Research Article

A Comparative Study of VPT in Newly Detected Type 2 Diabetic Individuals With and Without Subclinical Hypothyroidism.

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ABSTRACT

Background: Type 2 Diabetes (T_2D) and subclinical hypothyroidism (SCH), are common endocrine disorders. Presence of both will have a prominent, augmented complex interaction with obvious interdependence. Diabetic peripheral neuropathy (DPN) is a T_2D complication. VPT assessment is a quantitative testing and screening tool for DPN, is easy, accurate assessment. Objectives: To study, assess and compare VPT in normal, T_2D with and without SCH.

Study Design: A cross-sectional, observational, and comparative study design including 90 subjects; 3 groups of 30 each; Group-A: Healthy subjects, Group-B: Newly detected T_2D, Group-C: Newly detected T_2D and SCH.

Materials and Methods: Basic characteristics, Anthropometry, FBS, PPBS, HbA1c, TSH and VPT tests were conducted and the results were analyzed using ANOVA.

Results: FBS, PPBS, HbA1c of Group-B and C were statistically higher. TSH values of Group-C was statistically higher. Average VPT of Group-B and C were statistically higher.

Discussion: Groups (B, C) included newly detected T_2D. Free T4 levels were comparable. TSH was significantly higher in the Group-C. Higher VPT values indicated DPN in T_2D at diagnosis. T_2D with SCH has higher VPT values suggesting that SCH aggravates the pathogenetic mechanism causing DPN.

Conclusion: Microvascular changes in T_2D at diagnosis predisposes DPN and an altered VPT identifies those at risk. Early diagnosis will prevent nerve damage. Life style modification, treatment of T_2D and SCH at an earliest disease stage may even reverse DPN

Keywords: New T_2D, SCH, DPN, VPT

1. INTRODUCTION

1.1. Background:

Type 2 Diabetes Mellitus (T_2D) is the most prevalent endocrine disease. Hyperglycemia in DM arises due to the impaired secretion and/or altered insulin action. According to the International Diabetes Federation (IDF), approximately 415 million adults aged 20-79 years were living with DM in 2015, a number projected to rise to over 600 million by 2040 [1]. As the global burden of T_2D increases, so does the need to understand and manage its associated complications.

Subclinical hypothyroidism (SCH), another common endocrine condition, involves dysregulation of the hypothalamic-pituitary-thyroid (HPT) axis. It is characterized by elevated serum thyroid-stimulating hormone (TSH) levels with normal levels of free thyroid hormones [2]. Individuals with SCH are often asymptomatic, in contrast to overt hypothyroidism. However, SCH may still influence metabolic homeostasis and is

increasingly recognized as a potential modifier of other metabolic diseases, including T_2D.

Emerging evidence suggests a complex, bidirectional interaction between T2D and thyroid dysfunction [3]. SCH has been reported to be more prevalent among individuals with T2D than in the general population [4, 5]. Contributing risk factors include genetic predisposition, obesity, impaired glucose tolerance, insulin resistance, and sedentary lifestyle. Moreover, hypothyroidism may independently contribute to the development of T_2D [6].

Chronic hyperglycemia in T_2D can result in microvascular complications affecting multiple systems, including the peripheral nerves-manifesting as diabetic peripheral neuropathy (DPN) [7]. DPN commonly begins in the lower extremities and may progress to serious complications such as foot ulcers, joint destruction, and limb amputation. It may also cause autonomic dysfunction, presenting as urinary incontinence, orthostatic hypotension, gastrointestinal issues, and sexual dysfunction.

Subclinical hypothyroidism may contribute to metabolic syndrome, atherosclerosis, cardiovascular morbidity, and neuromuscular dysfunction. Thyroid hormones play a vital role in glucose homeostasis and insulin sensitivity. Therefore, SCH may influence the development or progression of DPN in individuals with T_2D.

Vibration perception threshold (VPT) testing using a biothesiometer is a reliable, quantitative method for detecting and assessing DPN [8]. A VPT value >25 V indicates a high risk of sensory loss. Despite several studies linking VPT abnormalities to T_2D and SCH independently, few have explored the association of SCH with VPT changes in newly diagnosed T_2D.

1.2. Study hypothesis:

"Newly diagnosed T_2D with SCH affects VPT values and may serve as an early predictor of DPN risk".

1.3. Objectives:

To assess and compare VPT in healthy individuals, newly diagnosed T_2D individuals with SCH, and newly diagnosed T_2D individuals without SCH.

