RESULTS

This study was done during the period from September 2006 to July 2007 on a study group and a control group. In the study group, there were thirty patients, (26 females and 4 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.

Personal factors are among the factors that strongly affect the occurrence and the development of autoimmune disease in general and Graves' disease in special.

In the present study the distribution of Graves' disease according to personal factors as age, sex and smoking was studied.

Table (5): Distribution of study group according to personal factors:

Age group	No.	%
15- 45 Y	28	93.33
> 45 Y	2	6.67
Z= 9.51	**P** <0.05	
Sex	No.	%
Male	4	13.33
Female	26	86.67
Z= 5.91	P <0.05	
Smoking.	No	%
Smoker	5	16.67
Non smoker	25	83.33
Z= 4.89	P <0.05	

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 distribution of study group according to smoking was statistically significant (P <0.05).

Table (6): Distribution of control group according to personal factors:

Age group	No.	%
15- 45 Y	9	90
>45 Y	1	10
Sex	No.	%
Male	2	20
Female	8	80
Smoking	No.	%
Smoker	1	10
Non smoker	9	90

The study showed that 9 (90%) of persons in the control group were in the age group from 15-45 years i.e. in the childbearing period, one person(10%) was above 45 years.

According to sex, there were 8(80%) females and 2 (20%) males in the control group. Out of the ten of the control group, only one was a smoker and 9 were non smokers

Table (7): Distribution of study group according to family history of autoimmune diseases:

Autoimmune diseases	No.	%
Graves'	3	10
Type -1 diabetus	2	6.67
Vitiligo	1	3.33
SLE	1	3.33
Total	7	23.33

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%).

Table (8): Distribution of study group according to associated medical conditions:

Associated medical conditions	No.	%
Type -1 DM	4	13.33
Type -2 DM	3	10
Hypertension	2	6.67
Hypertension + Type - 2 DM	3	10
Coronary insufficiency	1	3.33
Vetiligo	1	3.33
Total	14	46.67

Fourteen patients (46.67%) in the study have associated medical conditions, five out of them (16.67%) have an associated autoimmune disease, four (13.33%) have type-1 diabetes mellitus and one (3.33%) has viteligo. Three (10%) have type-2 diabetes mellitus, two (6.67%) have hypertension, three (10%) have hypertension and type-2 diabetes mellitus. One (3.33%) has coronary insufficiency.

Table (9): Distribution of study group according to the symptoms of Graves' disease

Symptoms of Graves' disease	No.	%
Nervousness and irritability	28	93.3
Weight loss with increased appetite	27	90
Palpitation	27	90
Heat intolerance	27	90
Night mares	25	83.3
Weakness and fatigue	24	80
Amenorrhea	4	13.33
Infertility	2	6.67

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%).

Table (10): Distribution of study group according to the signs of Graves' disease

Signs of Graves' disease	No.	%
Goiter	28	93.3
Tachycardia	27	90
Fine tremors	23	76.67
Warm moist skin	19	63.3
Exophthalmos	12	40
Pretibial myxedema	1	3.33

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

Table (11): Distribution of study group according to the line of treatment:

Line of treatment	No.	%
Anti thyroid drugs	25	83.33
Surgery	4	13.33
Radioiodine therapy	1	3.33

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

I- **Results of Flow cytometric measurment of expression of CD4 CD25 out of CD4 cell:**

Table (12): Difference between control and study group according to the mean and SD of the percentage of CD4 CD25 out of CD4 cells:

Variables	Study group	Control group	t	P
Mean	0.97	2.85	7.79	<0.001 HS
SD	0.55	0.92		

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)

Figure 19: Results of flow cytometric measurement of expression of CD4CD25 out of CD4 cells

