AIM OF THE WORK

The aim of the present work is to:

a. Evaluate CTX-II as a biochemical marker of knee osteoarthritis .

b. Investigate its association with ultrasonographic, conventional imaging and clinical findings in knee OA.

c. Assess the validity and clinical utility of ultrasound in knee osteoarthritis.

SUBJECTS

The study included 2 groups:

Group I: 50 adult patients with primary knee osteoarthritis , diagnosed according to the American College of Rheumatology (ACR) clinical and radiographic criteria of knee osteoarthritis. [172]

Group II: 20 adult healthy persons age and sex matched as a control group.

Exclusion criteria: patients with secondary osteoarthritis either due to inflammatory joint diseases (e.g ; rheumatoid arthritis) or traumatic causes were excluded from the study.

All patients were selected from internal medicine department, Rheumatology unit, Main Alexandria university hospital.

An informed consent was taken from all patients before the beginning of the study.

METHODS

Study design: Cross-sectional study

Our study was conducted on 50 patients with knee osteoarthritis and 20 adult persons as a control group admitted to rheumatology unit at Alexandria Main University Hospital.

All studied sample were subjected to the following after taking an informed consent:

A. Clinical evaluation:

1- History taking including:

- Demographic data: name, age, gender and occupation.
- History of the present complaints: morning stiffness, joint pain, swelling, restriction of movement.
- Disease duration and medication used by patients.

2- General examination included the following

- Vital signs.
- Body mass index.
- Head and neck examination.
- Cardiovascular examination.
- Chest examination.
- Abdominal examination.
- Skin and extremities.

3- Joint examination:

- Inspection of the joints were done as regards the presence of swelling, deformity, signs of inflammation and muscle status
- Tenderness and crepitus
- Presence of effusion
- Range of movement whether active or passive movement
- Presence of deformities.

4- Osteoarthritis clinical assessment:

a. The Western Ontario and McMaster Universities (WOMAC) questionnaire (Table IV)[173]

Functional disability resulting from knee or hip osteoarthritis usually is evaluated using the WOMAC function subscale, which is a questionnaire of 17 items related to daily activities Each item in the scale scored from 0 to 4:

0 (none) 1(mild) 2(moderate) 3(sever) 4(extreme)

(Table V) :The WOMAC questionnaire

Pain Subscale (5 Questions)
How much pain do you have … Walking on a flat surface? Going up or down stairs? At night while in bed? Sitting or lying? Standing upright?
Stiffness Subscale (2 Questions)
How severe is your stiffness after first waking in the morning?
How severe is your stiffness after sitting, lying down, or resting later in the day?
Function Subscale (17 Questions)
What degree of difficulty do you have with … Descending stairs? Ascending stairs? Rising from sitting? Standing? Bending to floor? Walking on a flat surface? Getting in or out of a car? Going shopping? Putting on socks or stockings? Rising from bed? Taking off socks or stockings? Lying in bed? Getting in and out of the bath? Sitting? Getting on or off the toilet? Heavy domestic duties? Light domestic duties?

b. Visual analogue scale for pain

VAS is a standard 100 mm horizontal scale on which the patient indicates the severity of pain by placing a mark between terminal points designed "no pain " and "extreme pain"**.**

c. Lequesne functional index

Lequesne et al developed an index of severity for osteoarthritis for the knee (ISK). This can be used to assess the effectiveness of therapeutic interventions. (Table VI) [174-176]

Sections for index:

(1) pain or discomfort

(2) maximum distance walked

(3) activities of daily living

Interpretation:
• minimum points for each section: 0
• maximum points for each section: 8
• minimum index score: 0
• maximum index score: 24

Index Score	Handicap
0	none
1 - 4	mild
5 - 7	moderate
8 - 10	severe
11 - 13	very severe
>= 14	extremely severe

Table (VI) : Lequesne's Algofunctional Index

Pain or Discomfort	Points*
During nocturnal bed rest	
None or insignificant	0
Only on movement or in certain positions	1
With no movement	2
Morning stiffness or regressive pain after rising	
≤1 min	0
>1 min, but <15 min	1
After standing for 30 min	0 or 1
While walking	
None	0
Only after walking some distance	1
After initial walking and increasingly with continued walking	2
With prolonged sitting (2 hr)	0 or 1
Maximum distance walked (even with pain)	
Unlimited	0
>1 km (>0.6 mile) but limited	1
About 1 km (0.6 mile) in about 15 min	2
500-600 m (1640-2952 ft or 0.31-0.56 mile) in about 8-15 min	3
300-500 m (987-1640 ft)	4
100-300 m (328-985 ft)	5
<100 m (<328 ft)	6
With one walking stick or crutch	1
With two walking sticks or crutches	2
Day-to-day activities	
Put on socks by bending forward	0 or 2
Pick up an object from the floor	0 or 2
Climb up and down a standard flight of stairs	0 or 2
Get into and out of a car	0 or 2

B. Laboratory evaluation:

Laboratory investigation included:

1. CBC
2. Blood urea, serum creatinine
3. Liver profile
4. ESR,CRP
5. Serum CTX-II
6. Synovial CTX-II

Sampling:

- Venous blood samples were collected from subjects after proper disinfection.
- Blood samples were withdrawn from the anti-cubital vein with minimal stasis.
- CRP was detected by latex agglutination. [177]
- ESR was estimated by Westergen method,the test was performed on venous blood diluted accurately in the proportion of 1 volume of sodium citrate to 4 volumes of blood. The result was expressed as ESR = X mm in 1 h. [178]
- **Serum CTX-II level:**

The level of CTX-II was measured in the serum of patients and control group by ELISA technique. The human CTX-II ELISA is an enzyme-linked immunosorbant assay for the quantitativ

Principle of the test

1- An anti-human CTX-II coating antibody is adsorbed onto microwells. (figure 7)

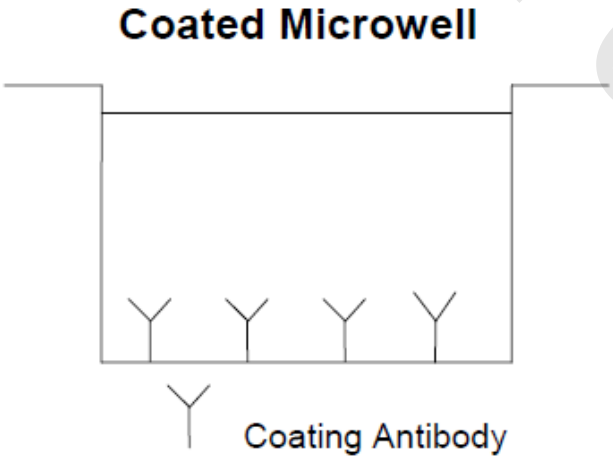

Figure (7): Principle of the human CTX-II ELISA test (Coated Microwell)

2. Human CTX-II present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human CTX-II antibody is added and binds to human CTX-II captured by the first antibody. (figure 8)

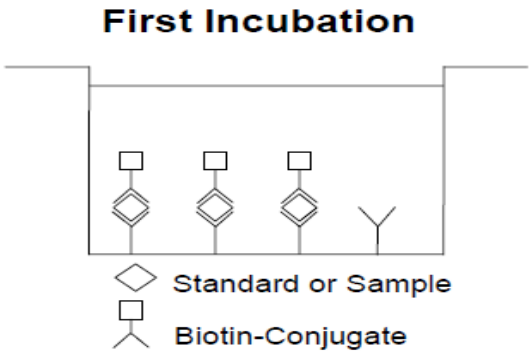

Figure (8) :Principle of the human CTX-II ELISA test (first Incubation).

3. Following incubation unbound biotin-conjugated anti-human CTX-II antibody WAS removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human CTX-II. (figure 9)

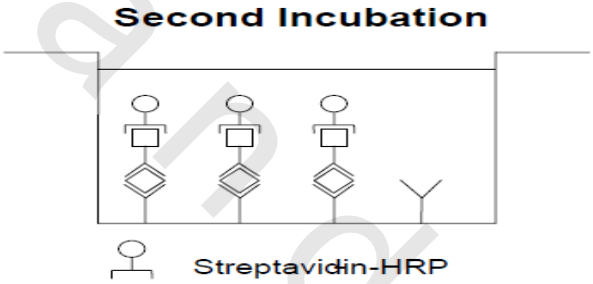

Figure (9) :Principle of the human CTX-II ELISA test (2nd Incubation)

4. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells. (figure 10)

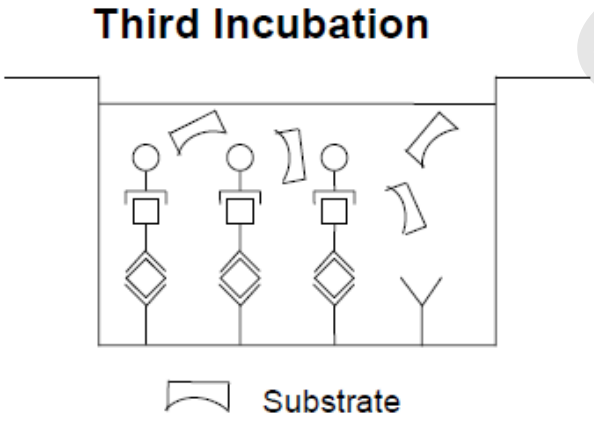

Figure (10): Principle of the human CTX-II ELISA test (3rd Incubation)

5. A coloured product is formed in proportion to the amount of human CTX-II present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human CTX-II standard dilutions and human CTX-II sample concentration determined. (figure 11)

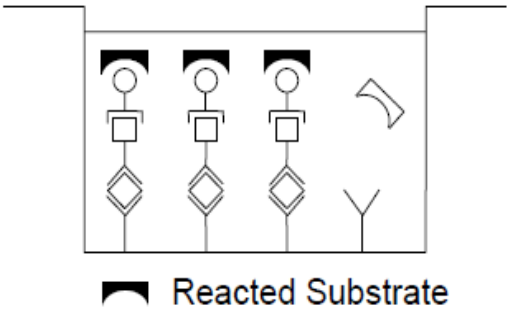

Figure (11): Principle of the human CTX-II ELISA test (terminal reaction)

Specimen collection and storage instructions

Serum samples should be aliquoted and must be stored frozen at -20 °C to avoid loss of bioactive human CTX-II

Calculation of results (Figure 12)

1. We calculated the average absorbance values for each set of duplicate standards and samples. Duplicates were within 20 per cent of the mean value.

2. We created a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human CTX-II concentration on the abscissa. We drew a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).

3. To determine the concentration of circulating human CTX-II for each sample, first we found the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, we extended a vertical line to the abscissa and read the corresponding human CTX-II concentration.

4. Samples had been diluted 1:2 (50 µl sample + 50 µl Sample Diluent), the concentration read from the standard curve were multiplied by the dilution factor (x 2).

5. It was suggested that each testing facility establishes a control sample of known human IL-6 concentration and runs this additional control with each assay.

6. A representative standard curve is shown below. This curve cannot be used to derive test results. Each laboratory was prepare a standard curve for each group of microwell strips assayed. Representative standard curve for human CTX-II ELISA. Human CTX-II was diluted in serial 2-fold steps in Sample Diluent. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

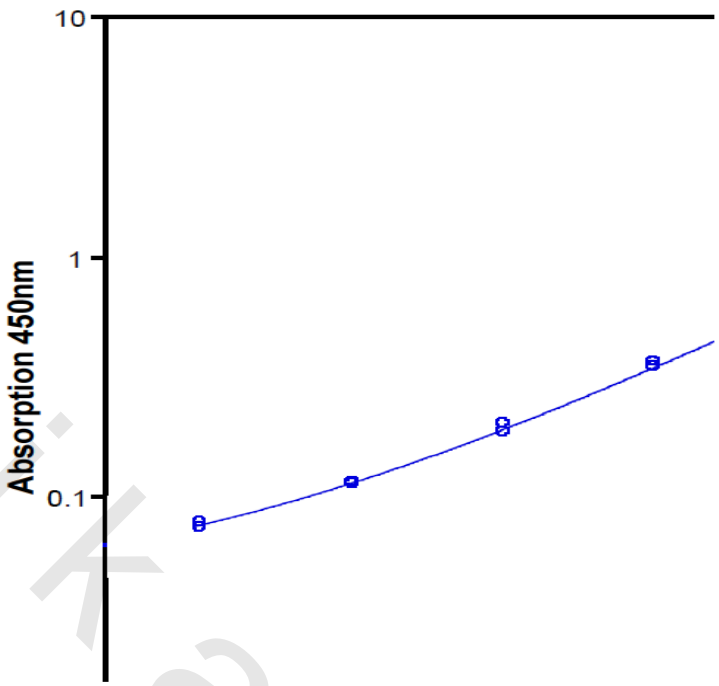

Figure (12): Calculation curve

C. Radiological evaluation

1- Plain radiography of both knees (X-ray knee)

Standardized knee radiograph were done, we had applied the Kellgren-Lawrence scale for grading of osteoarthritis.

