

clinical presentations, direct measures of serum levels of catecholamines and their metabolites demonstrate near-normal values in hyperthyroid patients and increased levels of catecholamines and their urinary metabolites in hypothyroid patients (1, 2). Many researchers (3, 4, 5) have tried to understand this paradox. In hyperthyroid patients there is reduced vagal activity and greater sympathetic responsiveness. The vagal inhibition might be due to action of thyroid hormone on CNS structures integrating autonomic functions while the increased responsiveness to catecholamines could be due to the increase β -adrenergic receptors and decrease in α -adrenergic receptors in a number of cells. In hypothyroidism, there is overall depression of the adrenergic responses at the peripheral level due to decrease in β -adrenergic receptors and increase in α -adrenergic receptors and there is increase in efferent sympathetic activity reaching virtually every tissue (5). The increase in sympathetic activity appears to be compensatory in nature and may come about in response to the deficient peripheral response to catecholamines.

In our study, we have assessed the autonomic status in hyperthyroid and hypothyroid patients and compared with healthy normal controls by a combination of tests not used earlier in thyroid patients. The valsalva ratio, standing to lying ratio (S/L ratio) and immediate heart rate response to standing (30:15 ratio) were used to assess the sympathetic status of the ANS.

METHODS

Female patients of hyperthyroidism and hypothyroidism (29 ± 7 years) were selected

from the medical OPD, Guru Teg Bahadur Hospital, Shahdara, Delhi, and Thyroid Clinic, Institute of Nuclear Medicine and sciences, Timarpur, Delhi. Age and sex matched subjects (30 ± 9) were selected from the employees of the associated University college of Medical Sciences and used as controls for the study. Consent for experimentation was obtained from all patients and healthy volunteers. Since the majority of the patients available were females, the study was confined to females only. Patients suffering from other conditions known to affect autonomic function tests, were excluded from the study e.g. coronary heart disease, electrolyte imbalance, diabetes mellitus. Based upon general history, clinical examination and serum levels of T3, T4 and TSH, the subjects were categorised into two groups: Hyperthyroid (Group II) and Hypothyroid (Group III). The healthy controls comprised Group I.

Test protocol

All subjects were tested under similar laboratory conditions. The subjects were allowed an hour to get familiarised with the experimental and environmental conditions. During this period, detailed history and medical examination were carried out and the nature of the tests was explained to the subjects beforehand to allay their apprehension. The investigative procedures involved tow types of measurements; electrocardiography and galvanic skin response measurement. A standard limb lead II ECG was recorded using the *Physiograph* (INCO) which was connected through ECG interface module to MP 100 WSW Biopac system. The R-R intervals were

calculated using the peak detector in *Acqnowledge*. The following autonomic Software programme function tests were carried out.

Standing to lying ratio

Each subject was made a stand quietly for two minutes and then lie down quickly without any support while continuous ECG was recorded from 20 beats before to 60 beats after lying down. The point at which the posture changed from standing to lying got marked automatically due to unavoidable associated noise. For calculating S/L ratio, longest R-R interval during the 5 beats before lying down and the shortest R-R interval during 10 beats after lying down was taken into consideration (7).

$$S/L = \frac{\text{Longest R - R interval during 5 beats before lying down}}{\text{Shortest R - R interval during 10 beats after lying down}}$$

30:15 ratio

In this test, each subject laid quietly for three minutes, then stood up unaided while continuous ECG was recorded. The time of standing was marked in the record. The 30:15 ratio was calculated by taking the ratio of R-R interval at beat 30 and 15 after standing (8).

$$30/15 \text{ ratio} = \frac{\text{R - R interval at 30}^{\text{th}} \text{ beat}}{\text{R - R interval at 15}^{\text{th}} \text{ beat}}$$

Valsalva ratio

Each subject was told to perform Valsalva manoeuvre for 15 sec by blowing into a mouth piece attached to an aneroid manometer and maintain a pressure of 40 mm Hg for 15 seconds. A continuous ECG

was recorded 60 sec. before the manoeuvre (resting period), during the manoeuvre (strain period, 15 sec) and 60 seconds subsequent to strain period. Valsalva ratio (9) was taken as the ratio of maximum R-R interval after the strain to that of shortest R-R interval during the strain. The maximum ratio of three trials was taken for the autonomic activity.

