05$). No correlation was observed between, vitamin B_{12} and FT4, folic acid and FT3, FT4 and TSH (table.8, figure.8). Mean values of FT3, FT4, TSH, vitamin B_{12}, folic acid are presented in table.9.

Table.1. Association between Vitamin B_{12} and folic acid with thyroid hormones in first trimesters in healthy pregnant women.

Parameter	Correlation of Vitamin B_{12} with (r)	P value	Correlation of folic acid (r) with	P value
FT_3	0.1721	0.1605	0.0724	0.5571
FT_4	0.1367	0.2665	0.0886	0.4723
TSH	-0.0475	0.7003	0.1569	0.2013

(*P<0.05 is significant, **P<0.01 is significant, ***P<0.001 is significant)

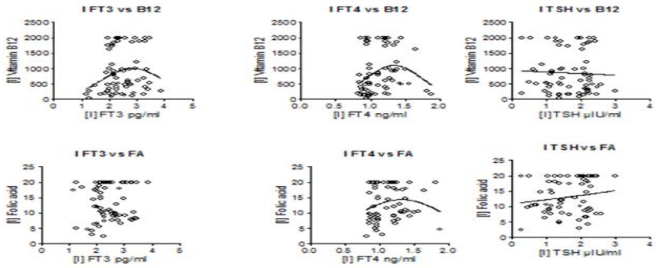


Figure.1. Association between Vitamin B_{12} and folic acid with thyroid hormones in first trimesters in healthy pregnant women

Table.2. Association between Vitamin B_{12} and folic acid with thyroid hormones in second trimesters in healthy pregnant women.

Parameter	Correlation of Vitamin B_{12} with (r)	P value	Correlation of folic acid (r) with	P value
FT_3	0.2564	0.0348*	0.0383	0.7563
FT_4	0.3076	0.0107*	0.2251	0.065
TSH	0.1753	0.1529	0.3576	0.0028**

(*P<0.05 is significant, **P<0.01 is significant, ***P<0.001 is significant)

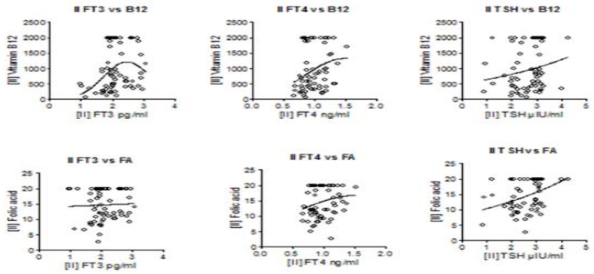


Figure.2. Association between Vitamin B_{12} and folic acid with thyroid hormones in second trimesters in healthy pregnant women

Table.3. Association between Vitamin B_{12} and folic acid with thyroid hormones in third trimesters in healthy pregnant women.

Parameter	Correlation of Vitamin B_{12} with (r)	P value	Correlation of folic acid (r) with	P value
FT_3	0.4047	0.0006***	0.1116	0.365
FT_4	0.36	0.0026**	0.1174	0.3403
TSH	0.2084	0.0881	0.3646	0.0022**

(*P<0.05 is significant, **P<0.01 is significant, ***P<0.001 is significant)

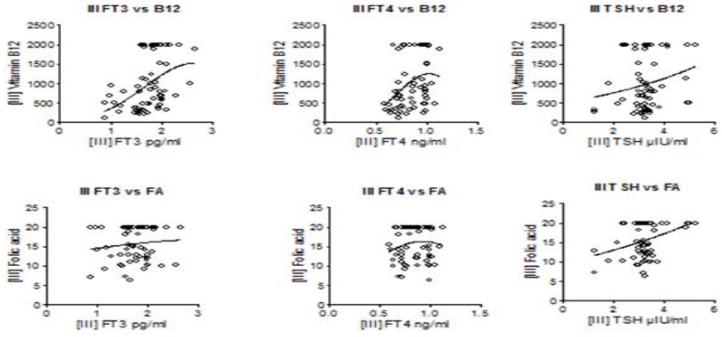


Figure.3. Association between Vitamin B_{12} and folic acid with thyroid hormones in third trimesters in healthy pregnant women

Table.4. Association of cord blood Vitamin B_{12} and folic acid with thyroid hormones of third trimester in healthy pregnant women.

