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Subclinical hypothyroidism in combination with vitamin D deficiency increases the risk of impaired left ventricular diastolic function

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Objective. Subclinical hypothyroidism and vitamin D deficiency are common. The diastolic function of patients with both subclinical hypothyroidism and vitamin D deficiency remains unknown. This study aimed to investigate diastolic dysfunction in patients with both subclinical hypothyroidism and vitamin D deficiency.

Subjects and Methods. This study included 254 patients. All patients underwent standard Doppler echocardiography. Patients who had risk factors for diastolic dysfunction or had used L-thyroxine and vitamin D within the previous 3 months were excluded. Vitamin D deficiency was defined as a 25-OH-vitamin D level lower than 20 ng/ml, and vitamin D sufficiency was defined as a 25-OH-vitamin D level ≥30 ng/ml. Subclinical hypothyroidism was defined as a TSH level of 4.5-10 mU/l when the free T4 concentration was normal.

Results. The patients were divided into 4 groups. Group 1 (n=71) included patients with subclinical hypothyroidism and vitamin D deficiency; Group 2 (n=66) included patients with subclinical hypothyroidism and vitamin D sufficiency; Group 3 (n=65) included euthyroid patients with vitamin D deficiency; and Group 4 (n=52) included euthyroid patients with vitamin D sufficiency. LAVI (31.3±3.2, 28.7±3.0, 28.4±3.4, and 27.9±3.9; $p<0.001$) and E/E' values (11.2±2.7, 8.9±2.7, 9.1±2.9, 8.8±2.5; $p<0.001$) were significantly higher in Group 1 than in Groups 2, 3 and 4. E' values were significantly lower in Group 1 than in Groups 2, 3 and 4.

Conclusion. The 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.

Key words: vitamin D deficiency, subclinical hypothyroidism, diastolic function, echocardiography

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List of abbreviations: A: velocity at atrial contraction; **ASE**: American Society of Echocardiography; **BMI**: body mass index; **Ca**: calcium; **CRP**: C-reactive protein; **DT**: mitral E velocity deceleration time; **E**: peak early filling velocity; **E'**: mitral annulus early diastolic velocity; **FPG**: fasting plasma glucose; **fT3**: free triiodothyronine; **fT4**: free thyroxine; **IVRT**: the isovolumic relaxation time; **LVDF**: left ventricular diastolic function; **LVDD**: left ventricular diastolic dysfunction; **LAVI**: left atrial volume index; **LVEF**: left ventricular ejection fraction; **LVMI**: left ventricular mass index; **SCH**: subclinical hypothyroidism; **SERCA**: sarcoplasmic reticulum calcium ATPase; **TSH**: thyroid-stimulating hormone; **25(OH)D**: 25-hydroxy-vitamin D

Subclinical hypothyroidism (SCH) is a condition that affects 4-20% of the adult population and is characterized by elevated thyroid stimulating hormone (TSH) in the context of normal thyroid hormone [i.e. free triiodothyronine (fT3) and free thyroxine (fT4)] concentrations (Fatourech 2009; Cooper and Biondi 2012). SCH can impair the left ventricular diastolic function (LVDF) by causing reduced expression of the sarcoplasmic reticulum calcium ATPase (SERCA), which reduces calcium re-uptake into the sarcoplasmic reticulum during diastole via decreased expression of SERCA and phospholamban activation (Kiss et al. 1994; Klein and Ojamaa 2001; Kahaly and Dillmann 2005; Biondi and Cooper 2008). Moreover, Wassen et al. (2002) have demonstrated an increased expression of type 3 deiodinase, which converts inactive T3 to its active form in cardiac tissue. Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), which stimulate the upregulation of type 3 deiodinase in cardiac tissue (Wassen et al. 2002; Abo-Zenah et al. 2008), are related to left ventricular diastolic dysfunction (LVDD) (Dinh et al. 2009; Melendez et al. 2010).

Some studies have demonstrated that SCH might cause LVDD, which can be reversed by levothyroxine replacement therapy (Monzani et al. 2001; Yazici et al. 2004). LVDD may have a negative impact in patients with SCH, because LVDD has been shown to be associated with a higher risk of morbidity and mortality in the general population (Pritchett et al. 2005).

Vitamin D deficiency is associated with an increased risk of cardiovascular disease and mortality from all causes (Schottker et al. 2013; Chowdhury et al. 2014). Experimental animal studies have demonstrated that vitamin D deficiency is related to myocardial hypertrophy (Chen et al. 2011; Gupta et al. 2012) and VDR knockout mice have impaired cardiac contractility and relaxation (Tishkoff et al. 2008).

