

## ORIGINAL ARTICLE

# Power Spectral Analysis of HRV for Evaluation of Sym-pathovagal Imbalance in Thyroid Dysfunctions

Manisha Mavai<sup>1</sup>, R.C.Gupta<sup>2</sup>, Keerti Mathur<sup>3</sup>, Urmila Choudhary<sup>4</sup>

<sup>1,2</sup>Department of Physiology, M. G. Medical College, Jaipur (Rajasthan),

<sup>3</sup>Senior Professor, Department of Physiology, S.M.S Medical College, Jaipur (Rajasthan)

<sup>4</sup>Senior Demonstrator, Department of Physiology, Dr.S.N. Medical College, Jodhpur (Rajasthan).

Email ID: manishamavai@gmail.com

### ABSTRACT

Thyroid hormone impacts on the cardiovascular system. The cardiovascular system is influenced by the autonomic nervous system and ANS abnormalities may increase cardiovascular morbidity and mortality. The aim of the study was to assess the cardiac autonomic functions by power spectral in patients with hypothyroidism, hyperthyroidism and to compare it with healthy controls. Autonomic functions were evaluated by measurement and analysis of frequency domain parameters of HRV by ECG method and scored in 50 hypothyroid, 50 hyperthyroid patients, 20-60 years and compared with 25 controls. The present study was conducted in the Dept. of Physiology, SMS Medical College, Jaipur with the collaboration of Dept. of Endocrinology of the Institute. Informed written consent was obtained from all the subjects included in the study. Biochemical estimation of TSH,  $fT_3$ ,  $fT_4$  was done. Results were presented as Mean  $\pm$  SD. For statistical analysis, One Way ANOVA (Post Hoc Tukey) test was used. Result shows that frequency parameters of HRV except LF nu and LF/HF ratio were reduced in subjects of hypothyroidism and hyperthyroidism as compared to controls suggesting sympathovagal imbalance with sympathetic overactivity and diminished parasympathetic activity.

**Keywords:** Heart rate variability, hypothyroidism, hyperthyroidism, PSA, LF, HF, LF/HF ratio

Received 04/09/2014 Accepted 21/11/2014

©2014 Society of Education, India

### How to cite this article:

Manisha M, R.C.Gupta, Keerti M, Urmila C. Power Spectral Analysis of HRV for Evaluation of Sym-pathovagal Imbalance in Thyroid Dysfunctions. Adv. Biores., Vol 5 [4] December 2014: 151-156. DOI: 10.15515/abr.0976-4585.5.4.151156

### INTRODUCTION

The autonomic nervous system is the part of nervous system that exerts a tight control on essential functions such as circulation, respiration, thermoregulation and hormonal secretion. Autonomic nervous system plays an important role in the human response to various internal and external stimuli, which can modify homeostasis [1]. The term dysautonomia refers to a change in autonomic nervous system function that adversely affects health [2].

HRV analysis is a sensitive tool for the detection of autonomic nervous system regulation of the heart [3]. It reflects the balance between the sympathetic and the parasympathetic tone: when the sympathetic tone is dominant the HRV is low and vice versa [4].

Thyroid disorders are commonly separated into two major categories, hyperthyroidism (caused by an overactive thyroid gland) and hypothyroidism (due to a poorly functioning thyroid gland), depending on whether serum thyroid hormone levels (T4 and T3) are increased or decreased, respectively. Both hypothyroidism and hyperthyroidism have potentially fatal systemic manifestations [5].

Deviation from normal thyroid status has profound influence on all body systems including cardiovascular system. Changes in serum thyroid hormone levels are usually associated with alteration in autonomic regulation of cardiovascular activity [6,7]. The thyroid hormones affect the cardiovascular system, not only through its metabolic effects on myocardial cells but also by modifying sympathetic tone. Even with proper treatment to normalize thyroid hormone levels, there are short-term and long-term effects on the cardiovascular system. Thyroid hormones exert effects on cardiac systolic and diastolic

function, peripheral vascular resistance and arrhythmogenesis [8]. Flynn *et al* [9] reported an increased risk for cardiovascular morbidity, mortality and dysrhythmias in exogenous hyperthyroidism.

The present study was undertaken to see whether there was any derangement in the various power spectral parameters of heart rate variability in thyroid disorders and to find out correlation if any, thyroid disorders and HRV parameters. Patients with no evidence of cardiac disease clinically may show deranged HRV as an early feature of cardiac involvement and early myocardial damage.

