CHAPTER III

MATERIAL AND METHOD

Objectives:

To determine the correlation between levels of thyroid hormones in the hyperthyroid population and the left ventricular systolic indices.

Study population:

Patients clinically suspected of having hyperthyroidism from the manifestations such as nervousness, emotional lability, insomnia, tremors. frequent bowel movements, excessive sweating and heat intolerance, skin changes, nail sign (Plummer's nail). ocular signs, wide pulse pressure, arrhythmia, cardiac murmurs or heart failure, were selected from the thyroid clinic and general medical clinics of the out patient department of Chulalongkorn University Hospital. If they conform to the inclusion and exclusion criteria mentioned below, they are then sent for echocardiogram to be followed by venepuncture to obtain the blood sample for hormone assay. Thirty patients were recruited into the study.

Test of thyroid function:

Serum free triiodothyroinine (FT3) and Free thyroxine index (FT4I) were used for this study. This is because the total hormone levels (Triiodothyronine, and thyroxine) do not reflect the true metabolic state. The free hormone concentrations correlate better with the metabolic state because they most consistently reflect the rate of hormone production and are influenced only by the alterations in hormone secretion.

The free T4 concentration (FT4) can be measured by equilibrium dialysis of serum enriched with a tracer quantity of labeled T4. Since this technique is cumbersome, an indirect assessment of hormone binding, the in vitro uptake test, simple to perform and usually provides the same information is preferred. Therefore free thyroxine index (FT4I) which is the product of the RT3U (resin T3 uptake) and tatal T4 is preferred by many labs. This is the method employed in the diagnosis of hyper or hypothyroidism in the endocrine laboratory of Chulalongkorn Hospital.

In this study, the commercial kits supplied by the Diagnostic Products Corporation were used to determine the serum hormone levels. In free T3 procedure, 125 I-labeled T3 analog competes for a fixed time with free T3 in the patient sample for sites on T3-specific antibody immobilized to the wall of a polypropylene tube. The tube is then decanted to isolate the antibody bound fraction and counted in a gamma counter, the counts being inversely related to the concentration of free T3 in the sample. The FT3 level is then interpolated from a

standard curve calibrated in FT3 concentrations. The system has been optimized to eliminate all bindings of the T3 analog tracer to endogenous proteins, while leaving essentially undisturbed the original equilibrium between free and protein-bound T3 in the patient sample. the tracer itself has no measurable affinity for thyroid-binding globulin (TBG), the principal thyroid hormone transport protein. To prevent binding of the tracer to albumin, blocking agents are present, at a concentration carefully adjusted to avoid displacement of native T3 from endogenous carrier proteins. To further minimize the risk of "stripping," the system employs an antibody, at low concentration, with an affinity for T3 slightly less than that of TBG, and operates under physiological conditions of temperature, pH and ionic strength.

The tracer has a high specific activity, with total counts of approximately 60,000 cpm at iodination. Maximum binding is approximately 45 to 55%. Near the upper limit of normal the assay has a coefficient of variance of approximately 5%, and can detect less than 0.2 pg/ml. No "end of run" effect has been observed. The antiserum is highly specific for T3, with low cross reactivity to other compounds.

To calculate the Free T4 Index (FT4I) of a sample, it is first necessary to determine its total T4 concentration. This is achieved by the Double Antibody Total T4 procedure in which 125 I-labeled T4 competes with T4 in the patient sample for antibody sites in the presence of blocking agents to prevent binding of radiolabeled T4 to thyroid hormone-binding proteins. After incubation for a fixed time,

separation of bound from free is achieved by the PEG- accelerated double-antibody method. Finally, the antibody-bound fraction is precipitated and counted. Patient sample concentrations are read from a calibration curve. The tracer has a specific activity, with a total counts of 100,000 cpm at iodination. Maximum binding is approximately 60%. Nonspecific binding and patient blanks are negligible. CVs are low and uniform, and no "end of run" effect has been observed. The antiserum is highly specific for T4, with very low cross reactivity to other compounds that might be present in patient samples. the procedure can detect as little as 0.3 microg./dl.

