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EFFECT OF SUBCLINICAL HYPOTHYROIDISM IN COMBINATION WITH VITAMIN D DEFICIENCY ON LEFT VENTRICULAR DIASTOLIC FUNCTION

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ABSTRACT The present study draw special attention to find the effect of effect of subclinical hypothyroidism in combination with vitamin D deficiency on left ventricular diastolic function. The aim of the study to assess the left ventricular diastolic function in patients of subclinical hypothyroidism with vitamin D deficiency. In this study all patients were above the age 18 years having subclinical hypothyroidism with vitamin D deficiency and also patients having normal vitamin D level with subclinical hypothyroidism were included in the present study. Apart from the detailed clinical history, relevant clinical examination was done in very participants. This study was done in the following two groups viz: Group –I: Subclinical hypothyroidism with vitamin D deficiency and Group –II: Subclinical hypothyroidism with normal vitamin D. the study found that left ventricular diastolic dysfunction was found in 81% of patients having subclinical hypothyroidism with vitamin D deficiency also the study revealed that the coexistence of subclinical hypothyroidism with Vitamin D deficiency can lead to further deterioration in left ventricular diastolic function.

KEYWORDS : Subclinical Hypothyroidism, Vitamin D Deficiency, Left Ventricular Diastolic Function

INTRODUCTION

Subclinical hypothyroidism is biochemically defined as an elevated serum thyrotropin level in combination with a serum free T_4 level that is within the population reference range. The incidence of subclinical hypo- thyroidism varies among populations and ranges from 3 to 15%, with a higher incidence associated with increasing age, female sex, and a suboptimal iodine status[1,2] . The relationship between serum thyrotropin and free T_4 is such that a small decrease in free T_4 can result in a relatively large increase in serum thyrotropin, which can subsequently lead to a thyrotropin level that is above the reference range while the free T_4 level is still within the reference range. In cases of progression to overt hypothyroidism, the thyrotropin level typically continues to increase and the free T_4 level falls below the reference range. In this respect, subclinical hypothyroidism can be seen as a mild form of thyroid failure, one that is caused by autoimmune thyroid disease in the majority of cases. A thyrotropin cutoff level of 10 mIU per liter is commonly used to distinguish between mild and more severe subclinical hypothyroidism[3,4,5]. Approximately 75% of patients with subclinical hypothyroidism have a thyrotropin level of less than 10 mIU per liter. Serum thyrotropin and free T_4 show substantial variability among healthy persons, whereas the range of variability within an individual healthy person tends to be relatively narrow.

Vitamin D deficiency

Optimal serum concentration of 25-hydroxyvitamin D considers only bone health and was defined as the concentration that maximally suppresses serum parathyroid hormone[6]. Vitamin D deficiency is defined as a calcidiol level of <20 ng/mL and insufficiency as 21–29 ng/mL[7,8]. Vitamin D is sufficient if >30 ng/mL, and vitamin D intoxication is considered if >150 ng/mL[9]. There are variations among professional bodies regarding the cut-off values for insufficient or deficient vitamin D level[10].

Vitamin D deficiency in association with Cardiac function

Vitamin D suppresses inflammation via several pathways, such as inhibition of prostaglandin and cyclooxygenase pathways, up regulation of anti-inflammatory cytokines, decrease of cytokine induced expression of adhesion molecules, reduction of matrix metalloproteinase 9,[11,12] and down regulation of the renin-angiotensin-aldosterone system. Vitamin D deficiency stimulates systemic and vascular inflammation, enabling atherogenesis[13], On the other hand, as already mentioned, hypertension is also associated with lack of vitamin D, due to activation of the renin-angiotensin-aldosterone system, enabling endothelial dysfunction, the first step in plaque formation. The proinflammatory nuclear factor kB mediates partly the association between endothelial dysfunction and low vitamin D status. A strong association was found between vitamin D deficiency and slow coronary flow, endothelial dysfunction and Subclinical atherosclerosis[14] in patients with normal or near-normal coronary arteries at coronary angiography. Decreased levels of vitamin D binding protein were found in the plasma of survivors of a myocardial infarction at young age, statistically correlated with the number of affected coronary arteries. Low vitamin D levels have been linked to inflammation, higher coronary artery calcium scores, increased mean platelet volume, and increased vascular stiffness[15]. Abnormally high mean platelet volume has been associated with cardiovascular diseases, considering the higher risk to block arteries, due to the ability to aggregate more rapidly with collagen, the higher thromboxane A2 level, and expression of more glycoprotein IIB and IIIA receptors than smaller platelets. The increased release of proinflammatory cytokines in patients with vitamin D deficiency increases oxidative stress and enables release of immature and activated platelets from the bone marrow, with an increased mean platelet volume.