## METHODS

The present study was conducted in the Dept. of Physiology, SMS Medical College, Jaipur with the requisite inputs from the Deptt. of Endocrinology of the Institute. The study was carried out after getting formal approval from Institutional Ethics Committee of SMS Medical College, Jaipur. Informed written consent was obtained from all the subjects included in the study.

Patients visiting Endocrinology Outdoor of the SMS Hospital were preliminary assessed clinically and biochemically to ascertain whether the patient has any endocrine disorder and if so, whether he or she is hypothyroid, hyperthyroid or euthyroid. The clinical assessment for the thyroid disorders was done by Endocrinologist using standard clinical protocol and if the subjects were suspected to be having clinical manifestations of thyroid malfunction was further subjected to biochemical investigation for confirmation of diagnosis.

The study included a total 125 subjects out of which 100 were those suffering from thyroid disorders age ranging from 20-60 years of either sex and 25 subjects of same age group as control. The patients included in the study were screened for hypo and hyperthyroid states, based on following clinical and biochemical parameters

### Clinical parameters:

Patients having history or complication of following symptoms were clinically considered as Hypothyroid - Heavy voice, bradycardia, puffiness of face, hair loss, constipation, lethargy, weight gain, decrease appetite, cold intolerance, snoring, muscle weakness, thin brittle fingernails, decreased sweating, muscle cramps, joint pain, dry, itchy skin.

Patients having history or complication of following symptoms were clinically considered as Hyperthyroid - Pulse rate, palpitation, tremor, weight loose, increase appetite, orbitopathy, anxiety, restlessness, diarrhoea, intolerance to heat, hair loss, muscle aches, weakness, fatigue, dyspnea, hyperactivity, irritability and sweating.

**Biochemical Parameters** - For confirmation of clinical diagnosis, the patients were further subjected to following biochemical parameter evaluation: FT<sub>3</sub>, FT<sub>4</sub>, TSH

### Inclusion Criteria:

Patient's readiness to participate in the study, confirmed thyroid patients by endocrinologist based on clinical, biochemical and laboratory findings in the age group of 20 to 60 years of either sex, never previously treated for endocrinal disease.

**Exclusion Criteria:** Subjects were excluded from the participation if they exhibited

Cardiac Problems, liver diseases, renal dysfunction, HIV / Immunodeficiency disorders, neurological disease, any other systemic disease that can affect autonomic activity i.e. diabetes, hypertension, patients taking any drug which affects autonomic activity were excluded

Based on the finding of the screening, the patients were grouped as follows-

**Group A** : 50 confirmed patients of hypothyroidism, newly diagnosed based on thyroid hormone profile, clinical manifestations and not on any thyroid hormone replacement therapy.

**Group B** : 50 confirmed patients of hyperthyroidism, newly diagnosed based on thyroid hormone profile, clinical manifestations and not on any anti thyroid therapy.

**Group C** : Age matched 25 control subjects were randomly selected from general population. The subjects of this group had no medication and were all in good health as determined by medical history, clinical examination, laboratory analysis including T<sub>3</sub>, T<sub>4</sub>, TSH.

**Laboratory Profile** - The fasting venous blood samples were collected by standard aseptic techniques. Serum was separated and assays were performed. To evaluate thyroid functions serum free thyroxine (FT<sub>4</sub>), free triiodothyronine (FT<sub>3</sub>) and thyroid stimulating hormone (TSH) were measured. FT<sub>3</sub>, FT<sub>4</sub> were measured because these are the active form of thyroid hormone and generally they are initially affected with TSH in thyroid disorders. Serum levels of FT<sub>3</sub>, FT<sub>4</sub> and TSH were determined by chemiluminescent immunoassay with the IMMULITE 2000 Systems Analyzer. IMMULITE 2000 can be expected to have reference ranges for FT<sub>3</sub>, FT<sub>4</sub>, TSH were 1.8-4.2 pg/mL, 0.89-1.76 ng/dL and 0.4-4.0 uIU/mL respectively.

**Assessment of HRV-** Heart rate variability can be measured over any length of recorded ECG, as per the guidelines of Task Force [3] at least 5 minutes of ECG must be recorded to quantify Sympathetic and Parasympathetic tone.

