Cardiac autonomic modulation and anti-TPO antibodies in Subclinical Hypothyroidism – Does any correlation exit?

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Research Article

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Abstract

Background

Heart rate variability (HRV) reflects the balance of activities of sympathetic and parasympathetic components of the autonomic nervous system. Anti-thyroid antibodies have long been associated with thyroid dysfunction and influence thyroid profile testing, the most common being anti-Thyroid Peroxidase (TPO) and anti-Thyroglobulin antibodies. Subclinical hypothyroidism (SCHypo) is characterized by elevated TSH with normal thyroid hormones.We hypothesized that autonomic function may be deranged in anti-TPO positive sub-clinical hypothyroid cases even before the onset of overt hypothyroidism.

Objectives

To investigate the association between anti-TPO antibodies (anti-TPOAb) positive SCHypo and sympathovagal imbalance (SVI).

Methodology:

The study was conducted on age and BMI matched subclinical hypothyroid patients (n = 52) and healthy controls (n = 20). Cardiac autonomic activity was assessed by short term HRV in the time (SDNN, RMSSD, pNN50) and frequency domain ($LFms^2$, $HFms^2$, LFnu, HFnu, TP and LF/HF ratio). Nonlinear geometric measures (SD1, SD2, SD1/SD2, TINN, HRV triangular index) were also evaluated. Biochemical evaluation of serum thyroid profile, anti-TPOAb was done in all the subjects.

Results

Decreased HRV was observed in anti-TPOAb positive group when compared to negative and control groups. Significant positive correlation of anti-TPOAb with TSH, LF nu, LF/HF and negative correlation with SDNN, RMSSD, pNN50, SD1, SD1/SD2, HFnu and TP of HRV was observed.

Conclusion

Anti-TPOAb positive SCHypo group exhibited modifications in HRV characterized by decreased parasympathetic modulation, as compared to controls. The findings were also suggestive of increased risk of autonomic dysfunction in TPOAb- positive patients than negative. Anti-TPO antibody was significantly correlated with TSH and SVI in SCHypo patients.

Introduction

SCHYPO is defined as a clinical entity in which the serum-free thyroxine (fT4) level remains within the reference range with an increased serum thyroid stimulating hormone (TSH) level (*Cooper DS et al., 2012*). Autoimmune thyroid disease (AITD) includes hyperthyroid Grave's disease, hypothyroid autoimmune thyroiditis, and subtle subclinical thyroid dysfunctions (*Yoo & Chung, 2016*). Thyroid autoimmunity is characterized by easily detectable production of thyroid autoantibodies, to thyroglobulin (TG) and thyroid peroxidase (TPO) *(Macdonald, Parsy-Kowalska, & Chapman, 2017).* The sensitivity and specificity of anti-TPO is higher than that of anti-TG for identifying autoimmune thyroid disease *(Spencer, 2000*). TPO-Abs are the circulating hallmark of autoimmune thyroid disease and are present in majority of cases. (*Roberts & Ladenson, 2004*). SCHypo has found to be associated in various studies with an increased number of cardiovascular risk factors and altered autonomic activity (Christine, 2017; Selmer, Hoshi 2019)

The thyroid gland and the Autonomic Nervous System (ANS) are closely linked by their effects on the cardiovascular system (*Reeves, Fisher, Newman, & Granger, 2016; Mavai 2018*). The ANS controls heart by complex mechanism of interactions between its two branches, which produces fluctuations in heartbeat intervals. The greater the beat-to-beat interval, the better the cardiovascular system functions, to adapt and respond to internal and external stimuli (*Billman, 2011*&*Maor et al., 2013*). Heart rate variability (HRV) is an easy, non-invasive, sensitive and widely applied method for cardiac autonomic assessment. Lowered HRV is associated with increased risk of mortality, and HRV has been proposed as a marker for disease (Task Force, 1996).

Autonomic disturbances are common in patients with overt thyroid diseases, even in subclinical conditions *(de Miranda et al., 2018; Mavai 2018*) and it has been suggested that clinical impairments in SCHYPO may precede cardiac dysfunctions (Hoshi 2019, 2020). In light of the above, this study was planned to assess the cardiac autonomic functions by HRV parameters in anti-TPOAb positive and anti-TPOAb negative subclinical hypothyroid subjects and to assess the association between presence of anti-TPOAb in SCHypo and sympathovagal imbalance (SVI).