The mechanisms responsible for this impairment could be explained by the following two mechanisms. First, active vitamin D causes the phosphorylation of phospholamban via the induction of protein kinases. The inhibitory effect of phosphorylated phospholamban on SERCA2 is then

removed. The activation of SERCA2 causes the reuptake of calcium by the sarcoplasmic reticulum, decreasing intracytosolic Ca2+ and leading to the development of myocardial relaxation. However, in vitamin D deficiency, phospholamban remains unphosphorylated; thus, the inhibitory effect of phospholamban on SERCA2 continues, increasing intracytosolic Ca2+ levels and disrupting myocardial relaxation. Similar mechanisms are present in SCH, and while the decrease in phospholamban activation and reduced expression of SERCA2 decrease Ca2+ re-uptake, intracellular Ca2+ increases and myocardial relaxation is deteriorated[16]. Diastolic dysfunction can result from excessive Ca entry into the cytosol, from a decrease in Ca efflux, or from inadequate Ca reuptake by the sarcoplasmic reticulum during diastole. As a result, in patients with SCH in combination with vitamin D deficiency, intracellular Ca2+ increases more than expected, causing myocardial relaxation and the impairment of diastolic function. Some previous studies give evidence that vitamin D deficiency leads to myocardial fibrosis and cardiac hypertrophy[17,18]. Similarly, cardiac hypertrophy has been observed in the hearts of VDR knockout mice. Moreover, the vitamin D receptor is located in the t-tubules of the sarcolemma and presumably regulates myocyte relaxation[19]. In experimental studies, activated vitamin D or related analogs augment diastolic relaxation, reduce end-diastolic pressures, reduce cardiac mRNA expression and lead to the regression of LVH[20,21].

AIMS AND OBJECTIVES

- To assess the left ventricular diastolic function in patients of subclinical hypothyroidism with vitamin D deficiency.

STATISTICAL ANALYSIS

Descriptive statistics for quantitative variables was summarized using the mean ± Standard deviation (SD) and the median with interquartile range as a appropriate. Categorical data was expressed as frequency and percentage and then mean comparison with p-value <0.05 was considered as significant.

Ethical Issues: Nil and **Financial Issues:** Nil

MATERIAL AND METHODS

After obtaining the ethical clearance from the Institution Ethical Committee (IEC), the present investigational study was carried out in the Department of Medicine, Government Medical College, Srinagar. The study was conducted over a period of two years.

1. **Study Design:** This is a prospective study to assess the left ventricular diastolic function in subclinical hypothyroid patients in combination with vitamin D deficiency and in those having subclinical hypothyroidism with normal vitamin D levels.
2. **Study Duration:** The duration for the present study is two year.
3. **Participants:** In this study all patients were above the age 18 years having subclinical hypothyroidism with vitamin D deficiency and also patients having normal vitamin D level with subclinical hypothyroidism were included in the present study.

Apart from the detailed clinical history, relevant clinical examination was done in very participants. This study was done in the following two groups viz:

1. Group –I: Subclinical hypothyroidism with vitamin D deficiency.
2. Group –II: Subclinical hypothyroidism with normal vitamin D.

INVESTIGATIONS

The following investigations to be done for the present study were stated as under:

1. Carotid Doppler
2. 2D Echo and Doppler study
3. Lipid Profile
4. CRP
5. Anti TPO Antibodies
6. Thyroid Panel (F – T_3, T_4 & TSH)
7. Vitamin D (Chemiluminescent method was used for detection of 25 dihyroxyvitamin D in biological samples).