$$\text{Valsalva ratio} = \frac{\text{Maximum R - R interval after strain}}{\text{Minimum R - R interval during the strain}}$$

Galvanic skin response (10)

GSR was recorded using the Biopac GSR amplifier module (GSR 100) which measures skin conductance via the constant voltage technique. The electrodes were applied on two index fingers and a constant voltage of 0.5 volts was passed through electrodes. The GSR was computed electronically in the module and the data was put through to MP 100 WSW. The test was performed 3 times with the adequate time of rest in between each trial. The recordings were taken at rest and while doing valsalva manoeuvre. The difference between the baseline and the maximum value obtained during valsalva manoeuvre was taken as the GSR.

In our study we have taken autonomic stress (Valsalva manoeuvre) as a stimulus for eliciting GSR, which is new and tried for first time in our study. We have taken autonomic stress as a stimulus for eliciting GSR on the basis as well as periphery (11, 12). When a person sweats, as when stressed, there is an increase in electrical current flow along the surface of the skin that is reflected in an increase in electrical current flow along the surface of the skin that is reflected in an increase in voltage.

The computer screen receives input from the galvanic skin response unit. It displays a voltage, which increases when current flow increases, such as when a person develops sweaty hands.

Statistical analysis

Statistical analysis comprised comparison, using unpaired Student's t-test of autonomic function tests between the different groups and regression analysis with individual tests between autonomic function tests and thyroid hormone levels.

RESULTS

The mean T3 (ng/dl), T4 (ug/dl) TSH (mU/L) levels in the hyperthyroid, hypothyroid and controls are given in Table I. The hormonal levels are not only distinctly different in the three groups but also show a gradation, which is related to the thyroid status. Statistical comparisons between the hormonal levels in the three groups also show the hormonal levels to be significantly different. The difference between the controls and the hypothyroid groups or the controls and the hyperthyroid was significant at $P < 0.05$ whereas the

TABLE I: Showing Thyroid function tests in hyperthyroid and hypothyroid patients and normal controls.

Thyroid function test (normal range)	Hyperthyroid Mean \pm SD	Hypothyroid Mean \pm SD	Controls Mean \pm SD
T3 (75-175ng/dl)	234.6 \pm 57.72	36.3 \pm 12.13	107.8 \pm 24
T4 (4-11mcg/dl)	21.8 \pm 6.36	1.32 \pm 0.6	7.57 \pm 2.25
TSH (0.3-5.0mU/L)	0.07 \pm 0.05	67.69 \pm 29.5	1.91 \pm 0.77

difference between the hyperthyroid and the hypothyroid group was significant at $P < 0.01$.

The mean values for the various parasympathetic i.e. S/L ratio (SLR), 30:15 ratio (LSR) and valsalva ratio (VLR) and the sympathetic function test (GSR) in the three groups are given in Table II. Comparisons were made in pairs, between hyperthyroid and controls, hypothyroid and controls, and between hyperthyroid and

TABLE II: Showing means and standard deviations of S/L Ratio (SLR), 30:15 Ratio (LSR) Valsalva Ratio (VLR) and Galvanic skin response (GSR).

	Hyperthyroid		Hypothyroid		Controls	
	Mean	SD	Mean	SD	Mean	SD
SLR	1.080	0.097	1.204	0.135	1.042	0.096
LSR	1.043	0.070	1.107	0.260	0.983	0.100
VLR	1.374	0.360	1.486	0.264	1.831	0.450
GSR (μ mhos)	0.231	0.178	0.261	0.301	0.310	0.310

hypothyroid groups. The mean value of S/L ratio in all the three groups were found to be in normal range i.e. > 1.04 . The mean value of 30:15 ratio hyperthyroid group was found to be in normal range i.e. > 1.04 , while the mean value in hypothyroid group was found to be < 1.04 but > 1.00 . The mean value of Valsalva ratio in all the three groups are > 1.21 , which can be taken as normal. Statistically, no significance could be obtained from the mean value of S/L ratio, 30:15 ratio, Valsalva ratio and GSR when compared in all the three groups. However, a regression analysis carried out between the autonomic tests and the thyroid hormone levels showed significant ($P < 0.05$) correlation's (Table III, and Figs. 1 to 3) between the S/L ratio (SLR) and the