Parameter	Correlation of Vitamin B_{12} with (r)	P value	Correlation of folic acid (r) with	P value
FT_3	0.4142	0.0004***	0.1341	0.2754
FT_4	##	##	##	##
TSH	0.2216	0.0693	0.3279	0.0063**

(*P<0.05 is significant, **P<0.01 is significant, ***P<0.001 is significant, ## cannot be analyzed. No correlation).

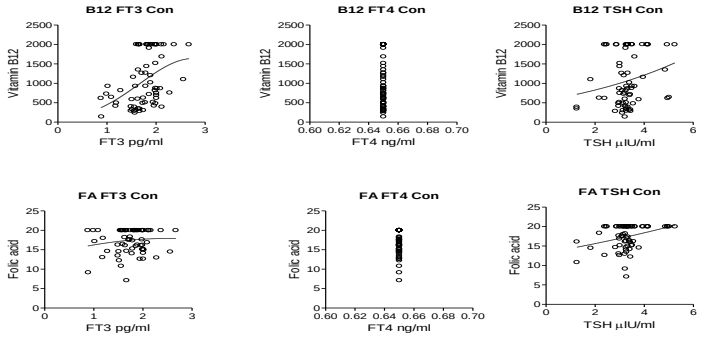


Figure.4. Association of cord blood Vitamin B_{12} and folic acid with thyroid hormones of third trimester in healthy pregnant women

Table.5. Association between Vitamin B_{12} and folic acid with thyroid hormones in first trimesters in pregnant women with Subclinical hypothyroidism.

Parameter	Correlation of Vitamin B12 with (r)	P value	Correlation of folic acid (r) with	P value
FT_3	0.3664	0.0004***	0.0347	0.7481
TSH	-0.1799	0.0934	0.0123	0.9092
FT_4	0.2342	0.0281*	0.1069	0.3215

(*P<0.05 is significant, **P<0.01 is significant, ***P<0.001 is significant).

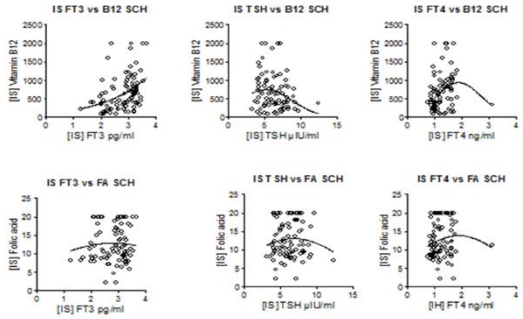


Figure.5. Association between Vitamin B_{12} and folic acid with thyroid hormones in first trimesters in pregnant women with Subclinical hypothyroidism.

Table.6. Association between Vitamin B_{12} and folic acid with thyroid hormones in second trimesters in pregnant women with Subclinical hypothyroidism.

Parameter	Correlation of Vitamin B_{12} with (r)	P value	Correlation of folic acid (r) with	P value
FT_3	0.2134	0.0459*	-0.033	0.76
TSH	-0.2123	0.047*	0.0085	0.937
FT_4	-0.0889	0.41	-0.0144	0.8938

(*P<0.05 is significant, **P<0.01 is significant, ***P<0.001 is significant).

Table 7: Association between Vitamin B_{12} and folic acid with thyroid hormones in third trimesters in pregnant women with Subclinical hypothyroidism.

Parameter	Correlation of Vitamin B12 with (r)	P value	Correlation of folic acid (r) with	P value
FT_3	0.2976	0.0049**	-0.006	0.9558
TSH	-0.1912	0.0743	-0.0511	0.636
FT_4	0.1876	0.08	0.0130	0.9042

(*P<0.05 is significant, **P<0.01 is significant, ***P<0.001 is significant).