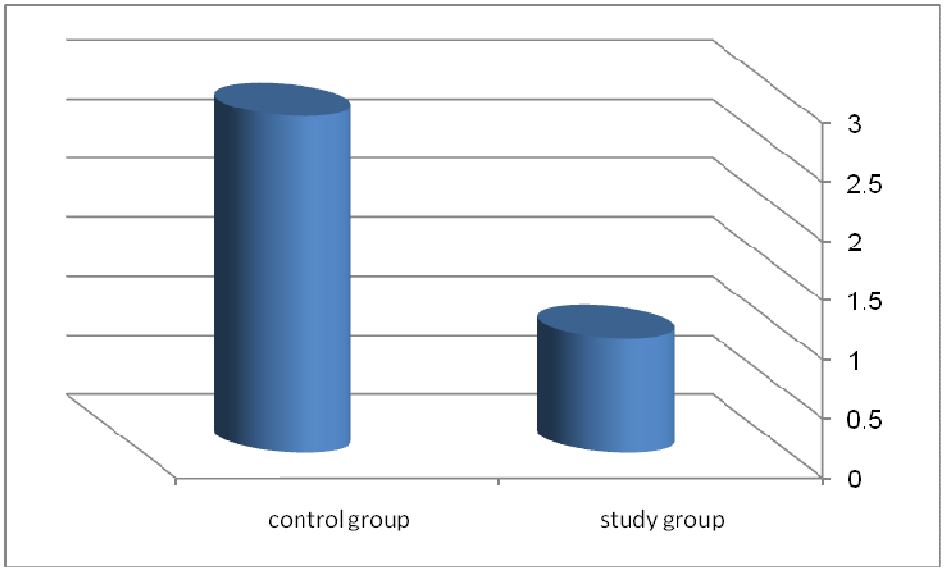


Figure 20: Results of flow cytometric measurement of expression of CD4CD25 out of CD4 cells in one of the cases in the study, the percentage of CD2 CD25 out of CD4 in this case was 0.20

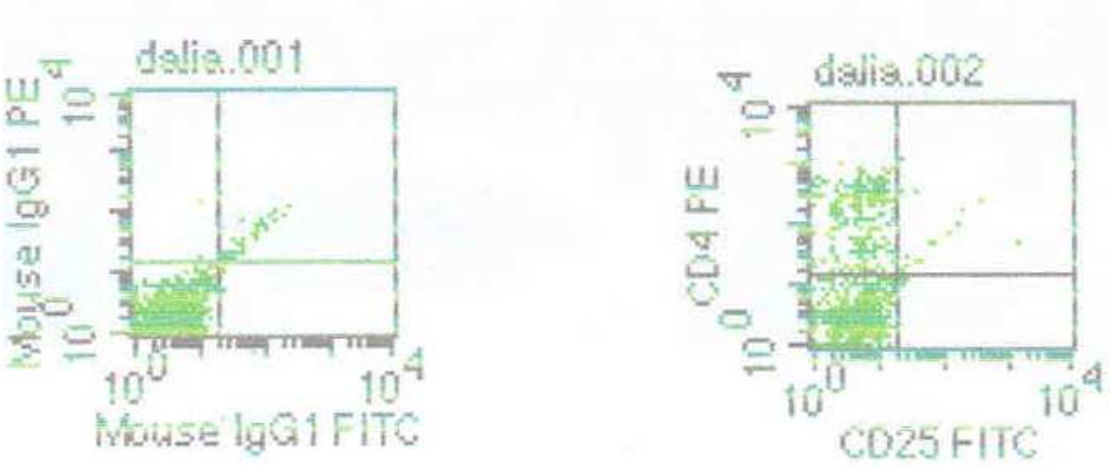
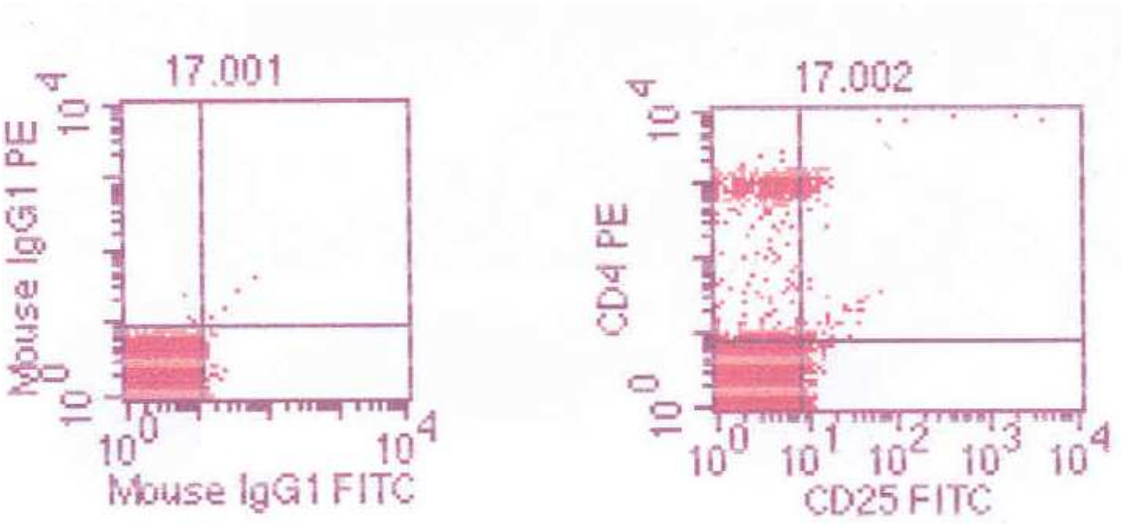


