Effect of Vitamin – D Supplementation On Serum TSH Levels in Pregnant Women and Its Co Relation to Age and Body Mass Index.

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Abstract: The main aim of the study is to investigate the association between vitamin D after insufficiency and thyroid hormone levels in pregnancy. The objective of the present study is to see how serum vitamin D levels are related to Thyroid stimulating hormone (TSH) during the first trimester of after pregnancy in relation to the age and BMI of the pregnant women. In this cross-sectional study 100 pregnant women (50as control group, 50 as vitamin D supplementation group) are included and categorized as per their age and BMI. Vitamin D supplementation is done under the supervision of the gynecologist at the start of 1st trimester and at the end of the first-trimester, serum TSH levels are estimated in them. The mean was analysed by two-way ANOVA (Two-way analysis of variance with Bonferroni’’t’’ test method). For all the statistics and graph plotting, SigmaPlot 13.0 (Systat software, USA) was used. P <0.05 is considered significant. The difference in the mean values among the different levels of the group is not significant enough to exclude the after possibility that the difference is just due to random sampling variability after allowing for the effects of differences in BMI Category. There is not a statistically significant difference (P = 0.506). According to the findings of this study, there is no correlation between vitamin D levels and TSH levels in relation to the age and BMI of pregnant women; therefore, more research should be done on groups of pregnant women divided by weeks of pregnancy and age, and vitamin D supplementation should be recommended.

Keywords: Age, BMI, Vitamin D, Thyroid Function, Pregnancy.

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1. INTRODUCTION

Vitamin D is essential for the health, growth and development of the foetus. In many nations, vitamin D deficiency (VDD) is a global and public health issue. Many people have been diagnosed with VDD including pregnant women. VDD in pregnancy is still a public health concern and growing data suggests that VDD causes a slew of negative consequences for pregnant women and their children. They’ve been identified as a high-risk population, with VDD prevalence rates ranging from 20 to 40%. Both vitamin D deficiency and thyroid dysfunction/autoimmunity can cause diseases like preeclampsia, gestational hypertension, gestational diabetes mellitus, premature delivery, low birth weight, and impaired neurodevelopment of offspring to prevent preeclampsia in pregnant women and normal skeletal development in foetus, an optimal amount of Vitamin D should be maintained during pregnancy. Though there is no direct relationship between vitamin D and TSH, there are enormous studies being carried out as vitamin D helps in absorption of calcium which is helpful to the mother and the developing foetus. It helps to maintain the serum calcium levels of both mother and foetus. Optimal functioning of thyroid gland is essential during pregnancy. It is proved that as age advances there is deterioration of Thyroid functioning according to various research findings and obesity also has negative consequences on thyroid gland functioning. Estimation of serum TSH levels helps to understand the status of thyroid gland and aberration of thyroid levels indirectly implies the functioning of thyroid gland which induces Hypothyroidism during pregnancy in most women in India. There are various factors or cause for dysfunction of the thyroid gland like multiple pregnancies, lactation, undernourishment, early marriages in rural areas of India which is a prerequisite to find the solution to optimize the thyroid functioning in pregnant women. So it is seen age and obesity has negative correlation with thyroid gland functioning. Optimum maintenance of serum calcium levels is essential to minimize the complications of pregnancy and also the health of the new-born. It is a known fact that vitamin D is an essential element in regulating serum calcium levels. Due to high demand of calcium during pregnancy and because of changing lifestyles of individuals vitamin D supplementation has become the new normal during Antenatal care. Vitamin D and serum calcium levels may have indirect implications on serum TSH levels and to minimize pregnancy induced hypothyroidism, it is important to identify various causes of it. Hence there is a need to know the relation between vitamin D on TSH levels and circulating TSH levels in pregnant women in comparison to age and BMI. As very few studies are in this line especially in rural areas of Telangana state which is a part of Southern India. Consideration of age and BMI in pregnant women is important to optimize the dosage of vitamin D supplementation for maintaining proper thyroid functioning. Many studies have shown the role of vitamin D in autoimmune thyroiditis, but few have looked at the role of vitamin D in pregnancy and conflicting results have been reported on the link between vitamin D levels in pregnancy and adverse effects on maternal and foetal health. Thus, it is recommended to review VDD in mothers and their children so that strategies can be implemented to prevent VDD during pregnancy and lactation, in order to avoid negative effects on the foetus, new born, and children, with the goal of preventing the development of chronic diseases in adulthood. On the other hand, excess vitamin D may cause the body to absorb too much calcium, increasing the risk of kidney stones and heart stroke. Normal thyroid function is critical for the development of the foetus. Thyroid hormone shortage or excess can occur during pregnancy. Thyroid disease can affect the mother and the infant. The aim of the present study is to see if there’s a link between vitamin D levels and circulating TSH levels in pregnant women.

