AIM OF THE WORK

The aim of the work is to study the level of TNF-α and CRP before & after programmed resistance exercise in Egyptian elderly subjects.

SUBJECTS AND METHODS

This study had been conducted on 30 elderly individuals aged from 60 to 78 years old , of both gender and with no history of any systemic metabolic diseases as ; diabetes , nor hypertension , nor hepatic nor renal diseases. The subjects were attended at the Department of Physical Medicine, Rheumatology & Rehabilitation in El Hadara Alexandria University Hospital .The study was done in the period from December 2013 to July 2014.

All subjects enrolled in the study were subjected to the followings :

i. Clinical evaluation:

1. History taking:
2. Complete clinical examination
3. Electrocardiogram (ECG).

ii. Routine laboratory investigations: including

A. Complete blood picture [163].
B. Random blood sugar(RBS)[164].
C. Renal function tests;
   - Blood urea.
   - Serum creatinine [165]
   - Assessment of glomerular filtration rate (GFR) by Modification of Diet in Renal Diseases (MDRD) [166].

iii. Specific laboratory investigations (inflammatory markers):

Both markers were measured twice before and after regular resistance exercise training for four weeks.

1. Tumor necrosis factor –alpha (TNF-ALPHA):[167,168]

eBioscience, high performance immunoassays Human TNF-alpha ,by ***Platinum ELISA*** . BMS223/4/ BMS223/4TEN .

Principle of the test:

1- An anti-human TNF-alpha coating antibody is adsorbed onto microwells.
2- Human TNF-alpha present in the sample or standard binds to antibodies adsorbed to the microwells.A biotin-conjugated anti-human TNF-alpha antibody is added and binds to human TNF-alpha captured by the first antibody.
3- Following incubation unbound biotin-conjugated anti-human TNF-alpha antibody is removed during a wash step.Streptavidin-HRP is added and binds to the biotin-conjugated anti-human TNF-alpha antibody.
4- Following incubation unbound Streptavidin-HRP is removed during a wash step,and substrate solution reactive with HRP is added to the wells.

5- A coloured product is formed in proportion to the amount of human TNF-alpha present in the sample or standard.The reaction is terminated by addition of acid and absorbance is measured at 450nm.A standard curve is prepared from 7 human TNF-alpha standard dilutions and human TNF-alpha sample concentration determined.

Specimen collection and storage instructions:

Serum samples were aliquoted and stored frozen at -20℃ to avoid loss of bioactive human TNF-alpha till assayed.

Test protocol summary:

1. The number of microwell strips required was determined.
2. Microwell strips were washed twice with Wash Buffer. Standard dilution on the microwell plate: 100 μl Sample Diluent were added to all standard wells. Standard were prepared into the first wells by using pipette 100 μl and then standard dilutions were created by transferring 100 μl from well to well.100 μl from the last well was discarded.

Alternatively, external standard dilution: in tubes; pipette 100 μl of these standard dilutions in the microwell strips.

3. 100 μl Sample Diluent were added , to the blank wells.
4. 50 μl Sample Diluent were added to sample wells.
5. 50 μl of the sample were added to the sample wells.
6. Biotin-Conjugate was prepared.
7. 50 μl Biotin-Conjugate were added to all wells.
8. Microwell strips were covered and incubated 2 hours at room temperature (18℃ to 25℃).
9. Streptavidin-HRP was prepared.
10. Microwell strips were washed 4 times with Wash Buffer.
11. 100 μl diluted Streptavidin –HRP were added to all wells.
12. Microwell strips were covered and incubated 1 hour at room temperature (18℃ to 25℃).
13. Microwell strips were washed 4 times with Wash Buffer.
14. 100 μl of TMB Substrate Solution were added to all wells.
15. The microwell strips were incubated for about 10 minutes at room temperature (18℃ to 25℃).
16. 100 μl Stop Solution were added to all wells.
17. Microwell reader was blanked and measured colour intensity at 450 nm.

Calculation of results:

A standard curve was created by plotting the mean absorbance for each standard concentration on the ordinate against the human TNF-alpha concentration on the abscissa. A best fit curve was drawed through the points of the graph .

