AIM OF THE WORK

The aim of this work was to study:

1. Thyroid dysfunction (hyperthyroidism or hypothyroidism) in seropositive versus sero negative rheumatoid arthritis patients.

2. Autoimmune thyroid markers (anti-TPO, anti-TG, anti TSH receptor antibodies)in seropositive versus seronegative RA patients and their relation to overt thyroid dysfunction.

3. Relation between autoimmune thyroid markers namely thyroid peroxidase antibodies (anti-TPO), thyroglobulin antibodies (anti-TG) and particularly TSH receptor antibodies (TRAbs) and rheumatoid arthritis auto antibodies namely Anti-cyclic citrullinated peptide (anti –CCP) &rheumatoid factor (RF).

SUBJECTS

The study was carried out on 70 consecutive patients with rheumatoid arthritis who were attending rheumatology outpatient clinic and inpatient in Alexandria Main University Hospital.

They were divided according to the results of their serological tests (RF and anti-CCP) into 2 groups:

Group I: Included 35 patients with seropositive rheumatoid arthritis (positive to one or both seromarkers).

Group II: Included 35 patients with seronegative rheumatoid arthritis (negative to both seromarkers)

A Third group (Group III): Including 20 healthy age matched individuals who are not suffering of any rheumatologic disorder were included as a control group.

Diagnosis of rheumatoid arthritis will depend on 2010 ACR-EULAR classification criteria [112]:

A score of ≥6/10 is needed for classification:

A. Joint involvement:

1 large joint	0
2-10 large joints	1
1-3 small joints (with or without large joints affection)	2
4-10 small joints (with or without large joints affection)	3
>10 joints (at least one small joint)	5

B. Serology (at least 1 test result is needed for classification)

Negative RF and negative ACPA	0
Low-positive RF or low positive ACPA	2
High positive RF or high positive ACPA	3

C. Acute phase reactants (at least 1 test result is needed for classification)

Normal CRP and normal ESR	0
Abnormal CRP or abnormal ESR	1

D. Duration of symptoms:

<6 weeks	0
≥ 6 weeks	1

Exclusion criteria:

1- Patients receiving anti thyroid drugs or known to have a history of any thyroid disease.

2- Patients receiving corticosteroids or any immunosuppressive drugs known to alter the autoimmune markers.

3- Patients having history of any autoimmune disease other than RA.

METHODS

Each patient was subjected to the following:

- Thorough history taking with emphasis on symptoms of thyroid dysfunction (hypo or hyper function of the thyroid gland) and symptoms of rheumatoid arthritis focusing on joint affection or any other systemic affection.

- Complete physical examination with emphasis on any signs of thyroid dysfunction and joint examination for tenderness, swelling, deformity and loss of function with disease activity score (DAS)for assessment of activity [131] as shown in figure (5) and (6)

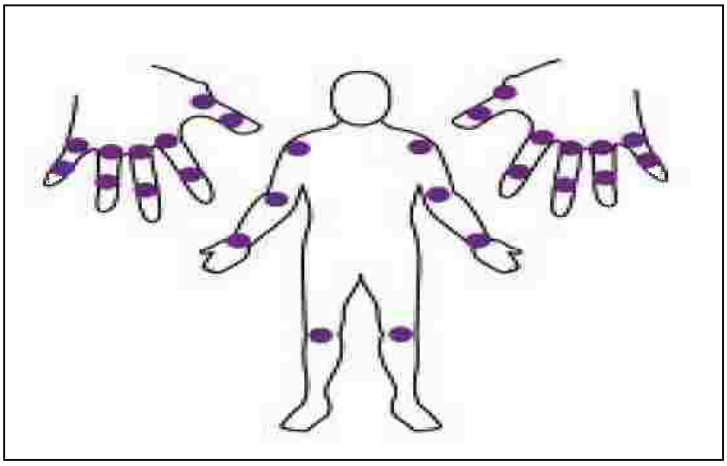


Figure (5): joints to be assessed in DAS calculation.

Right and left (5 metacarpophalangeal joints, 5 proximal interphalangeal joints, knee, shoulder, elbow, wrist)

After assessment the results are interpreted as follows:

Remission (<2.6)
Low Activity (2.6-3.2)
Moderate Activity (>3.2 - 5.1)
Severe Activity (>5.1)

Figure (6): interpretation of DAS results

- **Laboratory investigations:**

Venous blood samples were withdrawn from every subject after an overnight fast of 8 hours, patients were subjected to the following laboratory investigations:

I- Routine investigation:

i. Complete blood picture.
ii. Erythrocyte sedimentation rate.
iii. C-reactive protein.
iv. Renal function tests.
    -Serum urea.
    -Serum creatinine.
v. Liver function tests.
    -Serum Aspartate transaminase (AST).
    -Serum Alanine transaminase (ALT).

II- Rheumatologic assay:

i. Rheumatoid factor: [215,216]

Principle: (latex agglutination technique)

In this test blood is mixed with tiny rubber (latex) beads that are covered with human antibodies. If RF is present, the latex beads clump together (agglutinate).

Procedure:

1. Reagents and specimens were brought to room temperature before use.
2. One drop (50 ul) of the RF Positive Control was placed on the first field of the reaction slide. One drop (50 ul) of the RF negative Control was placed on the second field. The remaining fields were used for test specimens. Using pipettes provided, one drop of each specimen was placed on successive fields. The Pipette/Stir Sticks were retained for mixing step.
3. The RF latex Reagent was resuspended gently and one drop was added to each test field. The Pipette/Stir Stick was used to spread reaction mixture over entire test field.
4. The slide was rotated for 3 minutes and then read under direct light.

Expected values:

Agglutination (positive reaction) indicates the level of rheumatoid factor(s) in the undiluted test sample is approximately 15 IU/ml or greater.

ii. Anti-CCP antibody : [217]

Principle:

Highly purified cyclic citrullinated vimentin peptides (CCP) are bound to microwells. Antibodies against the coated antigen, if present in diluted sample, bind to the

respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated antihuman antibodies immunologically detect the bound antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm.

Procedure:

Microplate modules were prepared for all calibrators / controls and samples.

1. 100 µL of calibrators, controls and prediluted samples were pipetted into the wells.
2. Incubated for 30 minutes at room temperature (20 °C - 28 °C).
3. The contents of the microwells were discarded and washed 3 times with 300 µL of wash solution.
4. 100 µL of enzyme conjugate was dispensed into each well.
5. Incubated for 15 minutes at room temperature.
6. The contents of the microwells were dispensed and then wash 3 times with 300 µL of wash solution.
7. 100 µL of TMB substrate solution was dispensed into each well.
8. Incubated for 15 minutes at room temperature.
9. 100 µL of stop solution was added to each well of the modules.
10. Incubated for 5 minutes at room temperature.
11. The optical density was read at 450 nm (reference 600-690 nm) and the results were calculated. The developed colour was stable for at least 30 minutes. We read during this time.

Expected values:

Samples with results <25 U/ml are defined as negative. Samples ≥25 U/ml are defined as positive.

III-Hormonal assay using ELISA technique:

i. Serum TSH: [218-220]

Principle:

The micro titer wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site of the TSH molecule. An aliquot of specimen sample containing endogenous TSH is incubated in the coated well with enzyme conjugate, which is an anti-TSH antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase is proportional to the concentration of TSH in the sample. Having added the substrate solution, the intensity of color developed is proportional to the concentration of TSH in the specimen sample

Procedure:

1. 25 µL of each Standard, Control and sample were dispensed with new disposable tips into appropriate wells.
2. Incubated for 10 minutes at room temperature.
3. 100 µL Enzyme Conjugate was dispensed into each well and thoroughly mixed for 10 seconds. It is important to have a complete mixing in this step.
4. Incubated for 90 minutes at room temperature.
5. The contents of the wells were briskly shacked.
6. The wells were rinsed 5 times with diluted Wash Solution(300 µL per well), then the wells were sharply striked on absorbent paper to remove residual droplets
7. 100 µL of Substrate Solution was added to each well.
8. Incubated for 20 minutes at room temperature.
9. The enzymatic reaction was stopped by adding 100 µL of Stop Solution to each well.
10. The absorbance (OD) of each well was determined at 450 ± 10 nm with a microtiter plate reader.

Expected values:

Normal serum TSH ranges from 0.4-4.6 mIU/L. [221,222]

ii. Serum total T3: [223-226]

Principle:

In this assay, a second antibody (goat anti mouse IgG) is coated on a microtiter wells. A measured amount of patient serum, a certain amount of mouse monoclonal Anti T3 antibody, and a constant amount of T3 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, the mouse anti-T3 antibody is bound to the second antibody on the wells.

T3 and the enzyme conjugated-T3 compete for the limited binding sites on the anti-T3 antibody. After 60 minute incubation at room temperature, the wells are washed 5 times by water to remove unbound T3 conjugate. A solution of TMB is then added and incubated for 20 minutes at room temperature, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present, and is inversely related to the amount of unlabeled T3 standards assayed in the same way. The concentration of T3 in the unknown sample is then calculated.

Procedure:

1. The desired number of coated wells in the holder were secured. Data sheet with sample identification was prepared.
2. 50µL of standards, specimens, and controls (not included with this kit) was pipetted into appropriate wells.
3. 50µL of T3 Antibody Reagent was dispensed into each well and mixed thoroughly for 30 seconds.

4. 100µL of Working Conjugate Reagent was added into each well and mixed thoroughly for 30 seconds. It is important to have completed mixing in step 3 and 4.
5. Incubated at room temperature (18-25°C) for 60 minutes.
6. The incubation mixture was removed by flicking plate contents into a waste container.
7. The wells were rinsed and flicked 5 times with distilled or deionized water.
8. The wells were striked sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. 100µL of TMB Reagent was dispensed into each well and gently mix for 5 seconds.
10. Incubated at room temperature, in the dark, for 20 minutes.
11. The reaction was stopped by adding 100µL of Stop Solution to each well.
12. Gently mixed for 30 seconds to ensure that all of the blue color changes completely to yellow.
13. Read absorbance at 450nm with a microtiter plate reader within 15 minutes.

Expected values:

Normal value: 0.8 – 1.9 ng/ml.

iii. Serum total T4 : [227,228]

Principle:

To measure T4 by competitive immunoassay techniques, a sample of serum or plasma containing the T4 to be quantified is mixed with labeled T4 and T4 antibody.

The labeled T4 contains 8-anilino-1-napthalene sulfonic acid (ANS) to inhibit binding of T4 to serum proteins, which would otherwise interfere with the assay.

During incubation, a fixed amount of labeled T4 competes with the unlabeled T4 in the sample, standard, or quality control serum for a fixed number of binding sites on the specific T4 antibody.

Separation of the unbound T4 from antibody-bound T4 and the subsequent measurement of the labeled fraction of the bound phase complete the test. By comparing results of the unknown sample with those obtained from a series of T4 calibrators, an accurate measurement of the T4 concentration in the sample can be obtained.

In thisT4 EIA, antibody to T4 is coated on a solid phase (microtiter well). A measured amount of patient serum and a constant amount of T4 labeled with horseradish peroxidase are added. During incubation, T4 in the patient sample and enzyme-labeled T4 compete for the limited binding sites on the T4 antibody. After 60-minute incubation at room temperature, the solid phase is washed with water to remove unbound-labeled T4. A solution of tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCI, and the resulting yellow color is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of T4 in the patient sample. By reference to a series of calibrators processed in the same way, the concentration of T4 in the unknown sample is determined.

Procedure:

1. The desired number of coated wells were secured in the holder.
2. 25uL of standards, specimens, and controls (not included) was pipetted into appropriate wells.
3. 100 µL of Working Conjugate Reagent was added into each well.
4. Mixed thoroughly for 30 seconds.
5. Incubated at room temperature (18-25°C) for 60 minutes.
6. The incubation mixture was removed by flicking plate contents into a waste container.
7. The microtiter wells were rinsed and flicked 5 times with distilled H2O.
8. The wells were striked sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. 100µL of TMB Reagent was dispensed into each well and gently mixed for 5 seconds.
10. Incubated at room temperature, in the dark, for 20 minutes.
11. The reaction was stopped by adding 100µL of Stop Solution to each well.
12. Gently mixed for 30 seconds to ensure that all of the blue color changes completely to yellow.
13. Read absorbance at 450nm with a microtiter plate reader within 15 minutes.

Expected values:

Normal values: 4.7-12.8 ug/dl.

iv. Thyroid autoantibodies:

a- Serum anti thyroid peroxidase anti bodies (Anti -TPO).
b- Serum anti thyroglobulin antibodies (anti-TG).
c- Serum TSH receptor antibodies (TRAbs).

a- Anti-TPO:

Done using enzyme linked immunosorbant assay (ELISA). [229]

Principle:

Highly purified human thyroid peroxidase (TPO) is bound to microwells. Antibodies to this antigen, if present in diluted serum, bind in the microwells. Washing of the microwells removes unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically bind to the bound patient antibodies forming a conjugate/ antibody/ antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of color is directly proportional to the concentration of IgG antibodies present in the original sample.

Procedure:

1. A sufficient number of microplate modules were prepared to accommodate controls and prediluted patient samples.
2. 100 µl of controls and prediluted patient samples were pipetted in duplicate into the wells.
3. Incubated for 30 minutes at room temperature (20-28°C).
4. The contents of the microwells were discarded and wash 3 times with 300 µl of wash solution.
5. 100 µl of enzyme conjugate was dispensed into each well.
6. Incubated for 15 minutes at room temperature.
7. The contents of the microwells were discarded and washed 3 times with 300 µl of wash solution.
8. 100 µl of TMB substrate solution was dispensed into each well.
9. Incubated for 15 minutes at room temperature.
10. 100µl of stop solution was added to each well of the modules and incubated for 5 minutes at room temperature.
11. The optical density was read at 450 nm and the results were calculated.