2. MATERIALS AND METHODS:

2.1 Materials

A cross-sectional, observational, and comparative study was conducted at the Physiology Research Laboratory of a medical college and tertiary healthcare center in a rural area over 6 months. Ninety individuals meeting the eligibility criteria were enrolled and divided into three groups:

- **Group A (n=30):** Healthy controls without T_2D or SCH
- **Group B (n=30):** Newly diagnosed T_2D without SCH
- **Group C (n=30):** Newly diagnosed T_2D with SCH

All participants underwent a detailed clinical examination and completed a standardized questionnaire. Ethical clearance was obtained, and written informed consent was provided by all participants.

Inclusion criteria:

- Age >25 years
- Age matched males and females
- Newly diagnosed T_2D (within 1 year), with or without SCH

Exclusion Criteria:

- Age <25 years, overt thyroid disease, pregnancy, T_1D, serious systemic illness, steroid use, epilepsy, psychiatric or neurological conditions, smoking

2.2 Study Procedure:

A standard proforma was employed for a detailed clinical and laboratory evaluation. The gender, age (years), BP and the details of any co morbidities / illness were documented.

2.3 Anthropometric measurements:

Study subjects reported in comfortable dressing. Anthropometric measurements were done in standing and included height, weight, waist, and hip circumference. A height meter, horizontally wall mounted was used to measure height to the last complete 0.1 cm. An electronic, digital weighing scale was used to measure the weight to the last complete 0.1 kg. BMI was determined by the formula weight (kg)/height (m2). Using a measuring tape, WC (cm) was measured at the midpoint between the iliac crest and the lower ribs. HC (cm) was measured around the gluteal

region and the largest circumference recorded. Waist Hip ratio (WHR) was calculated.

2.4 Biochemical Parameters:

Following an overnight fast of over 12 hours, trained technicians were employed to apply aseptic precautions and draw 4 ml of peripheral venous blood from the antecubital vein. The tests were conducted at the biochemistry lab.

- 2 ml of venous blood was collected into a fluoride vacutainer and used for FBS (fasting blood sugar), PPBS (post prandial blood sugar) and HbA1c estimation. FBS, PPBS estimation was by glucose oxidase peroxidase method and Glycosylated hemoglobin (HbA_1c) by HPLC (High power liquid Chromatography) method.
- 2 ml of venous blood was collected into a plain vacutainer and used for thyroid hormones estimation. TSH was estimated by electrochemiluminescence immunoassay (ECLIA). The normal range of TSH is 0.4-4.20 µIU/ml. Primary hypothyroidism was diagnosed when the TSH was more than 4.2 µIU/ml and T4, FT4 were less than the normal values. SCH was diagnosed when the TSH was more than 4.2 µIU/mL and T4, FT4 were within the normal range [9].

2.5 Vibration perception threshold test:

VPT was assessed in all using digital biothesiometer (vibrosense), an instrument which measures the threshold of appreciation of vibration sense. The vibration frequency of the biothesiometer is 120 Hz, with an output range of 0-50 volts. In a lie down position, probe was placed on the six points on each foot (plantar aspect); one on the plantar aspect of the big toe, three points at the metatarsal's heads, one at the instep and one on the heel [10, 11]. To begin with, the probe was placed on the subject hand. Subject was requested to focus to appreciate and inform the sense of vibration when felt to note the VPT value. The stimulus range (volts / V) was gently intensified to reach a threshold and appreciate the stimulus. Vibratory threshold of $\leq$ 15 V is normal, 16-25V is grade 1 derangement and >25 V is grade 2, considered abnormal, a strong predictor of sensory loss [12, 13]. Single blinding was followed during subjects' assignment to the VPT assessors (technicians). An average of all 12 VPT values (6 per foot) was considered for analysis [13, 14].

2.6 Sample Size Estimation:

A total of 90 participants, with 30 individuals in each of the three groups, was determined to be sufficient based on a one-way ANOVA sample size estimation. The calculation assumed a significance level (α) of 0.05, a power of 80%, and a moderate effect size (Cohen's f = 0.333), informed by findings from Bharathi *et al.,* [15] who reported significant differences in vibration perception threshold (VPT) between diabetic neuropathy patients and healthy controls. The effect size was derived using the formula $f = \sqrt{(\eta^2 / (1 - \eta^2))}$, and the required total sample size was estimated using $n = ((k - 1)(Z_{1-\alpha/2} + Z_{1-\beta})^2) / (k \times f^2)$, where k represents the number of groups. This sample size was considered adequate to detect clinically meaningful differences in VPT across groups with sufficient statistical power.