To determine the concentration of circulating human TNF-alpha for each sample , first the mean absorbance value on the ordinate was found and a horizontal line was

extended to the standard curve .At the point of intersection ,a vertical line was extended to the abscissa and the corresponding human TNF-alpha concentration was read.

Expected values:

There were no detectable human TNF-alpha levels found.

2. Quantitative c- reactive protein(CRP): [169]

Cardio Phase ® hs CRP. BN /BN ProSpec ® System.

Principle of the method :

Polystyrene particles coated with monoclonal antibodies specific to human CRP are aggregated when mixed with samples containing CRP. These aggregates scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the relevant protein in the sample. The result is evaluated by comparison with a standard of known concentration.

Specimens: [170]

Serum samples centrifuged, aliquoted and stored at -20 □C till assayed.

Assay protocols on the BN systems:[171]

The assay protocol is given in the BN System Instruction Manual and software of the instrument .All steps are performed automatically by the system.

Establishment of the reference curve: [172]

Reference curve is generated by multi-point calibration .Serial dilutions of N Rheumatology Standard SL are automatically prepared by the instrument using N Diluent.

Reference interval:[173]

Expected values for healthy individuals are typically ≤ 3 mg/dl.

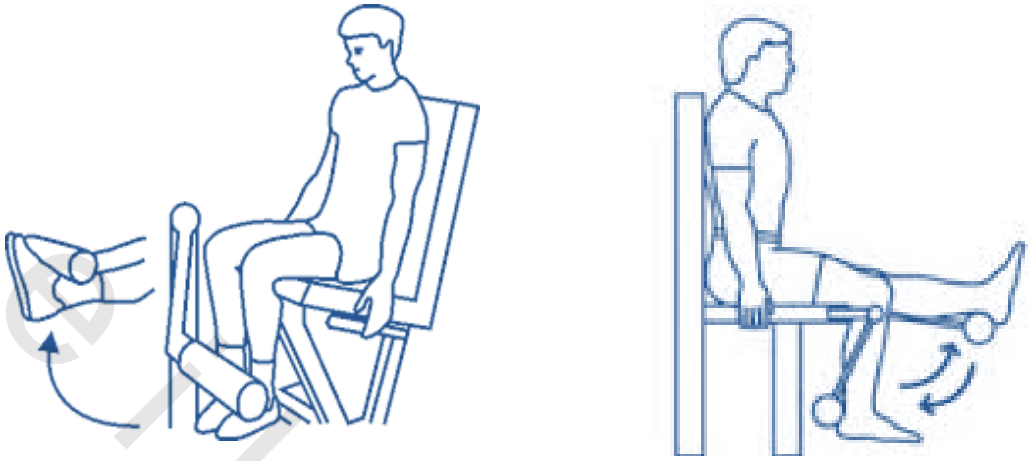
iv. Resistance exercise training:

Exercise protocol:

The exercise sessions was done three times per week for four weeks. The exercise program was involved a low-intensity resistance exercise training which had be performed for both upper and lower limbs [174].

Resistance exercise training sessions involved the followings: a ***warm-up period*** (5 min) on a selectively bicycle ergometer (Bike-Max) seated leg curl ***(Figure 9)***, at low intensity; ***specific resistance training period*** (30 min), upper body exercises performed using elastic bands and dumbbells; and lastly a ***cool-down period*** (5 min).

To minimise fatigue, exercises for the upper and lower limbs of the body were performed in a non consecutive way, with a rest period of approximately two minutes between each set. Training intensity was gradually increased during the first week. Training was continuously monitored by heart rate monitors.



(A) **(B)**

Figure 9(A and B): seated leg curl [174].

Exercise intensity:

Exercise intensity was determined as the max. heart rate was not exceed 70% of the predicted max. heart rate for subject‘ s age.

[max. ht. rate=220-age(years)×70%]

The exercise was consisted of:

- Resistance training for dorsiflexors (a cable is attached to the foot from the floor, and the thigh is raised);
- Front traction (a horizontally drawn cable is grasped and stretched forward);
- Vertical traction (a cable is grasped from above and stretched downward) [175].