2. MATERIALS & METHODS

2.1 Study design & study group

A cross-sectional study was carried out on 100 pregnant women aged between 20-35 years. The study protocol was reviewed and approved by the Institutional Ethics committee of SVS Medical College & Hospital, and an approval number (IEC approval No. 02/2019) was obtained before starting the study.

2.2 Inclusion criteria

The participants were randomly selected who got pregnancy and gestation age is 1st trimester were recruited and divided into two groups as vitamin D supplementation group and controls and also to see the role of age and BMI on TSH levels in relation to the vitamin D supplementation the groups are categorizing into two categories as per their age and body mass index (BMI). Age wise <30 years and >30years categories and BMI wise <30 sq.m and >30 sq.m categories. In each group fifty pregnant women are there.

![Flowchart of the study population](Fig 1: flowchart of the study population.)
2.3 Exclusion criteria

Pregnant women with endocrine disorders, hypertension, diabetes mellitus, a recent history of major surgeries and trauma were excluded from the study. The study protocol’s benefits were explained to all participants and clarified their willingness to withdraw from the study. A written informed consent document was obtained from all participants as well as from their guardians and adopted the recommendations of the Declaration of Helsinki guidelines (1975) and later amendments. All study group participants were interviewed about their pregnancy profile and 6000 IU of Vitamin D3 was intravenously infused for three months. The dosage was adopted the recommendations and policies of vitamin – D supplementation. No adverse effects were reported during the interventional period. Trained nurses carried out all the interventional procedures under the supervision of the fraternity from the gynaecology department. The effectiveness of Vitamin – D supplementation on TSH levels is measured after three months in both the groups by Siemens Centaur kit using CLIA method. All the study and control group pregnant women were healthy individuals who were screened routinely and did not have any thyroid problems.

2.4 Estimation of TSH

The Access TSH (3rd IS) assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of human thyroid-stimulating hormone (thyrotropin, TSH, hTSH) levels in human serum and plasma using the Access Immunonassay Systems. The Access TSH (3rd IS) assay is a two-site immunoenzymatic (“sandwich”) assay. A sample is added to a reaction vessel with mouse anti-hTSH-alkaline phosphatase conjugate, buffered protein solution and paramagnetic particles coated with immobilized mouse monoclonal anti-hTSH antibody. The hTSH binds to the immobilized monoclonal anti-hTSH antibody on the solid phase while the mouse anti-hTSH-alkaline phosphatase conjugate reacts with a different antigenic site on the hTSH. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with an illuminometer. The light production is directly proportional to the concentration of TSH in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

2.5 Reagents

R1a: Paramagnetic particles coated with mouse monoclonal anti-human TSH antibody suspended in TRIS buffered saline, with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin 300. R1b: TRIS buffered saline with surfactant, BSA, protein (murine), < 0.1% sodium azide, and 0.1% ProClin 300. R1c: Mouse monoclonal anti-human TSH alkaline phosphatase conjugate in ACES buffered saline, with surfactant, BSA matrix, protein (murine), < 0.1% sodium azide, and 0.25% ProClin 300. R1d: Mouse monoclonal anti-human alkaline phosphatase conjugate in ACES buffered saline, with surfactant, BSA matrix, protein (murine), < 0.1% sodium azide, and 0.25% ProClin 300.

2.6 Procedure

The TSH (3rd IS) assay is a two-site immunoenzymatic (“sandwich”) assay. A sample is added to a reaction vessel with mouse anti-hTSH-alkaline phosphatase conjugate, buffered protein solution and paramagnetic particles coated with immobilized mouse monoclonal anti-hTSH antibody. The hTSH binds to the immobilized monoclonal anti-hTSH antibody on the solid phase while the mouse anti-hTSH-alkaline phosphatase conjugate reacts with a different antigenic site on the hTSH. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with an illuminometer. The light production is directly proportional to the concentration of TSH in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

2.7 Classification criteria for BMI

Height and weight were measured on the subjects in standing position. The weighing scales and the measuring tapes were calibrated periodically. BMI was calculated from the formula, BMI = weight (kg) /height2 (mts2). BMI normal values are below 18.5 (underweight), 18.5–24.9 (normal), 25.0–29.9 (pre obesity), 30.0–34.9 (obesity class I), 35.0–39.9 (Obesity class II) and Above 40 (Obesity class III).

