SUMMARY

This study was performed to assess the relation between $CD4^+$ $CD25^+$ T regulatory cell and Graves' disease and its role in the pathogenesis of Graves' disease. The diagnosis of Graves' disease is confirmed by detecting thyroid autoantibodies. Also this study was performed to assess apoptosis of peripheral blood lymphocytes in patients with Graves' disease.

This study was done during the period from September 2006 to July 2007 on thirty patients (27 females and 3 males) suffering from Graves' disease. Their ages ranged from 18 to 49 years. They were selected from the General Surgery Department and the Outpatient Clinic of General Surgery Department of Benha University Hospital. Ten apparently healthy persons matched for age and sex were chosen to serve as a control group. Diagnosis and selection of cases were based on: complete history taking, clinical examination and laboratory investigations. It must be noted that the cases included in the study were either just diagnosed and in the beginning of the treatment or in relapse of symptoms during the treatment.

The following methodology was performed:

(A) Sample collection

From each selected patient and control, 7 ml blood were aseptically withdrawn by sterile venipuncture and collected in 3 test tubes:

- 5ml fresh heparinized blood were used for assessment of apoptosis.

- 1ml fresh blood on EDTA was used for determination the expression of CD4 and CD25 of T regulatory cell.
- 1ml fresh blood was left to clot and serum was separated by centrifugation and collected in aliquots for detection of thyroid autoantibodies.

(B)Flow cytometric measurment of expression of CD4 CD25 of T regulatory cell

It was carried out by using of PE conjugated mouse monoclonal anti-human CD4, FITC conjugated mouse monoclonal anti-human CD25 as commercial fluorescent dyes and Fluorescence Activated Cell Sorter (FACS), flow cytometer (Becton- Dickinson San Jose California, USA).

(C)Detection of thyroid autoantibodies

It was done by indirect fluorescent antibody testing to detect antimicrosomal (antithyroperoxidase) and antithyroglobulin thyroid autoantibodies by using substrate slides in which each slide well contains a fixed monkey thyroid section and properly equipped and aligned fluorescent microscope.

(D) Assessment of apoptosis:

Assessment of apoptosis of peripheral blood lymphocytes was done from peripheral blood by lymphocyte separation then DNA purification was carried out to the lymphocyte of the peripheral blood and agarose gel electrophoresis of DNA was

done. Cells undergoing apoptosis exhibited DNA fragmentation and appeared as ladder like pattern.

The results of the study showed the following

- The study showed that 28 (93.33%) of patients were in the age group from 15-45 years i.e. in the childbearing period, two patients (6.67%)were above 45 years. The distribution of study group according to age was statistically significant ($P < 0.05$).

- According to sex, the study showed that 26 of patients (86.67%) were females and 4 (13.33%) were males. The distribution of study group according to sex was statistically significant ($P < 0.05$).

- Five (16.67%) out of 30 patients were smokers, they were three males and two females, while the non smokers were 25 (83.33%).

- The total number of cases which has family history of autoimmune disease was 7(23.33%). Among them Graves disease has the highest incidence of occurrence 3 (10%).

- Fourteen patients (46.67%) in the study have associated medical conditions; five out of them (16.67%) have an associated autoimmune disease.

- According to the study the most common symptom of Graves' disease was nervousness and irritability, number of cases with nervousness and irritability was 28 (93.3%) and goiter was the

most common sign of Graves disease and the number of cases with goiter was 28 (93.3%).

- According to the line of treatment, 25 patients (83.33%) were run along antithyroid drugs, four patients (13.33 %) underwent subtotal thyroidectomy and only one patient (3.33) did radioiodine therapy.

- In the study group the mean and SD of the percentage of CD4 CD25 from CD4 cells were 0.97, 0.55 respectively. In the control group the mean and SD of the percentage of CD4 CD25 from CD4 cells were 2.85, 0.92 respectively.

- The difference between control and study group according to the mean and SD of the percentage of CD4 CD25 from CD4 cells was statistically highly significant ($P < 0.001$).

- According to presence or absence of thyroid autoantibodies, 27 patients (90%) out of 30 have positive thyroid autoantibodies and 3 (10%) of them have negative thyroid autoantibodies.

- According to the type of thyroid autoantibodies, eight patients (26.67%) had antimicrosomal (anti Tpo) only, four patients (13.33%) had anti TG only and 15 patients (50%) had both anti microsomal and anti TG thyroid autoantibodies.

- The difference between control and study group according to presence and absence of thyroid autoantibodies was statistically significant ($P < 0.05$).