Expected values:

(IU/ml)

Normal: < 50

Borderline: 50 - 75

Elevated: > 75

b- Anti-TG:

Using ELISA technique with the same principle and procedure as anti-TPO.[230]

Expected values:

(IU/ml)

Normal: < 100

Borderline: 100 - 150

Elevated: > 150

c- TSH receptor antibodies (TRAbs):

Done using ELISA technique. [231,232]

Principle:

In TRAb ELISA, TSH receptor autoantibodies in patient sera, calibrators and controls are allowed to interact with TSH receptor coated onto ELISA plate wells. After 2 hour incubation, the samples are discarded leaving TRAb bound to the immobilized TSH receptor. TSH biotin is added in a 2nd incubation step, where it interacts with immobilized TSH receptors, which have not been blocked by the bound TRAb from patient sera, calibrators or controls. The amount of TSH biotin bound to the plate is then determined in a 3rd incubation step by addition of streptavidin peroxidase, which binds specifically to biotin. Excess unbound streptavidin peroxidase is then discarded and the addition of tetramethylbenzidine (TMB) results in formation of a blue color.

This reaction is stopped by the addition of stop solution causing the well contents to turn from blue to yellow. The absorbance of the yellow reaction mixture at 450 nm is then read using an ELISA plate reader. A lower absorbance indicates the presence of TRAb in the test sample as TRAb inhibits the binding of TSH biotin to TSH receptor coated plate wells. The measuring range is 1 – 40 u/L.

Procedure:

1. 75 µL of start buffer (B) was pipetted into each well to be used, leaving the last well for a blank (see step 12).
2. 75 µL of patient sera, calibrators (C1-4) and controls (D1 and D2) was pipetted into respective wells (start with the 40 u/L calibrator and descended down the plate to the negative control and then test sera), leaving the last well blank.
3. The frame was covered and the wells were shaken for 2 hours at room temperature on an ELISA plate shaker (500 shakes per min.).
4. After incubation, the wells were washed once with diluted wash solution (J), and aspirated the wash by use of a plate washing machine or discard the wash by briskly inverting the frame of stripwells over a suitable receptacle. The inverted wells were tapped gently on a clean, dry, absorbent surface to remove excess wash solution (only necessary if washing plate by hand).
5. 100 µL of reconstituted TSH biotin (E) was reconstituted into each well.
6. The plate was covered, and incubated at room temperature for 25 minutes without shaking.
7. Repeat wash step 4.
8. 100 µL of diluted streptavidin peroxidase (G) was pipetted into each well (except blank) and incubated at room temperature for 20 minutes without shaking.
9. After incubation, samples were aspirated by use of a plate washing machine, or discarded by briskly inverting the frame of stripwells over a suitable receptacle. The wells were washed twice with diluted wash solution (J) followed by once with pure water (to remove any foam) and tap the inverted wells gently on a clean, dry, absorbent surface to remove excess wash solution (if a plate washing machine is used, the plate can be washed 3 times with diluted wash solution (J) only).

10. 100µL of TMB (I) was pipetted into each well (including blank) and incubated in the dark at room temperature for 30 minutes without shaking.
11. 50 µL stop solution (K) was pipetted to each well (including blank) and shaked the plate for approximately 5 seconds on a plate shaker.
12. The absorbance of each well was read at 450 nm using an ELISA plate reader, blanked against the well containing 100 µL of TMB (I) and 50 µL stop solution (K) only.

Expected values:

Negative ≤1.1u/L.

Equivocal 1.1 – 1.5 u/L.

Positive >1.5 u/L.

IV-Radiological assay:

Ultrasound neck using high resolution linear transducer (7.5 MHz) Magic agile device (Kontron system) for assessment of thyroid gland. [233]

Proper patient positioning is critical to performing high quality ultrasound. The patient was made to lie flat and adequate neck extension was achieved by placing pillows under the shoulders. Coupling gel was then placed on the transducer to enhance image generation. The transducer was then moved over the patient's neck to obtain a series images of the thyroid gland and other neck structures, reports include measurement of thyroid gland size, architecture, blood flow on Doppler evaluation, presence of nodules, nodule size and characteristics and any other periglandular pathology like neck lymph nodes or parathyroid glands. In addition, evidence of compression or displacement of adjacent structures like trachea or internal jugular vein should be assessed.[234,235]

The size of the thyroid is calculated in milliliters as the sum of the volumes of both lobes (isthmus is neglected). The volume of one thyroid lobe is calculated as:

V(ml)=width x depth x length x 0.479(cm)

Normal thyroid volume in females is less than 18 ml and in males less than 22 ml. The typical TUS appearance of autoimmune (Hashimoto's) thyroiditis includes diffuse heterogeneity all over the gland, hypo echogenic pattern (as compared to the echogenicity of the neck muscles), increase in vascularization of the gland. [236-238]

Statistical analysis of the data: [239]

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. [240]

Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's exact test or Monte Carlo correction.

The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test, also Histogram and QQ plot were used for vision test. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed data, comparison between more than two populations were analyzed using F-test (ANOVA) and Post Hoc test (Scheffe). For abnormally distributed data, Kruskal Wallis test was used to compare between different groups and pair wise comparison was assessed using Mann-Whitney test.

Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

An informed consent was taken from each subject and local ethical committee approval.

RESULTS

The study was conducted on 90 subjects, classified into 3 groups:

- **Group I:** 35 patients with seropositive rheumatoid arthritis.
- **Group II:** 35 patients with seronegative rheumatoid arthritis.
- **Group III:** 20 age and sex matched controls.

I- Demographic data:

1- Age:

Group I: Their age ranged from 23 to 73 years with a mean of 46.23±11.90.

Group II: Their age ranged from 20 to 70 years with a mean of 44.97±13.01.

Group III: Their age ranged from 23 to 69 years with a mean of 46.20 ± 15.88.

❖ No statistical significant difference was observed between the three groups as regards the mean age. (Table 1)(figure 7)

Table (1): A table showing the age of the three studied groups

	Group I (n=35)	Group II (n=35)	Group III (n=20)	Test of Sig.	P
Age					
Min. – Max.	23.0 – 73.0	20.0 – 70.0	23.0 – 69.0	F=0.094	0.910
Mean ± SD.	46.23 ± 11.90	44.97 ± 13.01	46.20 ± 15.88		

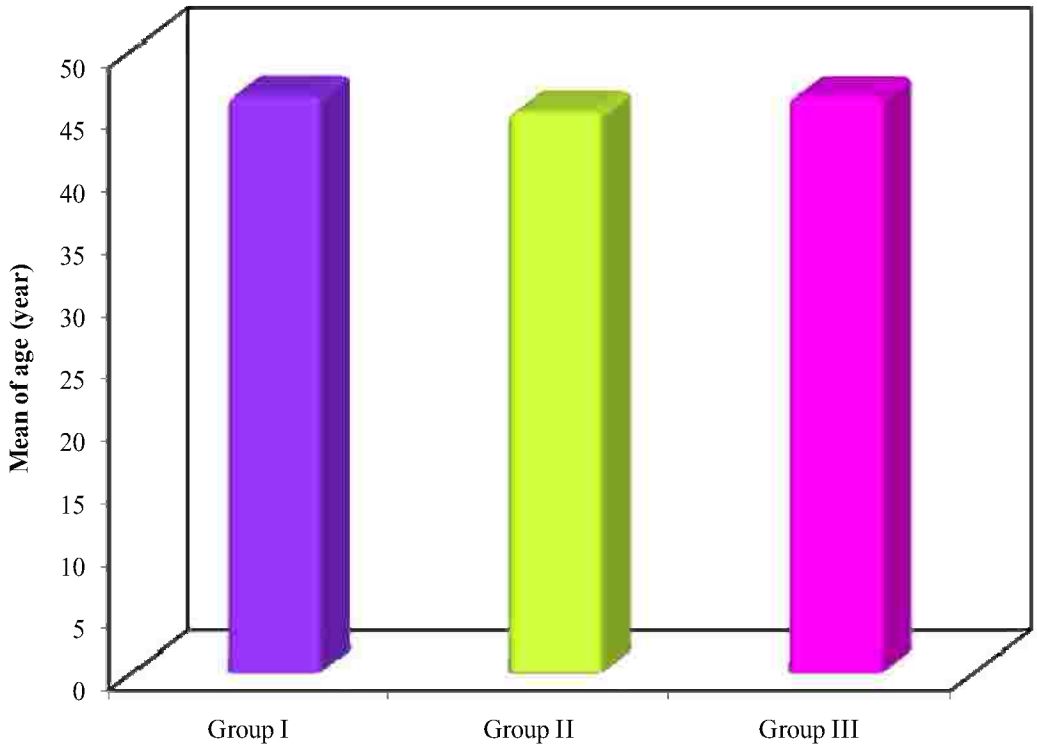


Figure (7): A figure comparing the age of the different studied groups.

2- Sex:

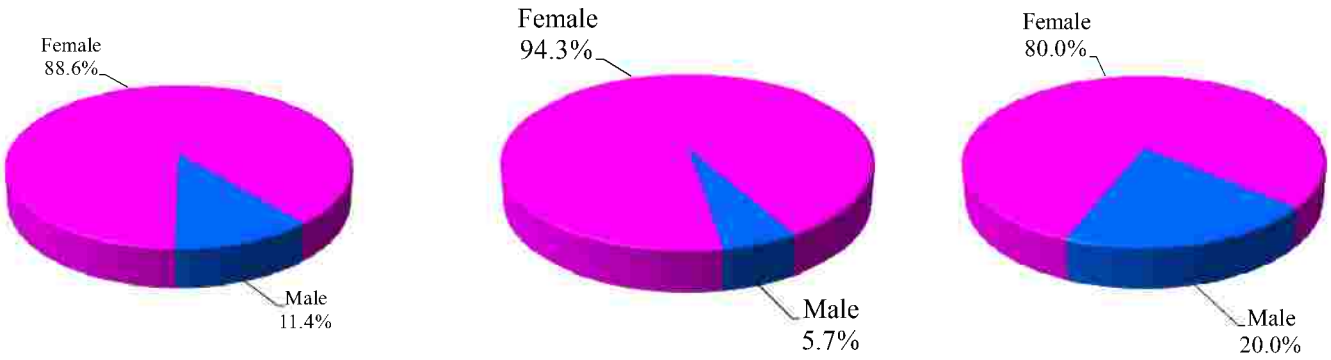
Group I: 88.6% were females while 11.4 %were males.

Group II: 94.3 % were females while 5.7% were males.

Group III: 80 % were females while 20 % were males. (Table 2) (Figure 8)

Table (2): A Table showing the percentage of males versus females among the three studied groups

	Group I (n=35)	Group II (n=35)	Group III (n=20)
Sex	**%**	**%**	**%**
Male	11.4	5.7	20.0
Female	88.6	94.3	80.0



Group I **Group II** **Group III**

Figure (8): A figure comparing the sex of the different studied groups.

II- Rheumatoid Arthritis (RA) duration:

Group I: RA duration ranged from 1 month to 30 years with a mean of 5.73±6.78.

Group II: RA duration ranged from 1 month to 12 years with a mean of 2.26±3.13.

❖ RA duration was significantly higher in group I VS group II. (Table 3) (Figure 9)

Table (3): A Table showing RA duration among the studied groups

	Group I (n=35)	Group II (n=35)	Test of Sig.	P
RA duration				
Min. – Max.	0.08 – 30.0	0.08 – 12.0	$^{KW}\chi^2$=5.604*	0.018*
Mean ± SD.	5.73 ± 6.78	2.62 ± 3.13		
Sig. bet. Grps	I-II*			

*significant

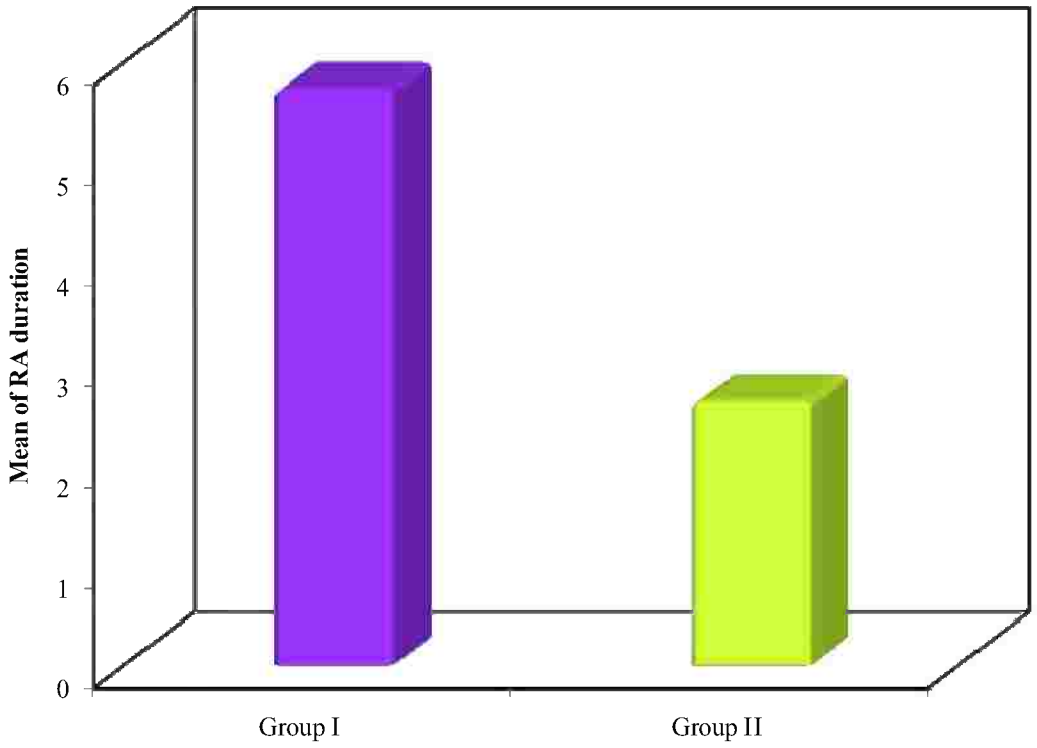


Figure (9): A figure comparing RA duration between the studied patients.