3. STATISTICAL ANALYSIS

All the data were expressed as least squares means ± SE. The least square means are expressed for the TSH levels in comparison with the age and BMI of the study groups. The mean were analyzed by two-way ANOVA (Two-way analysis of variance with Bonferroni’s test method). For all the statistics and graph plotting, SigmaPlot 13.0 (Systat software, USA) was used. P <0.05 is considered as significant.

4. RESULTS

Table 1: Baseline characteristics of the patient

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.773±0.175</td>
</tr>
<tr>
<td>Vitamin D supplementation</td>
<td>2.521±0.151</td>
</tr>
<tr>
<td>Age &lt;30 years</td>
<td>2.546±0.101</td>
</tr>
<tr>
<td>Age &gt;30 years</td>
<td>2.748±0.208</td>
</tr>
<tr>
<td>Control x Age &lt;30 years</td>
<td>2.646±0.137</td>
</tr>
<tr>
<td>Control x Age &gt;30 years</td>
<td>2.900±0.322</td>
</tr>
<tr>
<td>Vitamin D supplementation x Age &lt;30 years</td>
<td>2.446±0.148</td>
</tr>
<tr>
<td>Vitamin D supplementation x Age &gt;30 years</td>
<td>2.597±0.263</td>
</tr>
</tbody>
</table>

Table 1 represents TSH (mIU/L) levels in control and vitamin D supplementation groups in comparison with age categories in 1st trimester. The difference in the mean values among the different levels of Group is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Age Category.

There is not a statistically significant difference (P = 0.278).
Table - 2: TSH (mIU/L) levels in control and vitamin D supplementation groups in comparison with BMI categories in 1st trimester

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.615±0.139</td>
</tr>
<tr>
<td>Vitamin D supplementation</td>
<td>2.489±0.129</td>
</tr>
<tr>
<td>BMI &lt;30 sq.m</td>
<td>2.663±0.122</td>
</tr>
<tr>
<td>BMI &gt;30 sq.m</td>
<td>2.441±0.145</td>
</tr>
<tr>
<td>Control x BMI &lt;30 sq.m</td>
<td>2.780±0.149</td>
</tr>
<tr>
<td>Control x BMI &gt;30 sq.m</td>
<td>2.451±0.234</td>
</tr>
<tr>
<td>Vitamin D supplementation x BMI &lt;30 sq.m</td>
<td>2.545±0.193</td>
</tr>
<tr>
<td>Vitamin D supplementation x BMI &gt;30 sq.m</td>
<td>2.432±0.171</td>
</tr>
</tbody>
</table>

There is not a statistically significant interaction between Group and Age Category. (P = 0.824).

Table - 2 represents TSH (mIU/L) levels in control and vitamin D supplementation groups in comparison with BMI categories in 1st trimester. The difference in the mean values among the different levels of Age Category is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Group. There is not a statistically significant difference (P = 0.383). The effect of different levels of Group does not depend on what level of Age Category is present.

Table - 3: The influence of age and BMI on TSH levels of control and vitamin D supplementation groups in 1st trimester

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups for age category (Control and Vit D supplementation)</td>
<td>1</td>
<td>0.986</td>
<td>0.986</td>
<td>1.188</td>
<td>0.278</td>
</tr>
<tr>
<td>Age category (&lt; 30 and &gt; 30 years)</td>
<td>1</td>
<td>0.636</td>
<td>0.636</td>
<td>0.767</td>
<td>0.383</td>
</tr>
<tr>
<td>Group x age Interaction</td>
<td>1</td>
<td>0.041</td>
<td>0.041</td>
<td>0.049</td>
<td>0.824</td>
</tr>
<tr>
<td>Groups for BMI category (control and Vit D supplementation)</td>
<td>1</td>
<td>0.367</td>
<td>0.367</td>
<td>0.446</td>
<td>0.506</td>
</tr>
<tr>
<td>BMI category (&lt; 30 and &gt; 30 sq.m)</td>
<td>1</td>
<td>1.122</td>
<td>1.122</td>
<td>1.363</td>
<td>0.246</td>
</tr>
<tr>
<td>Group x BMI interaction</td>
<td>1</td>
<td>0.268</td>
<td>0.268</td>
<td>0.325</td>
<td>0.570</td>
</tr>
</tbody>
</table>