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. Heart rate Variability was analyzed in Time domain and Frequency domain measures by Software Version 1.1.

### **Procedure**

#### **Patient's preparation**

Following instructions were given to the patient for accurate measurement and analysis of Heart Rate Variability-

To avoid food preceding two hours of the testing, no coffee, nicotine or alcohol 24 hours prior to the testing, to avoid smoking 24 hours before testing, to wear loose and comfortable clothing.

#### **Recording**

For short term analysis of HRV, ECG was recorded in supine position for 5 min after 15 min of supine rest. Recording was done in noise free room and room temperature was maintained at 24-28°C. Subject is instructed to close the eyes and to avoid talking, moving hands, legs and body, coughing during test, sleeping

#### **Acquisition**

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. The detection of R wave was done by HRV Soft version 1.1 developed by AIIMS, New Delhi. The procedure calculated heart rate variability (HRV) in time domain and frequency domain measures.

#### **Processing (R wave detection and RR intervals)**

The detection of R wave was done by software. Different software use different algorithms to detect the R-wave (Fig-a) and differ in the features. They all have basic similarity in that they all compute R-R intervals after the R wave detection. Abnormal beats and areas of artifact were automatically and manually identified and excluded from the recording.

#### **Quantification of HRV**

The analysis of HRV can be done by different methods like Time domain, frequency domain, nonlinear, periodic and non periodic oscillation pattern. In the present study we applied spectral analysis of HRV to evaluate cardiac autonomic control.

#### **Frequency Domain methods**

The periodic oscillation of a given heart rate at various frequencies were examined by Frequency Domain method [10]. The frequency components of HRV are analyzed by using many methods. Fast Fourier Transform (FFT) is one of the commonly employed methods. Frequency domain measures of HRV provide information on the frequency distribution of the components of HRV using power spectral density analysis.

**Selected Frequency domain parameters of HRV:** Total Power, LF(ms<sup>2</sup>), HF(ms<sup>2</sup>), LF (nu), HF (nu), LF/HF ratio. Spectral analysis of HRV is characterized by three main components:

The high frequency (HF) (0.15 - .4 Hz) component measures the influence of the vagus nerve in modulating the sinoatrial node. The low frequency (LF) (0.04 - 0.15 Hz) component provides an index of sympathetic effects on the heart, particularly when these are measured in normalized units. The very low frequency (VLF) component reflects the influence of several factors on the heart, including chemoreceptors, thermoreceptors and renin - anigotensin system. Almost all of the variability from a short term spectral analysis of HRV is captured in these three components. However, physiological explanation for VLF in short term analysis of HRV is not well defined.

The LF and HF indices were presented both in absolute units (ms<sup>2</sup>) and normalized units (nu). The normalized units assume a balanced behaviour of the sympathetic (normalized LF) and parasympathetic nervous system (normalized HF). Power of LF and HF are established in short term analysis of HRV. The mean values of all variables were compared by using Oneway ANOVA (Post Hoc Tukey) test. Results were presented as Mean ± SD. All the statistical analysis was performed using SPSS Version 20 and Microsoft Excel 2007. A p<0.05 was considered statistically significant.

### **RESULT**

As depicted from Table-1 mean values of ft3, ft4 were decrease and increase significantly in hypothyroids and hyperthyroids than controls. Mean value of TSH was significantly high and low (p<0.001) in hypothyroid and hyperthyroid subjects respectively compared to controls.

**Table- 1**Thyroid Hormone Profile

Parameter	Group A	Group B	Group C	p value
fT3	1.95±0.83	6.07±5.27	2.59±0.47	<0.001
fT4	0.83±0.34	7.55±8.28	1.30±0.22	<0.001
TSH	13.96±7.68	0.09±0.9	2.41±1.15	<0.001

T3: tri-iodothyronine, T4: thyroxine, TSH- thyroid stimulating hormone

Group A: Hypothyroid; Group B: Hyperthyroid; Group C: Control

The spectral analysis results are summarized in Table 2,3

The mean values of Frequency Domain measures of HRV TP, HF power (ms<sup>2</sup>), HF nu, LF power (ms<sup>2</sup>) were reduced significantly (p<0.001) in study groups (A, B) whereas LF nu and LF/HF ratio were significantly increased (p<0.001) in study groups when compared with Control group – C.