K-L grading: [179]

- **Grade 1 (doubtful OA)**: doubtful narrowing of joint space and possible osteophytic lipping.
- **Grade 2 (minimal OA)**: definite osteophyte and possible narrowing of joint space
- **Grade 3 (moderate OA)**: moderate multiple osteophyte, definite narrowing of joint space, some sclerosis and possible deformity of bone contour
- **Grade 4 (severe OA)**: large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour.

2- Musculoskeletal ultrasound

Ultrasound images were acquired with ACUSON X 300 SIEMENS by using 7-10 mhz linear transducer

Positioning of the patient

Supine position for ventral and lateral scans

Prone position for dorsal scans

Knee joint in neutral position and/or 30° flexion

Maximal flexion for imaging of the intercondylar sulcus

Dynamic examination of the suprapatellar pouch with relaxed and contracted quadriceps muscle

Standard scans

1. Suprapatellar longitudinal scan.
2. Suprapatellar transverse scan in neutral position.
3. Suprapatellar transverse scan in maximal flexion.
4. Infrapatellar longitudinal scan.
5. Infrapatellar transverse scan.
6. Medial longitudinal scan.
7. Lateral longitudinal scan.
8. Posterior medial longitudinal scan.
9. Posterior lateral longitudinal scan.
10. Posterior transverse scan.

Defintion of sonographic findings

Effusion : Abnormal hypoechoic or anechoic intra-articular material that can be displaced and compressed, but does not exhibit a Doppler signal.

Synovial hypertrophy / proliferation : Abnormal hypoechoic intra-articular tissue that is non –displaceable and poorly compressible and may exhibit a Doppler signal.

3- Ultrasound Guided Aspiration

Ultrasound guided aspiration of the knee joint was done for some patients.

Sterile technique was designed to avoid the introduction of skin bacteria into the joint in all procedures

A mark was made just posterior to the medial or lateral aspect of the patella in the recess behind the patella, An 18-gauge to 22-gauge needle was directed posteriorly and slightly inferiorly, and fluid was aspirated after advancing the needle ½ to 1½ inches into the joint space under ultrasound imaging.

Statistical analysis of the data[180]

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0.[181]

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.

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 normally distributed data, comparison between two independent population were done using independent t-test. For abnormally distributed data, comparison between two independent population were done using Mann Whitney test while.

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.

RESULTS

The present study was conducted on 50 patients with knee osteoarthritis fulfilling the ACR criteria for diagnosis of the disease and 20 age- sex matched healthy individual as a control group.

Demographic characteristics of studied patients:

The present study was conducted on 2 groups:

- Group **I** (50 patients with knee OA), 20 of them were males (40%) and 30 were females (60%).
- Group **II** (20 healthy persons), 7 of them were males (35%) and 13 were females (65%). **Table (VII), Figure(13,14)**

The median age of group **I** was 53 years ranged from 45 to 65 years,while the median age of group **II** was 55 years ranged from 40 to 63 years and the median. There was no statistically significant difference between the studied groups as regards their age (p=0.645) and sex (p=0.698) **Table (VII), Figure (15)**

The median BMI of group A was 29.5 ranged from 26 to 31.5,while that of group B was 28 ranged from 23.5to 30. There was statistically significant difference between the studied groups as regards their BMI (P 0.001) **Table (VII), Figure (16)**

The disease duration in group **I** ranged from 1 year to 12 years, with a mean of 5.7±3.3 standard deviation and a median of 5 years. **Table (VII)**

Table (VII): Comparison between the two studied groups according to demographic data

	Group I (n = 50)		Group II (n = 20)		Test of sig.	p
	No.	%	No.	%		
Sex						
Male	20	40.0	7	35.0	χ^2= 0.151	0.698
Female	30	60.0	13	65.0		
Age						
Min. – Max.	45.0 – 65.0		40.0 – 63.0			
Mean ± SD.	54.02 ± 5.24		54.70 ± 6.33		t= 0.462	0.645
Median	53.0		55.0			
BMI						
Min. – Max.	26.0 – 31.50		23.50 – 31.0			
Mean ± SD.	29.40 ± 1.26		28.68 ± 1.97		t= 1.832	0.071
Median	29.50		29.25			
Disease duration						
Min. – Max.	1.0 – 12.0		-			
Mean ± SD.	5.70 ± 3.32		-		-	-
Median	5.0		-			

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

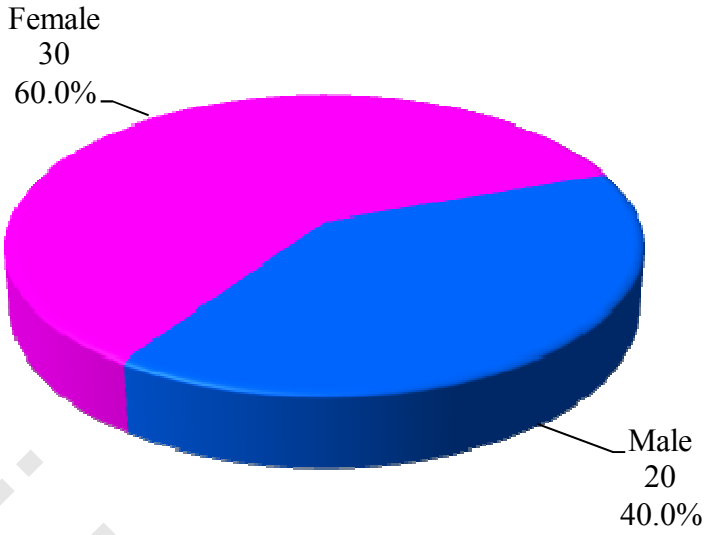

Figure (13): distribution of group I according to sex

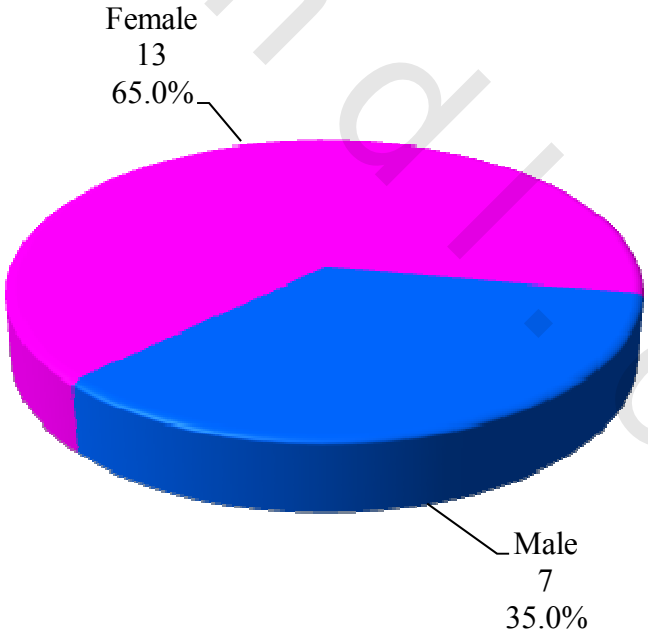

Figure (14): distribution of group II according to sex

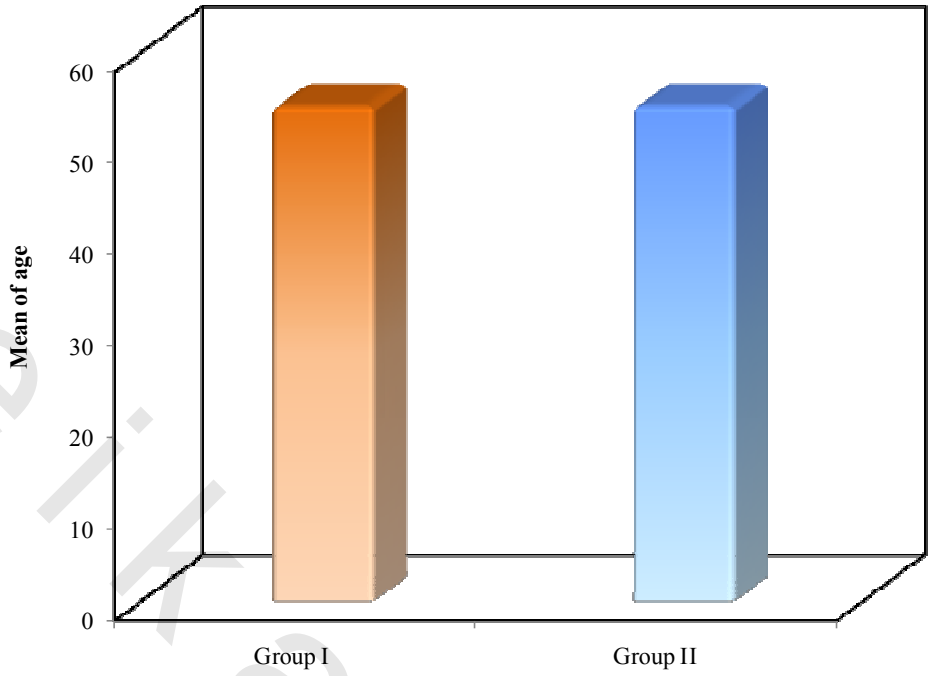

Figure (15): Comparison between the two studied groups according to age

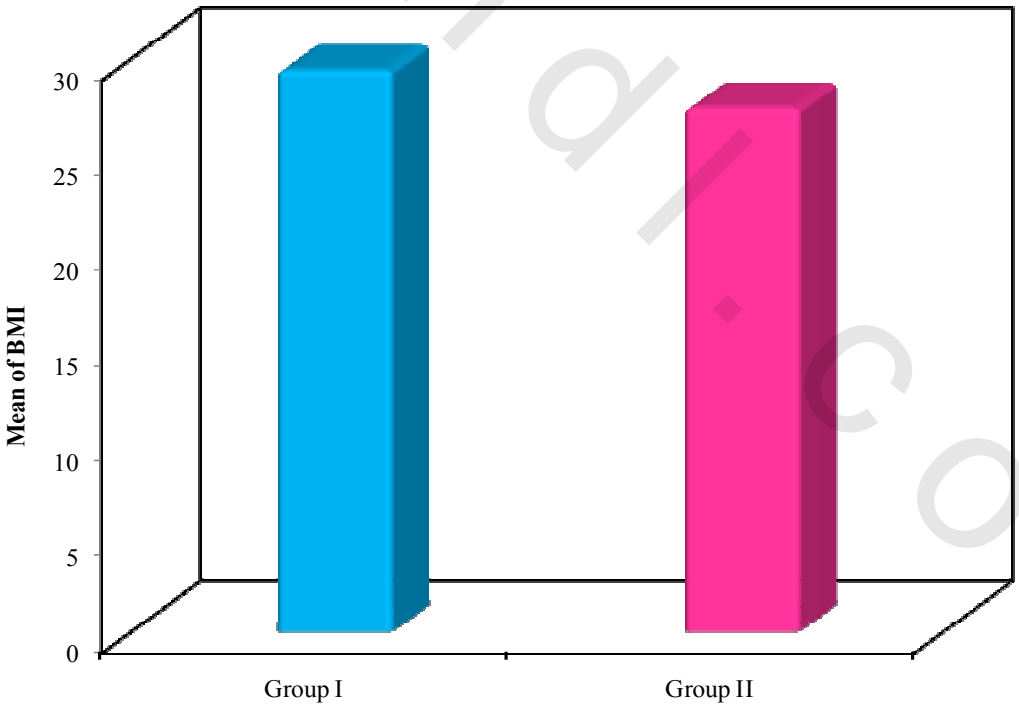

Figure (16): Comparison between the two studied groups according to BMI

Descriptive analysis of the studied cases according to medications

The medications used by the patients were in form of paracetamol (30 patients), non steroidal anti-inflammatory drugs (40patients), disease modifying osteoarthritis drugs (42 patients), Intra articular steroid injection (7 patients) and Intra articular Hyaluronic acid injection (4 patients). **Table (VIII), Figure (17)**