Two way analysis of variance with Bonferroni ‘t’ test

Table - 3: The influence of age and BMI on TSH levels of control and vitamin D supplementation groups in 1st trimester. The difference in the mean values among the different levels of Group is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in BMI Category. There is not a statistically significant difference (P = 0.506). The difference in the mean values among the different levels of BMI Category is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Group. There is not a statistically significant difference (P = 0.246). The effect of different levels of Group does not depend on what level of BMI Category is present. There is not a statistically significant interaction between Group and BMI Category. (P = 0.570).
ANOVA with Bonferroni’s test. A Significantly different from SE (n – control = 52; vitamin supplementation= 50). Two RM ANOVA with Bonferroni’s test. A Significantly different from the respective before group (within groups comparison). BSignificantly different from the respective before group (between groups comparison).

5. DISCUSSION

In the present study, the TSH levels are not dependent on the vitamin D supplementation and when compared with age and BMI, the TSH levels are not statistically significant after the vitamin D supplementation and these findings are in line with the previous studies.13,14 Table 3. Due to its activity in the thyroid gland and even in patients with thyroid disorders and thyroid autoantibody, several types of research have shown that vitamin D has an effect on thyroid hormone. Few research, however, has looked into the link between vitamin D and thyroid hormones during pregnancy. As a result, this research was undertaken on a group of pregnant women2,15-17. Many studies have linked an increase in TSH levels to a higher risk of preterm birth, placental abruption, foetal death, and impaired neurological development in children, as well as a link between blood pressure severity and thyroid hormone levels18,19. Both vitamin D and thyroid hormone bind to steroid hormone receptors, which are identical to one another20. A study found no link between vitamin D, FT4, or FT3 during pregnancy, however there was a significant link between vitamin D and decreased TSH in pregnant women with appropriate vitamin D levels (greater than 30 ng/ml)13. Despite vitamin D shortage among these women, no link was established between 25-hydroxy vitamin D and thyroid function in a pregnancy research4. Vitamin D levels and thyroid hormones during pregnancy have been studied in various ways, with varying findings table1. Another case-control research found no link between vitamin D levels and hypothyroidism in non-pregnant and healthy women21. Studies on vitamin D and thyroid function have yielded mixed results thus far. These discrepancies may be attributable to diverse study designs, differing definitions of vitamin D deficiency, and a lack of agreement on serum levels of vitamin D among vitamin D deficient people. As a result, concluding that vitamin D insufficiency is a risk factor for thyroid problems and vice versa is difficult, and for a better conclusion, studies should use a single technique22. However, if earlier studies show that vitamin D insufficiency is a risk factor for hypothyroidism and autoimmune thyroiditis, and if those disorders occur concurrently during pregnancy, the potential harm to both the mother and her foetus is substantial. As a result, it is recommended that both problems be corrected in order to reduce any potential hazards23. Some of the previous studies reported findings similar to the present study, but they reported that BMI was not related to serum TSH levels in any of the trimesters23-25. The serum TSH level was considerably higher in the overweight group, according to Han C et al26 Table 3. According to Ashoor G et al., with higher BMI scores, FT4 declined while FT3 increased, however there were contradictory effects of FT3 and FT4 on maternal weight27. Mannisto T et al. found a positive connection between BMI and serum TSH level, which is not similar to the current study28. BMI was found to be favourably linked with serum FT3 levels but not with serum FT4 levels. Obese patients have lower FT4 readings than normal weight patients29 Table 1. These data imply that changes in body weight and BMI are linked to differences in normal thyroid function in euthyroid pregnant women. The causes could be straightforward or complex, but the biological mechanism is yet unknown20.

6. CONCLUSION

According to the findings of this study, there is no correlation between vitamin D levels and TSH levels in relation to the age and BMI of pregnant women; therefore, more research should be done on groups of pregnant women divided by weeks of pregnancy and age, and vitamin D supplementation should be recommended. Because of the harmful impact of Vitamin D deficiency and thyroid hormone abnormalities on the mother and foetus, further research is needed to better screen these diseases during pregnancy.
7. ACKNOWLEDGEMENT

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10. REFERENCES