Gender, Region and Age distribution of study patients

Gender	N	Percent
Male	66	44.0
Female	84	56.0
Total	**150**	**100.0**
Region	**N**	**Percent**
Rural	102	68.0
Urban	48	32.0
Total	**150**	**100.0**
Age	**N**	**Percent**
18-25 years	21	14.0
26-30 years	27	18.0
31-35 years	47	31.3
36-40 years	55	36.7
Total	**150**	**100.0**

Thyroid Function Test in study patients (N=150)

		Range	Group I		Group II	
			N	Percent	N	Percent
TSH	Normal	0.35 to 5.50μIU/mL	0	0.0	0	0.0
	High	5.51 to 9μIU/mL	81	100.0	69	100.0
	Total		81	100.0	69	100.0
Ft3	Normal	0.61 to 1.81ng/mL	81	100.0	69	100.0
	High	1.82 & above ng/mL	0	0.0	0	0.0
	Total		81	100.0	69	100.0
Ft4	Normal	5.01 to 12.45 μg/mL	81	100.0	69	100.0
	High	12.46 & above μg/mL	0	0.0	0	0.0
	Total		81	100.0	69	100.0

Subclinical hypothyroidism with vitamin D deficiency in relation to Vitamin D, CRP and Anti TPO

		Range	Group I	
			N	Percent
Vitamin D	Deficiency	less than 20ng/mL	81	100.0
	Normal	20-40ng/mL	0	0.0
	Total		81	100.0
CRP	Negative	Less than 5mg/L	61	75.30
	Positive	6mg/L to 10 mg/L	20	24.70
	Total		81	100.0
ANTI TPO	High	61U/mL to 100/mL	41	50.6
	Normal	Less than 60U/mL	40	49.4
	Total		81	100.0

Subclinical hypothyroidism with normal vitamin D in relation to Vitamin D, CRP and Anti TPO

		Range	Group II	
			N	Percent
Vit D	Deficiency	less than 20ng/mL	0	0.0
	Normal	20-40ng/mL	69	100.0
	Total		69	100.0
CRP	Negative	Less than 5mg/L	59	85.50
	Positive	6mg/L to 10 mg/L	10	14.50
	Total		69	100.0
ANTI TPO	High	61U/mL to 100/mL	10	14.5
	Normal	Less than 60U/mL	59	85.5
	Total		69	100.0

Distribution of Lipid Profile in study patient

		Range	Group I		Group II		p-value
			N	Percent	N	Percent	
CHOL	High	201mg/dL to 300mg/dL	16	19.8	15	21.7	.052*
	Normal	50-200mg/dL	65	80.2	54	78.3	
	Total		81	100.0	69	100.0	
TGL	High	260 mg/dL	63	77.7	52	75.4	.054*
	Normal	50-200 mg/dL	18	22.3	17	24.6	
	Total		81	100.0	69	100.0	
HDL	High	≥140mg/dL	11	13.6	17	24.6	.251
	Normal	30-90mg/dL	70	86.4	52	75.4	
	Total		81	100.0	69	100.0	
LDL	High	≥150mg/dL	10	12.3	7	10.1	.312
	Normal	≤100mg/dL	71	87.7	62	89.9	
	Total		81	100.0	69	100.0	

**significant at 0.05 level*

Comparison between vitamin D deficiency and Normal Vitamin D levels with subclinical hypothyroid patients

	Vitamin D Deficiency (N=81)		Normal vitamin D (N=69)		p-value
	Mean ±S.D.	*Std. Error Mean*	*Mean ±S.D.*	*Std. Error Mean*	
TSH	7.40±1.506	.167	7.03±1.272	.153	.000**
FT3	.95±.218	.024	.91±.284	.034	.000**
Ft4	6.02±1.458	.162	6.09±1.411	.170	.000**
CRP	6.39±.274	.021	6.12±.295	.243	.128
ANTI TPO	21.95 ±23.990	2.666	1.48±1.009	.121	.000**
Carotid Doper I	.3015 ±.37223	.04136	.1651 ±..28269	.03403	.014**
Carotid Doper (L)	.2898 ±.35555	.03951	.1690 ±..28955	.03486	.026*
CHOL	182.44 ±34.471	3.830	170.09 ±44.057	5.304	.000**
TGL	136.99 ±40.301	4.478	137.97 ±41.698	5.020	.214
HDL	64.85 ±21.121	2.347	66.10 ±21.082	2.538	.053*
LDL	79.99 ±22.337	2.482	74.17 ±20.272	2.440	0.52*