Statistical analysis of the data:[176]

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0.[177] Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The distributions of quantitative variables were tested for normality. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For abnormally distributed data .To compare between the different periods using Wilcoxon signed ranks test. Correlations between two quantitative variables were assessed using Spearman coefficient. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

RESULT

1. Demographic data: (table 5 & figure 10-13)

In our study, the 30 elderly subjects included 11 males (36.7%) and 19 females (63.3%),their age ranged from 60-78 years with mean age value 66.17± 5.57 as there are 17 subjects(56.7%) between 60-65 years; 7 subjects (23.3%) between 66-70 years;and 6 subjects (20%) above 70 years old. Among the elderly subjects ,there were 17 worker (56.7%) and 13 non-workers (43.3%);also 24 subjects were non smokers (80%) and 6 only were smokers (20%).

Table (5): Distribution of the studied cases according to demographic data.

	No.	%
Gender		
Male	11	36.7
Female	19	63.3
Age		
60 – 65	17	56.7
66 – 70	7	23.3
>70	6	20.0
Min. – Max.	60.0 – 78.0	
Mean ± SD.	66.17 ± 5.57	
Median	65.0	
Occupation		
Worker	17	56.7
Non worker	13	43.3
Habits		
Non smoker	24	80.0
Smoker	6	20.0