When intergroup comparison was done between Test Group – A and Control Group – C, significant difference (p<0.05) was found for mean values of TP, HF power (ms<sup>2</sup>), LF power (ms<sup>2</sup>) whereas non-significant difference (p>0.05) was found for mean values of LF nu, HF nu, LF/HF ratio. Whereas on comparison between Test Group – B and Control Group – C statistically significant (p<0.05) was observed for all measures. When the comparison was done between Test Group – A and B, statistical significant difference (p<0.05) was observed for mean values of almost all frequency measures except total power (TP) and LF power (ms<sup>2</sup>) (**Table 2 & 3**).

**Table 2.** Spectral heart rate variability parameters of thyroid patients and controls

Groups	TP	LF (ms <sup>2</sup> )	HF (ms <sup>2</sup> )
<b>A (n=50)</b>	1497.21 ± 860.01	439.44 ± 379.25	619.88 ± 910.17
<b>B (n=50)</b>	1182.01 ± 670.36	470.23 ± 805.09	246.63 ± 227.27
<b>C (n=25)</b>	3564.28 ± 1245.83	1094.09 ± 604.02	1443.03 ± 710.89

Statistical Analysis

Groups	P value		
<b>A vs B vs C</b>	0.000	0.000	0.000
<b>A vs B</b>	0.077	0.806	0.006
<b>A vs C</b>	0.000	0.000	0.000
<b>B vs C</b>	0.000	0.000	0.000

Statistical analysis were done by One way ANOVA and Post Hoc Tukey test

TP = Total Power, LF = Low frequency, HF = High frequency, ms<sup>2</sup> = squared millisecond

**Table 3.** Spectral heart rate variability parameters of thyroid patients and controls

Groups	LF nu	HF nu	LF/HF ratio
<b>A (n=50)</b>	48.66 ± 16.83	51.16 ± 17.00	1.22 ± 0.89
<b>B (n=50)</b>	59.21 ± 16.37	40.74 ± 16.30	1.94 ± 1.47
<b>C (n=25)</b>	42.87 ± 15.60	56.93 ± 15.37	0.91 ± 0.65

Statistical Analysis

Groups	p value		
<b>A vs B vs C</b>	0.000	0.000	0.000
<b>A vs B</b>	0.002	0.002	0.002
<b>A vs C</b>	0.152	0.154	0.259
<b>B vs C</b>	0.000	0.000	0.000

All values are expressed as Mean ± Standard deviation. P values <0.05 are Significant. Statistical analysis were done by One way ANOVA and Post Hoc Tukey Test .

LF nu.= LF power in normalized unit, HF nu = HF power in normalized unit, LF/HF= ratio of low frequency and high frequency

Group A: Hypothyroids; Group B : Hyperthyroids; Group C : Controls

## DISCUSSION

Thyroid hormones play an important role on the physiological chemistry of heart and vascular systems in healthy subjects [11]. These hormones have relevant effects on the cardiovascular system. Many signs and symptoms recognized in patients with overt hyperthyroidism and hypothyroidism are due to the increased or reduced action of thyroid hormone on the heart and the vascular system, respectively, and the related hemodynamic derangements [12]. The thyroid hormones exert effects on the heart and peripheral circulation, playing an important role in the regulation of the function of sino-atrial node, the systolic and diastolic function of the myocardium and peripheral resistance. They act directly by influence

on protein-synthesis, the properties of cell membranes and indirectly by interactions with autonomic nervous system, causing increase in cardiac output and decrease in systemic vascular resistance [13].

This study found both hypothyroid and hyperthyroid patients to have autonomic dysfunction with the altered sympathetic reactivity and parasympathetic activity. During supine rest, the body is in a relaxed state with high parasympathetic activity and low sympathetic activity [14]. Thus in a normal supine HRV, the contribution of HF component (parasympathetic activity) to the total spectral power is about two-third as compared to one-third contribution by LF and VLF components [10], so any degree of reduction or impairment in high frequency (HF) power in patients compared to control subjects indicates decreased vagal activity in hypothyroid and hyperthyroid patients even if HF remains more than LF power in the same subjects.