Materials And Methods

The study was commenced after obtaining formal approval from Institutional Ethics Committee. Informed written consent was obtained from all the subjects included in the study. Age and BMI matched subclinical hypothyroid patients and healthy controls, 20-50 years of age, never previously treated for any endocrine disease and ready to participate were included in the study. Participants were stratified into anti-TPOAb positive (*n*= 35), anti-TPOAb negative (*n*= 17) and control (n=20) groups. SCHYPO was defined as patients with a TSH level of 4.5–10mU/ml and normal Individuals with history or complaints of any cardiac, hepatic or renal dysfunction, HIV / Immunodeficiency disorders, neurological disease, any other systemic disease that may affect autonomic activity i.e. diabetes, hypertension and those taking any drug which affects autonomic activity were excluded. Serum levels of fT_3, fT_4, TSH, anti-TPOAb levels were measured by using the chemiluminescent immunoassay method (IMMULITE 2000 Systems Analyzer). The serum fT_3, fT_4, TSH levels for theeuthyroid state was between 1.8-4.2 pg/mL, 0.89-1.76

ng/dL and 0.4-4.0 µIU/mL.The level of anti-TPOAb was <35 IU/mL, respectively.The presence of antithyroid antibodies was determined by levels of serum antibodies to TPO. TPO antibodies were considered positiveabove these values.

Based on the above mentioned criteria, the following recruitment /screening plan was charted: **see figure**.

Based on the finding of the screening, the patients were grouped as Anti-TPOAb positive Subclinical Hypothyroid (35), Anti-TPOAb negative Subclinical Hypothyroid (17) age and BMI matched 25 healthy subjects (asymptomatic with normal clinical examination and biochemical tests and not on any medication) randomly selected from general population. Confirmation of clinical diagnosis was done by biochemical evaluation of FT_3, FT_4, TSH, anti-TPO Antibody.

Heart Rate Variability assessment was done by recording 5 minutes ECG by RMS ECG (DECG 1/ 63041/ ADBXB). The analogue signals were converted to digital signals by National Instrument Software NI-DAQ Version 8.0. The analysis was performed using linear methods, analyzed in the time domain, frequency domain and by the Geometric measures. Linear analysis of HRV for time domain (SDNN, RMSSD, pNN50%) and frequency domain (Total Power, LF (ms^2), HF(ms^2), HF in normalized unit (nu), LF (nu), LF/HF ratio) parameters was performed by HRV analysis software version 1.1 (Bio-signal Analysis group, Kuopio, Finland).

Time domain analysis included the squared root of the mean of the sum of the squares of successive normal R–R interval differences (RMSSD) and the percentage number of pairs of adjacent normal R–R intervals differing by more than 50 ms in the entire recording (pNN50) were used as indexes of Parasympathetic activity. We also computed the standard deviation of all normal R–R intervals(SDNN).

In the frequency domain following indices: total power (TP) of HRV spectrum, the power in the high (HF) and the low frequency (LF) bands (TP: 0–0.4 Hz; LF: 0.04– 0.15 Hz; HF: 0.15–0.4 Hz) in absolute units (ms^2),HFnu, LFnu and the LF/HF ratio were then used in the analyses (Task Force, 1996).

In the geometric measures, Poincaré plot was evaluated quantitatively through the computation of the SD indexes of the plot. Poincare indices, SD1 (standard deviation of the instantaneous beat-to-beat variability), SD2 (standard deviation of the long-term continuous RR intervals); SD1/SD2 ratio; and TINN((triangular interpolation of NN intervals); HRV index were analyzed (Billman, 2011; Hsu et al., 2012; Rossi et al., 2015).

Experimental Protocol:

1. For HRV, the participants underwent 5 min of rest in supine position. Recording was done in quiet and comfortable room, temperature was maintained at 24-28°C. Subject was instructed to close the

eyes and to avoid talking, moving hands, legs and body, coughing during test, sleeping.

2. The subjects were instructed to avoid food at least two hours before the procedure, abstain from coffee, nicotine or alcohol 24 hours prior to testing and to wear loose and comfortable clothing. They were asked to report at 10.00 am. Their age, height, and body weight were recorded. Body Mass Index (BMI) was calculated by weight (kg) divided by the square of height (meter) (Quetelet's Index).
3. All standard limb leads were applied and the lead with upright R wave was selected for recording. The ECG signals were continuously amplified, digitized and stored in the computer for offline analysis. Processing including R wave and RR interval detection was done by the software. Abnormal beats and areas of artifact were automatically identified and excluded from the recording.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). Descriptive characteristics are presented as means and standarddeviations (mean± SD). Kolmogorov–Smirnov test was done to assess the normal distribution of variables. For parametric data, the level of significance among the groups was tested by One-way ANOVA followed by post-hoc Bonferroni test and for non-parametric data; Kruskal-Wallis One way ANOVA with post hoc Dunn's multiple comparison test was used. Spearman correlation tests were done to study associations between various variables. For all the analyses statistical significance was defined at the level of $p < 0.05$.