Table (VIII): Distribution of the studied cases according to medications (n=50)

Medications	No.	%
Para cetamol	30	60.0
NSAIDs (non steroidal anti-inflammatory drugs)	40	80.0
DMOAD (disease modifying osteoarthritis drugs)	42	84.0
Intra articular steroid injection	7	14.0
Intra articular Hyaluronic acid injection	4	8.0

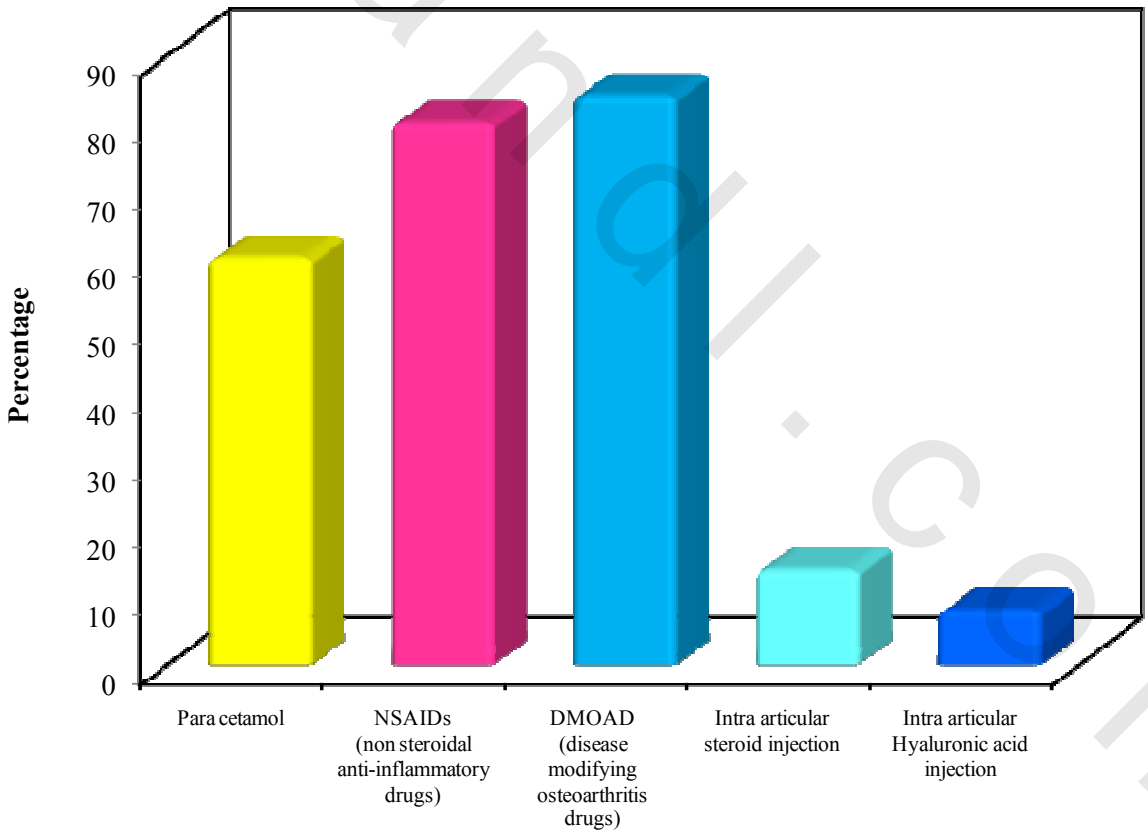

Figure (17): Distribution of the studied cases according to medications (n=50)

Clinical findings in group (I)

Group **I**

27 patients had swelling (54%), 23 patients had tenderness (46%),20 patients had effusion (40%), 37 patients had crepitus (74%) and 14 patients had deformities (28%) **Table (IX), Figure (18)**

Table (1X): Distribution of studied cases according to clinical findings

	No.	%
Swelling	26	52.0
Tenderness	23	46.0
Effusion	20	40.0
Crepitus	37	74.0
Deformities	14	28.0

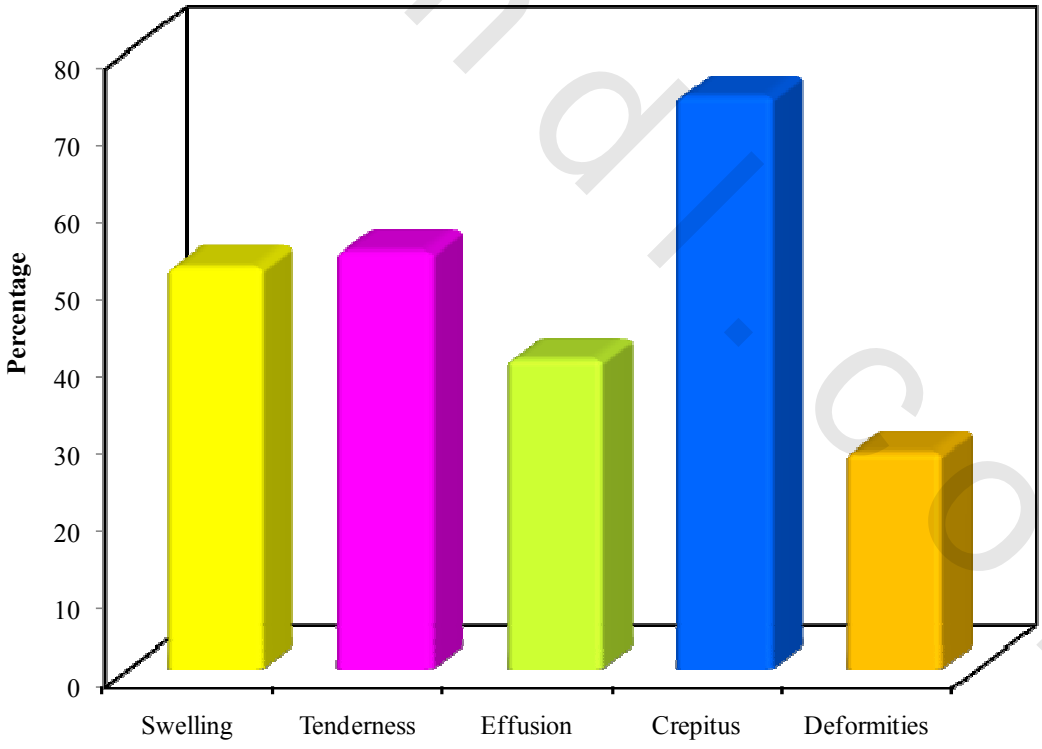

Figure (18) : Distribution of studied cases according to clinical findings

Descriptive analysis of the studied groups according to VAS (visual analogue scale):

VAS is a standard 100 mm horizontal scale on which the patient indicates the severity of pain by placing a mark between terminal points designed "no pain " and "extreme pain**".**

There was statistically significant difference between the studied groups as regards their VAS (P< 0.001). **Table (X), Figure 19**

Table (X): Comparison between the two studied groups according to VAS

	Group I (n = 50)	Group II (n = 20)	Z	P
VAS (mm)				
Min. – Max.	20.0 – 65.0	1.0 – 10.0		
Mean ± SD.	43.30 ± 14.62	5.35 ± 2.76	6.523*	<0.001*
Median	42.50	5.50		

p: p value for Mann Whitney test
*: Statistically significant at p ≤ 0.05

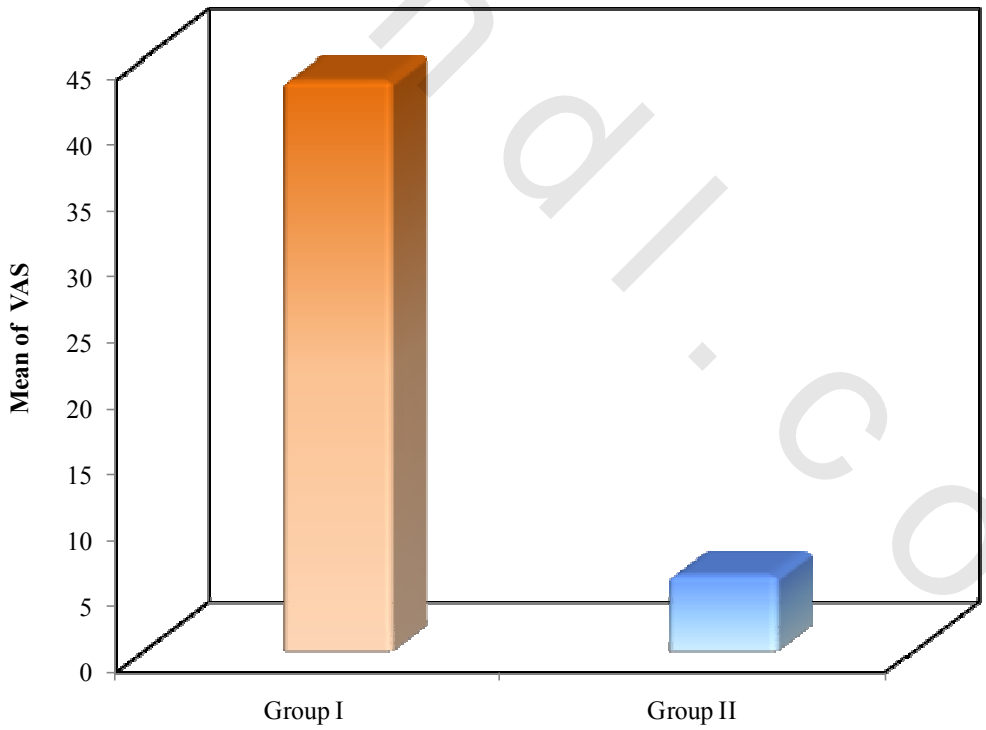

Figure (19) Comparison between the two studied groups according to VAS

Functional assessment of knee OA:

The functional level was assessed in group A and group B using WOMAC scale for function.the median WOMAC scale 1n patients was 42 (ranged from 20 – 65) and that of the control group was 7. There was significant decrease in the functional level in patients in comparison to the control group ($p < 0.001$). **Table (XI), Figure 20**

Table (X1): Comparison between the two studied groups according to WOMAC

	Group I (n = 50)	Group II (n = 20)	Z	P
WOMAC				
Min. – Max.	20.0 – 65.0	1.0 – 13.0		
Mean ± SD.	41.80 ± 14.45	6.80 ± 3.53	6.521*	<0.001*
Median	42.50	6.50		