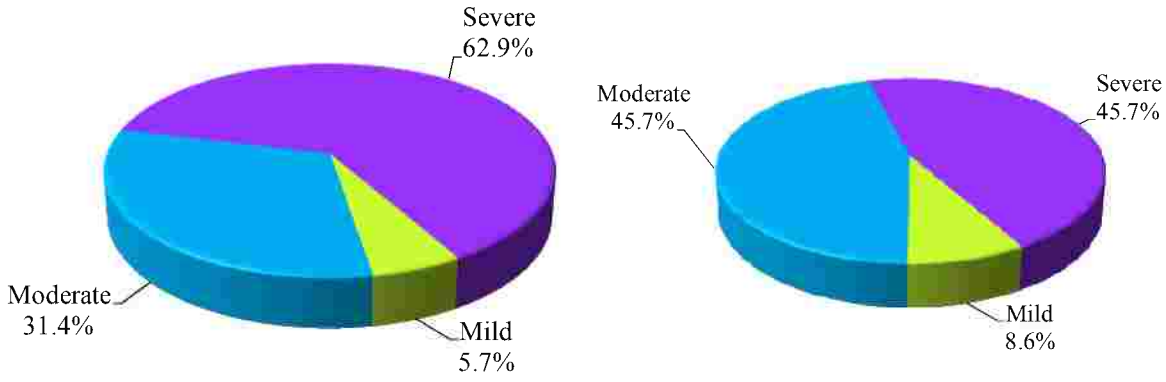
III- Disease activity score 28 (DAS 28):

Group I: According to the DAS 28 62.9% were severe, 31.4% were moderate while 5.7% were mild degree.

Group II: According to the DAS 28 45.7% were severe, 45.7% were moderate while 8.6% were mild degree. (Table 4) (Figure 10)

Table (4): A Table showing the DAS 28 among the studied groups

	Group I (n=35)		Group II (n=35)	
	No.	**%**	**No.**	**%**
DAS 28 score				
Mild	2	5.7	3	8.6
Moderate	11	31.4	16	45.7
Severe	22	62.9	16	45.7



Group I **Group II**

Figure (10): Comparison between the studied groups according to DAS 28 score**.**

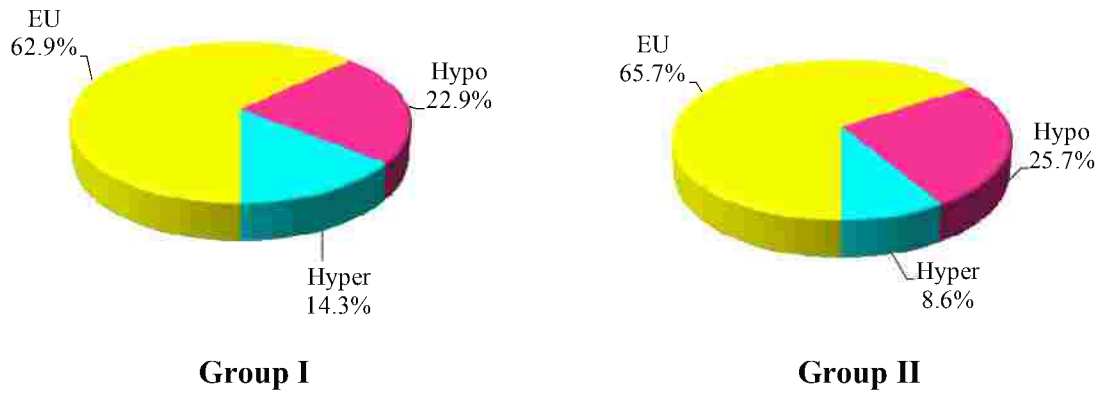
IV-Clinical picture of thyroid disorder:

Group I: 62.9 % of patients were clinically eu-thyroid, 22.9% were clinically hypothyroid while 14.3%were clinically hyperthyroid.

Group II: 65.7 % of patients were clinically eu-thyroid, 25.7 % of patients were clinically hypothyroid while 8.6 % of patients were clinically hyperthyroid. (Table 5)(Figure 11)

Table (5): A Table showing the clinical picture of thyroid disorders among the studied groups

	Group I (n=35)		Group II (n=35)	
	No.	**%**	**No.**	**%**
Clinically				
EU	22	62.9	23	65.7
Hypo	8	22.9	9	25.7
Hyper	5	14.3	3	8.6



Group I **Group II**

Figure (11): A figure comparing the clinical picture of thyroid disorders between the studied groups.

V-Laboratory:

A- Rheumatoid profile:

1) Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP):

It was found that ESR and CPR in group I ranged from 13 to 143 with a mean of 46.11±30.79 and 4 to 126 mg/L with a mean of 23.86±26.31 respectively, while in group II ESR and CRP ranged from 15 to 100 with a mean of 45.11±30.21and 1.02 to 176 mg/L with a mean of 26.46±41.6 respectively and in group III ESR and CRP ranged from 7 to 36 with a mean of 21.15±9.67 and 3 to 11mg/L with a mean of 5.19±2.08 respectively.

- A significant statistical difference was observed between the studied groups as regards ESR, where ESR is higher in group I than group III and it is higher in group II than group III while as regards CRP a significant statistical difference was observed between the studied groups where it was higher in group I than group III and higher in group II than group III. (Table 6) (figures 12,13)

Table (6): A Table showing ESR and CRP among the studied groups

	Group I (n=35)	Group II (n=35)	Group III (n=20)	$^{KW}\chi^2$	P
ESR					
Min. – Max.	13.0 – 143.0	15.0 – 100.0	7.0 – 36.0	12.606*	0.002*
Mean ± SD.	46.11 ± 30.79	45.11 ± 30.21	21.15 ± 9.67		
Sig. bet. Grps	I-III**,II-III**				
CRP					
Min. – Max.	4.0 – 126.0	1.02 – 176.0	3.0 – 11.0	26.480*	<0.001*
Mean ± SD.	23.86 ± 26.31	26.46 ± 41.60	5.19 ± 2.08		
Sig. bet. Grps	I-III***,II-III***				

$^{KW}\chi^2$: Chi square test for Kruskal Wallis
*significant

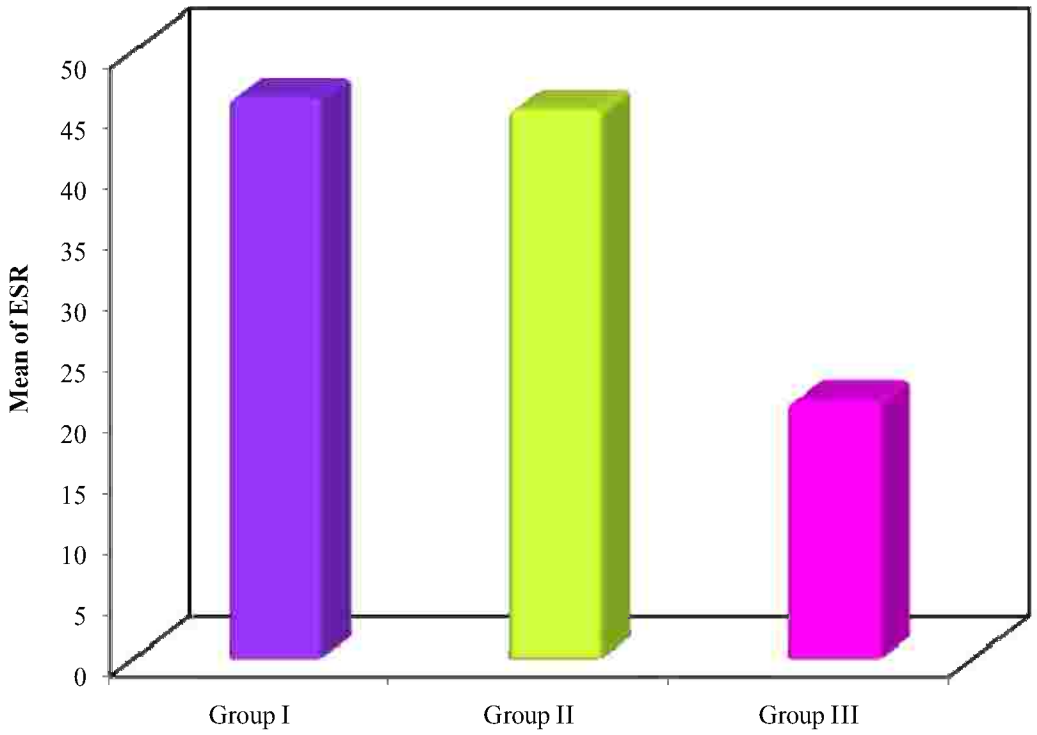


Figure (12): A figure comparing the ESR between the studied groups.

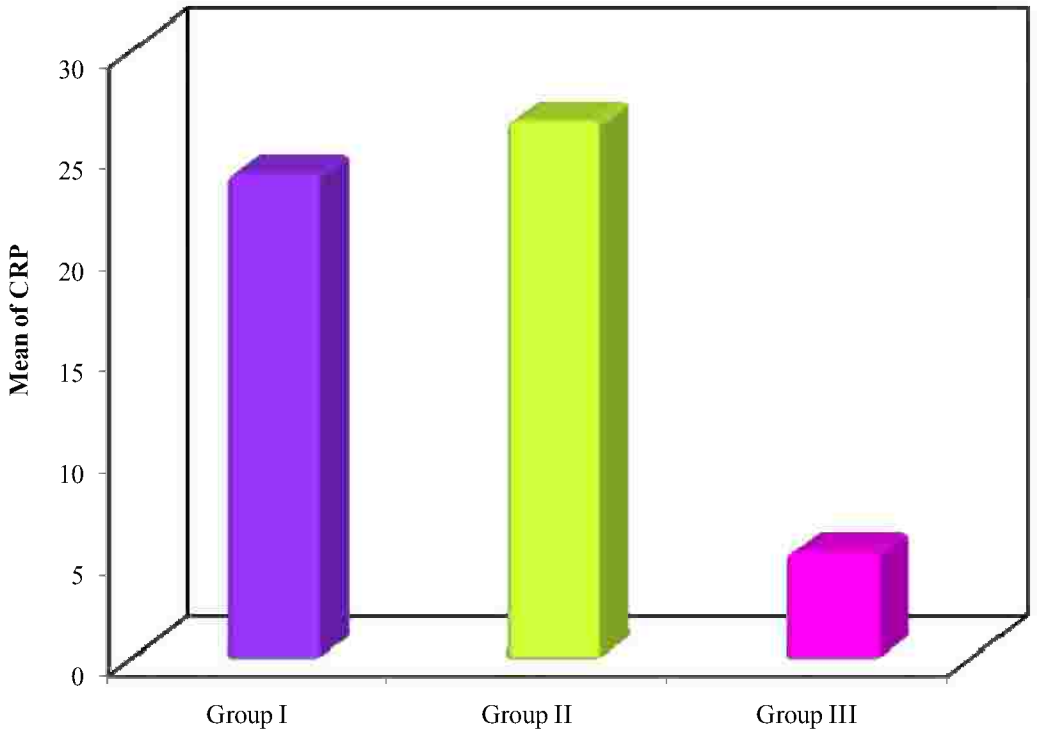


Figure (13): A figure comparing CRP between the studied groups.

2) Rheumatoid factor (RF) and Anti-Cyclic citrullinated peptide (Anti-CCP):

It was found that RF and Anti-CCP in group I ranged from 8 to 724 IU/ml with a mean of 112.49±181.77 and 13.5 to 531U/ml with a mean of 128.47±149.74 respectively, while in group II RF and Anti-CCP ranged from 2.7 to 14.6 with a mean of 8.29±3.49 and 0.7 to 22 U/ml with a mean of 9.27±6.08 respectively and in group III RF and Anti-CCP ranged from 3 to 16 IU/ml with a mean of 6.24±3.30 and 0.6 to 18 U/ml with a mean of 7.12±4.99 respectively.

- A significant statistical difference was observed among the studied groups regarding RF where it was higher in group I than group II and group III ,and it was higher in group II than group III, while as regards Anti-CCP a significant statistical difference was observed between the studied groups where it was higher in group I compared to group II and group III. (Table 7) (Figure 14,15)

Table (7): A Table showing mean serum RF and Anti-CCP among the studied groups

	Group I (n=35)	Group II (n=35)	Group III (n=20)	$^{KW}\chi^2$	P
RF(IU/ml)					
Min. – Max.	8.0 – 724.0	2.70 – 14.60	3.0 – 16.0	57.444*	<0.001*
Mean ± SD.	112.49 ± 181.77	8.29 ± 3.49	6.24 ± 3.30		
Sig.bet. groups	I-II***,I-III***,II-III*				
Anti-CCP (IU/ml)					
Min. – Max.	13.50 – 531.0	0.70 – 22.0	0.60 – 18.0	$^{KW}\chi^2$= 60.396***	<0.001** *
Mean ± SD.	128.47± 149.74	9.27 ± 6.08	7.12 ± 4.99		
Sig. bet. Groups	I-II***,I-III***				

$^{KW}\chi^2$: Chi square test for Kruskal Wallis test
*significant

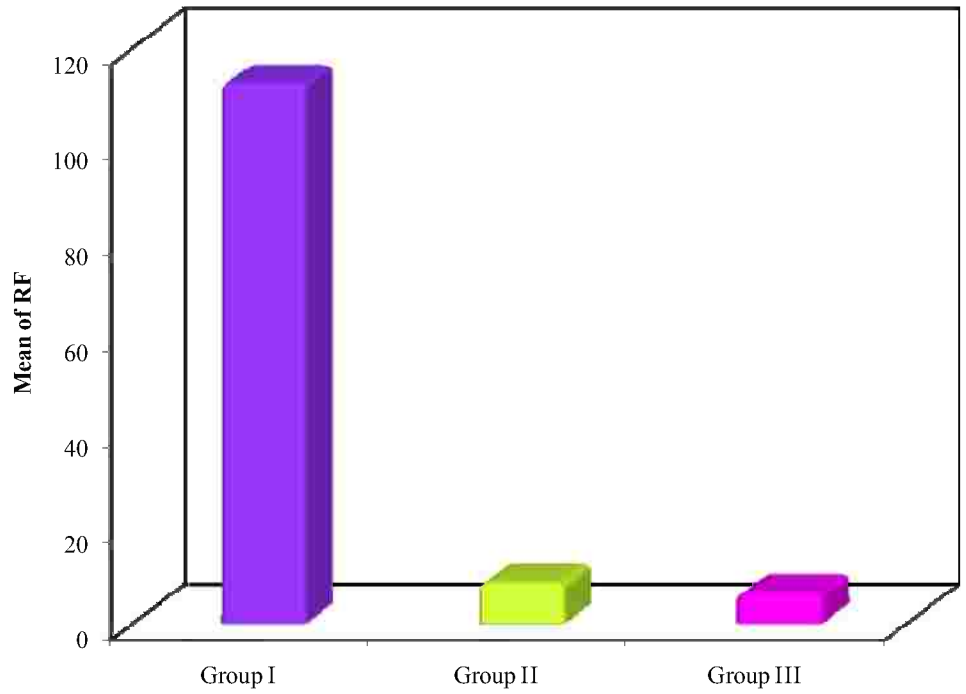


Figure (14): A figure comparing between mean RF among the studied groups.