**Significant at 0.05 level*
***Significant at 0.01 level*

Comparison of Echo Cardiographic findings between vitamin D deficiency and Normal Vitamin D levels with subclinical hypothyroid patients

	Vitamin D Deficiency (N=71)		Normal vitamin D (N=48)		p-value
	Mean±S.D.	*Std. Error Mean*	*Mean±S.D.*	*Std. Error Mean*	
E (cm/s)	85±9	.021	86±.5	.020	.231
A (cm/s)	80±8	.592	78±7	. 152	.324
E` (cm/s)	8.3±1.8	. 231	10.4±2.3	.021	.000**
IVRT (ms)	112±.20	.020	107±.16	.031	.000**
DT (ms)	181±31	.143	176±29	.127	.000**
E/A	1.07±0.21	.314	1.12±0.25	.215	.264
E/E`	10.3±.2.4	.012	8.4±.2.9	.014	.000**

**Significant at 0.05 level*
***Significant at 0.01 level*

DISCUSSION

Subclinical hypothyroidism is a common clinical problem with a prevalence of about 3 to 15% globally, and is associated with cardiovascular morbidity and mortality. The global prevalence of vitamin D deficiency varies among different regions with some regions as high as 40 to 98%. The recent studies showing additive effect of vitamin D deficiency on cardiovascular morbidity and mortality in patients with subclinical hypothyroidism. It is prudent to study the effect of vitamin D deficiency and subclinical hypothyroidism on cardiovascular system. The present study was aimed to assess the diastolic function in patients having only subclinical hypothyroidism and in the patients having both subclinical hypothyroidism and vitamin D deficiency.

In our study a total of 150 patients were studied. All patients were in the age group of 18 to 40 with 44% being males and 56% were females, sixty nine patients that is 46% were having subclinical hypothyroidism with normal vitamin D level and eighty one patients that is 56% were having both subclinical hypothyroidism and vitamin D deficiency. In patients with subclinical hypothyroidism the diastolic function was assessed by using echocardiography with the following parameters:

E cm /sec = 86±5
A cm/sec = 78±7
E` cm /se = 10.4±2.3
IVRT ms = 107±16
DT ms = 176±29
E/A = 1.12±0.25
E/E` = 8.4±2.9

Among the patients having only subclinical hypothyroidism 48 patients that is 70% had diastolic dysfunction as evidenced by above parameters.

Our results are similar with the study done by **Pankaj Kumar**[51] **et al.,** in 2015, where they studied the effect of subclinical hypothyroidism on left ventricular function. They studied a total of 200 patients and found that about 75% of patients had diastolic dysfunction. The results of their study showed that in patients with subclinical hypothyroidism, the diastolic parameters on ECHO were altered mainly the increased intraventricular relaxation time and reduction of E/A ratio. This study also showed that almost all the patients had normal systolic function.

Meena[48] **C.l. et al. (2012)** also concluded that subclinical hypothyroidism significantly affects LV and RV structure, systolic, diastolic and global function, and LV and RV mechanics. Levothyroxine replacement therapy significantly improved cardiac structure, function, and mechanics in the SHT patients.

Bernadette[36] **et al, (2008)** also found that in subclinical hypothyroid patients there is impairment in left ventricular relaxation time.

Biondi Serafino[22] **F., et al** also found significant prolongation of IVRT in subclinical hypothyroid patients.

Marco Ziun[56] **et al (2016)** also came to conclusion that in young sub clinical hypothyrioid patients there is impairment in diastolic function moreover, it could be the prelude to more serious cardiac impairment, given that LVDD often precedes and/or causes systolic dysfunction.

Yuthika Malhotra[61] et al found that SCH patients had a higher prevalence of LVDD than controls (13.43% versus 1.49%; p = 0.017). LVDD showed a significant association with gender (p = 0.004) and serum FT4 (p = 0.001). E velocity, E' velocity, A' velocity, iso-volumetric relaxation time (IVRT), E/A, and E'/A' ratios were significantly lower, while A velocity, deceleration time (DT), E/E' ratio, left atrial (LA) volume index, and peak tricuspid regurgitation (TR) velocity were significantly higher in cases than controls (p < 0.05 each). The E/A ratio correlated significantly with age, serum very low-density lipoprotein

(VLDL), triglycerides (TG), thyroid stimulating hormone (TSH), free triiodothyronine (FT3), and high-density lipoprotein (HDL) ($p < 0.05$ each). E' velocity correlated significantly with age, serum total cholesterol, VLDL, and TG ($p < 0.05$ each), DT with serum total cholesterol ($p = 0.047$), and LA volume index with age ($p = 0.021$). Age ($p = 0.016$) and serum HDL ($p = 0.029$) were independent predictors of E/A ratio. Gender was an independent predictor for LVDD ($p = 0.003$). Echocardio graphic indices for LVDD showed significant improvement after 6 months of L-thyroxine therapy ($p < 0.05$ each).