: p value for Mann Whitney test
*: Statistically significant at $p \leq 0.05$

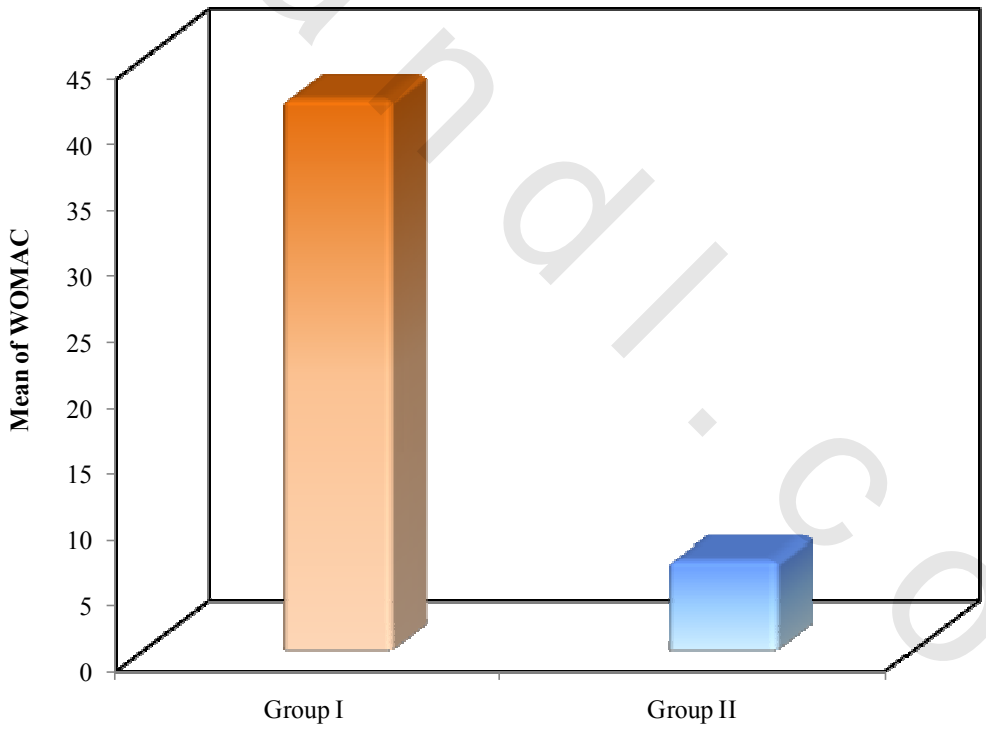

Figure (20): Comparison between the two studied groups according to WOMAC

Descriptive analysis of the studied groups according to lequsene index

The lequsene index was assessed in group A and group B, the mean 1n patients was 11.4 (ranged from 8-17) and that of the control group was 0.5 ranged from (0- 2). there was significant difference between 2 groups as regard their index ($p < 0.001$) **Table (XII), Figure 21**

Table (XII): Comparison between the two studied groups according to lequsene index

	Group I (n = 50)	Group II (n = 20)	Z	p
Lequesne index				
Min. – Max.	8.0 – 17.0	0.0 – 2.0		
Mean ± SD.	11.48 ± 2.43	0.50 ± 0.61	6.549*	<0.001*
Median	11.50	0.0		

Z: Z for Mann Whitney test
*: Statistically significant at $p \leq 0.05$

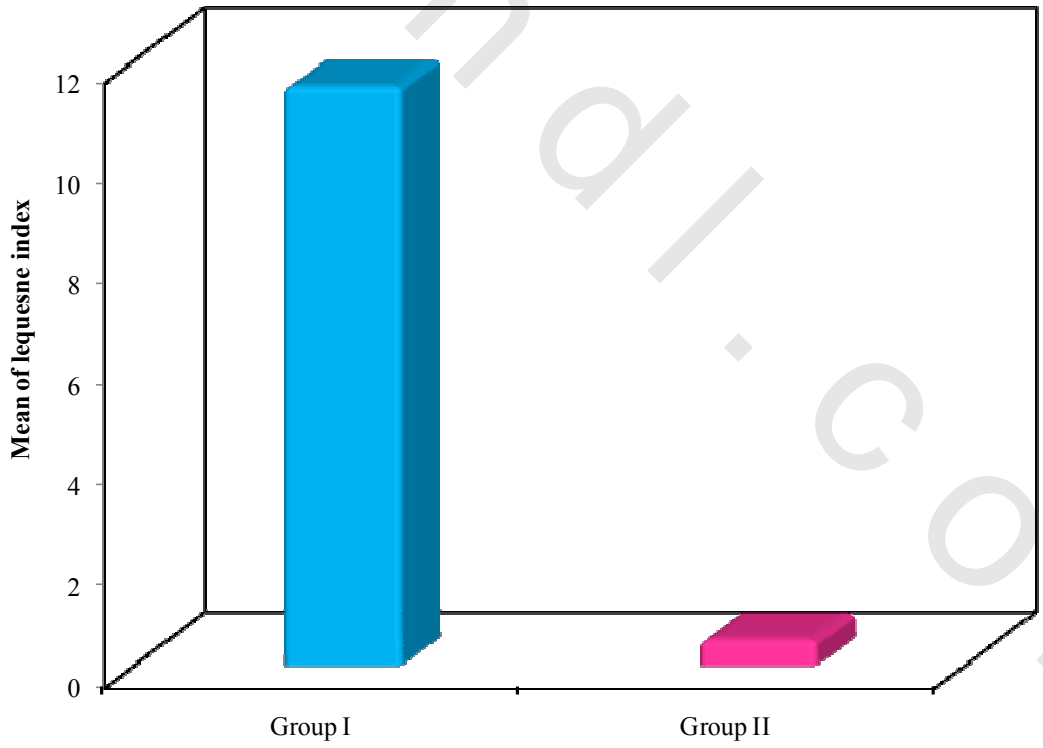

Figure (21): Comparison between the two studied groups according to lequsene index

Descriptive analysis of the studied groups according to ESR

In group **1**, the median ESR was 18 mm/h ranged from 10 to 26 mm/h, while the median ESR of group II was 11mm/h ranged from 6 to 16 mm/h **Table (XIII), Figure (22)**

There was statistically significant difference between group **I** and group **II** as regards to ESR (P = 0.001).

Table (XIII): Comparison between the two studied groups according to ESR

	Group I (n = 50)	Group II (n = 20)	Test of sig.	P
ESR				
Min. – Max.	10.0 – 26.0	6.0 – 16.0		
Mean ± SD.	18.40 ± 3.66	11.10 ± 2.81	t= 8.015*	<0.001*
Median	18.0	11.0		

t: Student t-test
*: Statistically significant at p ≤ 0.05
p: p value for Chi-square test

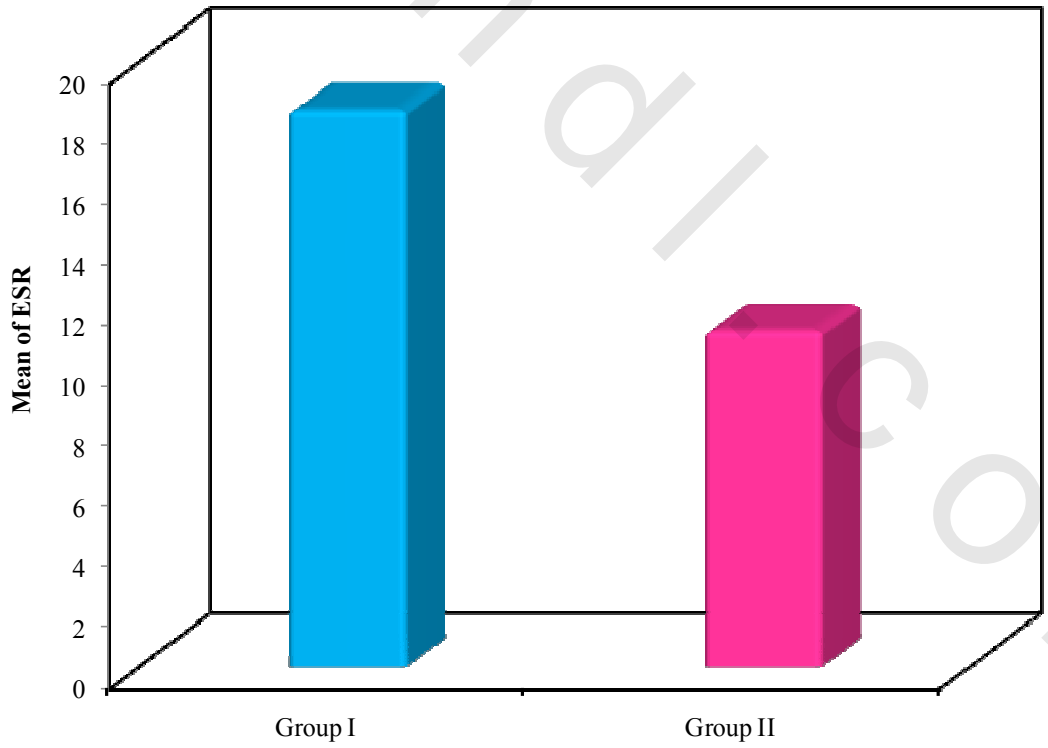

Figure (22): Comparison between the two studied groups according to ESR

Descriptive analysis of the studied groups according to CRP

In group **1**, the median CRP was 3.5 ranged from 1 to 7, while the median CRP of group II was 1 ranged from 0 to 2. **Table (XIV), Figure (23)**

There was statistically significant difference between group **I** and group **II** as regards to CRP (P < 0.001).

Table (XIV): Comparison between the two studied groups according to CRP

	Group I (n = 50)	Group II (n = 20)	Test of sig.	P
CRP				
Min. – Max.	1.0 – 7.0	0.0 – 2.0		
Mean ± SD.	3.68 ± 1.41	1.20 ± 0.52	Z=6.124*	<0.001*
Median	3.50	1.0		

p: p value for Chi-square test

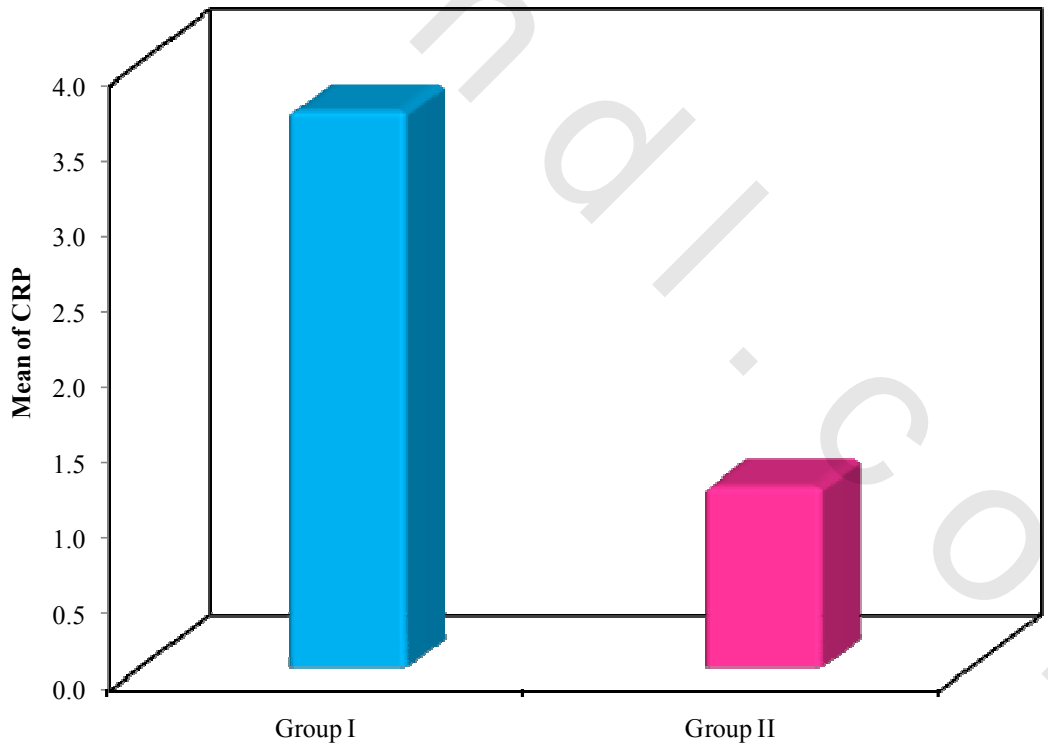

Figure (23): Comparison between the two studied groups according to CRP

Comparison between the two groups according to readings of Serum CTX-II

In the present study the readings of serum CTX-II among the cases of knee osteoarthritis ranged from 120- 600 pg/ml with a mean 249.8 pg/ml and standard deviation 125.5 and a median 200 pg/ml

While among the control group the readings of serum CTX-II ranged from 110 -200 pg/ml with a mean 150.5 and standard deviation 38.8 and a median 130 pg/ml.