The data suggest that the coexistence of SCH with vitamin D deficiency can cause further impairment of the LVDF. The purpose of this study was to evaluate diastolic function using Doppler echocardiography in patients with SCH accompanied by vitamin D deficiency.

Subjects and Methods

Subjects. Two hundred fifty-four (n=254) participants were recruited from internal medicine and endocrinology clinics between January 2012 and December 2013 in this cross-sectional study. Subjects were included in the study if they were ≥18 years old. Subjects were excluded if they had overt hypothyroidism or overt hyperthyroidism, subclinical hyperthyroidism of any cause, angina pectoris or acute coronary artery disease, pregnancy, vitamin D insufficiency, a history of oral calcium, vitamin D or levothyroxine use in the past six months, or a current or recent history of congestive heart failure, diabetes mellitus, valvular heart disease, any cardiac arrhythmia, or chronic kidney disease. Written informed consent for data collection was obtained from all participants. Our study was approved by the local ethics committee (IRB Number: 99950669/1140).

We defined vitamin D deficiency as a 25-hydroxy vitamin D [25(OH)D] level of <20 ng/ml and vitamin D sufficiency as a 25(OH)D level of ≥30 ng/ml (Lee et al. 2008).

Subclinical hypothyroidism was defined as an elevated serum concentration of thyroid stimulating hormone (TSH=4.5-10 μIU/ml) in combination with normal serum concentrations of fT4 and fT3. Euthyroidism was defined as normal serum TSH (normal range: 0.34-4.25 μIU/ml), fT3 (normal range: 2.4-4.2 pg/ml) and fT4 (normal range: 0.8-1.7 ng/dl) concentrations.

The subjects were divided into four groups. Group 1 was comprised of subjects with concomitant vitamin D deficiency and subclinical hypothyroidism. Group 2 was comprised of subjects with concomitant subclinical hypothyroidism and vitamin D sufficiency. Group 3 was comprised of subjects with concomitant vitamin D deficiency and euthyroidism. Group 4 was comprised of subjects with concomitant vitamin D sufficiency and euthyroidism.

Transthoracic echocardiography was recorded using a Philips iE33 xMATRIX® device (Philips Medical Systems, Bothell, WA, USA) with the X5-1 transducer and the Doppler technique. All images were obtained in the standard parasternal and apical position using 2D, M-mode, and Doppler echocardiographic techniques, according to the American Society of Echocardiography (ASE) guidelines (Lang et al. 2005). Echocardiographic assessment was performed by an experienced cardiologist. The left ventricular (LV) end-diastolic and end-systolic dimensions, as well as the interventricular septum and posterior wall thicknesses, were obtained using M-mode echocardiography (Lang et al. 2005). The left ventricular ejection fraction (LVEF) was measured using biplane Simpson's method, according to the recommendation of the ASE (Lang et al. 2005). The left atrial (LA) volume was estimated using Simpson's method, as recommended by the ASE guidelines (Lang et al. 2005). The pulse Doppler sample volume was placed at the mi-

tral valve tips in the apical four-chamber view to record the LV inflow velocity. From the LV inflow velocity, the early diastolic peak flow velocity (*E*), late diastolic peak flow velocity (*A*), *E*/*A* ratio, and deceleration time of the *E* wave velocity were measured and calculated from three consecutive cardiac cycles (Nagueh et al. 2009). The early diastolic mitral annular velocity (E') and the E/E' ratio were measured at the septal annulus during tissue Doppler imaging. Isovolumic contraction and relaxation times were also calculated from the LV inflow and outflow tract velocities.

Clinical characteristics. Venous blood samples were obtained from the antecubital region between 8:00 and 8:30 am after a 10- to 12-hour overnight fast for all study subjects. Serum creatinine, albumin, uric acid, fasting plasma glucose (FPG), C-reactive protein (CRP), TSH, fT3, fT4, calcium (Ca) and phosphorus (P) levels were measured via standard laboratory techniques using an autoanalyzer (Cobas 6000 analyzer series, Roche Diagnostics, Mannheim, Germany), and serum 25(OH) D levels were measured using high-performance liquid chromatography (Shimadzu UFLC system, Kyoto, Japan).

Statistical analysis. The SPSS statistics 17.0 software program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics for quantitative variables were summarized using the mean ± standard deviation (SD) and the median with interquartile range as appropriate. Categorical data are expressed as frequencies and percentages and were compared using the Pearson Chi-square test. Comparisons between groups were performed using analysis of variance (ANOVA) (with the post-hoc Tukey test for unequal groups) or the Kruskall-Wallis test (the difference between the means of two variables was calculated using the Mann-Whitney U test). A two-tailed p value < 0.05 was considered statically significant.