Sanjha Llic[23] et al. also concluded that SCH significantly affected LV, RV structure, systolic, diastolic and global function, RV and LV mechanics.

In our study 81 patients (56%) had both SCH and concomitant Vitamin D deficiency. All these patients went Echocardi ography to assess diastolic function with following parameters.

E cm /sec = 85±9
A cm/sec = 80±8
E` cm /se = 8.3±1.8
IVRT ms = 112±20
DT ms = 181±31
E/A = 1.07±0.21
E/E` = 10.3±2.4

Among the patients who had both SCH and concomitant Vitamin D deficiency 71(81%) had diastolic dysfunction as evident by echocardiography. Thus our study shows that concomitant Vitamin d deficiency with SCH had additive deleterious effect on diastolic function as compared to patients having SCH only.

The main finding of our study is that patients who have subclinical hypothyroidism in combination with vitamin d deficiency were at the higher risk of LVDD, as vitamin d deficiency itself is responsible for impairment in myocardial relaxation. This is in concordance with previously published study by **Kathleen Nolte[63], et al., (2019)** who concluded that lower 25 (OH)D levels were associated with reduced functional capacity in patients with DD or HF pEF and were significantly predictive for an increased rate of cardiovascular hospitalizations, also after adjusting for age, NT-proBNP, and selected baseline characteristics and co-morbidities.

So combination of subclinical hypothyroidism with vitamin D deficiency leads additive effect on diastolic dysfunction. Currently vitamin d deficiency is a global health problem and subclinical hypothyroidism is present in 4 to 20 percent the general population. in this study LVDF was evaluated in patients who have SCH in combination with vitamin D deficiency ,in this group we demonstrated that patients who have both SCH and vitamin D deficiency have lower E values and increased IVRT, in comparison to patients who have only SCH, thus in this study we demonstrated that patients who have vitamin d deficiency with SCH Have a tendency to impair diastolic function, this observed decrease in early mitral annulus velocity E' is a reflection of the impairment in myocardial relaxation.

When there occurs impairment in relaxation, mitral valve opening is delayed and IVRT is prolonged. In this study we found that lower valve of e prime and high IVRT value of patients who have vitamin D deficiency in combination with subclinical hypothyroidism indicates detoriation of myocardial relaxation. In our study we also found that patients with vitamin D deficiency in combination with SCH had E/E' on higher side as compared to the patients who have only subclinical hypothyroidism, DT is also increased. **Yilmaz[9] H, et al., (2015)** also found that coexistence of subclinical hypothyroidism with vitamin D deficiency can lead to further deterioration in the LV diastolic function via the regulation of intracellular calcium and induction of inflammatory activity. Therefore, close follow-up of the diastolic functions of these patients could be beneficial.

In this study we also found that 24% of patients had grade 2 diastolic dysfunction in the SCH with vitamin D deficiency group. These 24% of patients had TSH level on higher side 9±1 and vitamin D in the range of 7±2 from this observation we come to the conclusion that more the TSH and lower the vitamin D level accelerate impairment of left ventricular relaxation or myocardial relaxation, thus causing grade 2 diastolic dysfunction.

SUMMARY AND CONCLUSION

The present study was conducted in postgraduate department of medicine, Government medical college Srinagar, over a period of 2 years to assess diastolic function in subclinical hypothyroid patients with vitamin D deficiency

- The mean age of patients in our study was 44 years
- There were 44% males and 56% females
- In our study left ventricular dysfunction was found in 70% of patients having subclinical hypothyroidism
- Left ventricular diastolic dysfunction was found in 81% of patients having subclinical hypothyroidism with vitamin D deficiency
- Thus the coexistence of subclinical hypothyroidism with Vitamin D deficiency can lead to further deterioration in left ventricular diastolic function.

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