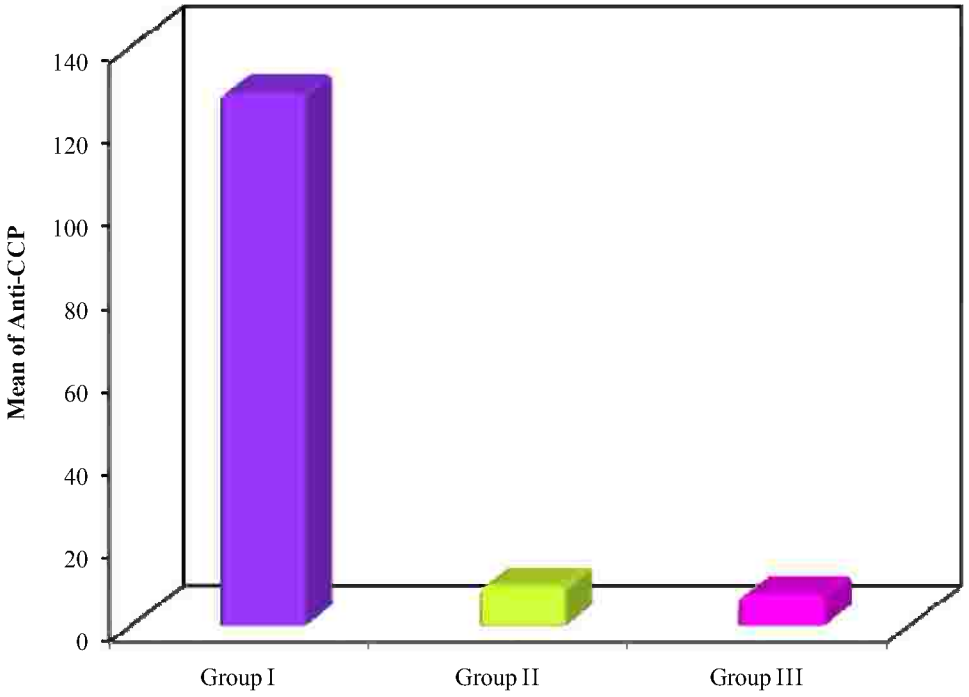


Figure (15): A figure comparing mean serum Anti CCP between the studied groups**.**

❖ A significant statistical difference was observed among the studied groups as regards % of RF and Anti-CCP positive patients (p value <0.001) where in group I 85.7% were positive for both RF and Anti-CCP and 14.3% were negative for both, and in group II 100% were negative for both RF and Anti-CCP while in group III 100% were negative for Anti-CCP and 95% were negative for RF.(Table 8)(Figure 16)

Table (8): Comparison between the three studied groups according to RF and anti CCP

	Group I (n=35)		Group II (n=35)		Group III (n=20)		χ^2	P
	No.	%	No.	%	No.	%		
RF (IU/ml)								
<15 Normal	5	14.3	35	100.0	19	95.0	66.813*	<0.001*
≥15 +ve	30	85.7	0	0.0	1	5.0		
Anti CCP (IU/ml)								
<25 Normal	5	14.3	35	100.0	20	100.0	70.714*	<0.001*
≥25 +ve	30	85.7	0	0.0	0	0.0		

χ^2: Chi square test
*: Statistically significant at $p \leq 0.05$

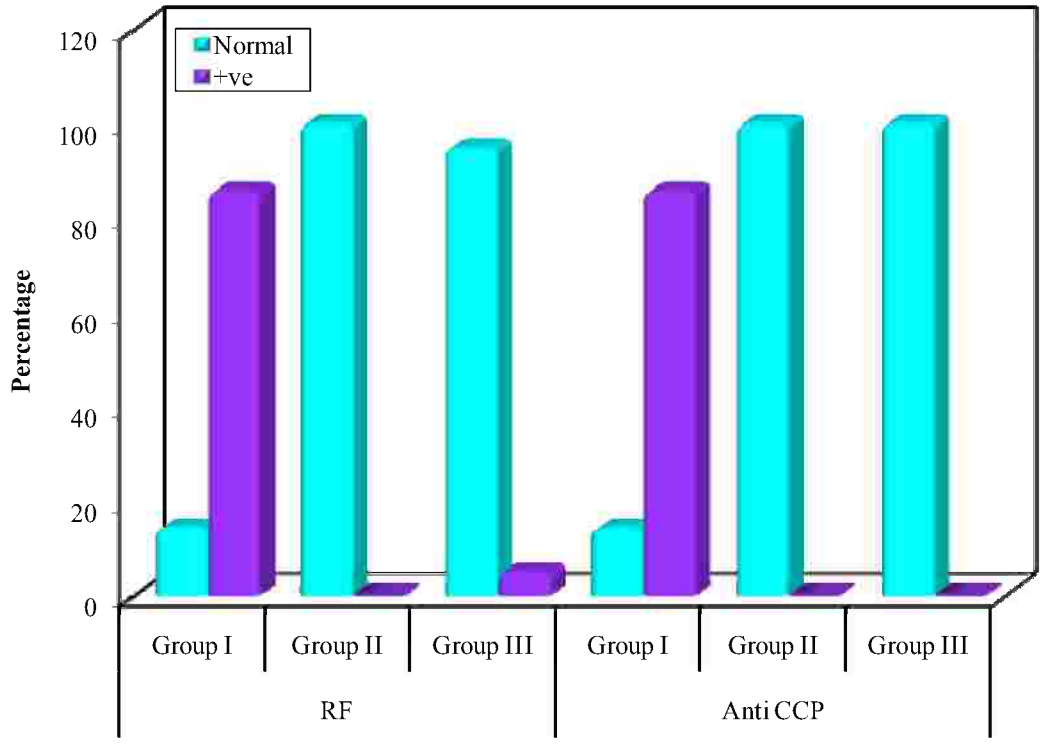


Figure (16): A figure showing comparison between the three studied groups according to RF and anti CCP.

B- Thyroid profile:

1) Thyroid function tests (TSH,T3 and T4):

It was found that in group I TSH,T3 and T4 ranged from 0.01 to 20 mIU/L with a mean of 3.38±5.12 and 0.8 to 2.4 ng/ml with a mean of 1.46±0.44 and 2.9 to 20.3 ug/dl with a mean of 9.32±2.95 respectively, while in group II TSH,T3 and T4 ranged from 0.01 to 20 mIU/L with a mean of 2.55±3.43 and 0.6 to 3.1 ng/ml with a mean of 1.31±0.47 and 3.1 to 12 ug/dl with a mean of 8.22±1.89 respectively and in group III TSH, T3 and T4 ranged from 0.6 to 5.7mIU/L with a mean of 1.89±1.11 and 0.9 to 1.9 ng/ml with a mean of 1.41±0.3 and 5.3 to 11.7 ug/dl with a mean of 8.3±1.64 respectively.

- There was no significant statistical difference observed among the studied groups as regards serum TSH, T3 and T4. (Table 9) (figure 17)

Table (9): A Table showing mean serum TSH, T3 and T4 among the studied groups

	Group I (n=35)	Group II (n=35)	Group III (n=20)	Test of Sig.	P
TSH(mIU/L)					
Min. – Max.	0.01 – 20.0	0.01 – 20.0	0.60 – 5.70		
Mean ± SD.	3.38 ± 5.12	2.55 ± 3.43	1.89 ± 1.11	$^{KW}\chi^2$=0.249	0.883
T3(ng/ml)					
Min. – Max.	0.80 – 2.40	0.60 – 3.10	0.90 – 1.90	F=1.179	0.312
Mean ± SD.	1.46 ± 0.44	1.31 ± 0.47	1.41 ± 0.30		
T4(ug/dl)					
Min. – Max.	2.90 – 20.30	3.10 – 12.0	5.30 – 11.70	F=2.289	0.107
Mean ± SD.	9.32 ± 2.95	8.22 ± 1.89	8.30 ± 1.64		

$^{KW}\chi^2$: Chi square test for Kruskal Wallis test
F: F test (ANOVA)
* significant

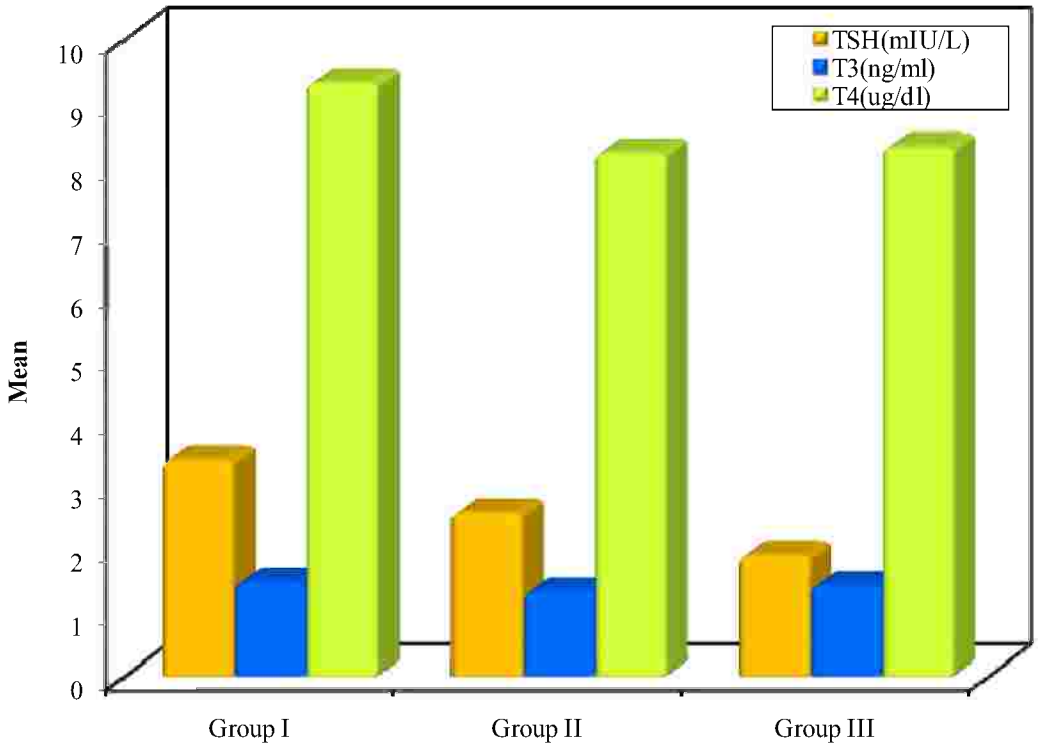


Figure (17): A figure showing mean serum TSH, T3 and T4 among the studied groups.

It was found that:

Group I: As regards TSH 86.6% had normal TSH,28.6% were hypothyroid and 2.9 % were hyperthyroid, and as regard T3 85.7 % had normal T3 while 14.3% had abnormal T3 ,while as regards T4 85.7% had normal T4 and only 14.3 % had abnormal T4.

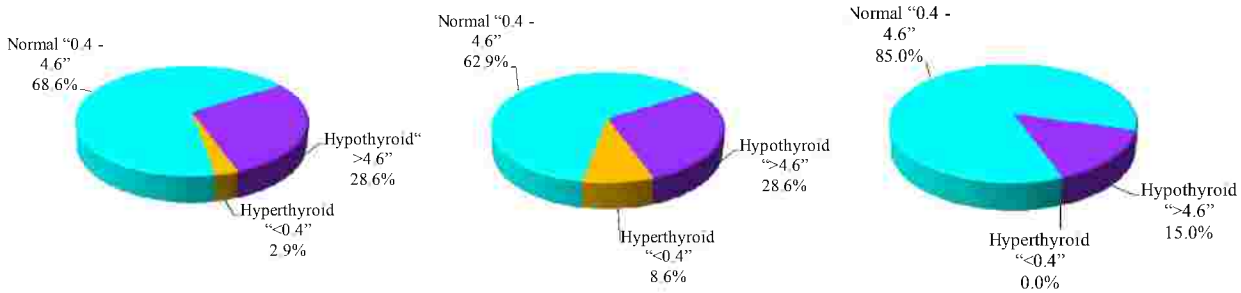
Group II: As regards TSH 62.9% had normal TSH, 28.6% were hypothyroid and 8.6% were hyperthyroid, and as regards T3 91.4% had normal T3 while 8.6% had abnormal T3, while as regards T4 94.3% had normal T4 and only 5.7% had abnormal T4.

Group III: As regards TSH 85% had normal TSH, 15% were hypothyroid, while as regards T3 and T4 100% were normal for both of them.(Table 10) (Figure 18,19,20).