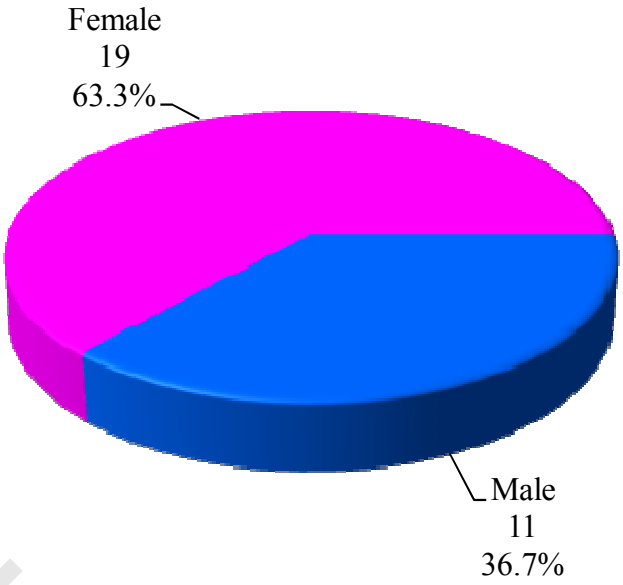


Figure (10): Distribution of the studied cases according to sex

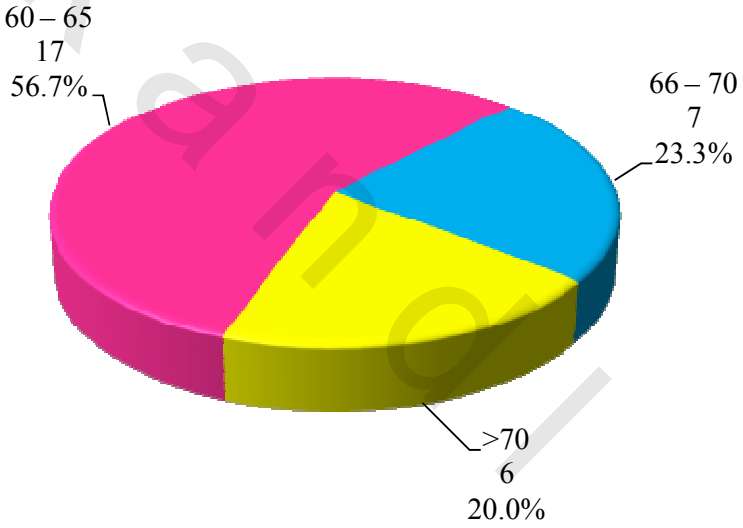


Figure (11): Distribution of the studied cases according to age

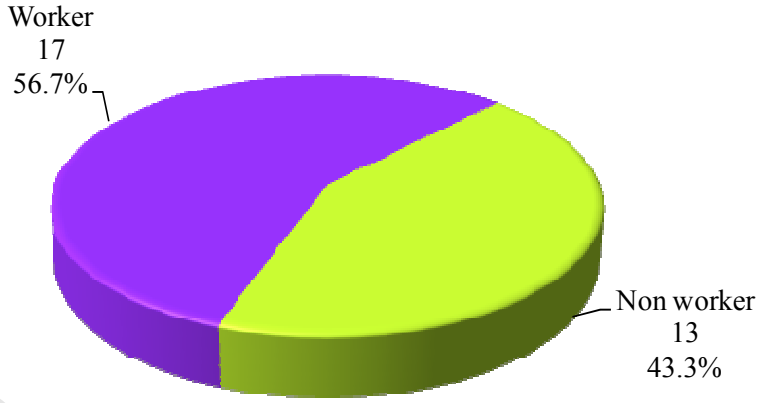


Figure (12): Distribution of the studied cases according to occupation

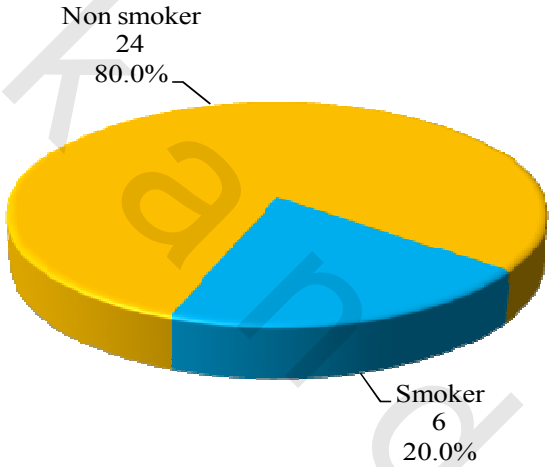


Figure (13): Distribution of the studied cases according to habits

2. Clinical assessment of the studied subjects (diagnosis): (table 6, figure 14)

Among the thirty individuals selected in this study,there were 21 diagnosed as post immobilization stiffness(70%),six others diagnosed as mild knee osteoarthritis (20%) and only one individual with past medical history of medial leminescus tear (3.3%) ;and another one diagnosed as carpal tunnel syndrome (3.3%) and finally a last one complained of chronic low back pain due to old disc prolapse (3.3%).

Table (6): Distribution of the studied cases according to diagnosis

Diagnosis	No.	%
Post immobilization stiffness	21	70.0
Knee osteoarthritis(mild)	6	20.0
Medial leminescus tear	1	3.3
Left carpal tunnel syndrome	1	3.3
Low back pain due to disc prolapse	1	3.3