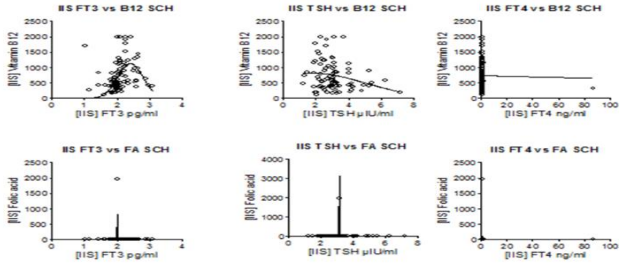
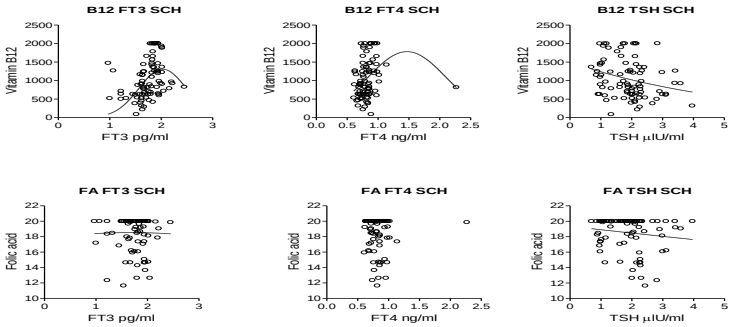


Figure.6. Association between Vitamin B_{12} and folic acid with thyroid hormones in second trimesters in pregnant women with Subclinical hypothyroidism



Figur.7. Association of cord blood Vitamin B_{12} and folic acid with thyroid hormones of third trimester in pregnant women with Subclinical hypothyroidism.

Table.8. Association of cord blood Vitamin B_{12} and folic acid with thyroid hormones of third trimester in pregnant women with Subclinical hypothyroidism.

Parameter	Correlation of Vitamin B_{12} with (r)	P value	Correlation of folic acid (r) with	P value
FT_3	0.3289	0.0019**	0.0015	0.9888
FT_4	0.1404	0.1945	0.0081	0.94
TSH	-0.2641	0.0134*	-0.1316	0.2243

(*P<0.05 is significant, **P<0.01 is significant, ***P<0.001 is significant).

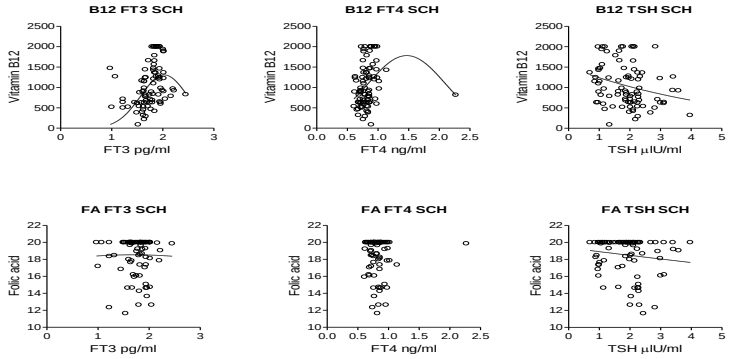


Figure.8. Association of cord blood Vitamin B_{12} and folic acid with thyroid hormones of third trimester in pregnant women with Subclinical hypothyroidism.

Table.9. FT_3, FT_4, TSH, folic acid and vitamin B_{12} values in pregnant women with Subclinical hypothyroidism.

Parameter	First trimester	Second trimester	Third trimester	Cord blood
$FT_{3\ (pg/ml)}$	2.82±0.51	2.10±0.34	1.75±0.25	
$FT_{4(ng/dl)}$	1.25±0.35	1.93±9.17	0.82±0.18	
TSH(µlU/ml)	6.22±1.67	3.06±1.02	1.90±0.68	
Vitamin B_{12}	668.13±479.5	738.17±486.17	787.05±472.68	1046.8±516.53
Folic acid	12.66±4.9	35.96±207.93	14.19±4.37	18.51±2.22

(Data presented are mean ± SD)

Table.10. FT_3, FT_4, TSH, folic acid and vitamin B_{12} values in healthy pregnant women.