TABLE III: Showing relationship of S/L Ratio (SLR), 30:15 Ratio (LSR), Valsalva Ratio (VLR) and Galvanic skin response (GSR) with Log(T3), Log(T4), Log(TSH).

Variable	Regression equation	P-value (t-Test)	Significant (at 5% level)
SLR with Log (T3)	$SLR=(0.064*Log(T3))+1.40$	0.017	Significant
SLR with Log (T4)	$SLR=(0.041*Log(T4))+1.18$	0.034	Significant
SLR with Log (TSH)	$SLR=(0.017*Log(TSH))+1.10$	0.034	Significant
LSR with Log (T3)	---	0.576	Not Significant
LSR with Log (T4)	---	0.767	Not Significant
LSR with Log (TSH)	---	0.536	Not Significant
VLR with Log (T3)	---	0.253	Not Significant
VLR with Log (T4)	---	0.336	Not Significant
VLR with Log (TSH)	---	0.267	Not Significant
GSR with Log (T3)	---	0.725	Not Significant
GSR with Log (T4)	---	0.582	Not Significant
GSR with Log (TSH)	---	0.721	Not Significant

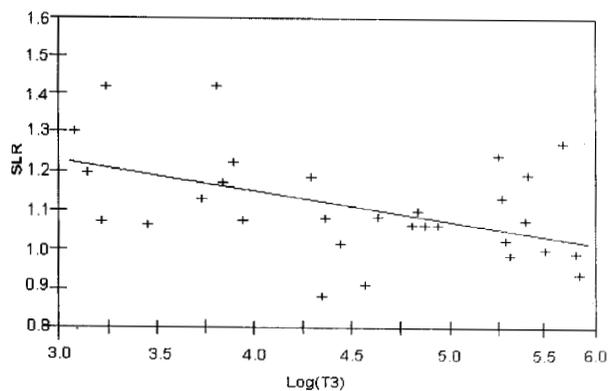


Fig. 1: Graph showing relationship of Log (T3) with S/L ratio (SLR) Regression equation $SLR = (-0.064 * Log(T3) + 1.40$.

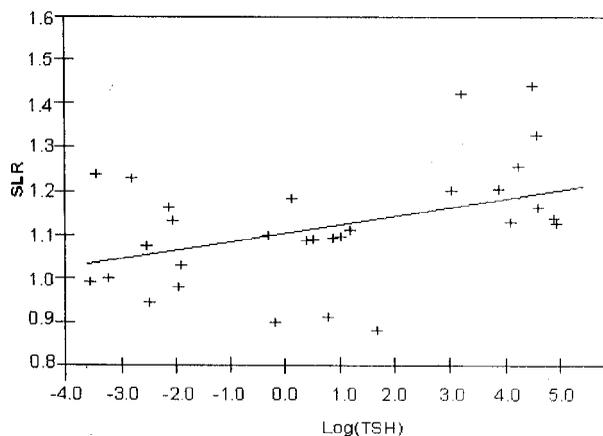


Fig. 3: Graph showing relationship of Log (TSH) with S/L ratio (SLR) regression equation $SLR = (0.017 * Log (TSH) + 1.10$