Results

The study population consisted of 52 SCHypo patients and 20 controls. Based on the presence of thyroid autoantibody (anti-TPOAb) status, subjects were divided into two groups: anti-TPOAb positive group (n=35) and anti-TPOAb negative group (n=17).

Table 1: Age, BMI and thyroid profile and anti- TPOAb parameters of control, anti- TPOAb negative and anti- TPOAb positive Subclinical hypothyroid subjects

Parameters (Mean ± SD)	Control (n=20)	Anti TPOAb negative SCHypo (n=17)	Anti TPOAb positive SCHypo (n=35)	*p*-value	Intergroup comparison *p* value
Age(yrs.)	34.44±10.0	32.12±8.49	34.05±7.25	>0.05	ns*†‡
BMI(kg/m^2)	21.3±1.11	22.33±2.62	22.34±1.68	>0.05	ns*†‡
FreeT3(pg/mL)	2.59±0.47	2.89±0.25	2.78±0.72	>0.05	ns*†‡
Free T4(ng/dL)	1.21±0.19	1.12±0.22	1.10±0.31	>0.05	ns*†‡
TSH(uIU/mL)	2.41±1.15	6.47±2.20	6.68±3.12	<0.001	<0.001*†, ns‡
Anti-TPO(IU/mL)	22.96±4.6	25.39±8.12	597.4±454.8	<0.001	ns* , <0.001†‡

Data presented in Mean±SD. P<0.05 were considered statistically significant. Intergroup comparison was done using one-way ANOVA followed by post hoc Bonferroni multiple comparison test (Age, BMI) and Kruskal–Wallis followed by Dunns multiple comparison test (Thyroid profile and Anti-TPO Ab). ns: not significant. *Control vs anti-TPOAb negative, † Control vs anti-TPOAb positive, ‡ anti-TPOAb positive vs anti-TPOAb negative. BMI - Body mass index; TSH - Thyroid stimulating hormone; Anti TPOAb – Anti Thyroid Peroxidase antibodies; SCHypo – Subclinical Hypothyroidism

Table 1 shows comparison of the age, body mass index (BMI), thyroid hormone profile and anti-TPOAb levels among the three groups. TSH difference was statistically significant between control and test groups (p<0.001) whereas, age, BMI, free T3, free T4 were comparable. Anti-TPO antibody was found to be statistically significant higher in antibody positive group as compare to antibody negative and controls (p<0.001).

Table 2: Time and frequency domain measures of HRV in control, anti-TPOAb negative and anti- TPOAb positive Subclinical hypothyroid subjects

Parameter (Mean ± SD)	Control (n=20)	Anti TPOAb negative SCHypo (n=17)	Anti TPOAb positive SCHypo (n=35)	*p*-value	Intergroup comparison *p* values
SDNN (ms)	53.43±14.22	48.02±9.42	42.2±6.43	<0.01	ns*‡, <0.01†
RMSSD(ms)	54.03±20.35	50.12±9.63	43.15±17.79	>0.05	ns*†‡
pNN50(ms)	25.63±7.79	20.52±18.81	11.17±6.4	<0.001	ns*‡, <0.001†
LF(ms^2)	994.8±632.3	865.2±460.3	618.5±310.7	>0.05	ns*‡†
HF(ms^2)	1298±681.7	1058±801.5	922.1±316.7	>0.05	ns*†‡
LF nu	40.89±9.41	45.61±8.55	47.25±10.72	>0.05	ns*†‡
HF nu	61.33±14.81	55.68±12.0	50.20±13.36	<0.05	ns*‡, <0.05†
LF/HF	0.75±0.36	1.01±0.40	1.2±0.52	<0.01	ns*‡, <0.01†
TP	3482±1183	2724±768.1	2178±956.9	<0.001	ns*‡, <0.001†
SD1(ms)	36.82 ± 16.68	25.47 ± 14.59	17.71 ± 11.24	<0.0001	ns*‡, <0.0001†
SD2(ms)	40.51 ± 20.85	46.70 ± 20.11	59.19 ± 26.78	<0.05	ns*‡, <0.05†
SD1/SD2	1.18 ± 0.79	0.56 ± 0.33	0.47 ± 0.55	<0.0001	ns*‡, <0.0001†
HRV Index	0.40 ± 0.26	0.31 ± 0.24	0.27 ± 0.21	>0.05	ns*‡†
TINN	338.6 ± 256.6	222.4 ± 102.7	161.6 ± 71.56	<0.01	ns*‡, <0.01†