Table (10): A Table showing the percentage of TSH, T3 and T4 among the studied groups

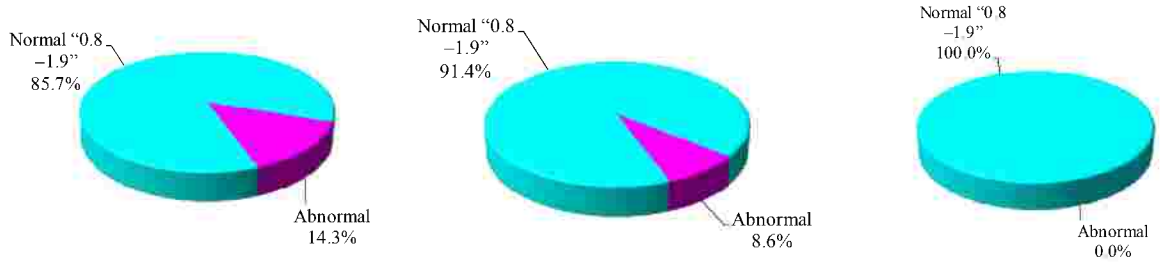
	Group I (n=35)		Group II (n=35)		Group III (n=20)	
	No	**%**	**No**	**%**	**No**	**%**
TSH						
Hyperthyroid "<0.4"	1	2.9	3	8.6	0	0.0
Normal "0.4-4.6"	24	68.6	22	62.9	17	85
Hypothyroid ">4.6"	10	28.6	10	28.6	3	15
T3 (ng/ml)						
Normal "0.8 – 1.9"	30	85.7	32	91.4	20	100
Abnormal	5	14.3	3	8.6	0	0.0
T4 (µg/dL)						
Normal "4.7 – 12.8"	30	85.7	33	94.3	20	100
Abnormal	5	14.3	2	5.7	0	0.0

χ^2: Value for Chi square test
MC: Monte Carlo test



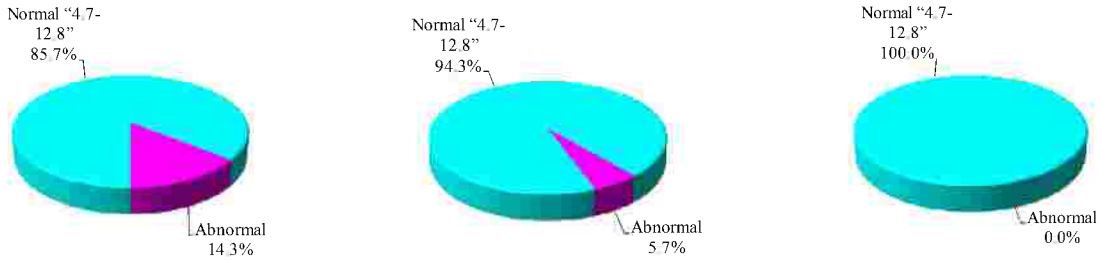
Group I **Group II** **Group III**

Figure (18): A figure showing the percentage of TSH among the studied groups.



Group I **Group II** **Group III**

Figure (19): A figure showing the percentage of T3 among the studied groups.



Group I **Group II** **Group III**

Figure (20): A figure showing the percentage of T4 among the studied groups.

2) Thyroid autoantibodies (Anti-TPO, Anti-TG and TRAbs):

It was found that in group I anti-TPO ,anti-TG and TRAbs ranged from 312.4 to 9221.9 IU/ml with a mean of 1301.9±1716 and 170.1 to 8164.8 IU/ml with a mean of 1750±1866.2 and 0.43 to 5.42u/L with a mean of 1.03±1.03 respectively, and in group II anti-TPO ,anti-TG and TRAbs ranged from 131.3 to 9596.3 IU/ml with a mean of 799.4±1597.7 and 10.1 to 5371.3 IU/ml with a mean of 898.1±988.11 and 0.24 to 1.77 u/L with a mean of 0.69±0.37 respectively while in group III anti-TPO ,anti-TG and TRAbs ranged from10.9 to 66.6 IU/ml with a mean of 30.26±12.96 and 10.7 to 401.7 IU/ml with a mean of 61.94±87.31 and 0.08 to 1.45 u/L with a mean of 0.56±0.38 respectively.

- A significant statistical difference was observed between the studied groups as regards Anti-TPO which was higher in group I than group II and III, and higher in group II than group III.
- A significant statistical difference was observed between the studied groups as regards Anti-TG where it was higher in group I than group II and III, and it was higher in group II than group III.
- A significant statistical difference was observed among the studied groups as regards TRAbs where it was higher in group I compared to group II ,and it was higher in group I compared to group III.(Table 11) (Figure 21,22)

Table (11): A Table showing the mean serum anti-TPO, anti-TG and TRAbs among the studied groups

	Group I (n=35)	Group II (n=35)	Group III (n=20)	Test of Sig.	P
Anti-TPO (IU/ml)					
Min. – Max.	312.4 – 9221.9	131.3 – 9596.3	10.90 – 66.60	$^{KW}\chi^2$= 55.384***	<0.001***
Mean ± SD.	1301.9±1716.0	799.4±1597.7	30.26 ± 12.96		
Sig. bet. Grps	I-II***, I-III***, II-III***				
Anti-TG (IU/ml)					
Min. – Max.	170.1 – 8164.8	10.10 – 5371.3	10.70 – 401.7	$^{KW}\chi^2$= 47.500***	<0.001***
Mean ± SD.	1750.0±1866.2	898.1± 988.11	61.94 ± 87.31		
Sig. bet. Grps	I-II**, I-III***, II-III***				
TRAbs (U/L)					
Min. – Max.	0.43 – 5.42	0.24 – 1.77	0.08 – 1.45	$^{KW}\chi^2$= 10.221**	0.006**
Mean ± SD.	1.03 ± 1.03	0.69 ± 0.37	0.56 ± 0.38		
Sig. bet. Grps	I-II*,I-III**				

$^{KW}\chi^2$: Chi square test for Kruskal Wallis test
* significant

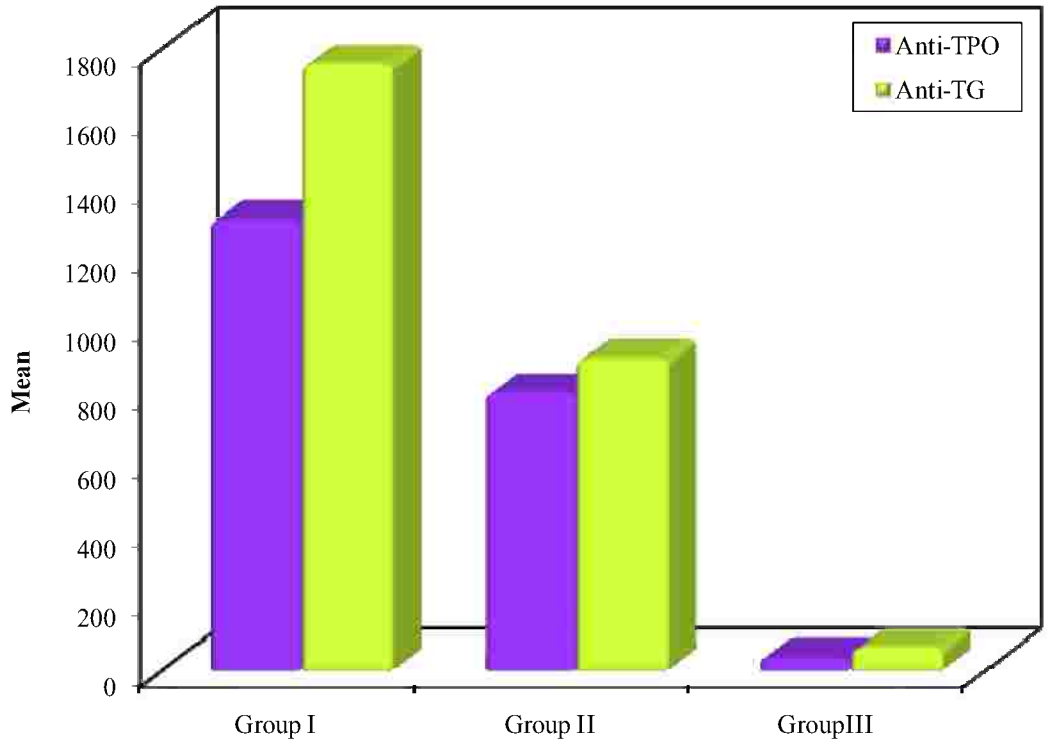


Figure (21): A figure showing the mean serum anti-TPO, anti-TG among the studied groups.

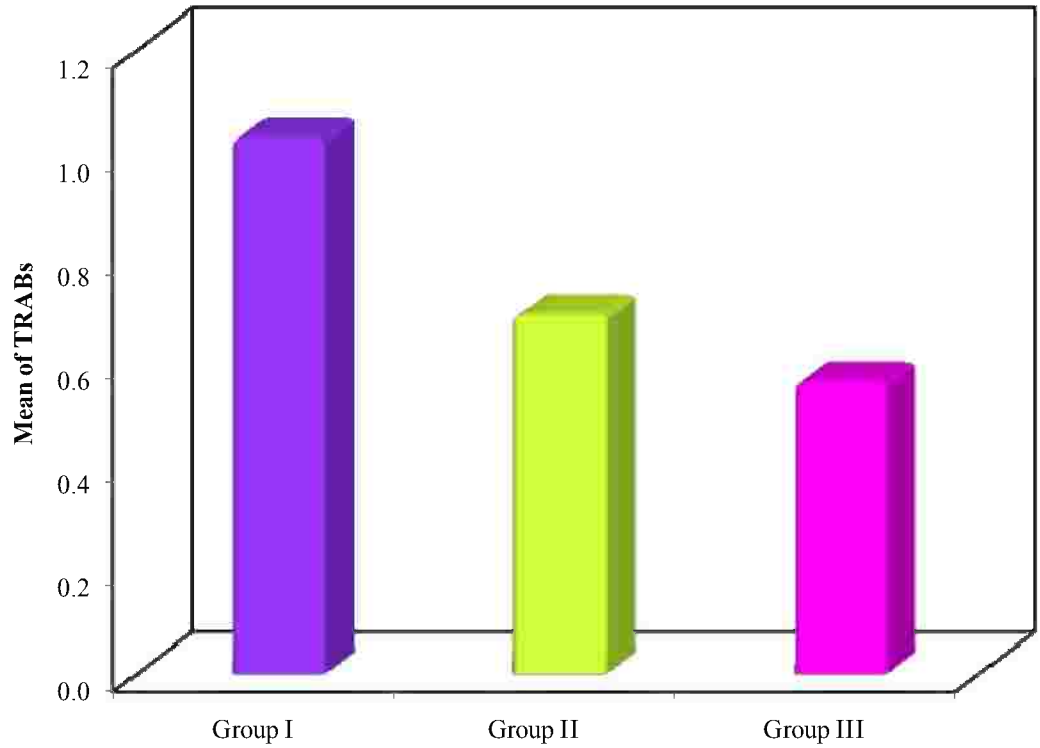


Figure (22): A figure showing the mean TRAbs among the studied groups.

It was found that:

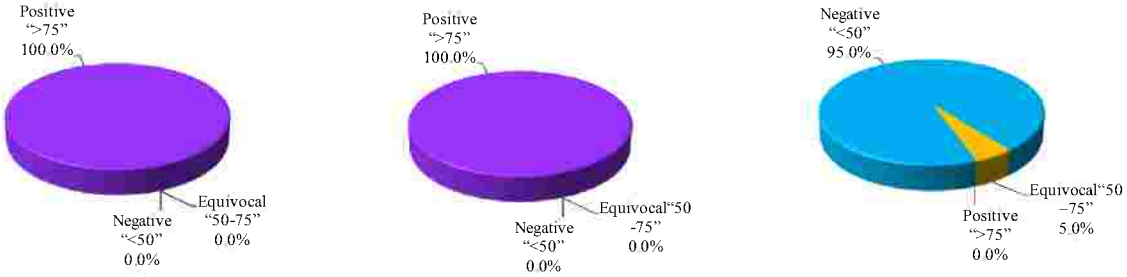
Group I: 100 % were positive for anti-TPO and anti-TG while as regards TRAbs, 91.4% were negative, 5.7% were positive and 2.9% were equivocal.

Group II: 100 % were positive for anti-TPO, as regards anti-TG 82.9% were positive, 8.6% were equivocal and 8.6% were negative, while as regards TRAbs, 80% were negative, 14.3% were equivocal and 5.7% were negative.