Parameter	First trimester	Second trimester	Third trimester	Cord blood
$FT_{3\ (pg/ml)}$	2.44±0.57	2.11±0.45	1.76±0.35	
$FT_{4(ng/dl)}$	1.13±0.23	0.99±0.18	0.84±0.13	
TSH(µlU/ml)	1.65±0.62	2.64±0.64	3.30±0.72	
Vitamin B_{12}	857.31±665.18	960.43±693.31	999.03±680.48	1069.11±661.53
Folic acid	13.19±5.54	14.75±4.97	15.74±4.35	17.40±3.11

(Data presented are mean ± SD)

DISCUSSION

During pregnancy, changes in the thyroid hormones are trimester specific and are associated with fetal neuronal development defects (Offie, 2006). It was reported that decrease in the folate levels will increase homocysteine levels and elevated levels of homocysteine are commonly observed in patients with hypothyroidism. The importance of folic acid in pregnancy is well established and is implemented all around the world. In the present scenario the knowledge and awareness about the importance of folic acid during pregnancy had increased in common public, through social networks. Women are taking folic acid supplementation prior to the conception as most of them are planned pregnancies. This may add up to prescribed supplementation of folic acid after conception i.e 5mg/day, many folds higher than required. This way dumping up of folic acid alone without monitoring the status or levels of other micronutrients like vitamin B_{12}, vitamin B_6, etc.; may lead to certain silent complications that may be imprinted in growing fetus and may express later in life or may lead to epigenetic changes in the mother itself. Few studies reported a strong correlation between hypothyroidism and Vitamin B_{12} deficiency (Anne, 2008; Fahd, 2016). It was also noticed that the generalized symptoms often present in hypothyroid patients, prevailed even after the adequate replacement doses of thyroxine and were corrected by B_{12} replacement therapy. Concluding the high prevalence of Vitamin B_{12} deficiency in hypothyroidism and suggested that, all hypothyroid patients should be screened for vitamin B_{12} levels irrespective of thyroid antibody status. A study by Sittig (2011) stated that the high folate levels effect thyroid function and thyroid hormone- mediated signaling in the hippocampus. They noticed that increased folate supplementation had a suppressive effect on thyroid hormones (T3 and T4), that had possibly led to motivational deficits and memory impairments in adolescent rats. Few studies stated that low freeT4 and also subclinical hypothyroidism has been associated with depressive symptoms, behavioral problems and attention deficit behavior in human at adolescence (Dron, 2008; Haviland, 2006). This available information regarding the importance of vitamin B_{12} in hypothyroidism and the effect of excess folic acid on thyroid hormones dragged our interest of research in knowing the association of folic acid and vitamin B_{12} in pregnant women with subclinical and overt hypothyroidism. Our study is different from other studies as in his study we aimed to know the association of the micronutrients like folic acid and vitamin B_{12} with thyroid hormones during pregnancy. However, the studies on association of Vitamin B_{12} and folic acid with thyroid hormones in healthy pregnant women and pregnant women with Subclinical hypothyroidism are relatively less. In the present study the association of vitamin B_{12} and folic acid with thyroid profile varied in healthy pregnant women and pregnant women with Subclinical hypothyroidism.

Limitations: As the study was conducted at one centre, generalization of the results may not be possible.

4. CONCLUSION

We have observed significant positive correlation was observed between vitamin B_{12} and FT3 and vitamin B_{12} and FT4 in first trimester. Significant positive correlation was observed between vitamin B_{12} and FT3 and significant negative correlation was observed between vitamin B_{12} and FT4 in second trimester. Significant positive correlation was observed between vitamin B_{12} and FT3 in third trimester. Significant positive correlation was observed between cord blood vitamin B_{12} and FT3 of third trimester, significant negative correlation was observed between cord blood vitamin B_{12} and TSH of third trimester in pregnant women with subclinical hypothyroidism.

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