In the present study LF nu was significantly increased in hyperthyroid subjects compared to both control ( $p < 0.001$ ) and hypothyroid subjects ( $p = 0.002$ ), which indicates significantly increased sympathetic activity in hyperthyroidism as increased LFnu is an index of increased cardiac sympathetic drive [3]. There was also statistically non-significant increase in LFnu in hypothyroid patients compared to control subjects ( $p > 0.05$ ). This indicates slightly increased sympathetic activity in these patients also. Our findings are consistent with the result observed by Cacciatori *et al* [15], that thyroid hormone deficiency is associated with increased sympathetic influence on the heart but commented that hypothyroid and controls did not differ in the reflex responses as evaluated by traditional autonomic function tests. They suggested that power spectral analysis was more sensitive than these tests, though it does not corroborate with the report of Xing H *et al* [16] that the autonomic imbalance in hypothyroid patients was due to a higher level of vagal tone.

LF-HF ratio is the most sensitive indicator of sympathovagal balance [3]. It has gained wide acceptance as a tool to assess cardiovascular autonomic regulation where increase in LF/HF ratio is assumed to reflect a shift to "sympathetic dominance" and decreases in this index correspond to a "parasympathetic dominance" [17]. In the present study LF/HF ratio was increased in both hypothyroid and hyperthyroid patients, which indicate presence of sympathovagal imbalance (SVI). Increase in LF/ HF ratio in resting supine condition of an individual indicates increased sympathetic and reduced parasympathetic activity [10]. In the present study, as the LF/HF ratio was increased in both hyperthyroid and hypothyroid groups, SVI in both the dysfunctions were due to joint disruption of sympathetic and vagal inputs to the heart. The increase in LF-HF ratio was maximum in hyperthyroid patients than controls ( $p < 0.001$ ), whereas non-significant increase in hypothyroid patients than controls ( $p > 0.05$ ) indicating sympathetic over activity in the presence of severe depression in parasympathetic activity in hyperthyroidism. Similarly Inukai *et al* [18]; Chen *et al* [19] observed significantly higher LF/HF ratio in untreated hyperthyroid patients. Again Ahmad *et al* [20]; Syamsunder *et al* [21] reported higher LF/HF ratio in hypothyroid group, but it was significant.

The result of the present study revealed that total power was reduced in hypothyroid and hyperthyroid subjects, which is consistent to the findings of some other studies [19, Chen JL *et al* (2006), Karthik *et al* [22], Ahmad *et al* [20], Syamsunder *et al* [21]. Its lower value indicates lower modulation of the cardiac autonomic nervous activities on heart [3]. The increased LF/HF ratio in the presence of reduced total power of HRV indicates poor cardiovascular status of the subject [10, 22] and strongly suggests enhanced sympathetic inputs in thyrotoxicosis. Therefore, the present study reveals a poor cardiovascular prognosis in thyroid malfunctions, especially, in subjects suffering from hyperthyroidism.

In this study significantly lower HF nu in hyperthyroid subjects as compared to controls ( $p < 0.001$ ) and hypothyroid subjects ( $p = 0.002$ ) was observed, which is consistent to the findings of Karthik *et al* [22], indicating diminished vagal inputs to the heart. This was also supported by markedly decrease in TP of HRV in hyperthyroid group, as TP in general indicates the vagal modulation of cardiac functions [3, 10].

## CONCLUSION

Thyroid patients had markedly reduced HRV, which manifests as deranged autonomic functions. As HRV was found to be reduced in hypothyroid and hyperthyroid subjects, as compared to controls which indicate poor cardiovascular status of the subjects and predisposing them to cardiovascular morbidities and mortalities? Our study revealed that hyperthyroids showed profound decrease HRV as compare to hypothyroids, which predicts poor cardiovascular prognosis in thyroid malfunctions, especially, in subjects suffering from hyperthyroidism.

## REFERENCES

1. Mazzeo AT, Monaca E, Di Leo R, Vita G, Santamaria LB. Heart rate variability : a diagnostic and prognostic tool in anaesthesia and intensive care. *Acta Anaesthesiol Scand*. 2011; 55(7): 797-811.