Group III:95% were negative for anti-TPO and 5%were equivocal, as regards anti-TG, 85% were negative,10% equivocal and 5% positive while as regards TRAbs ,95% were negative,5% were equivocal.(Table 12) (Figure 23,24,25)

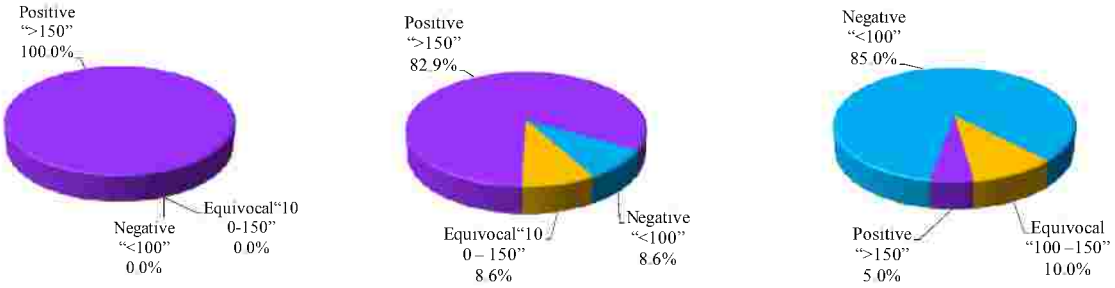
Table (12): A Table showing the percentage of anti-TPO, anti-TG and TRAbs among the studied groups

	Group I (n=35)	Group II (n=35)	Group III (n=20)
	%	%	%
Anti-TPO (IU/ml)			
Negative "<50"	0.0	0.0	95.0
Equivocal "50 – 75"	0.0	0.0	5.0
Positive ">75"	100.0	100.0	0.0
Anti-TG (IU/ml)			
Negative "<100"	0.0	8.6	85.0
Equivocal "100 – 150"	0.0	8.6	10.0
Positive ">150"	100.0	82.9	5.0
TRAbs			
Negative "<1.1"	91.4	80.0	95.0
Equivocal "1.1-1.5"	2.9	14.3	5.0
Positive ">1.5"	5.7	5.7	0.0



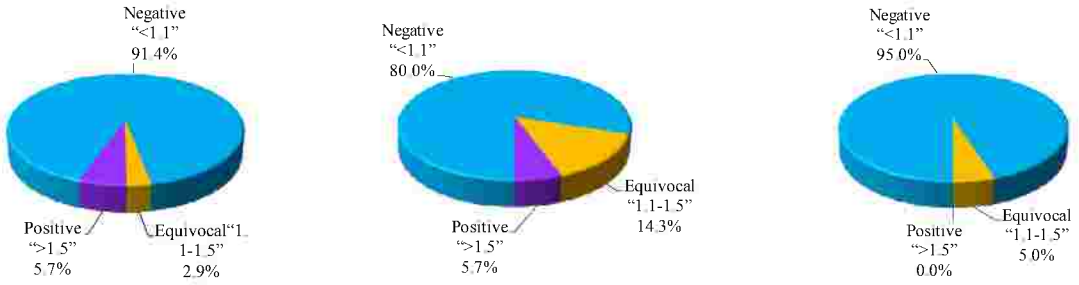
Group I **Group II** **Group III**

Figure (23): A figure showing the percentage of anti-TPO among the studied groups.



Group I **Group II** **Group III**

Figure (24): A figure showing the percentage of anti-TG among the studied groups.



Group I **Group II** **Group III**

Figure (25): A figure showing the percentage of TRAbs among the studied groups.

VI-Ultrasound findings of thyroiditis:

Group I: Evidence of thyroiditis was found in 34.3 % of this group while 65.7 % had no thyroiditis on ultrasonography.

Group II: Evidence of thyroiditis was found in 51.4 % of this group while 48.6 % had no thyroiditis on ultrasonography.

Group III: 100 % of this group had no thyroiditis on ultrasonography.

- A significant statistical difference was observed between the studied groups as regards ultrasound evidence of thyroiditis(between group I and group III and between group II and group III) while there was no significant statistical difference between group I and group II .(Table 13) (Figure 26)

Table (13): A Table showing thyroiditis on ultrasound among the studied groups

	Group I (n=35)		Group II (n=35)		Group III (n=20)		χ^2	P
	No.	%	No.	%	No.	%		
Thyroiditis								
No	23	65.7	17	48.6	20	100.0	15.171**	0.001**
Yes	12	34.3	18	51.4	0	0.0		
Sig. bet. Grps	I-III**, II-III***							

*significant

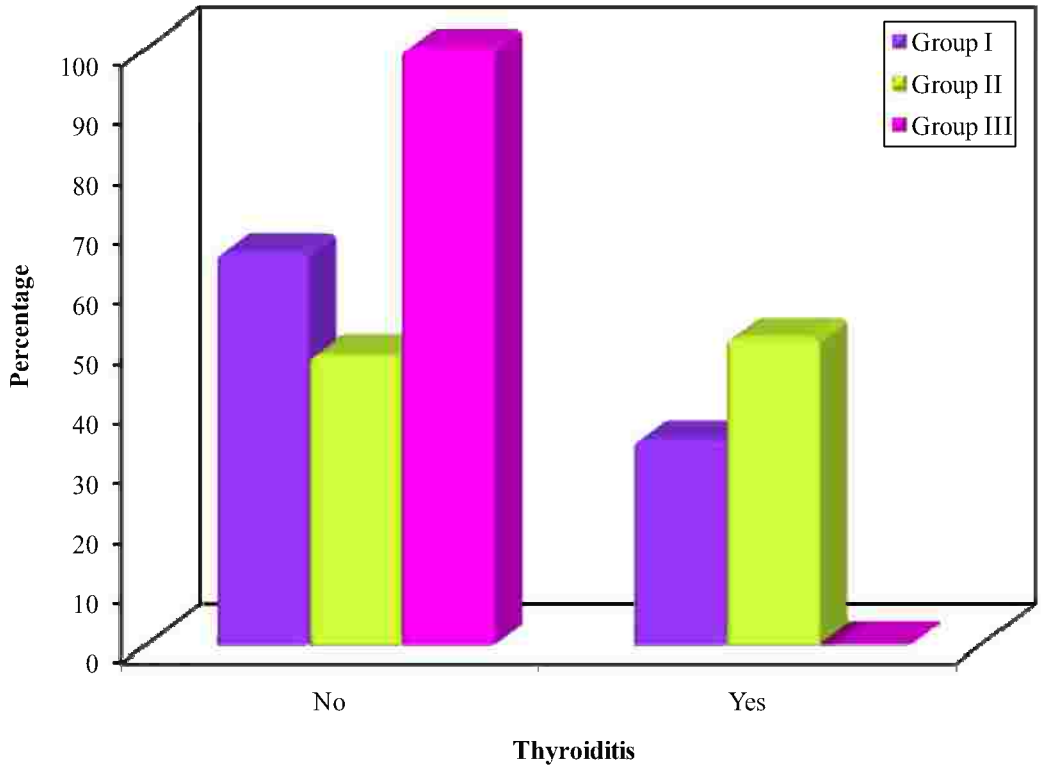
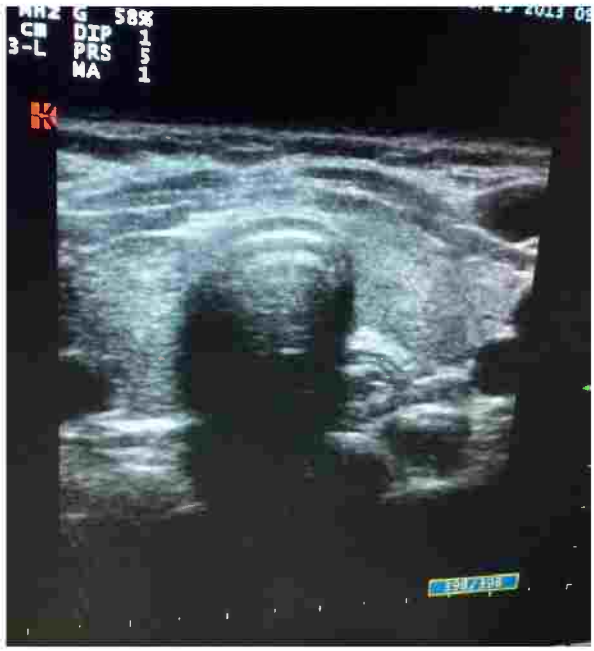
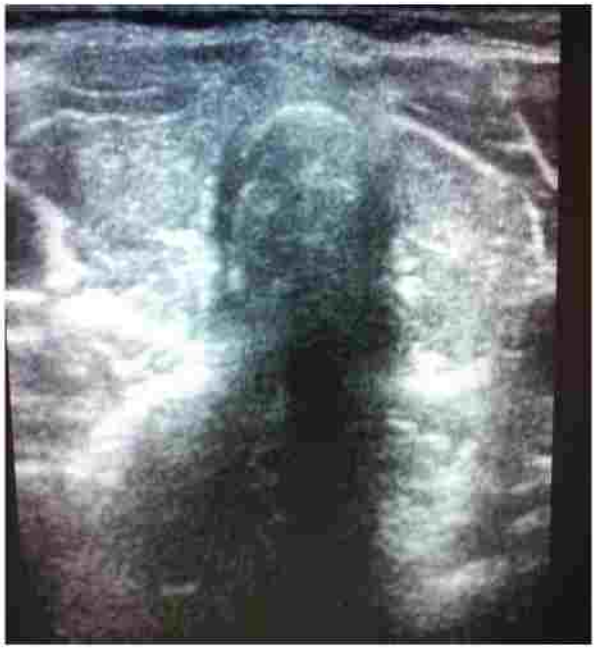


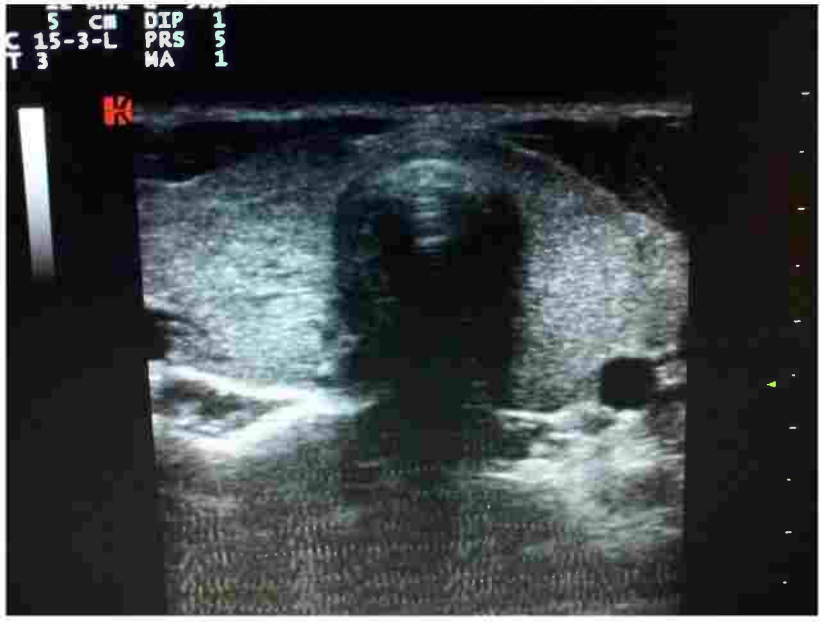
Figure (26): A figure comparing ultrasound finding of evidence of thyroiditis among the studied groups.



(A)



(B)



(C)

Figure (27): **A**:ultrasound picture of a 35 year old female patient showing diffuse heterogeneity of the thyroid gland , Rt lobe volume 6.1,lt lobe volume 6.5,no definite nodules…picture of thyroiditis (patient no. 18 of group I). **B**: ultrasound picture of thyroid gland of a 42 year old female patient showing diffuse heterogeneity of the gland…picture of thyroiditis (patient no.39 of group II). **C**: Ultrasound picture of normal thyroid gland regarding size and echogenicity of 24 year old female patient (patient no.84 of group III)

❖ Correlation between DAS 28 and clinical picture of thyroid disorder:

Group I: It was found that 100 % of patients with mild DAS were clinically euthyroid, and 63.6%, 27.3% and 9.1% of patients with moderate score were clinicaly euthyroid, hyperthyroid and hypothyroid respectively, while 59.1%, 31.8% and 9.1% of patients with severe degree were clinically euthyroid, hypothyroid and hyperthyroid respectively.

Group II: It was found that 33.3% of patients with mild DAS 28 were clinically euthyroid, 33.3% were hypothyroid and 33.3% were hyperthyroid while as regards patients with moderate score 68.8%, 25% and 6.2% were clinically euthyroid, hypothyroid and hyperthyroid respectively and in patients with severe degree 68.8%, 25% and 6.2% were clinically euthyroid, hypothyroid and hyperthyroid respectively.(Table 14) (Figure 28)

Table (14): A table showing relation between DAS- 28 score with clinical picture of thyroid disorder among the studied groups

Clinically	DAS-28 Score						χ^2	^{MC}p
	Mild		Moderate		Severe			
	No.	%	No.	%	No.	%		
Group I	**n = 2**		**n = 11**		**n = 22**			
Eu	2	100.0	7	63.6	13	59.1		
Hypo	0	0.0	1	9.1	7	31.8	3.941	0.403
Hyper	0	0.0	3	27.3	2	9.1		
Group II	**n = 3**		**n = 16**		**n = 16**			
Eu	1	33.3	11	68.8	11	68.8		
Hypo	1	33.3	4	25.0	4	25.0	3.263	0.600
Hyper	1	33.3	1	6.2	1	6.2		

χ^2: value for Chi square
MC: Monte Carlo test

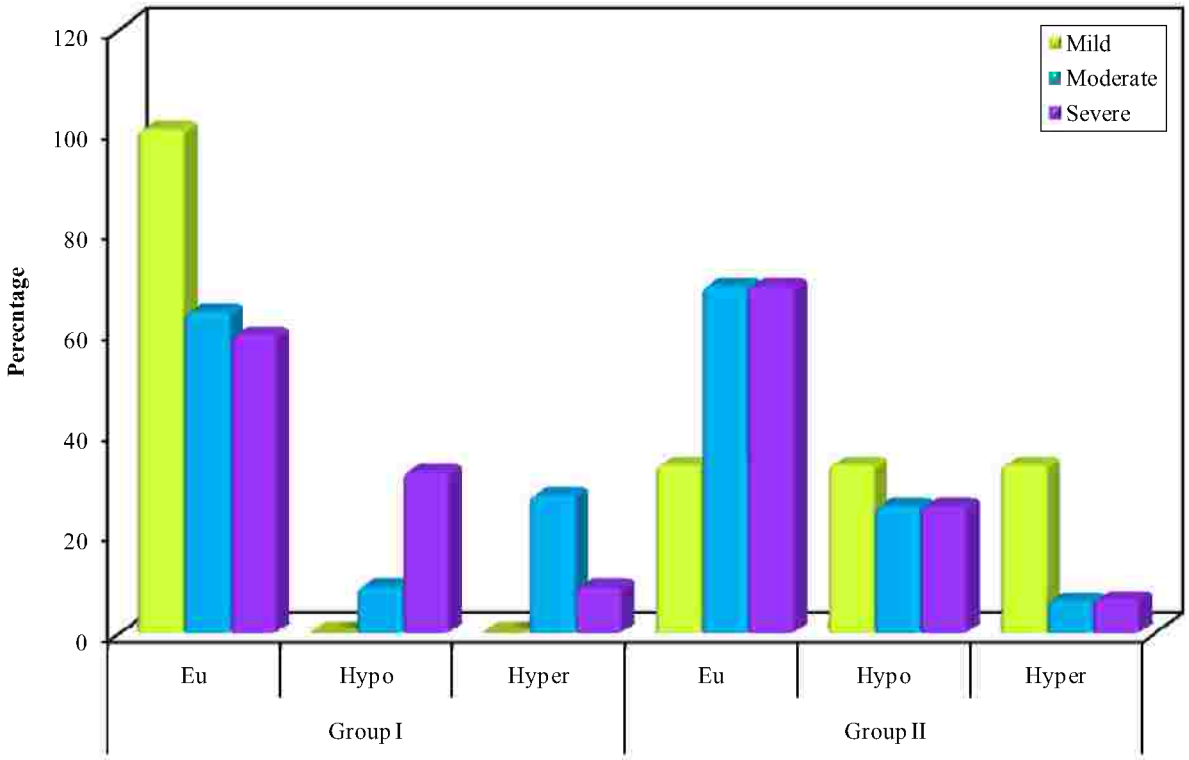


Figure (28): Relation between DAS- 28 score with clinical picture of thyroid disorder among the studied groups.