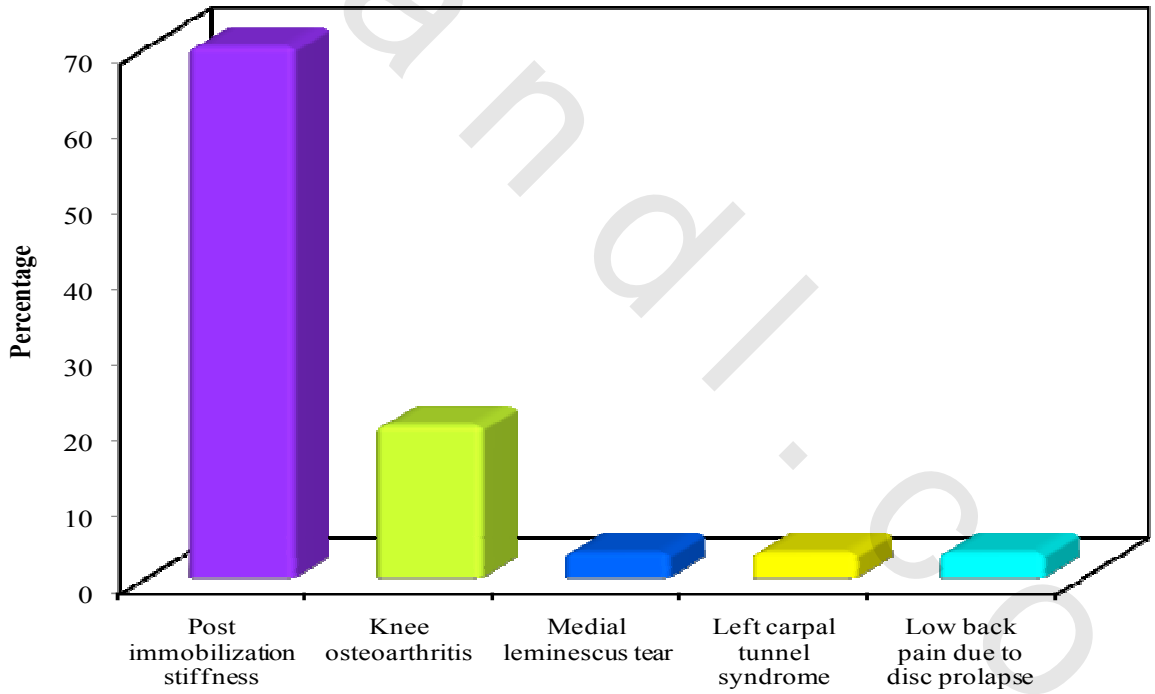


Figure (14): Distribution of the studied cases according to diagnosis

3. Laboratory findings in the studied subjects:

A. Complete blood count (CBC) : (table 7)

- **Haemoglobin Concentration (Hb) :**

Haemoglobin concentration ranged between 9 –15.5 g/dl in the selected individuals .The mean value of Hb concentration was 11.74 ± 1.45 g/dl.

- **Red Blood Cell Count (RBCs):**

RBCs count ranged between 3.01 – 5.59 $\times 10^6$/µl with mean value 4.22 ± 0.56 $\times 10^6$/ µl in the studied subjects.

- **White Blood Cells Count (WBCs):**

WBCs count ranged between 3.11 – 11.9 $\times 10^3$/ µl with mean value 7.31 ± 2.34 $\times 10^3$/ µl in the selected subjects.

- **Platelets Count :**

The platelet count ranged between 150 – 369 $\times 10^3$/ µl with mean value 233.77 ± 69.81 $\times 10^3$/ µl in the studied individuals.

Table (7): CBC parameters among the studied individuals .

	Min. – Max.	**Mean ± SD.**	**Median**
HB (g/dl)	9.0 – 15.50	11.74 ± 1.45	11.45
RBCells ($\times 10^6$/ µl)	3.01 – 5.59	4.22 ± 0.56	4.24
WBCells ($\times 10^3$/ µl)	3.11 – 11.90	7.31 ± 2.34	7.43
Platelets ($\times 10^3$/ µl)	150.0 – 369.0	233.77 ± 69.81	217.0

B. Random blood sugar (RBS):(table 8)

The random blood glucose ranged between 80– 184 mg/dl ,with a mean value of 122.7 ± 34.74 mg/dl in the studied subjects.

Table (8): Random Blood Sugar parameters in the studied individuals.

	Min. – Max.	Mean ± SD.	Median
Random Blood Sugar (mg/dl)	80.0 – 184.0	122.70 ± 34.74	110.50

C. Renal function tests:(table 9)

- **Blood urea:**

The blood urea ranged between 10.9 – 102 mg/dl in the studied individuals ,with mean value of 39.7 ± 24.08 mg/dl .

- **Serum creatinine:**

The level of serum creatinine ranged between 0.23 – 1.1 mg/dl ,with mean value 0.22 ± 0.73 mg/dl detected in the selected subjects.

- **Glomerular filtration rate :**

The level of GFR ranged between 60 – 435 ml/min and mean value 112.34 ± 435 ml/min .

Table (9): Renal functions tests parameters among the studied subjects.