Data presented in Mean±SD. P<0.05 were considered statistically significant. SDNN - standard deviation of normal to normal interval, RMSSD- the square root of the mean of squares of the differences between adjacent NN intervals, pNN50 - the proportion derived by dividing NN50 by the total number of NN intervals, LF - low frequency power(ms^2), HF - high frequency power(ms^2) , LFnu - normalized low frequency power, HFn - normalized high frequency power, LF/HF - Ratio of LF to HF, TP- Total power, SD1/SD2 – Ration of SD1 to SD2 (Poincare index), TINN Triangular interpolation of NN interval; Anti TPOAb – Anti Thyroid Peroxidase antibodies; SCHypo – Subclinical Hypothyroidism. Intergroup comparison was done using Kruskal–Wallis followed by Dunns multiple comparison test. ns: not significant, *Control vs anti-TPOAb negative, † Control vs anti-TPOAb positive, ‡ anti-TPOAb positive vs anti-TPOAb negative.

Table 2 demonstrates the HRV indices analyzed in the time, frequency domain and nonlinear geometric measures.

TPOAb -positive patients had significantly lowerSDNN (p<0.01), pNN50 (p<0.001) and TINN (P < 0.01)values compared to controls,though,RMSSD and HRV triangular index (P >0.05)werefound to benon-significant. Furthermore, in Ab- positive SCHYPOHF nu, SD1turned lower (HF p<0.05, SD1 p<0.0001) and SD2 turned higher (p<0.05) than controls. TP (p<0.001) was significantly decreased in TPOAb-positivegroup than controls. TheLF/HF, SD1/SD2 ratio exhibitedsignificant change (LF/HFp<0.01, SD1/SD2 p<0.0001) in anti-TPOAb positive patients in comparison to controls.Although not significant, there was a trend of an increase inLF nu, LF/HF, SD2 and decrease in SDNN, RMSSD, pNN50,HFnu, SD1, SD1/SD2, total power (TP), HRV index and TINN in TPOAb-positive group as compare to TPOAb-negativegroup, as illustrated in Table 2.

Table 3: Correlation of anti-TPOAb with TSH & HRV parameters

Parameters	R	p value
TSH	0.963	**<0.0001***
LF(ms^2)	-0.248	0.169
LFnu	0.635	**<0.0001***
HF(ms^2)	-0.146	0.425
HFnu	-0.606	**0.002***
TP	-0.639	**<0.0001***
LF/HF	0.757	**<0.0001***
SDNN	-0.999	**<0.0001***
RMSSD	-0.999	**<0.0001***
pNN50	-0.999	**<0.0001***
SD1	-0.786	**<0.0001***
SD2	0.831	**<0.0001***
SD1/SD2	-0.999	**<0.0001***
HRV Index	-0.178	0.3462
TINN	-0.356	0.0535

P<0.05 were considered statistically significant. TSH: Thyroid Stimulating Hormone; LF low frequency power(ms^2), HF high frequency power(ms2), LFnu normalized unit low frequency power, HFnu normalized high frequency power, LF/HF ratio of LF to HF, TP total power, SDNN standard deviation of normal to normal interval, RMSSD the square root of the mean of squares of the differences between adjacent NN intervals, pNN50 the proportion derived by dividing NN50 by the total number of NN intervals,SD1, SD2 and SD1/ SD2 ratio (Poincare index),, TINN triangular interpolation of NN interval.

Spearman rank Correlation test was used. '*' denotes significant correlation. Minus r values depicts negative correlation and plus sign shows positive correlation.

Table 3 presents correlation of TPO with TSH and HRV indices. We found a positive correlation of Anti-TPOAbwith TSH, LF nu and LF/HF, (p<0.05) and negative with HF nu, TP,SDNN, RMSSD and pNN50 (p<0.05). When we correlatedanti TPOAb with nonlinear geometricalmeasures, we found a negative correlation withSD1, SD1/SD2 (p<0.05); HRV index,TINN(p>0.05) and positive with SD2 (p<0.05), as descripted in Table 3.