Results

Baseline characteristics, such as age, gender, body mass index (BMI), smoking, creatinine, albumin, uric acid, FPG, Ca, and P, did not differ among the groups (Table 1). The levels of fT3 and fT4 were lower in Groups 1 and 2 than in Groups 2 and 3, but fT3 and fT4 were within normal limits in all groups (Table 1). CRP levels were higher in Groups 1 and 3 than in Groups 2 and 4 (Table 1).

No significant differences in LVEF, LV end-diastolic diameter, LV end-systolic diameter, LV mass index, or relative wall thickness were observed among the groups. The E' value of Group 1 was significantly lower than

Table 1
Baseline and clinical characteristics among groups

Parameter	Group 1 (n=71)	Group 2 (n=66)	Group 3 (n=65)	Group 4 (n=52)	p value
Age, years	38±12	36±15	39±14	37±13	0.821
Gender, Female n (%)	60 (84.5)	55 (83.3)	54 (83.0)	44(84.6)	0.763
BMI (kg/m²)	25.6±2.9	25.1±3.1	24.7±2.6	24.4±2.8	0.781
Smoker, n(%)	23 (32.3)	22 (33.3)	22 (33.8)	17 (32.6)	0.802
Creatinine (mg/dl)	0.71±0.19	0.68±0.23	0.72±0.17	0.70±0.21	0.619
Albumin (g/dl)	4.4±0.4	4.5±0.3	4.4±0.3	4.3±0.4	0.837
Uric acid (mg/dl)	4.6±1.2	4.8±1.4	5.0±1.5	5.1±1.6	0.541
FPG (mg/dl)	98±5	95±7	93±8	94±6	0.479
Ca (mg/dl)	9.1±0.4	9.9±0.6	9.3±0.7	9.8±0.7	0.716
Phosphate (mg/dl)	3.2±0.5	3.5±0.7	3.3±0.6	3.4±0.8	0.507
TSH (μIU/ml)	8.1±2.2[b,c]	7.9±2.5[#,x]	2.4±1.2	2.5±1.4	<0.001
fT3 (pg/ml)	2.91±0.30[b,c]	2.80±0.22[#,x]	3.51±0.64	3.62±0.71	<0.001
fT4 (ng/dl)	0.99±0.21	1.09±0.32	1.64±0.26	1.59±0.43	<0.001
25 (OH) D (ng/ml)	8.9±6.6[a,c]	41.2±9.5[#]	9.1±7.2[δ]	42.8±8.9	<0.001
CRP (mg/l)	7.6±2.4[a,b,c]	3.1±2.3[x]	2.8±1.9[δ]	0.5±0.3	<0.001

[a]p <0.01 between Group 1 and 2 with post hoc Tukey test; [b]p<0.01 between Group 1 and 3 with post hoc Tukey test; [c]p<0.01 between Group 1 and 4 with post hoc Tukey test; [#]p<0.01 between Group 2 and 3 with post hoc Tukey test; [x]p<0.01 between Group 2 and 4 with post hoc Tukey test; [δ]p<0.01 between Group 3 and 4 with post hoc Tukey test

the corresponding values of Groups 2, 3 and 4 (Table 2). In contrast, the E/E' and LAVI values of Group 1 were significantly higher than those of Groups 2, 3 and 4 (10.2±2.7, 7.9±2.7, 8.1±2.9, and 7.8±2.5 for E/E', respectively; p<0.001; and 31.3±3.2, 28.7±3.0, 28.4±3.4, and 27.9±3.9 for LAVI, respectively; p<0.001). The IVRT values were significantly higher in Groups 1 and 2 than in Groups 3 and 4. Deceleration time, E, A, E/A, E', E/E' and LAVI were similar among Groups 2, 3 and 4 (Table 2).

Discussion

The main finding of the present study is that in patients who have SCH in combination with vitamin D deficiency, the risk of LVDD is higher. We demonstrated that the diastolic function of patients with isolated SCH and isolated vitamin D deficiency does not differ from patients without vitamin D deficiency and SCH.