❖ Correlation between DAS 28 and serum TSH level:

Group I: It was found that 50% of patients with mild DAS had normal TSH and 50% were hypothyroid while in patients with moderate degree 72.7%, 18.2% and 9.1% had normal, high and low TSH level respectively, and in patients with severe degree 68.2% and 31.8% had normal and high level of serum TSH respectively.

Group II: It was found that 66.7% of patients with mild DAS had high TSH and 33.3% had low TSH, while in patients with moderate degree 81.3% and 18.8% had normal and high TSH levels respectively ,and as regards patients with severe degree 56.3%,31.3% and 12.5% had normal, high and low TSH respectively.(Table 15) (Figure 29)

Table (15): A Table showing the relation between DAS 28 and serum TSH level among the studied groups

TSH	DAS-28 Score						χ^2	^{MC}p
	Mild		Moderate		Severe			
	No.	%	No.	%	No.	%		
Group I	**n = 2**		**n = 11**		**n = 22**			
<0.4 hyperthyroid	0	0.0	1	9.1	0	0.0		
0.4-4.6 normal	1	50.0	8	72.7	15	68.2	4.371	0.382
>4.6 hypothyroid	1	50.0	2	18.2	7	31.8		
Group II	**n = 3**		**n = 16**		**n = 16**			
<0.4 hyperthyroid	1	33.3	0	0.0	2	12.5		
0.4-4.6 normal	0	0.0	13	81.3	9	56.3	8.725	0.069
>4.6 hypothyroid	2	66.7	3	18.8	5	31.3		

χ^2: value for Chi square
MC: Monte Carlo test
*: Statistically significant at $p \leq 0.05$

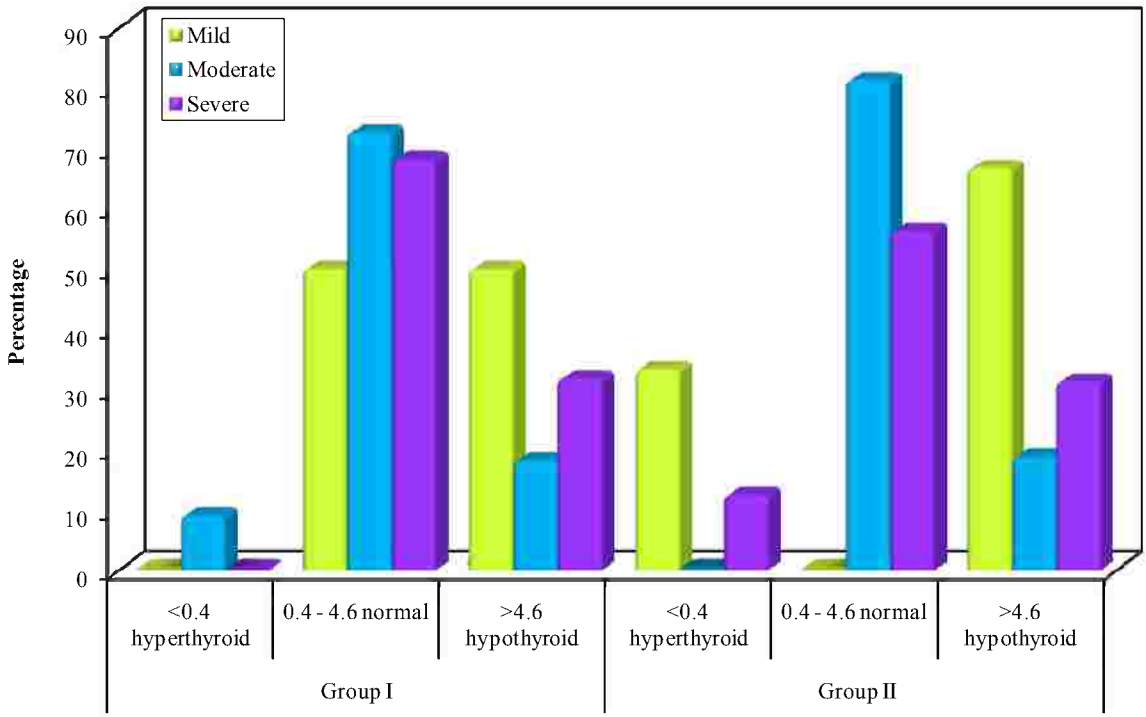


Figure (29): A Figure showing the relation between DAS 28 and serum TSH level among the studied groups.

❖ Correlation between DAS score and mean serum TSH:

There was found no statistical significant correlation between DAS score and mean serum TSH among the studied groups (p=0.83 and 0.69 respectively). (Table 16)

Table (16): Correlation between DAS score with mean serum TSH

		DAS score	
		Group I	**Group II**
TSH	r_s	0.037	-0.070
	P	0.835	0.691

r_s: Spearman coefficient

❖ Correlation between DAS score and thyroid autoantibodies(anti-TPO, anti-TG and TRAbs):

No statistical significant correlation was observed among the studied groups as regards DAS score with the level of thyroid antibodies. (Table17)

Table (17): Correlation between DAS score with Anti-TPO, Anti-TG and TRAbs

		DAS score	
		Group I	**Group II**
Anti TPO	r_s	-0.068	0.087
	P	0.700	0.619
Anti TG	r_s	-0.101	0.003
	P	0.566	0.989
TRAbs	r_s	-0.218	-0.252
	P	0.207	0.144

r_s: Spearman coefficient

❖ Relation between thyroid auto antibodies and overt thyroid dysfunction:

It was found that in group I 100% of patients who were clinically euthyroid were anti-TPO positive and 100% were anti-TG positive while only 9.1% of them were TRAbs positive while those with hyperthyroidism or hypothyroidism , 100% of them were anti-TPO and 100% anti-TG positive and no one was positive for TRAbs .

As regards group II 100% of the euthyroid patients were positive for anti-TPO,82.6% were positive for anti-TG and no one was positive for TRAbs, while patients with hyper or hypothyroidism 100% of them were anti-TPO positive,83.3%were anti-TG positive and only 16.7% were positive for TRAbs.(Table 18)(Figure 30)

Table (18): A Table showing the relation between thyroid auto antibodies and overt thyroid dysfunction

	Group I				Group II			
	EU (n=22)		Hypo+ Hyper (n=13)		EU (n=23)		Hypo+ Hyper (n=12)	
	No.	%	No.	%	No.	%	No.	%
Anti TPO	22	100.0	13	100.0	23	100.0	12	100.0
Anti TG	22	100.0	13	100.0	19	82.6	10	83.3
TRAbs	2	9.1	0	0.0	0	0.0	2	16.7

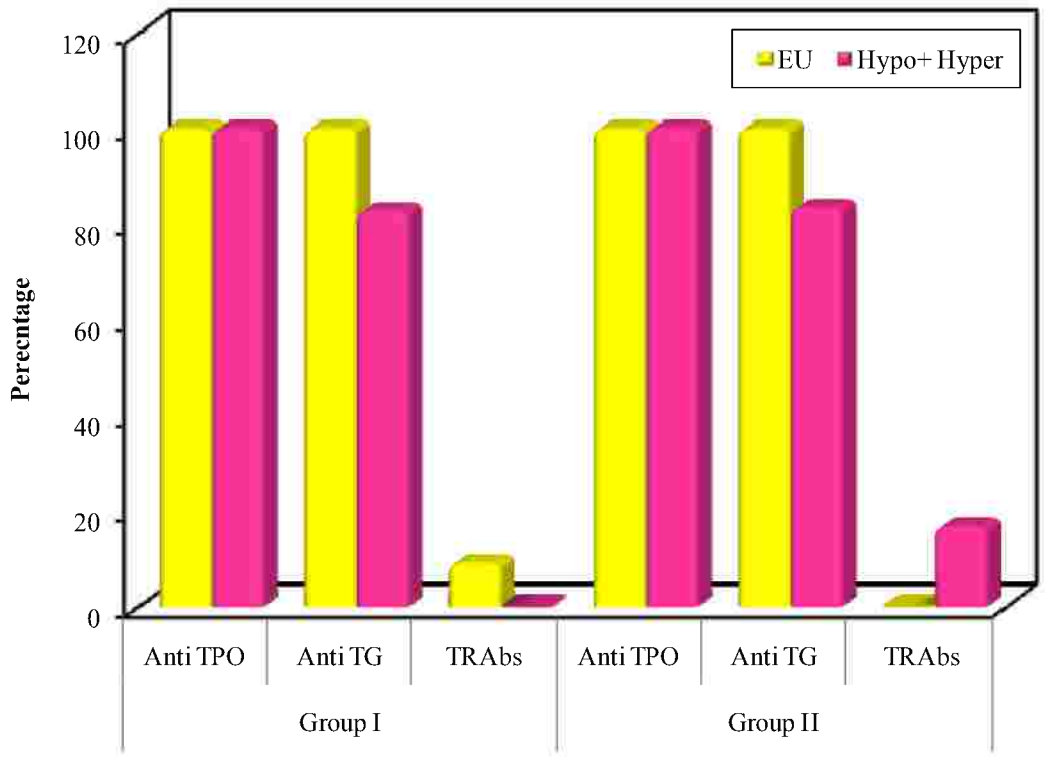


Figure (30): A figure showing the relation between thyroid autoantibodies and overt thyroid dysfunction.

❖ Relation between thyroid auto antibodies and RF and Anti-CCP:

It was found that among the RF positive patients, 100% were anti-TPO positive,100% were anti-TG positive and 3.3% were TRAbs positive, while among the Anti-CCP positive patients,100% were anti-TPO positive,100% were anti-TG positive and 6.7% were TRAbs positive.(Table 19)(Figure 31)

Table (19): Relation between thyroid autoantibodies and RF and anti-CCP

	RF+ve				Anti CCP+ve			
	Group I (n=30)		Group III (n=1)		Group I (n= 30)		Group III (n=0)	
	No.	%	No.	%	No.	%	No.	%
Anti TPO								
Negative	0	0.0	1	100.0	0	0.0	0	0.0
Positive	30	100.0	0	0.0	30	100.0	0	0.0
Anti TG								
Negative	0	0.0	1	100.0	0	0.0	0	0.0
Positive	30	100.0	0	0.0	30	100.0	0	0.0
TRAbs								
Negative	28	93.3	1	100.0	27	90.0	0	0.0
Positive	1	3.3	0	0.0	2	6.7	0	0.0

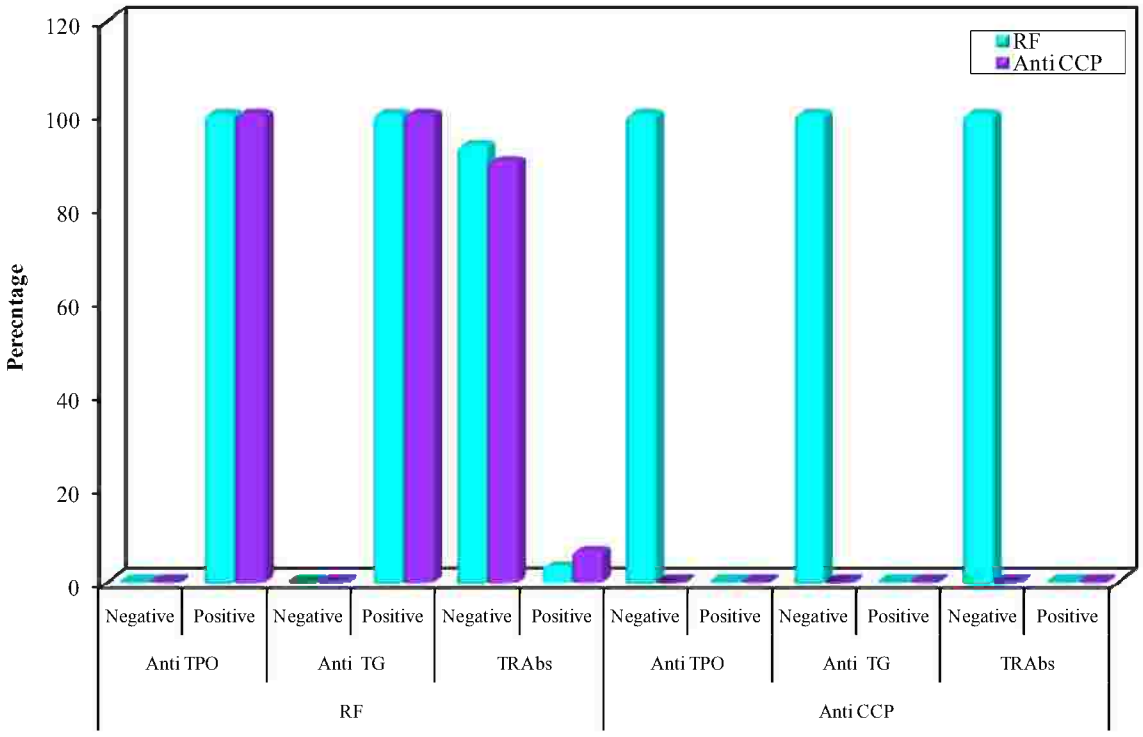


Figure (31): A figure showing the relation between thyroid autoantibodies and RF and anti-CCP.