The readings of serum CTX-II among cases of knee osteoarthritis were higher in comparison to that of control group and this difference was of high statistical significane ($p<0.001$).**Table (XV), Figure (24)**

Table (XV): Comparison between the two studied groups according to serum CTX-II level

	Group I (n = 50)	**Group II (n = 20)**	**Z**	**P**
Serum CTX-II				
Min. – Max.	120.0 – 600.0	110.0 – 200.0		
Mean ± SD.	249.80 ± 125.56	150.50 ± 38.86	Z=4.069*	<0.001*
Median	200.0	130.0		

Z: Z for Mann Whitney test
*: Statistically significant at $p \leq 0.05$

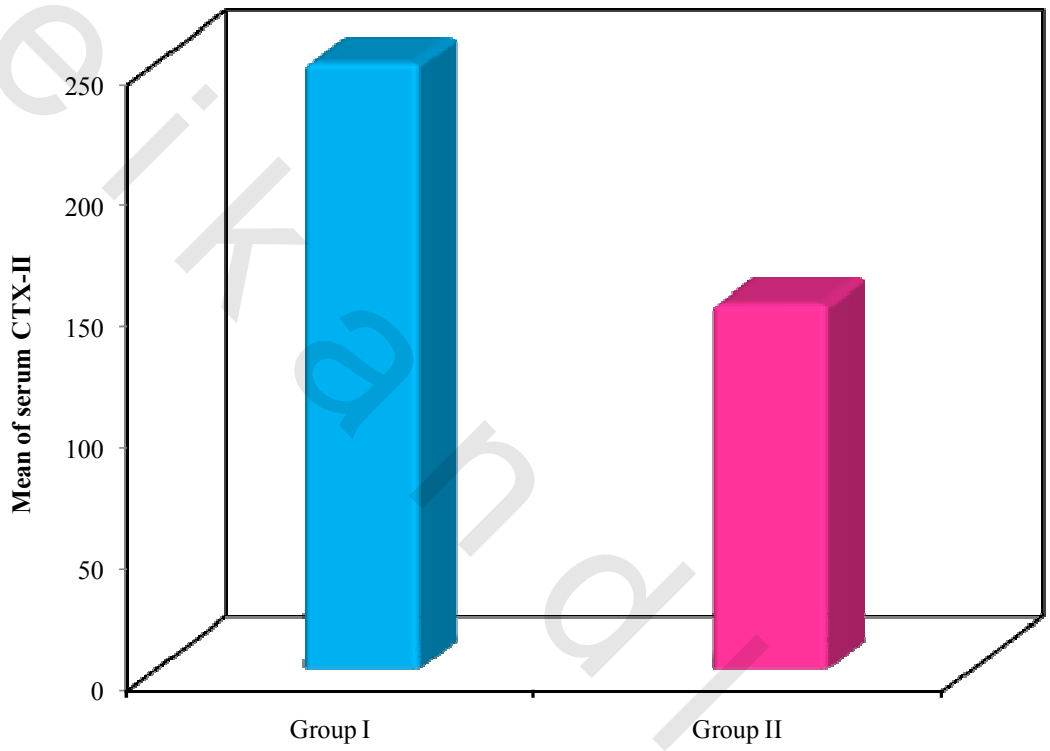

Figure (24): Comparison between the two studied groups according to serum CTX-II level

Distribution of the studied cases according to Synovial CTX-II level

Synovial fluid samples were collected from 15 patients (30%). The level of synovial CTX- II ranged from 200-400 pg/ml with a mean of 238.67 and standard deviation 70 and median 200 pg/ml **Table (XVI), Figure (25)**

Table (XV1): Distribution of the studied cases according to Synovial CTX-II level (n = 15)

	Min. – Max.	Mean ± SD.	Median
Synovial CTX-II	200.0 – 400.0	238.67 ± 70.70	200.0

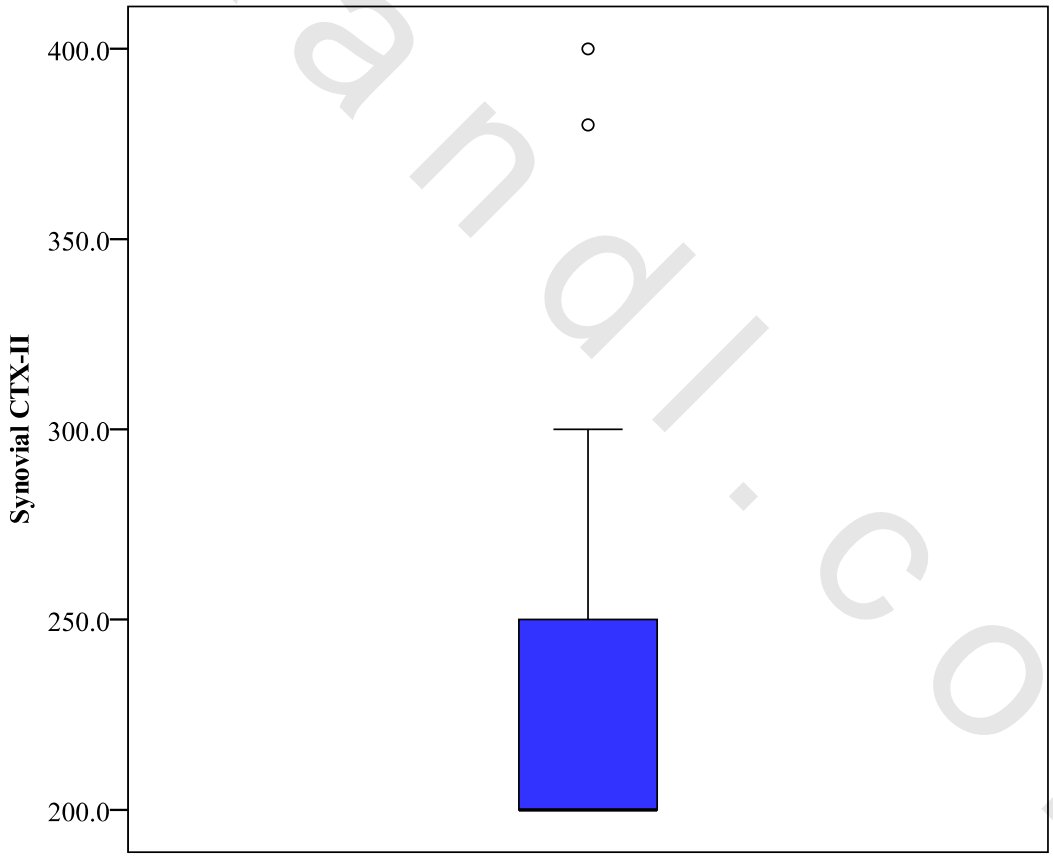

Figure (25): Distribution of the studied cases according to Synovial CTX-II level

Characteristic of patients with Synovial CTX (n = 15)

Synovial CTX-II was measured in 15 patients , 5 were males and 10 were females . mean age was 53.53 years , mean ESR was 20 mm/h, mean CRP was 4.6 unit , mean WOMAC , VAS, Lequsene were (49.3 , 50 ,13.4) respectively .

Table (XVII): Characteristic of patients with Synovial CTX (n = 15)

	No.	%
Sex		
Male	5	33.3
Female	10	66.7
Effusion	15	100.0
Synovitis	11	73.3
Beker cyst	8	53.3
Age (years)	53.53 ± 5.33	
BMI	30.20 ± 0.92	
ESR	20.67 ± 3.98	
CRP	4.67 ± 1.63	
OA grading	3.13 ± 0.74	
WOMAC	49.33 ± 11.93	
VAS	50.0 ± 13.23	
Lequsense	13.47 ± 2.17	
Serume CTX11	391.33 ± 135.69	
Cartilage thickness	2.27 ± 1.03	

Radiological findings

OA was diagnosed by X –ray imaging according to kellgren and Lawrence grading

In group **I**, 10 patients (20%) had doubtful OA, 18 patients (36%) had minimal OA, 15 patients (30%) had moderate OA and 7 patients (14%) had severe OA. **Table (XVII1), Figure (26)**

Table (XII1): Distribution of group I according to radiological score of osteoarthritis

	No.	%
OA		
Grade 1	10	20.0
Grade 2	18	36.0
Grade 3	15	30.0
Grade 4	7	14.0

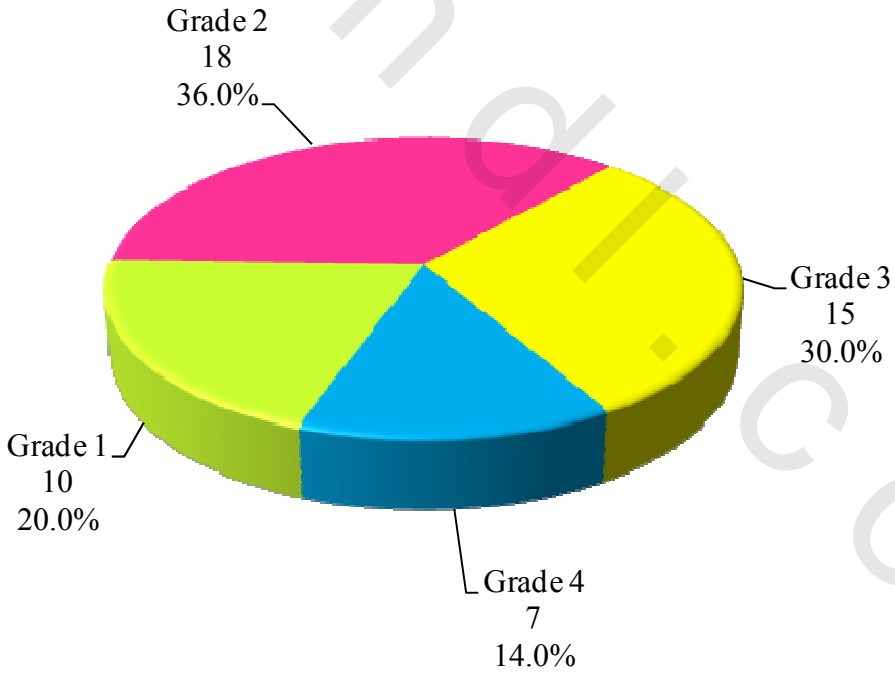

Figure (26): Distribution of group I according to radiological score of osteoarthritis

Musculoskeletal Ultrasound findings

1- Effusion

In group **1**, 30 patients (60%) had effusion detected by ultrasound and this was statistically significant with p value less than 0.001. **Table (XIX), Figure (27)**

2- Synovitis

In group **1**, 26 patients (52%) had low grade synovitis detected by ultrasound and this was statistically significant with p value less than 0.001. **Table (XIX), Figure (27)**

3- Baker's cyst

In group **1**, 10 patients (20%) had baker cyst detected by ultrasound and there was no statistically significant difference, with p value = 0.053. **Table (XIX), Figure (27)**

4- Femoral condyle cartilage thickness

In group **1**, the measurements of cartilage thickness ranged from 1- 5 mm with a mean 2.9 mm and standard deviation 1.04 and a median 3mm.

While among the control group, the measures ranged from 5.5 -6.5 mm with a mean 6 mm and standard deviation 0.26 and a median 6mm.