Figure 21: Results of flow cytometric measurement of expression of CD4CD25 out of CD4 cells in one of the controls in the study, the percentage of CD2 CD25 out of CD4 in this case was 3.90



II-Results of detection of thyroid autoantibodies

Table (13): Distribution of study group according to presence and absence of autoantibodies:

Study group	No.	%
Patients with positive thyroid autoantibodies	27	90
Patients with negative thyroid autoantibodies	3	10
Z= 7.3	P <0.05	

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 auto antibodies. The distribution of study group according to presence or absence of thyroid autoantibodies was statistically significant (P <0.05).

Table (14): Distribution of study group according to the type of thyroid autoantibodies in the study group.

Type of thyroid auto antibodies	No.	%
Anti microsomal (anti Tpo)	8	26.67
Anti TG	4	13.33
Anti TG + anti Tpo	15	50

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 antimicrosomal and anti TG thyroid autoantibodies.

The titer of positive cases that had antimicrosomal thyroid autoantibodies ranged between 1:40 and 1:320.

The titer of positive cases that had anti TG thyroid autoantibodies ranged between 1:20 and 1:160.

Figure 22: Distribution of autoantibodies in study group

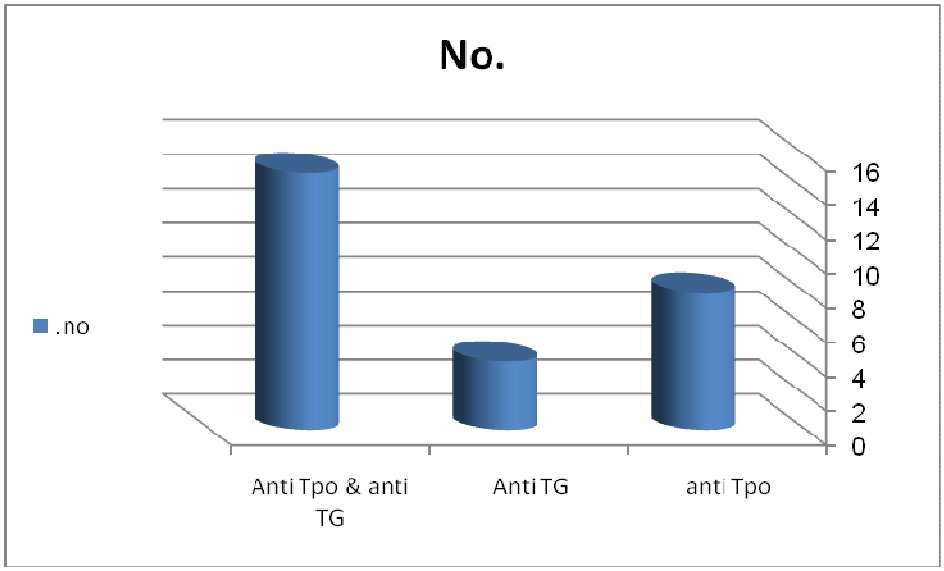


Figure 23: A case of positive antimicrosomal auto thyroid antibodies in the fluorescent microscope (x400).