To determine the T3 uptake, the liquid phase T3 uptake test is used. The patient's sample is incubated for a fixed period of time with radioactively labeled triiodothyronine (125 I-T3) in the presence of an exogenous binder, namely charcoal. After separation of bound from free, the radiolabeled T3 is counted in a gamma counter, and the counts are compared to those of a standard. A human serum-based calibrator with a percent uptake value in the euthyroid range is employed as the standard. the recommended normal range for the liquid phase T3 uptake kit is 24.2-33.4% T3U. Given a normal range for a total T4 of 4.5-12.5 microg./dl, this generates a normal range for FT4I of 1.1-4.2.

Having determined the T4 and T3U, multiply the total T4 concentration in micrograms per deciliter by the sample's percent uptake value, and divide the result by 100 to yield the FT4I.

Measurements of left ventricular systolic indices:

This study utilized the following indices as parameters of left ventricular function:

1. The duration of the electromechanical systole (QS2). It is the interval that encompasses the entire systolic period from the onset of the QRS complex on the electrocardiogram to the closure of the aortic value on the M-mode echocardiogram.

2. The pre-ejection period (PEP). IT is the interval from the onset of ventricular depolarization to the beginning of ejection and is derived from the onset of the QRS complex to the opening of the aortic valve on the M-mode echocardiogram, or alternatively by subtracting the LVET from the QS2.

3. The left ventricular ejection time (LVET). It is the phase of systole during which the left ventricle ejects blood into the arterial system and encompasses the period from the opening to the closure of the aortic valve. This interval is measured directly from the M-mode echocardiogram.

4. The PEP/LVET ratio. This ratio reflects the over all changes induced by left ventricular dysfunction. The normal PEP/LVET is 0.34.

5. The velocity of posterior wall contraction. This reflects the inotropic state of the left ventricle. It is is measured

directly from M-mode echocardiogram as the amplitude of the left ventricular wall motion during systole divided by the time period taken to reach that amplitude.

6. The left ventricular ejection fraction (EF). This is the percentage of blood ejected by the left ventricle during systole.

The PEP, LVET, QS2 are then corrected for heart rate as described by Lewis et al (1977) and Weissler (1986) by the following regression equations.

Sex	Equation
M	QS2I = 2.1 HR + QS2
F	QS2I = 2.0 HR + QS2
M	LVETI = 1.7 HR + LVET
F	LVETI = 1.6 HR + LVET
M	PEPI = 0.4 HR + PEP
F	PEPI = 0.4 HR + PEP

All these values, both corrected and directly measured were used for the purpose of finding the correlations.

The intervals were obtained by simultaneous fast speed recording of electrocardiographic lead II and M-mode echocardiogram

under direct 2-D echocardiographic guidance to obtain the optimal M-mode plane for the aortic box. The instrument used was an Aloka SSD 870 model equipped with high speed thermal printer for real time M-mode recording. The paper speed was set to run at 100 m.m. per second as recommended by Spodick, Lewis and Weissler. The STI were derived from the mean of 10 consecutive beats as recommended by Lewis and Weissler. All measurements were taken to the nearest 5 millseconds(msec.).

Inclusion Criteria:

1. Patients suspected to have hyperthyroidism from clinical manifestations who attended the thyroid clinic and the general medical clinic of the Chulalongkorn University Hospital.

Exclusion Criteria :

1. Patient with any concomittent heart disease.

2. Patients who were receiving or had received any one of the following drugs which influence the left ventricualr function: digitalis, beta-blockers, beta-stimulants, calcium-channel blocker, vasopressors, diuretics.

3. Patients with any degree of heart failure.

4. Patients whose surface EKG showed left bundle branch block.

5. Patients aged over 35 years to exclude any significant coronary arterial disease that may influence the left ventricular function.

6. Patients who were pregnant.

Study Design:

Cross-sectional analytic study.

Statistics used:

Determination of coefficient of corrleation.

Significance is considered if p value is 0.01 or less.