2.7 Statistical Analysis:

The results of all three groups were analyzed and compared using Analysis of Variance (ANOVA) test and descriptive statistics. Effect Size Interpretation (Cohen's d) to quantify the comparison was performed. Further Bonferroni post hoc analysis was performed for variables with a statistically significant p-value in the overall ANOVA. Bonferroni post hoc analysis was performed after an ANOVA to determine which specific group means differ significantly from each other. The findings of Bonferroni post hoc analysis supported the ANOVA results.

3. RESULTS AND DISCUSSION:

3.1 Results:

The primary attributes of the subjects of the 3 groups, age, calculated BMI (Wt / Ht^2), WC, HC, W/H ratio were similar and did not show any statistical difference ($p > 0.05$, Table - 1).

The blood glucose values (FBS, PPBS, HbA1c) of Group - B and Group - C (Cases) were higher than the blood glucose values of Group - A and showed statistical difference ($p < 0.05$, Table - 1).

The TSH values of Group - C (Cases - 2) was higher than the TSH values of the other 2 groups and showed statistical difference ($p < 0.05$, Table - 1)

The Free T_4 values of the subjects of the 3 groups, was similar and did not show any statistical difference ($p > 0.05$, Table - 1).

The average VPT of Group - B and Group - C (Cases) were higher than the VPT values of Group - A and showed statistical difference ($p < 0.05$, Table - 1).

Table - 1: ANOVA Results and Effect Size of the 3 Groups comparison

Variable	Group - A (Mean ± SD)	Group - B (Mean ± SD)	Group - C (Mean ± SD)	p-value	Effect Size
Age	41.07 ± 4.94	41.80 ± 3.87	40.50 ± 3.12	0.524	Small
BMI	23.96 ± 4.16	22.93 ± 4.89	23.58 ± 2.79	0.607	Small
WC	95.66 ± 10.70	96.53 ± 12.65	99.63 ± 11.65	0.389	Small
HC	98.67 ± 6.82	97.08 ± 11.59	99.73 ± 11.88	0.611	Small
W/H	0.97 ± 0.13	1.01 ± 0.19	1.01 ± 0.18	0.606	Small
FBS	96.34 ± 9.33	118.78 ± 11.01	122.87 ± 15.42	0.000 *	Large
PPBS	128.71 ± 10.59	182.26 ± 26.57	191.33 ± 36.17	0.000 *	Large
HbA1c	5.08 ± 0.45	6.88 ± 0.72	6.96 ± 0.71	0.000 *	V Large
Free T4	1.26 ± 0.34	1.31 ± 0.16	1.30 ± 0.20	0.690	Small
TSH	1.97 ± 0.69	2.19 ± 0.83	5.59 ± 1.68	0.000 *	Large
VPT	5.71 ± 0.35	8.75 ± 0.22	10.82 ± 0.41	0.000 *	E Large

* p value < 0.05. Effect size interpretation: Small: 0.2, Medium: 0.5, Large: 0.8+

Table - 2: Qualitative Bonferroni Post Hoc comparisons

Variable	Group A vs Group B	Group A vs Group C	Group B vs Group C	Significance
FBS	A (96.34) vs B (118.78)	A (96.34) vs C (122.87)	B (118.78) vs C (122.87)	A significantly (p value < 0.001) differs from both B and C
PPBS	A (128.71) vs B (182.26)	A (128.71) vs C (191.33)	B (182.26) vs C (191.33)	A significantly (p value < 0.001) differs from both B and C
HbA1c	A (5.08) vs B (6.88)	A (5.08) vs C (6.96)	B (6.88) vs C (6.96)	A significantly (p value < 0.001) differs from both B and C
TSH	A (1.97) vs B (2.19)	A (1.97) vs C (5.59)	B (2.19) vs C (5.59)	C significantly (p value < 0.001) differs from both A and B
VPT	A (5.71) vs B (8.75)	A (5.71) vs C (10.82)	B (8.75) vs C (10.82)	All groups significantly (p value < 0.001) differ from each other

Bonferroni post hoc analysis was performed for variables with a statistically significant p-value in the overall ANOVA. Qualitative Bonferroni post hoc comparisons based on the means and SDs was performed to determine which specific group means differ significantly from each other groups (Table - 2). Bonferroni Post-hoc analysis revealed that all pairwise group comparisons were statistically significant ($p < 0.001$), thus supporting the ANOVA results.