Discussion

The study compared the autonomic activity in TPO Ab-positive' and -negative groups. The anti-TPO antibody- positive group demonstrated greater alterations in autonomic modulation, primarily characterized by diminished parasympathetic activity. Literature search could not retrieve any study on anti-TPOAb status of SCHypo patients and HRV. However research has shown significantl higher sympathetic and lower parasympathetic modulation at baseline conditions in SCHypo (Hoshi 2019).

No significant difference was observed in TSH levels between anti-TPOAb positive and anti-TPOAb negative patients, however there was a significant difference in the TSH levels between the control and SCHypo groups. Contrary to this finding, *Brown J* (2016) evidenced significantly higher serum TSH in antibody-positive than in antibody-negative individuals *(Brown et al., 2016*).

In the current study, significant increase in the LF–HF ratio in the anti-TPOAb positive SCHYPO patients was recorded in comparison to the control group, however no significant difference was observed between control and anti-TPOAb negative patients. The LH–HF ratio is a sensitive marker of SVI (*Malliani A, 2005 &Task Force, 1996*), hence the increase in LF–HF ratio represents considerable SVI in anti-TPOAb positive SCHYPO. In the Poincaré indices (SD1 and SD2) of HRV, width of SD1 reflects the parasympathetic modulation and the SD2 length reflects the sympathetic activity (Hsu et al., 2012). In our study, SD1 value was profoundly decreased (p<0.0001) and SD2 was increased (p<0.05) in antibody positive SCHYPO group than controls, which can be used as a sensitive indicator of sympathovagal changes. Akin to HF-LF ratio, the SD1-SD2 ratio also reflects the sympathovagal balance. The significant decrease in SD1/SD2 in Anti TPO-Ab positive group further confirms SVI in TPO-Ab positive SCHYPO.

In anti-TPOAb positive SCHypo patients, a significant decrease in HFnu indicates decreased parasympathetic activity in these patients, as HFnu is an index of cardiac vagal drive (*Malliani A, 2005 & Task Force, 1996*). This was supported by a significant reduction in TP of HRV in antibody-positive SCHypo patients compared to controls, as TP in general indicates the magnitude of vagal modulation of cardiac function (*Malliani A, 2005 & Task Force, 1996*).

The time-domain indices of HRV - SDNN, RMSSD and pNN50 were significantly decreased in anti-TPOAb positive SCHypo patients than controls , further confirming the decreased vagal tone in these patients. The reduction in parasympathetic activity in TPO-positive group compared to healthy individuals can be further verified by the decrease in the SD1 indices. Hence, anti-TPO positive SCHypo group showed lower parasympathetic modulation than control group. A decrease vagal activity is related with an increased risk for all-cause morbidity and mortality and for the development of numerous other risk factors (Thayer & Lane, 200~~7~~).

In this study, no significant differences in cardiac vagal activity was observed in the two SCHypo groups, however TPOAb-positive group showed lower values in magnitude for parasympathetic indices in comparison to TPOAb-negative group. This points towards sympathovagal imbalance (SVI) in anti-TPOAb positive SCHypo group. Thus, from the present study, it may be presumed that SVI in anti-TPO positive SCHYPO could be due to diminished vagal activity. In another study, o reduced time domain indices and lower HF values in frequency domain analysis in SCHypo patients was founs in comparison to controls (*Hoshi 2019*).

In our study, anti- TPOAb were found to correlate positively with TSH and HRV indices. To the best of our knowledge, no study has assessed the correlation of anti-TPOAb with TSH levels and HRV indexes (linear as well as nonlinear) in SCHypo patients. It has been proved that Anti-TPOAb negative autoimmune thyroiditis has a milder course while the presence of anti-TPOAb is associated with an increased risk of overt hypothyroidism (*Radetti et al., 2012*). Furthermore, presence of antibodies is linked with increased risk of atherosclerotic disease and myocardial infarction. Hence, anti-TPOAb positive SCHypo patients are more prone to cardiac risk than anti-TPOAb negative SCHypo patients, this has been proved further by this study. Low parasympathetic activity has been also proposed as an important risk factor for cardiovascular disease and mortality (Mavai et al., 2018) .

The limitations of the present study are that we could not assess the cardiac autonomic reactivity by Conventional Autonomic Function Tests (CAFT). Also, we have not studied the cardiac dysfunctions by echocardiography and their possible correlation with anti-TPOAb levels in subclinical hypothyroid subjects. Future studies should be planned to confirm our findings in large sample size.

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Declaration

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