2. Goldstein DS, Robertson D, Esler M, Straus SE, Eisenhofer G. Dysautonomias: clinical disorders of the autonomic nervous system. *Ann Intern Med.* 2002; 137(9): 753-763.
3. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology Heart rate variability. Standards of measurement, physiological interpretation and clinical use. *European Heart Journal.*1996; 17: 354–381.
4. Ravenswaaij-Arts CM, Kollee LA, Hopman JC, Stoeltinga GB, van Geijn HP. Heart rate variability. *Ann Intern Med* 1993;118: 436–47.
5. Mittal A, Sathian B, Kumar A, Chandrasekharan N, Dwedi S. The Clinical implications of thyroid hormones and its association with lipid profile: A comparative study from western Nepal, *Nepal Journal of Epidemiology* 2010; 1 (1): 11–16p.
6. Foley CM, McAllister RM, Hasser EM. Thyroid status influences baroreflex function and autonomic contributions to arterial pressure and heart rate. *Am J Physiol Heart Circ Physiol.* 2001 May; 280(5): H2061-2068.
7. Bhat AN, Kalsotra L, Yograj S. Autonomic reactivity with altered thyroid status. *JK Science.* 2007; 9(2): 70-74.
8. Osman F, Gammage MD, Franklyn JA. Thyroid disease and its treatment: short-term and long-term cardiovascular consequences. *Curr Opin Pharmacol.* 2001; 1(6): 626-631
9. Flynn RW, Bonellie SR, Jung RT, Mc Donald TM, Morris AD, Leese GP. Serum thyroid-stimulating hormone concentration and morbidity from cardiovascular disease and fractures in patients on long-term thyroxine therapy. *J Clin Endocrinol Metab.* 2010; 95(1): 186–193.
10. Malliani A. Heart rate variability: from bench to bedside. *Eur J Intern Med.* 2005; 16(1): 12-20.
11. Mansourian AR. A review of literature on the adverse effects of hyperthyroidism on the heart functional behavior. *Pak J Biol Sci.* 2012; 15(4): 164-176.
12. Klein I, Ojamaa K. Thyroid hormone and the cardiovascular system. *N Engl J Med.* 2001; 344(7): 501–509.
13. Zysko D, Katedry, Kliniki Kardiologii AM. Thyroid hormones and the heart. *Pol Merkur Lekarski.*1999; 6(33):157-60.
14. Mourot L, Bouhaddi M, Perrey S, Cappelle S, Henriët MT, Wolf JP, Rouillon JD, Regnard J. Decrease in heart rate variability with overtraining: assessment by the Poincaré plot analysis. *Clinical Physiology & Functional Imaging.* 2004; 24(1):10-18.
15. Cacciatori V, Gemma ML, Bellavere F, Castello R, De Gregori ME, Zoppini G, Thomaseth K, Moghetti P, Muggeo M. Power spectral analysis of heart rate in hypothyroidism. *Eur J Endocrinol.* 2000 Sep; 143(3): 327-333.
16. Xing H, Shen Y, Chen H, Wang Y, Shen W. Heart rate variability and its response to thyroxine replacement therapy in patients with hypothyroidism. *Chin Med J (Eng).* 2001; 114(9): 906-908.
17. Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front Physiol.* 2013; 4: 26.
18. Inukai T, Takanashi K, Kobayashi H, Fujiwara Y, Tayama K, Aso Y, Takemura Y. Power spectral analysis of variations in heart rate in patients with hyperthyroidism or hypothyroidism. *Horm Metab Res.* 1998; 30(8): 531-535.
19. Chen JL, Chiu HW, Tseng YJ, Chu WC. Hyperthyroidism is characterized by both increased sympathetic and decreased vagal modulation of heart rate: evidence from spectral analysis of heart rate variability. *Clin Endocrinol (Oxf).* 2006 June; 64(6): 611-616.
20. Ahmed M, Begum N, Ferdousi S, Begum S, Ali T. Power spectral analysis of heart rate variability in hypothyroidism. *J Bangladesh Soc Physiol.* 2010 December; 5(2): 53-59.
21. Syamsunder AN, Pal GK, Pal P, Kamalanathan CS, Parija SC, Nanda N. Association of sympathovagal imbalance with cardiovascular risks in overt hypothyroidism. *N Am J Med Sci.* 2013 Sep; 5(9): 554-561.
22. Karthik S, Pal GK, Nanda N, Hamide A, Bobby Z, Amudhraj D, Pal P. Sympathovagal imbalance in thyroid dysfunctions in females: correlation with thyroid profile, heart rate and blood pressure. *Indian J Physiol Pharmacol.* 2009; 53(3): 243-252.