3.2. Discussion:

VPT values were assessed and compared among three groups: healthy individuals (Group A), newly detected T_2D patients without SCH (Group B), and newly diagnosed T2D patients with SCH (Group C). The baseline characteristics such as age, BMI, WC, WHR were comparable across the groups, showing no statistically significant differences. This suggests that the observed results are likely influenced by the presence of T_2D and SCH rather than by anthropometric differences.

As expected, blood glucose parameters (FBS, PPBS, HbA1c) were significantly higher in Groups B and C compared to Group A. This finding aligns with the study design, as Groups B and C included individuals with newly diagnosed T_2D, while Group A comprised non-diabetic controls. The elevation in blood glucose levels in Groups B and C thus reflects the diabetic status of these participants.

Free T_4 levels were comparable across all three groups, with no statistically significant differences observed. As FT4 is the unbound, biologically active form of thyroxine, it readily enters tissues to exert its physiological effects. FT4 is considered a more accurate indicator of thyroid function than total T_4, especially when used alongside TSH measurements.

In this study, TSH levels were significantly elevated in Group C compared to Groups A and B. Group C participants (with SCH) demonstrated elevated TSH levels despite having normal FT4 concentrations, which is the hallmark diagnostic feature of SCH.

SCH is more prevalent among women of reproductive age and has been associated with a range of adverse outcomes, including infertility,

miscarriage, CVD, and psychiatric disorders. [16,17,18] Despite these associations, individuals with SCH often remain asymptomatic and are frequently diagnosed incidentally. Consequently, the decision to treat SCH varies widely and is often influenced more by clinician preference and clinical judgment than by universally accepted evidence-based guidelines.

VPT values, though in normal range, were significantly higher in Groups B and C (newly diagnosed T_2D with and without SCH, respectively) compared to Group A (healthy controls), with a largest effect size indicating a higher incidence of DPN in these individuals and reinforcing the utility of VPT in differentiating neuropathy severity. Furthermore, VPT values were highest in Group C, suggesting that the presence of subclinical hypothyroidism (SCH) may exacerbate neuropathic changes in individuals with T_2D.

These findings reinforce the notion that DPN can be present even at the time of T2D diagnosis. This is consistent with existing evidence that the pathophysiological processes underlying T2D, including chronic hyperglycemia and microvascular dysfunction, often begin several years prior to clinical diagnosis [7, 19, 20]. The significantly elevated VPT values in Group C compared to Group B suggest that SCH may contribute to or worsen the development of DPN in T2D patients.

These results are in alignment with findings from other studies conducted in Asian and Chinese populations, which have similarly reported a higher prevalence and severity of DPN in patients with coexisting SCH and T_2D [21, 22, 23].

Appreciating the pathogenesis of T_2D and its complications, particularly DPN, is inherently complex. The findings of this study suggest that SCH may play a significant additive role in the development of DPN among individuals with T_2D. Several mechanisms have been proposed in the literature to explain this relationship. These include mitochondrial diseases / dysfunction, apoptosis of the Schwann cell, higher oxidative stress, chronic inflammation, dyslipidemia, persistent hyperglycemia, and insulin resistance, all of which may be exacerbated by SCH.

The observation that VPT values were significantly higher in T2D with SCH compared to those without SCH supports the hypothesis that thyroid dysfunction contributes to the pathophysiological processes leading to neuropathy. This highlights the need to consider routine thyroid function screening in newly diagnosed T_2D. Early identification and management of SCH may help mitigate the risk or progression of DPN and reduce associated neurological complications.

Furthermore, VPT assessment proves to be a valuable, non-invasive, and reliable tool for the early detection of peripheral neuropathy. Incorporating VPT testing into the initial evaluation of T_2D, particularly those with coexisting SCH and may allow for earlier interventions and better long-term outcomes.

4. CONCLUSION:

Manifestation of the microvascular changes in T_2D at diagnosis appears to predispose DPN. Elevated VPT values will identify individuals at a higher risk of developing neuropathic symptoms. An early diagnosis of DPN as suggested by altered VPT values will help prevent worsening of nerve damage. Life style modification including a healthy diet, physical exercise, optimal glycemic control combined with early identification and treatment of SCH, may help delay or potentially reverse the onset of DPN. Given this observed observation, thyroid function screening and VPT test should be considered essential components of initial diabetes evaluation and management protocols.

Limitations: Considering the magnitude of the disease issue, the sample size considered appears small. Scope for further study with a larger sample can yield better results and indications.

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