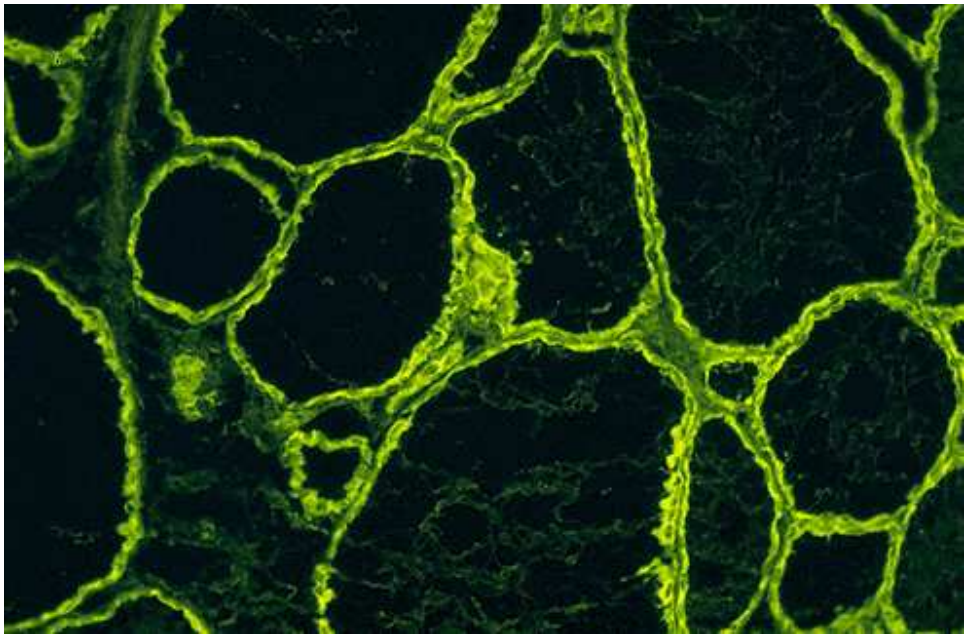


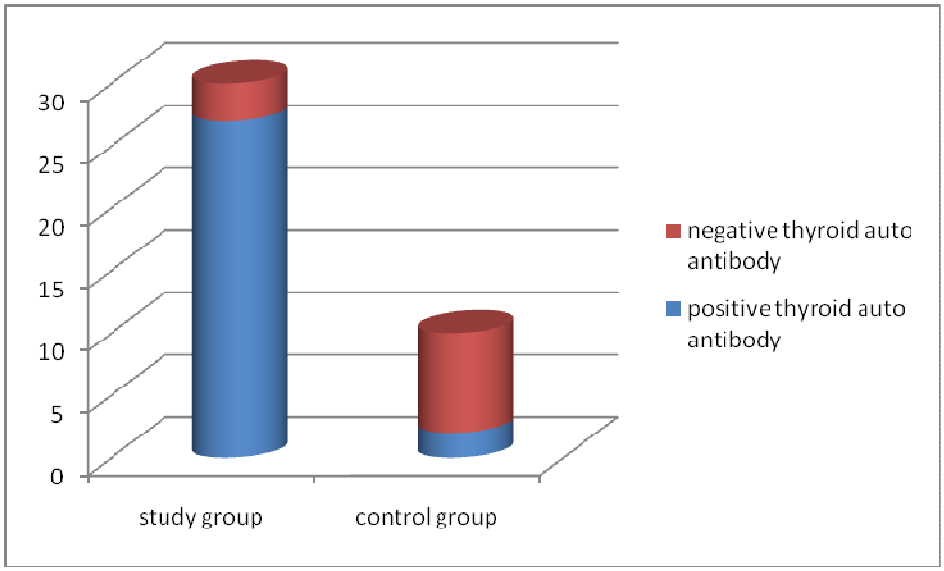
Figure 24: A case of positive antithyroglobulin autothyroid antibodies in the fluorescent microscope (x400).

Table (15): Difference between control and study group according to presence and absence of thyroid autoantibodies.