	Min. – Max.	Mean ± SD.	Median
Bl.Urea(mg/dl)	10.90 – 102.0	39.70 ± 24.08	34.0
S.Creatinine (mg/dl)	0.23 – 1.10	0.22 ± 0.73	0.73
GFR by MDRD (ml/min)	60.0 – 435.0	112.34 ± 435.0	93.50

D. Serum level of specific inflammatory markers:

i. Tumor Necrosis Factor –Alpha (TNF-Alpha):(n=29)(tables 10,11 &figures 15,16)

After excluding one case due to the extreme unexplained level of TNF-alpha which was 26 pg/ml. TNF-alpha level measured before exercise was ranged between 0– 10.4 pg/ml and ranged between 0– 8.6 pg/ml when measured after the resistance exercise training (RET).While the mean value was 3.07 ± 3.06 pg/ml and 2.23 ± 2.37 pg/ml before and after exercise training respectively.This difference was statistically significant (p= 0.036) as $p \leq 0.05$.

Table (10): Study the level of TNF-alpha Before and After resistance exercise training.

TNF-Alpha	Before exercise	After exercise
Min. – Max.	0.0 – 10.40	0.0 – 8.60
Mean ± SD.	3.07 ± 3.06	2.23 ± 2.37
Median	2.20	1.40
Z(p)	2.099*(0.036*)	

Z: Z for Wilcoxon signed ranks test
*: Statistically significant at $p \leq 0.05$
n=29

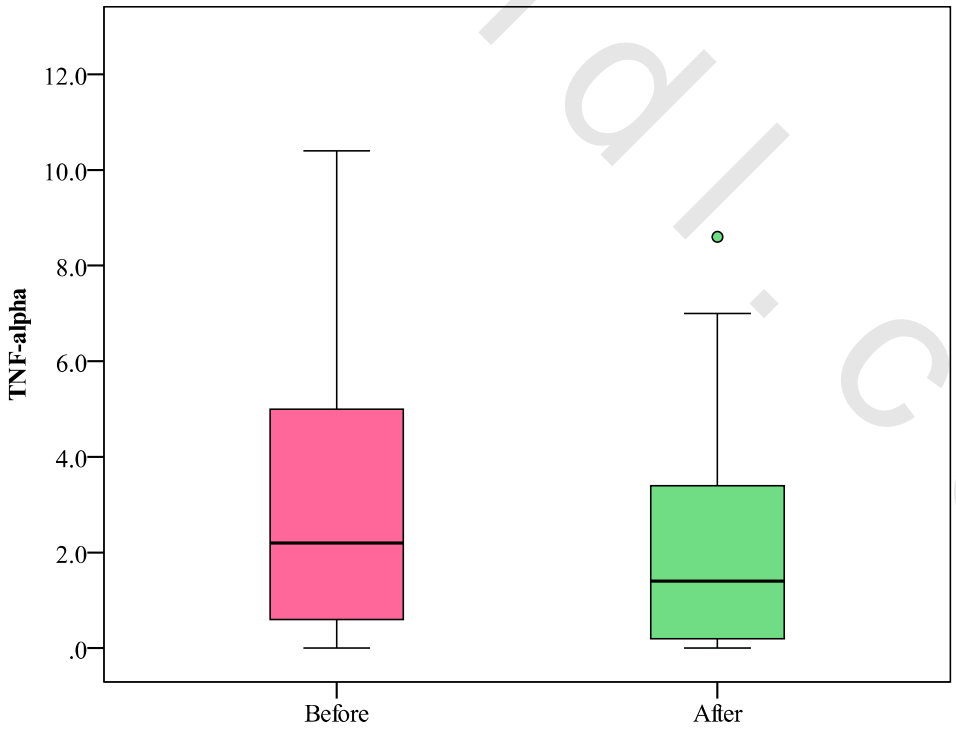


Figure (15): Study the level of TNF-alpha Before and After resistance exercise training.

Through these 29 subjects, the level of TNF-alpha was presented by three different courses before and after RET; as there were 17 studied subjects (58.6%) who showed a decreased course,while 6 selected individuals (20.7%) showed increased course and 6 others (20.6%) with unchanged course.