❖ Correlation between RF and thyroid autoantibodies:

There was a significant statistical positive correlation between RF and Anti-TPO among the total patients (seropositive and seronegative) with rheumatoid arthritis (r=0.318) (p=0.007), and between RF and TRAbs among the total patients(r=0.300)(p=0.021) while there was no statistical significant correlation between RF and Anti-TG(r=0.234)(p=0.052) (Table 20) (figure 32,33).

Table (20): Correlation between mean RF with Anti-TPO, Anti-TG and TRAbs

		Rh Factor
		Total patients
Anti-TPO	r_s	0.318
	P	0.007*
Anti-TG	r_s	0.234
	P	0.052
TRAbs	r_s	0.300
	P	0.012*

r_s: Spearman coefficient
*significant

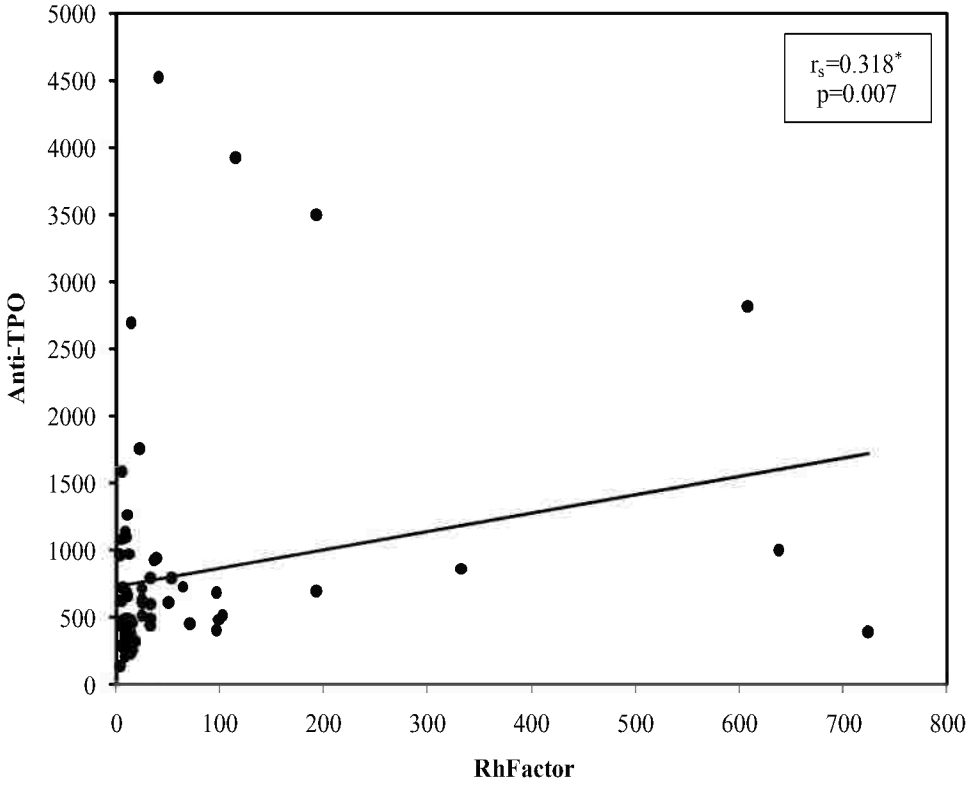


Figure (32): A figure showing the correlation between RF and Anti-TPO.

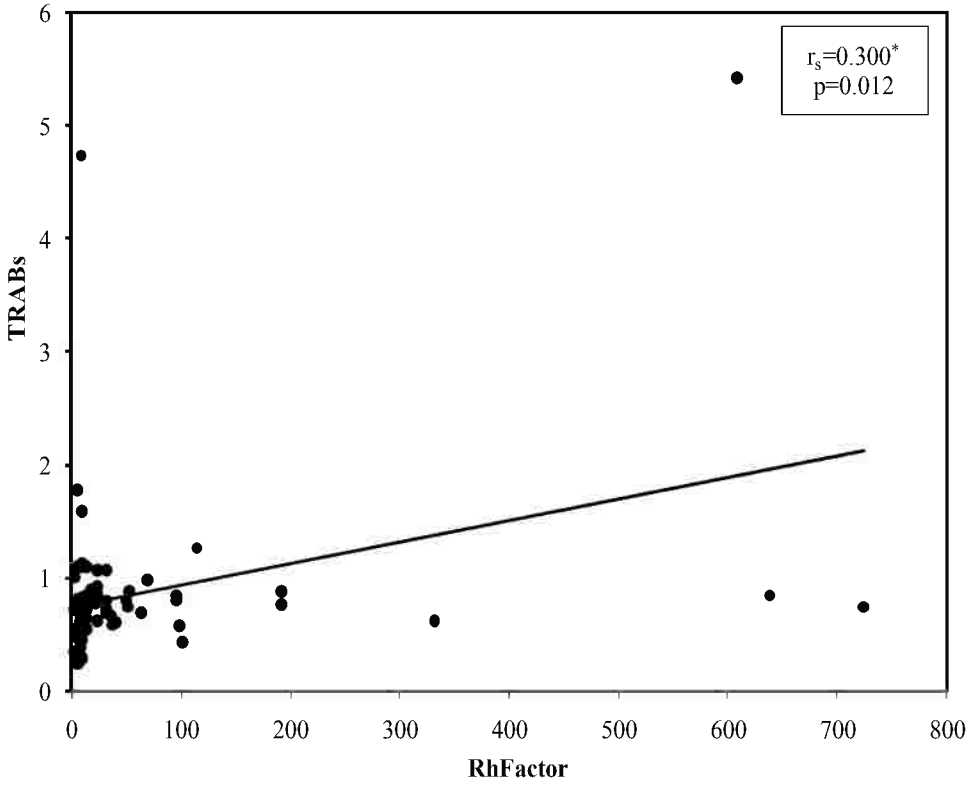


Figure (33): A figure showing the correlation between RF and TRAbs.

❖ Correlation between Anti-CCP and thyroid autoantibodies:

There was a significant statistical positive correlation between Anti-CCP and Anti-TPO among the total patients (seropositive and seronegative) with rheumatoid arthritis (r=0.336) (p=0.004), and between Anti-CCP and TRAbs among the total patients (r=0.252) (p=0.035) while there was no statistical significant correlation between Anti-CCP and Anti-TG (r=0.204) (p=0.90) (Table 21) (figure 34,35)

Table (21): Correlation between Anti-CCP with Anti-TPO, Anti-TG and TRAbs

		Anti-CCP
		Total patients
Anti TPO	r_s	0.336
	P	0.004*
Anti TG	r_s	0.204
	P	0.90
TRAbs	r_s	0.252
	P	0.035*

r_s: Spearman coefficient
*significant

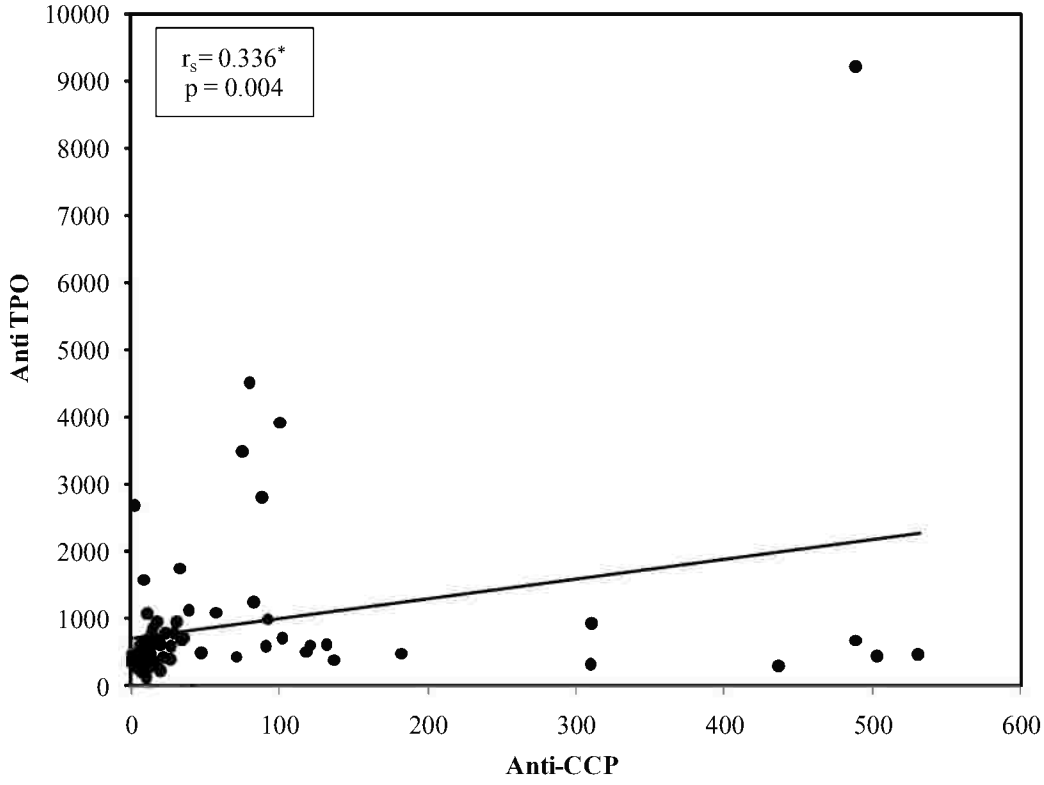


Figure (34): A figure showing correlation between Anti-CCP and Anti-TPO.

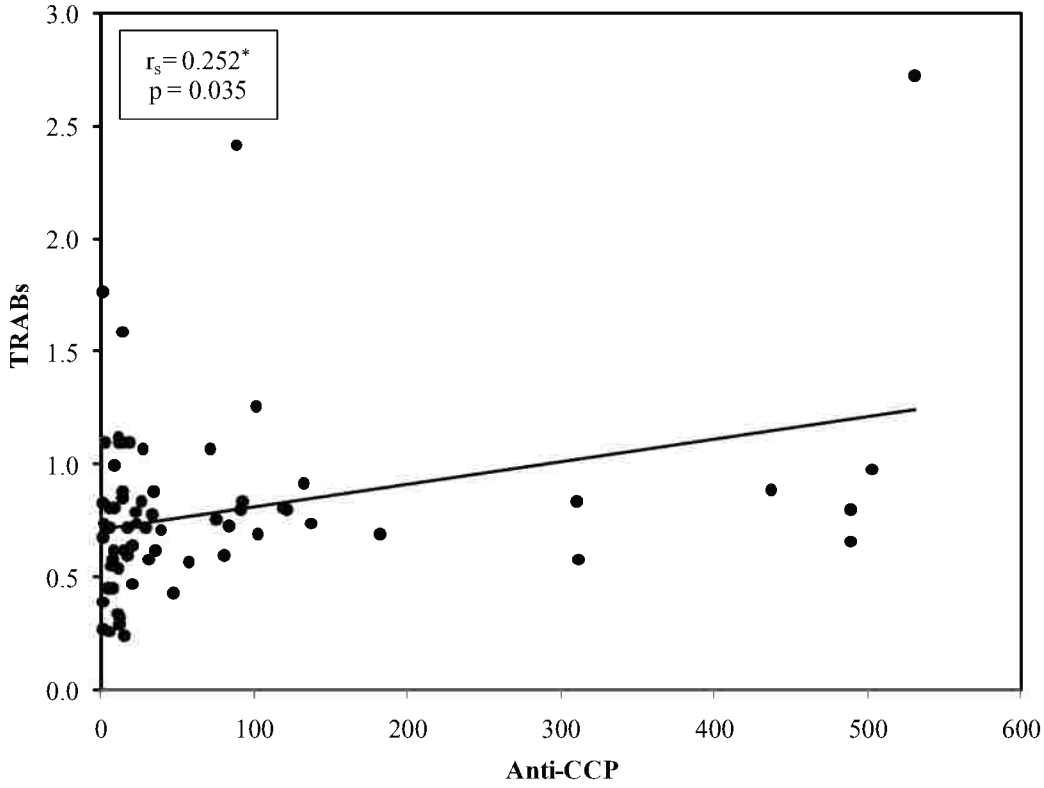


Figure (35): A figure showing correlation between Anti-CCP and TRAbs.

❖ Correlation between RA duration and anti-TPO and anti-TG antibodies level:

No significant statistical correlation was observed between RA duration and anti-TPO (p=0.36 and .65 respectively) and anti-TG antibodies titer (p= 0.8 and 0.44 respectively) among RA patients (group I or group II). (Table 22)

Table (22): Correlation between duration of RA with Anti-TPO, Anti-TG

		Duration of RA	
		Group I	**Group II**
Anti TPO	r_s	-0.157	-0.079
	P	0.367	0.652
Anti TG	r_s	0.043	0.135
	P	0.805	0.440

r_s: Spearman coefficient
*significant

❖ Correlation between mean serum TSH level and anti-TPO and anti-TG antibodies level:

No significant statistical correlation was observed between mean serum TSH and anti-TPO (p=0.81 and 0.63 respectively) and anti-TG antibodies titer (0.16 and 0.06 respectively) among RA patients (group I or group II). (Table 23)

Table (23): Correlation between TSH with Anti-TPO, Anti-TG

		TSH	
		Group I	**Group II**
Anti TPO	r_s	0.042	-0.084
	P	0.810	0.630
Anti TG	r_s	0.241	0.342
	P	0.162	0.064

r_s: Spearman coefficient
*significant