Presence and absence of thyroid auto antibodies	Study group (n=30)		Control group (n=10)		X^2	P
Positive thyroid auto antibodies	No.	%	No.	%	15.09	P<0.05
	27	90	2	**20**		
Negative thyroid auto antibodies	3	10	8	80		
Total	30	100	10	100		

The study showed that, 27 patients (90%) out of 30 have positive thyroid autoantibodies and 3 (10%) of them have negative thyroid auto antibodies. While there are 2 (20%) of the persons of the control group had Positive thyroid autoantibodies [one had positive anti TPO and the other had positive anti TG] and 8 (80%) of the persons of the control group had negative thyroid autoantibodies. The difference between control and study group according to presence and absence of thyroid autoantibodies was statistically significant (P <0.05).

Figure 25: Difference between control and study group according to thyroid autoantibodies



III-Results of apoptosis:

Table (16): Distribution of study group according to presence or absence of DNA apoptic bands in gel electrophoresis.

Study group	No.	%
Patients with positive apoptic bands in gel electrophoresis	9	30
Patients with negative apoptic bands in gel electrophoresis	21	70
Z= 2.3	P <0.05	

Number of patients with positive apoptic bands in gel electrophoresis was 9 (30%) and number of patients with negative apoptic bands in gel electrophoresis was 21(70%).The distribution of study group according to presence or absence of DNA apoptic bands in gel electrophoresis was statistically significant (P <0.05).

Table (17): Difference between control and study group according to presence or absence of DNA apoptic bands in gel electrophoresis.

Presence or absence of DNA apoptic bands in gel electrophoresis.	Study group (n=30)		Control group (n=10)		X^2	P
Positive DNA apoptic bands in gel electrophoresis.	No	%	No.	%	3.87	<0.05
	9	30	0	0		
Negative DNA apoptic bands in gel electrophoresis	21	70	10	100		
Total	30		10			

All persons in the control group show negative DNA apoptic bands in gel electrophoresis. The difference between control and study group according to presence or absence of DNA apoptic bands in gel electrophoresis was statistically significant (P <0.05).

Figure 26: Difference between control and study group according to presence or absence of DNA apoptic bands

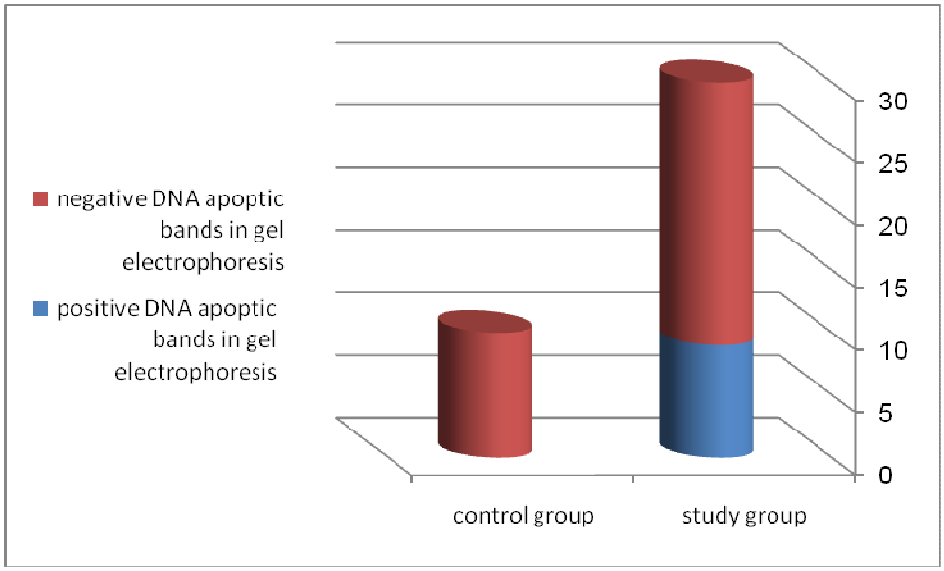


Figure 27: DNA bands in agarose gel electrophoresis.

1 2 3 4

Lanes 1, 2 show positive DNA apoptic bands in 2 cases in the study.

Lane 3 show negative DNA apoptic bands in a case in the study.

Lane 4 show negative DNA apoptic bands in a control in the study.