Table (11): Study the course of TNF-alpha level After resistance exercise training.

TNF-alpha	No.	%
Decreased	17	58.6
Un changed	6	20.7
In Creased	6	20.7

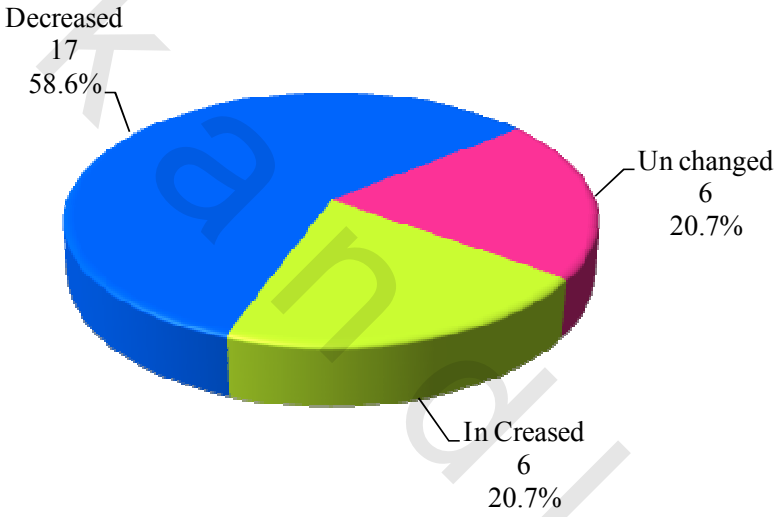


Figure (16): Study the course of TNF-alpha level After resistance exercise training.

ii. C- Reactive Protein (CRP):(tables 12,13 and figures 17,18)

The CRP level was ranged between 1.83 – 25.2 mg/L before RET while after exercise training ranged between 0.6– 51 mg/L . In our selected individuals ,the mean value was 6.57 ± 5 and 6.03 ± 8.84 mg/L before and after RET respectively. This difference was statistically significant (p=0.009) as p≤ 0.05.

Table (12):Study the level of CRP Before and After resistance exercise training.

CRP	Before exercise	After exercise
Min. – Max.	1.83 – 25.22	0.62 – 51.0
Mean ± SD.	6.57 ± 5.0	6.03 ± 8.84
Median	4.70	3.36
Z(p)	2.597*(0.009*)	