Currently, vitamin D deficiency/insufficiency is a global public health problem and SCH is present in 4% to 20% of the general population (Holick 2007; Holick and Chen 2008). The coexistence of SCH and vitamin D deficiency might be more frequent than expected. It remains unknown how cardiac function will be affected in patients under both of these conditions. In the current study, LVDF was evaluated for the first time in patients who have SCH deficiency in combination with vitamin D deficiency. We demonstrated that patients who have both vitamin D deficiency and SCH have lower E' values and increased E/E' and LAVI values in comparison to patients in the other groups; these differences were statistically significant. Thus, in the current study, we demonstrated for the first time that patients who have vitamin D deficiency in combination with SCH have a tendency to experience diastolic dysfunction. The observed decrease in early mitral annulus velocity (E') is a reflection of the impairment in myocardial relaxation (Lang et al. 2005; Nagueh et al. 2009). LAVi and E/E' can be used to estimate the LV diastolic filling pressures (Lang et al. 2005; Nagueh et al. 2009). When myocardial relaxation is impaired, mitral valve opening is delayed and IVRT is prolonged. In the current study, while the low (E') and high IVRT values of patients who have vitamin D deficiency in combination with SCH indicates the deterioration of myocardial relaxation, high LAVI and E/E values suggest high left atrial pressure. However, according to the ASE guidelines, LAVI values of ≥34 ml/m^2 in patients with an E/E' ratio between 9 and 14 suggest that the left atrial pressure is increased. In contrast, in the current study, the mean E/E' ratio of 10.2 and the mean LAVI value of 31.3 suggest that the left atrial pressure is normal. We believe that the main impairment is related to myocardial relaxation.

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 (Green et al. 2006). The inhibitory effect of phosphorylated phospholamban on SERCA2 is then removed (Gustavsson et al. 2013). 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

Table 2
Comparison of diastolic index among groups

Parameter	Group 1	Group 2	Group 3	Group 4	p value
E (cm/s)	85±9	86±5	87±6	86±7	0.749
A (cm/s)	80±8	75±9	77±6	76±5	0.438
E' (cm/s)	8.2±1.8[a,b,c]	11.3±2.2	10.9±2.7	11.1±2.9	<0.001
IVRT (ms)	111±19[b,c]	108±15[#,x]	78±18	75±11	<0.001
DT (ms)	183±32	176±29	181±26	175±22	0.213
E/A	1.07±0.21	1.13±0.25	1.11±0.28	1.12±0.22	0.189
E/E'	10.2±2.7[a,b,c]	7.9±2.7	8.1±2.9	7.8±2.5	<0.001
LAVI (ml/m^2)	31.3±3.2[a,b,c]	28.7±3.0	28.4±3.4	27.9±3.9	<0.001
LVMI (g/m2)	92±11	90±13	89±16	87±15	0.527

[a]p <0.01 between Group 1 and 2 with post hoc Tukey test; [b]p<0.01 between Group 1 and 3 with post hoc Tukey test; [c]p<0.01 between Group 1 and 4 with post hoc Tukey test; [#]p<0.01 between Group 2 and 3 with post hoc Tukey test; [x]p<0.01 between Group 2 and 4 with post hoc Tukey test

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 (Kiss et al. 1994; Klein and Ojamaa 2001; Kahaly and Dillmann 2005; Biondi and Cooper 2008). 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 (Chen et al. 2011; Gupta et al. 2012). 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 (Tishkoff et al. 2008). 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 (Simpson et al. 2007; Tishkoff et al. 2008; Bae et al. 2011).

Second, in the current study, in patients with isolated vitamin D deficiency and SCH, CRP levels were significantly higher in comparison to patients who were euthyroid and had sufficient vitamin D levels; in addition, these values were significantly lower in the group that had vitamin D deficiency in combination with SCH. According to the results of the present study, while both SCH and vitamin D deficiency lead to chronic inflammatory processes in patients with both conditions, chronic inflammation continues to increase. Similarly, vitamin D deficiency and SCH were associated with low-grade inflammation (Kvetny et al. 2004; Laird et al. 2014; Zanetti et al. 2014). In this inflammatory environment, the level of inflammatory cytokines, particularly IL-6 and TNF-α, increases and causes an increase in type 3 deiodinase expression in cardiac tissue (Wassen et al. 2002; Abo-Zenal et al. 2008). The resulting increases in the level of inflammatory cytokines and type 3 deiodinase expression are related to the observed impairment of LV diastolic function (Dinh et al. 2009; Melendez et al. 2010).

The present study has some limitations. Because this study was a cross-sectional study, it is not possible to establish a cause/result relationship. Because we did not measure inflammatory cytokines (i.e. IL-6, TNF-α) or perform serial measurements during certain periods, it may not be possible to completely evaluate inflammation in the groups. In addition, blood samples were collected only once due to the limited budget of the study. In the future, patients should be evaluated at different times of the year to study the effect of seasonal variation.

Conclusion. The 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.

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