Table (18): Mean and SD in different variables in study and control groups:

Variables	N	Minimum	Maximum	Mean	SD. Deviation
Age in cases	30	18.00	49.00	32.0667	8.4606
Age in control group	10	21	45	31.8	7.7
TSH	30	< 0.01	0.08	0.02.333	0.01729
FT4	30	3.03	8.02	6.3687	1.4970
FT3	30	1.90	9.60	5.2050	1.6804
Percentage of CD4CD25 out of CD4 cells in study group	30	0.20	2.70	0.9683	0.5550
Percentage of CD4CD25 out of CD4 cells in control	10	1.09	3.90	2.8480	0.9234

group					

The study showed that ages of the studied group ranged between 18-49 years, mean ± SD of age in the studied group = 32.0667 ± 8.4606.

The study showed that ages of the control group ranged between 21-45 years, mean ± SD of age in the studied group = 31.8 ± 7.7. So the control group was matched according to the age to the studied group.

The level of TSH in ml IU| ml in the studied group ranged between 0.08- < 0.01, mean ± SD of the level of TSH = 0.02.333 ± 0.01729.

The level of FT4 in the studied group ranged between 3.03-8.02 ng|dl, mean ± SD of the level of FT4 = 6.3687 ± 1.4970.

The level of FT3 in the studied group ranged between 1.90- 6.90 ng|dl, mean ± SD of the level of FT3 = 5.2050 ± 1.6804.

The percentage of CD4 CD25 out of CD4 cells in the studied group ranged between 0.20-2.70, mean ± SD of the percentage of CD4 CD25 out of CD4 cells = 0.9683 ± 0.5550.

The percentage of CD4 CD25 out of CD4 cells in the control group ranged between 1.09-3.90, mean ± SD of the percentage of CD4

CD25 out of CD4 cells = 2.8480± 0.9234.

Table (19): Relation between the level of FT3 and presence or absence of DNA apoptic bands in gel electrophoresis

Presence or absence of DNA apoptic bands in gel electrophoresis	Range of FT3 level in ng dl	X ± SD	t	P
Presence of DNA apoptic bands	3.8 - 9.6	6.56 ± 1.56	3.4	< 0.05
Absence of DNA apoptic bands	1.9 – 6.8	4.6 ± 1.4		

The study showed that the relation the level of FT3 and presence of DNA apoptic bands in gel electrophoresis was statistically significant

(P <0.05)

Table (20): Relation between the level of FT4 and presence of DNA apoptic bands in gel electrophoresis

Presence or absence of DNA apoptic bands in gel electrophoresis	Range of FT4 level in ng dl	X ± SD	t	P
Presence of DNA apoptic bands	5.02 – 8.02	7.44 ± 0.99	2.88	< 0.05
Absence of DNA apoptic bands	3.03 – 7.9	5.9 ± 1.46		

The study showed that the relation the level of FT4 and presence of DNA apoptic bands in gel electrophoresis was statistically significant

(P <0.05).

Table (21): Relation between the percentage of CD4 CD25 out of CD4 cells in the studied group and presence of DNA apoptic bands in gel electrophoresis.

Range of the percentage of CD4CD25 out OF CD4 in the studied group	Presence or absence of DNA apoptic bands in gel electrophoresis	X ± SD	t	P
0.2 – 1.02	Presence of DNA apoptic bands	0.47 ± 0.27	3.94	< 0.05
0.65 – 2.7	Absence of DNA apoptic bands	1.18 ± 0.51		

The relation between the percentage of CD4 CD25 out of CD4 cells in the studied group and presence of DNA apoptic bands in gel electrophoresis was statistically significant (P <0.05).

Table (22): Relation between the percentage of CD4 CD25 out of CD4 cells in the studied group and Presence or absence of thyroid auto antibodies

Range of the percentage of CD4CD25 out OF CD4 in the studied group	Presence or absence of thyroid auto antibodies	X ± SD	t	P
0.2 – 1.96	Positive thyroid auto antibodies	0.87 ± 0.45	3.39	< 0.05
1.26 – 2.7	Negative thyroid auto antibodies	1.85 ± 0.75		

The relation between the percentage of CD4 CD25 out of CD4 cells in the studied group and presence of thyroid autoantibodies was statistically significant (P <0.05).