There was statistically significant difference between the 2 groups as regard the cartilage thickness with p value less than 0.001. **Table (XIX), Figure (28)**

Table (X1X): Comparison between the two studied groups according to ultrasound findings

	Group I (n = 50)		Group II (n = 20)		χ^2	P
	No.	%	No.	%		
Effusion						
Absent	20	40.0	20	100.0	21.000*	<0.001*
Present	30	60.0	0	0.0		
Synovitis						
Absent	24	48.0	20	100.0	16.545*	<0.001*
Present	26	52.0	0	0.0		
Baker`s cyst						
Absent	40	80.0	20	100.0	4.667	FEp=0.053
Present	10	20.0	0	0.0		
Cartilage thickness						
Min. – Max.	1.0 – 5.0		5.50 – 6.50			
Mean ± SD.	2.90 ± 1.04		6.03 ± 0.26		Z= 6.650*	<0.001*
Median	3.0		6.0			

χ^2: Chi square test
FE: Fisher Exact test
Z: Z for Mann Whitney test
*: Statistically significant at $p \leq 0.05$

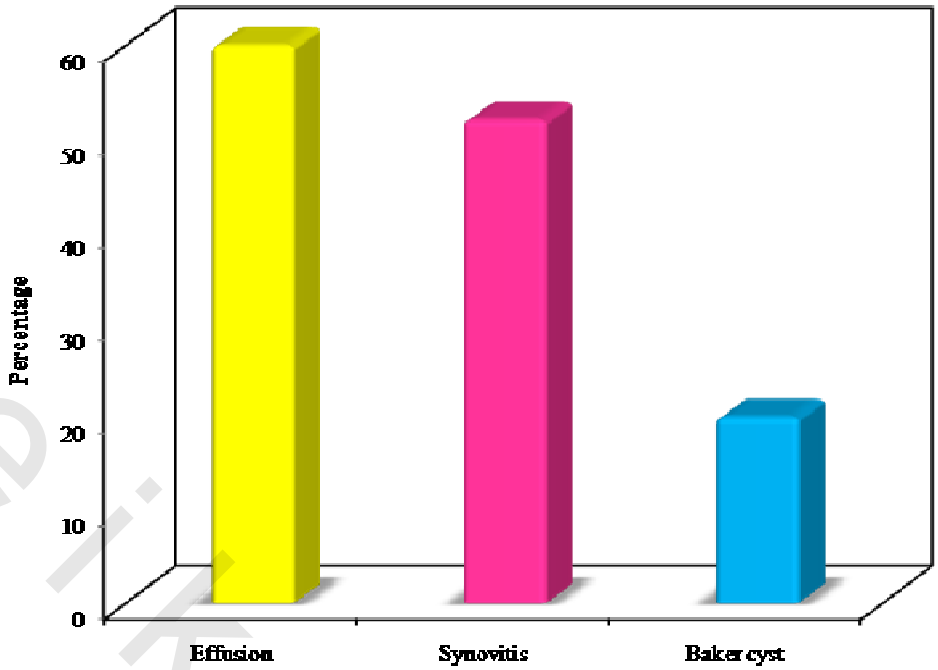

Figure (27): Comparison between the two studied groups according to ultrasound findings

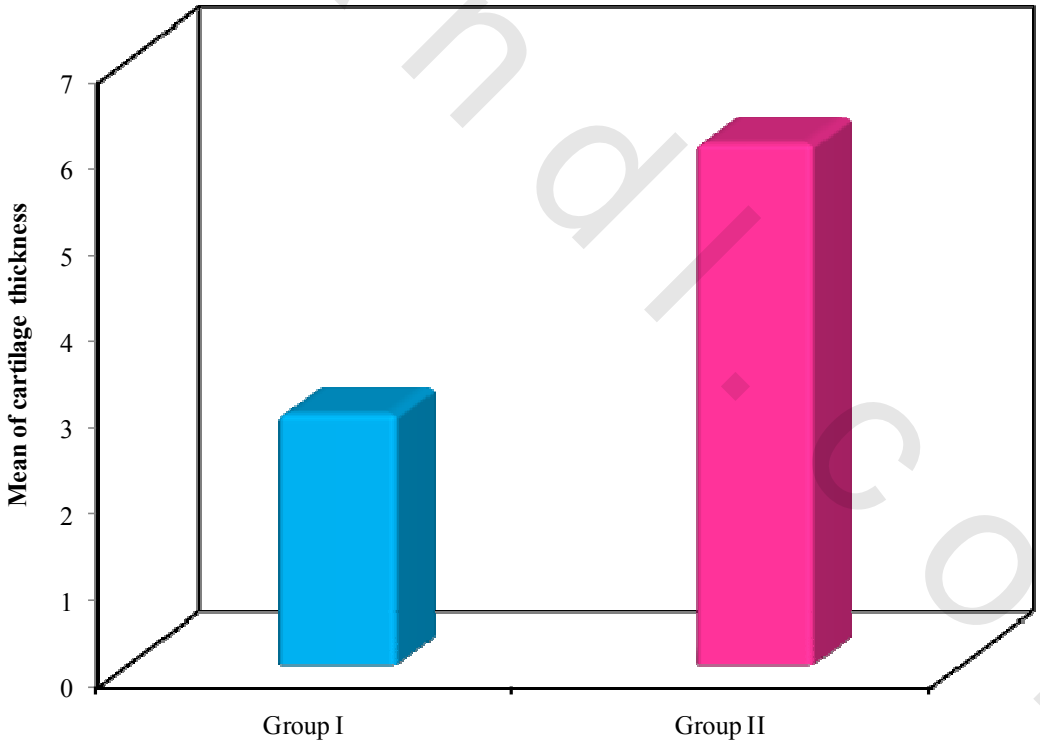

Figure (28): Comparison between the two studied groups according to cartilage thickness

Correlation between serum CTX-II and OA grading (K-L scale)

In the present study there was positive correlation of high statistical significance between serum CTX-II and radiological grading of osteoarthritis (r =+0.348, p =0.013) **Table (XX), Figure (29)**

Table (XX) : Correlation between Serum CTX-II with OA grading

	Serum CTX-II	
	r_s	P
OA	0.348*	0.013

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

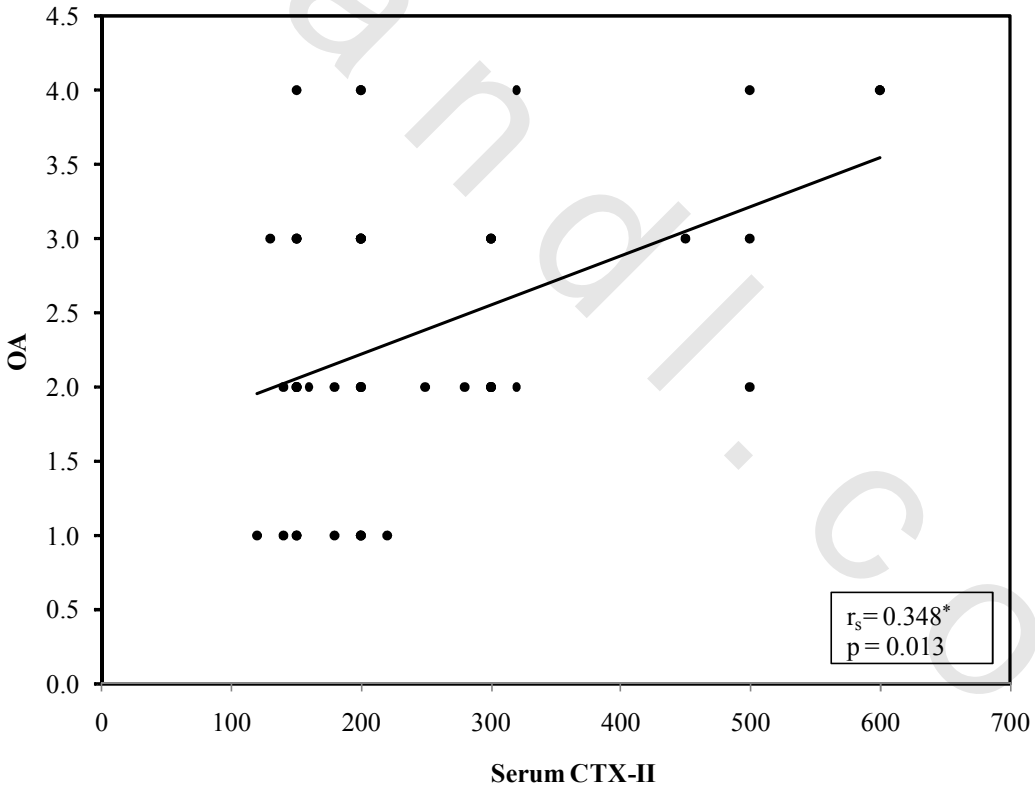

Figure (29): Correlation between Serum CTX-II with OA in group I.

Level of serum CTX-II in different grades of osteoarthritis

In grade 1: readings of CTX-II ranged from 120-220 pg/ml with a median of 190 pg/ml, this readings were lower than readings in grade 4, while of little difference with grade 2 and grade 3. so So there was high statistical significance according to CTX-II level between grade 1 and grade 4 (p=0.028). But there was no statistical significance difference between grade 1 and grade 2, 3 (p=0.108), (0.087) respectively

In grade 2: readings of CTX-II ranged from 140 to 500 pg/ml with a median 200,while in grade 3 the readings ranged from 130 to 500 pg/ml with a median 200. In grade 4 the readings ranged from 150 to 600 pg/ ml with a median 320. **Table (XXI), figure(30**)

Table (XXI):Level of serum CTX-II in different grades of OA in group I

	OA grading				$^{KW}\chi^2$	P
	Grade 1 (n =10)	**Grade 2 (n =18)**	**Grade 3 (n =15)**	**Grade 4 (n=7)**		
Serum CTX-II						
Min. – Max.	120.0 – 220.0	140.0 – 500.0	130.0 – 500.0	150.0 – 600.0		
Mean ± SD.	176.0 ± 33.4	235.0 ± 92.9	262.0 ± 127.4	367.1 ± 196.3	6.887	0.076
Median	190.0	200.0	200.0	320.0		
Sig.bet. Grps	$p_1 = 0.108, p_2 = 0.087, p_3 = 0.028^*$					

$KW\chi^2$: Chi square for Kruskal Wallis test
p_1 : p value for Mann Whitney test for comparing between grade 1 and grade 2
p_2 : p value for Mann Whitney test for comparing between grade 1 and grade 3
p_3 : p value for Mann Whitney test for comparing between grade 1 and grade 4

*: Statistically significant at $p \leq 0.05$

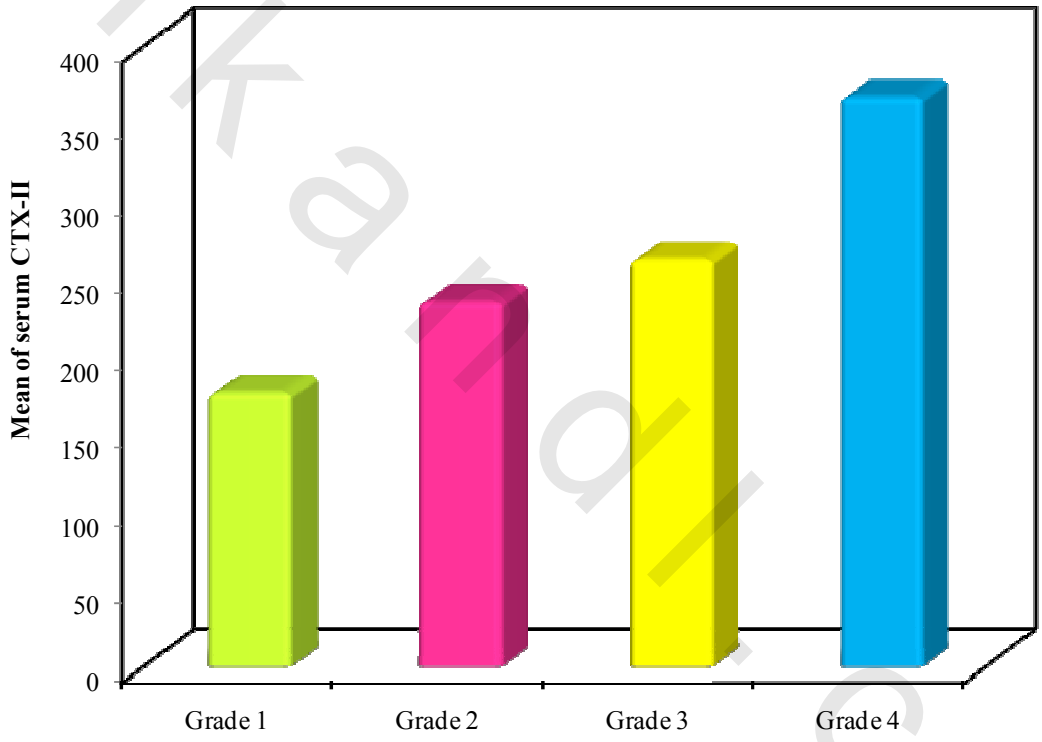

Figure (30): Level of serum CTX-II in different grades of OA in group I

Comparison between serum CTX-II level and ultrasound findings in cases group

In cases with effusion, the median serum ctx-II was 275 ranged from 140-600 with a mean 299 and standard deviation 137, while in cases without effusion the mean was 175.5 and 46 standard deviation. there was statistically significant difference between them (p <0.001) **Table (XXII), figure(31,32)**