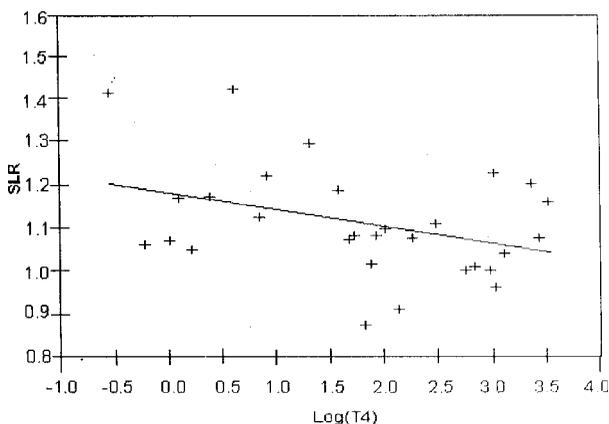


Fig. 2: Graph showing relationship of Log (T4) with S/L ratio (SLR) regression equation $SLR = (-0.041 * Log(T4) + 1.18$.

logarithm of the thyroid hormonal levels. The correlation was negative for log [T3] and log [T4], while it was positive for log [TSH]. 30 : 15 ratio (LSR), Valsalva Ratio (VLR) and the Galvanic Skin Response (GSR) did not show any significant correlation.

DISCUSSION

Work done over the past years indicates the presence of autonomic nervous dysfunction in a variety of systemic

disorders. Thyrotoxicosis is characterised by profound sympathovagal imbalance brought about by increased sympathetic activity and diminished vagal tone (13). The inhibition of cardiac motor neurones might be resulting from thyroid hormone action on CNS structures integrating autonomic function (14). Contrary to the clinical picture, thyroid hormone deficiency in hypothyroid patients is associated with an increased sympathetic influence on the autonomic cardiovascular system (15). The changes in sympathetic function could be explained by a secondary adaptation to an altered cardiovascular responsiveness.

We have assessed autonomic parasympathetic status in thyroid patients by tests different from those used by earlier workers. Although the results obtained in our study for different parasympathetic tests in three different groups were not statistically significant, yet the correlation between autonomic function and thyroid hormone levels indicates a statistically significant difference P -value < 0.05 , in one autonomic function (S/L, ratio). This correlation indicates that there is decreased parasympathetic activity with increased T3 and T4, which is in a agreement with earlier reports. The number of subjects in each group was very small (10) in our study, and so it may not have reflected the true picture of the thyroid dysfunction as shown by the lack of gradation running through the three different groups of subjects for assessment of parasympathetic status. However the negative correlation of the S/L ratio with the logarithms of T3 and T4 levels (Figs. 1 and 2) would suggest that over a wide range of hormonal levels, as occurs when the whole gamut of dysthyroidism is considered, the parasympathetic function does become exaggerated. When difference in the average

hormonal concentrations are modest, as in the groups of subjects in this study, the effect may not show up statistically. This seems especially likely in view of the fact that most clinical effects of thyroid hormones are related linearly and not logarithmically to the hormonal concentration. Significant results with other autonomic indices might have been obtained if large number of cases were assessed. The Galvanic Skin Response (GSR) was the only test done in our study to test the sympathetic activity. In our study by comparing mean values of GSR in all the three groups we could not find any statistical significant changes. Our results are not in agreement with the studies done earlier. Researchers have found lower onset of latency and duration of skin conductance response and very high skin conductance levels in thyrotoxic (11, 16) patients and lower skin conductance levels, lower fluctuation rates and prolonged onset latencies in hypothyroid patients (12, 17). The abnormal electrodermal activity (EDA) is due to change in hypothalamic-pituitary-thyroid axis functioning. The change in thyroid status produces marked changes in metabolism of several biogenic amine in brain which are responsible for the change in EDA (18). In our study we have tried a novel stimulus i.e. autonomic stress (Valsalva manoeuvre) for eliciting GSR. The number of patients in each group is very small to asses any change, especially in these type of measurement having so much of intra-group and inter-group variation. Sex difference cannot be imputed for the divergence of our results from earlier works since most of them too are exclusively only females. It is possible, however, that the phases of menstrual cycle may affect autonomic functions. Ideally, these factors should have been taken into consideration

too. However, given the paucity of patients available to us for the study, such considerations would have fragmented the size of the groups further and made any meaningful statistical analysis well nigh impossible.