Z: Z for Wilcoxon signed ranks test
*: Statistically significant at p ≤ 0.05

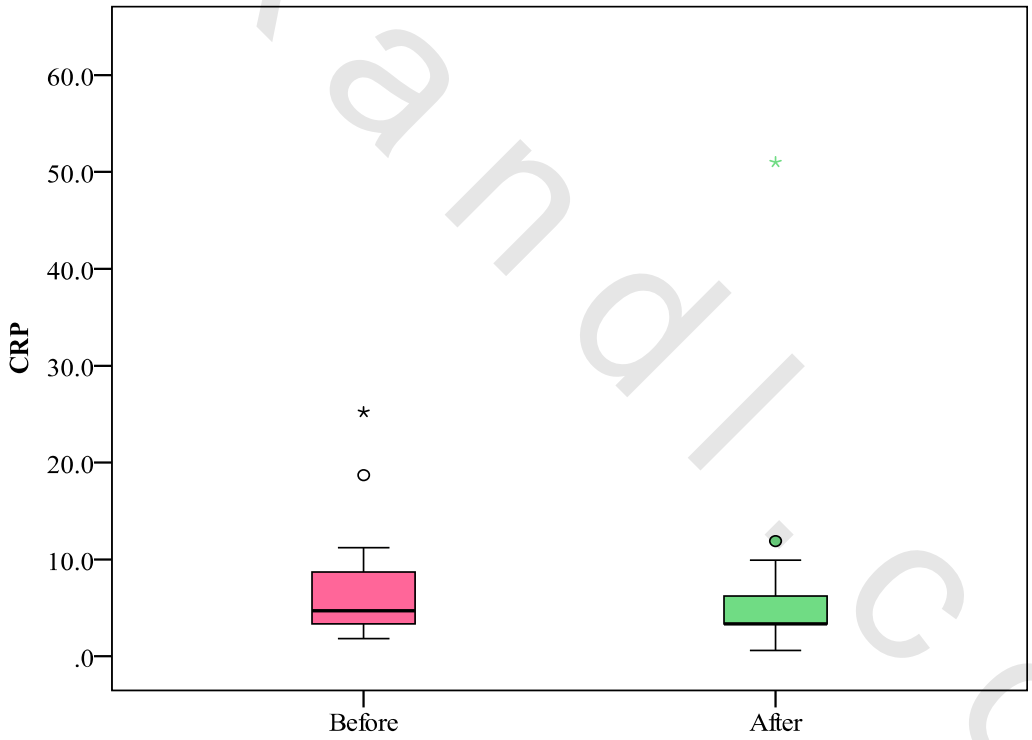


Figure (17): Study the level of CRP before and after resistance exercise training.

In this study,the course of CRP was decreased in 18 studied individuals (60%),increased in 7 subjects(23.3%),and only 5 others (16.6%) had unchanged course of CRP level.

Table (13): Study the course of CRP level After resistance exercise training.

CRP	No.	%
Decreased	18	60
Unchanged	5	16.6
Increased	7	23.3

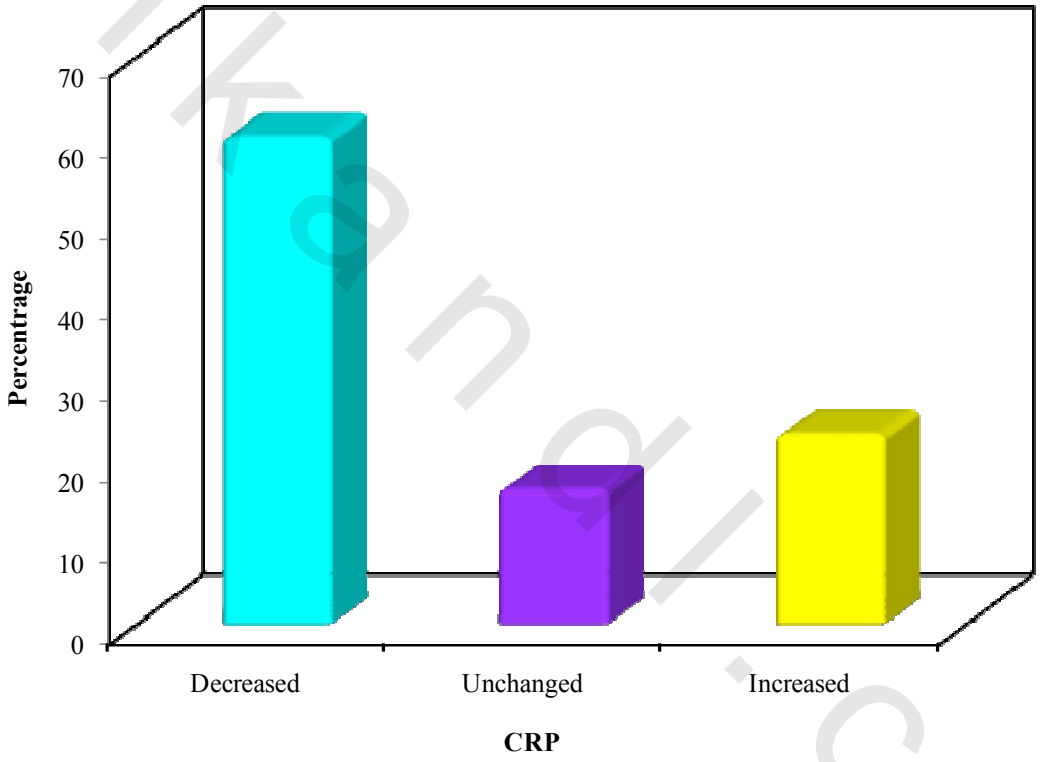


Figure (18): Study the course of CRP level After resistance exercise training