In cases with synovitis, the median serum ctx-II was 200 ranged from 140-600 with a mean 290 and standard deviation 154, while in cases without synovitis the mean was 205 and 61 standard deviation. there was no statistically significant difference between them (p 0.076) **Table (XXII), figure(31,32**)

In cases with baker`s cyst, the median serum ctx-II was 300 ranged from 140-600 with a mean 328 and standard deviation 153, while in cases without baker`s cyst the mean was 230 and 111 standard deviation. There was statistically significant difference between them (p 0.002) **Table (XXII), figure(31,32**)

Table (XXII): Comparison between serum CTX-II level with Effusion, Synovitis and Baker cyst in group I (n = 50)

	N	Serum CTX-II			Z	P
		Min. – Max	Mean ± SD.	Median		
Effusion						
Absent	**20**	120.0 – 300.0	175.50 ± 46.62	150.0	4.058*	<0.001*
Present	**30**	140.0 – 600.0	299.33 ± 137.36	275.0		
Synovitis						
Absent	**24**	120.0 – 300.0	205.42 ± 61.78	200.0	1.777	0.076
Present	**26**	140.0 – 600.0	290.77 ± 154.11	200.0		
Baker`s cyst						
Absent	**40**	120.0 – 600.0	230.25 ± 111.39	200.0	2.331*	0.020*
Present	**10**	160.0 – 600.0	328.0 ± 153.54	300.0		

Z: Z for Mann Whitney test
*: Statistically significant at p ≤ 0.05

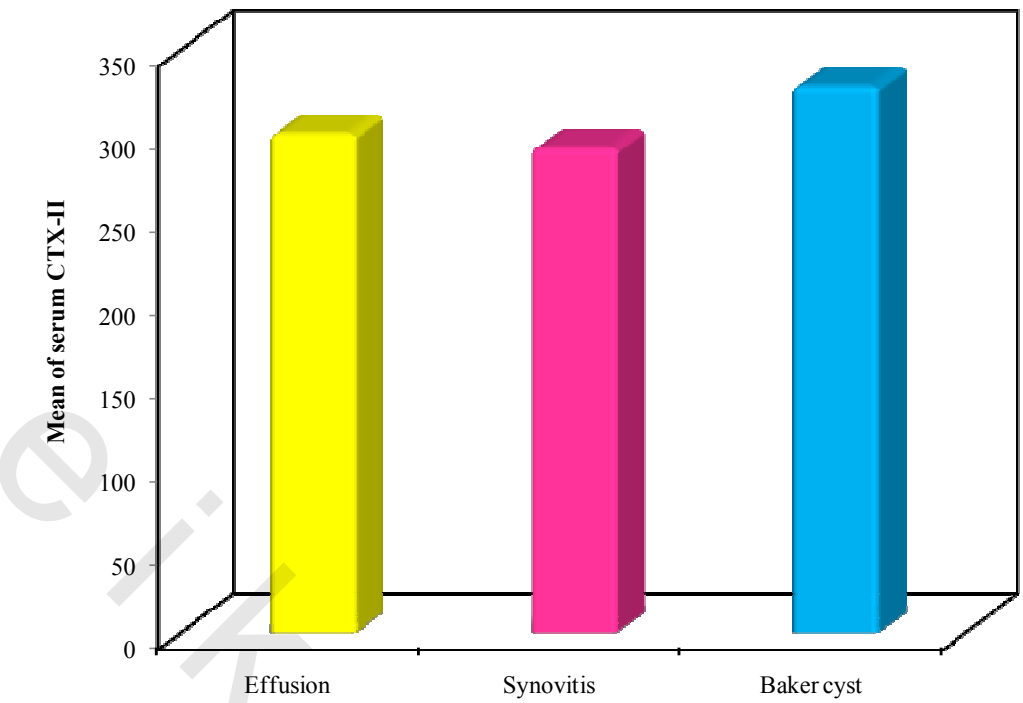

Figure (31): Relation between serum CTX-II with Effusion, Synovitis and Becker cyst in group A

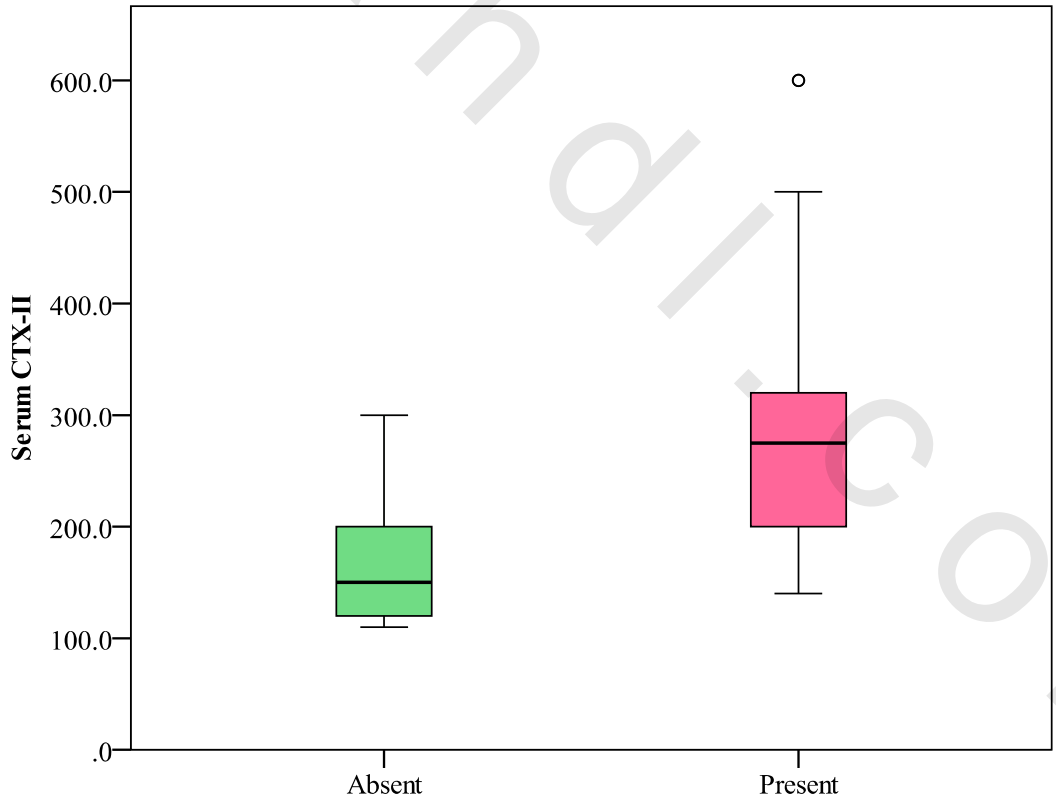

Figure (32): Relation between serum CTX-II with Effusion, Synovitis and Baker cyst in group I

Correlation between Serum CTX-II with different parameters in group I

Serum CTX-II was significantly positive correlated with ESR (r = +0.44, p = 0.001),CRP (r = +0.541, p = 0.001), OA grade (r = +0.348, p = 0.013) and Lequesne index (r = +0.378, p = 0.007). No correlation was found between CTX-II and VAS, WOMAC and cartilage thickness. **Table (XXIII), figure(33,34,35**)

Table (XXIII):Correlation between Serum CTX-II with different parameters in group I (n = 50)

	Serum CTX-II	
	r_s	P
Age	-0.225	0.116
ESR	0.440*	0.001
CRP	0.541*	<0.001
OA	0.348*	0.013
WOMAC	0.099	0.496
VAS	0.155	0.284
Lequesne	0.378*	0.007
BMI	0.217	0.130
Synovial ctx	0.430	0.109
Cartilage thickness	-0.230	0.109