Conclusion

The results obtained in our study show a negative correlation of the S/L ratio with the logarithms of T3 and T4 (Figs. 1 and 2) suggests that over a wide range of hormonal levels, as occurs when the whole gamut

of dysthyroidism is considered, the parasympathetic function becomes exaggerated. When the difference in the average hormonal concentrations are modest, as in the groups of subjects in this study, the effect may not show up statistically. This seems especially likely in view of the fact that most clinical effects of thyroid hormones are related linearly and not logarithmically to the hormonal concentration. The GSR, which is one of the measures of sympathetic activity, was found not to be affected in patients taken in our study.

REFERENCES

1. Bayliss RIS, Edwards OM. Urinary secretion of free catecholamine in graves disease. *Endocrinology* 1971; 49: 167-173.
2. Coulombe P, Dussault JH, Walker P. Plasma Catecholamine Concentrations in Hyperthyroidism and Hypothyroidism. *Metabolism* 1976; 25: 973-978.
3. Maciel BC, Gallo L, Jr, Marin Neto JA, Maciel LMZ, Alves MLD, Pacola GMF, Iazgi N. The role of autonomic nervous system in the resting tachycardia of human hyperthyroidism. *Clin Science* 1987; 72: 239-243.
4. Maciel BC, Gallo L, Jr, Marin Neto JA, Maciel LMZ, Martin Leb. Autonomic control of heart rate during dynamic exercise in human hyperthyroidism. *Clin Science* 1988; 75: 209-215.
5. Kollai B, Kollai M. Reduced cardiac vagal excitability in hyperthyroidism. *Brain Res Bull* 1988; 20: 785-790.
6. Bilezikian JP, Loeb JN. The influence of hyperthyroidism on alpha and beta-receptor systems and adrenergic responsiveness. *Endocr Rev* 1983; 4: 378-396.
7. Rodrigues EA, Ewing DJ. Immediate heart rate response to lying down: simple test for cardiac parasympathetic damage in diabetes. *Br Med J* 1983; 800: 287-291.
8. Ewing DJ, Hume L, Campbell IW, Murray A, Neilson JMM, Clarke BF. Autonomic mechanisms in the initial heart rate response to standing. *J Appl Physiol* 1980; 49: 809-814.
9. Kalbfleish JH, Stowe DF, Smith JJ. Evaluation of the heart rate response to theValsalva manoeuvre. *Am Heart J* 1978; 95: 707-715.
10. Heard HE. The psychogalvanic response in the study of sympathetic activity. *Br J Surg* 1964; 51: 629-635.
11. Morakinyo VO, Atkin RCB, Zcalley AK, Irvine WJ. Comparison of anxiety in thyrotoxic and neurotic patients using skin conductance measurement. *Clin Endocrinol* 1972; 1: 355-362.
12. Marcisz C. Studies on the effect of thyroid function on the skin electrical resistance. *Po. Arch Med Wevn* 1982; 68: 33-42.
13. Burggraaf J, Tulen JH, Lalezari S, Schoemaker RC, De Meyer PH, Meinders AE, Cohen AF, Pijl H. Sympathovagal imbalance in hyperthyroidism. *Am J Physiol Endocrinol Metab* 2001 Jul; 281(1): E190-E199.
14. Kollai B, Kollai M. Reduced cardiac vagal excitability in hyperthyroidism. *Brain Res Bull* 1988; 20: 785-789.
15. Cacciatori V, Gemma ML, Bellavere F, Castello R, De Gregori ME, Zoppini G. Power spectral analysis of heart rate ion hypothyroidism. *Eur J Endocrinol* 2000 Sep; 143(3): 327-334.
16. Dolu N, Suer C, Ozesmi C, Kelestimur F, Ozcan Y. Electrodermal activity in nonmedicated hypothyroid patients having no depressive symptoms. *Biol Psychiatry* 1997; Dec 1; 42(11): 1024-1029.
17. Dolu N, Suer C, Ozesmi C, Kelestimur F, Ozcan Y. Electrodermal activity in hypothyroid patients and healthy subject. *Thyroid* 1999 Aug; 9(8): 787-790.
18. Singhal RL, Rastogi RB, Hrdina PD. Brain biogenic amines and altered thyroid function. *Life Sci* 1975; 17: 1617-1622.