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

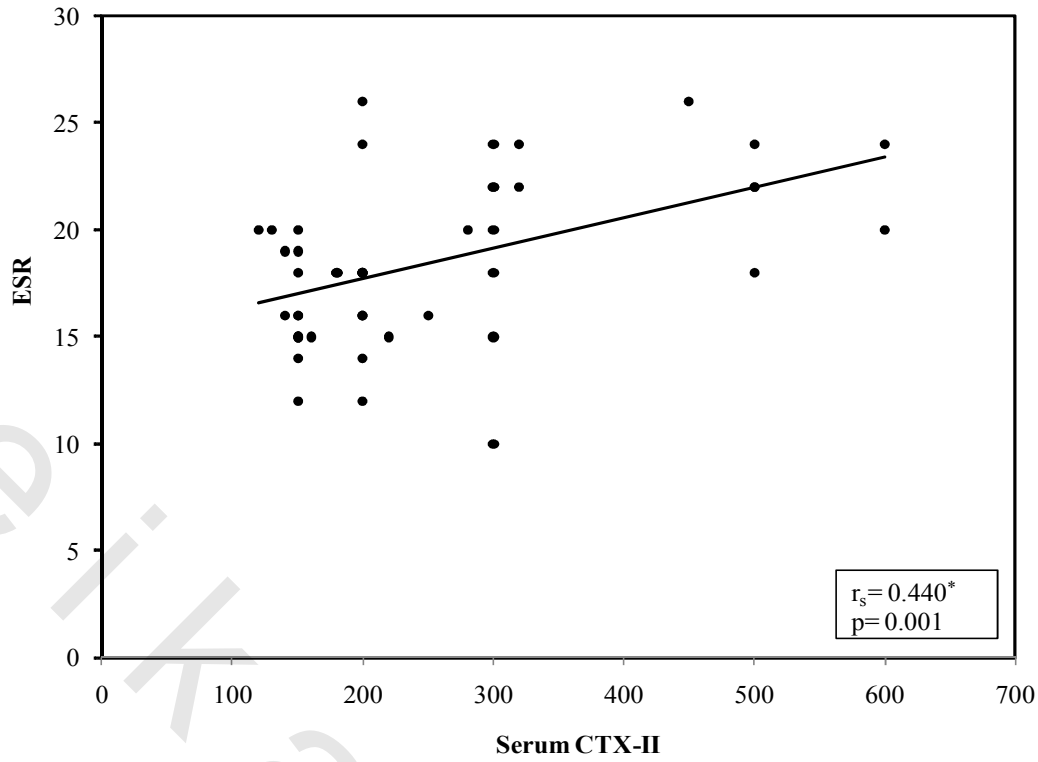

Figure (33): Correlation between Serum CTX-II with ESR in group I

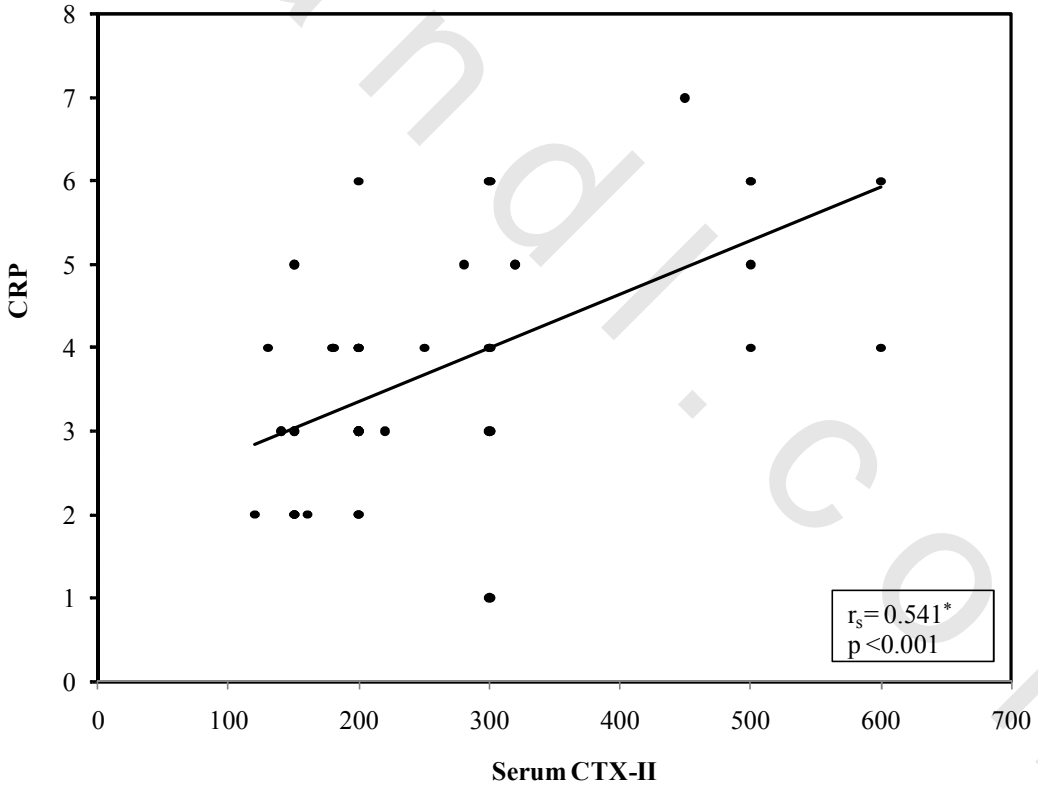

Figure (34): Correlation between Serum CTX-II with CRP in group I

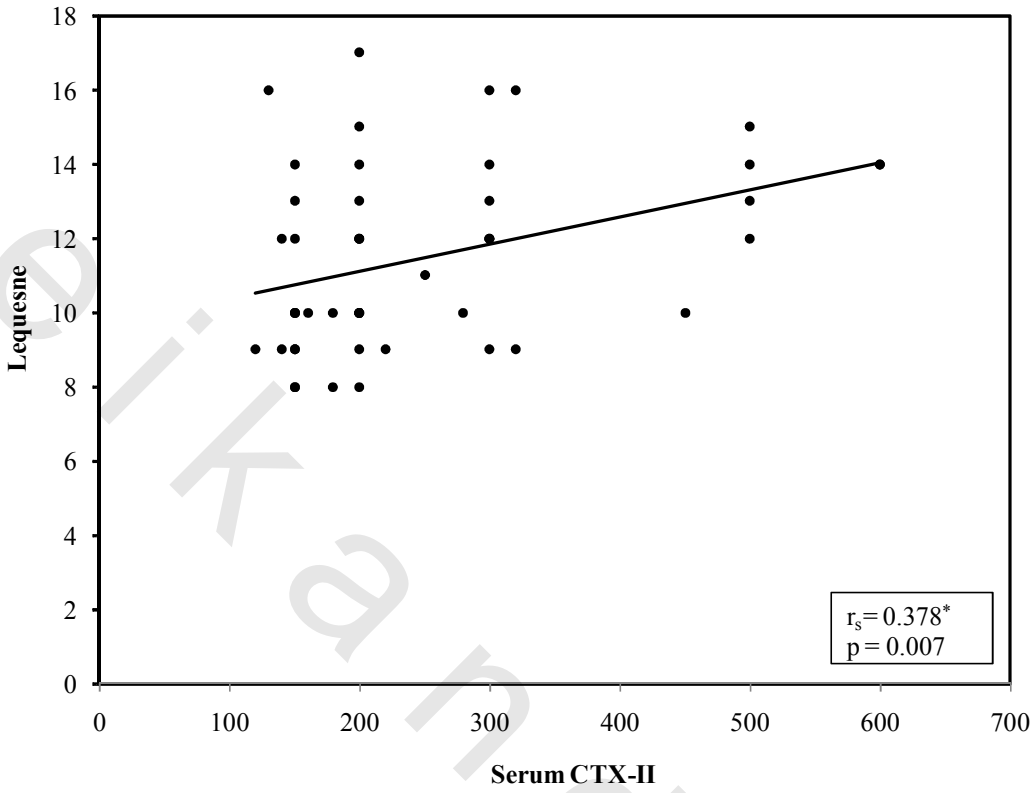

Figure (35): Correlation between Serum CTX-II with lequesne in group I

Relation between OA grading with WOMAC, VAS and Lequesne in group I

In grade 1 OA: mean of WOMAC,VAS, Lequesne was (24.5, 34, 9.4) respectively.

In grade 2 OA: mean of WOMAC,VAS, Lequesne was (36.3, 32, 9.4) respectively.

In grade 3 OA : mean of WOMAC,VAS, Lequesne was (51.3, 54, 12.9) respectively.

In grade 4 OA : mean of WOMAC,VAS, Lequesne was (60, 60.7, 14.4) respectively.

Table (XIVX): Relation between OA grading with WOMAC, VAS and Lequesne in group I

	OA grading				$^{KW}\chi^2$	P
	Grade 1 (n =10)	**Grade 2 (n =18)**	**Grade 3 (n =15)**	**Grade 4 (n=7)**		
WOMAC						
Min. – Max.	20.0 – 30.0	20.0 – 50.0	20.0 – 65.0	55.0 – 65.0		
Mean ± SD.	24.50 ± 3.69	36.39 ± 8.88	51.33 ± 10.43	60.0 ± 2.89	34.477*	<0.001*
Median	25.0	40.0	55.0	60.0		
VAS						
Min. – Max.	25.0 – 45.0	20.0 – 45.0	25.0 – 65.0	55.0 – 65.0		
Mean ± SD.	34.0 ± 6.99	32.22 ± 8.26	54.67 ± 10.77	60.71 ± 4.50	30.474*	<0.001*
Median	35.0	30.0	55.0	60.0		
Lequesne						
Min. – Max.	8.0 – 10.0	8.0 – 14.0	10.0 – 17.0	13.0 – 16.0		
Mean ± SD.	9.40 ± 0.70	10.39 ± 1.79	12.93 ± 2.40	14.14 ± 0.90	25.431*	<0.001*
Median	9.50	10.0	13.0	14.0		

$KW\chi^2$: Chi square for Kruskal Wallis test
*: Statistically significant at $p \leq 0.05$

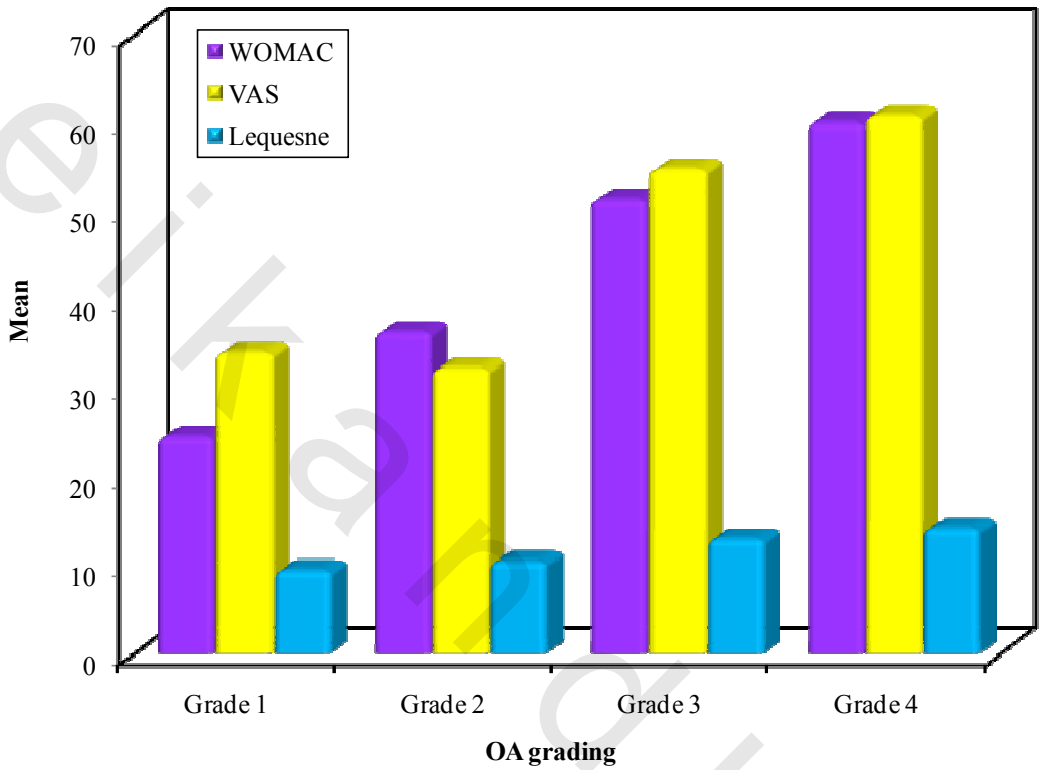

Figure (36): Relation between OA grading with WOMAC, VAS and Lequesne in group A

Correlations between cartilage thickness with WOMAC, VAS and lequesne Index

In the present study there was negative correlation of high statistical significance between cartilage thickness with WOMAC (r_s -0.705, P <0.001), VAS (r_s -0.596, P <0.001) and lequesne(r_s -0.650, P <0.001) **Table (XVX), Figure (37)**

Table (XVX): Correlations between cartilage thickness with WOMAC, VAS and lequesne

	Cartilage thickness	
	r_s	p
WOMAC	-0.705	<0.001*
VAS	-0.596	<0.001*
Lequesne	-0.650	<0.001*

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

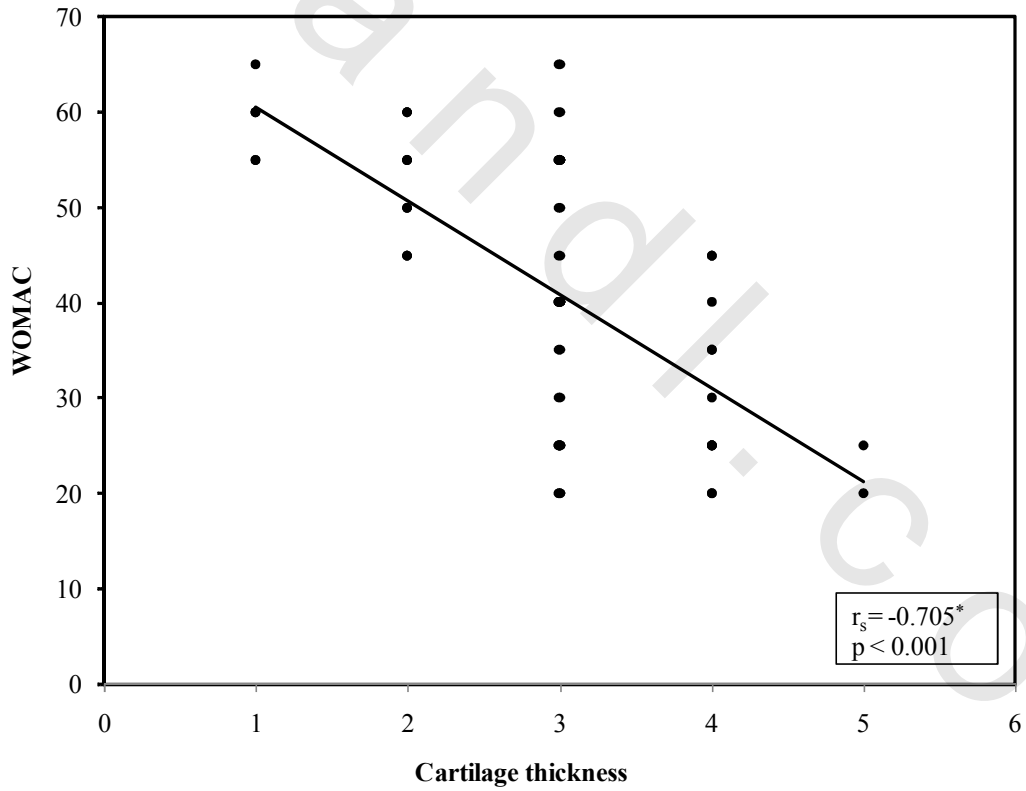

Figure (37): Correlations between cartilage thickness with WOMAC in group I

Relation between OA grading and cartilage thickness

In grade 1 OA, the mean cartilage thickness was 4 mm, in grade 2 OA the mean was 3.28mm, while in grade 3 OA the mean was 2.47mm and in grade 4 OA the mean thickness was 1.29mm.**Table (XXVI), Figure (38)**

Table (XXVI): Relation between OA and cartilage thickness

	OA grading				$^{KW}\chi^2$	P
	Grade 1 (n =10)	**Grade 2 (n =18)**	**Grade 3 (n =15)**	**Grade 4 (n=7)**		
Cartilage thickness						
Min. – Max.	3.0 – 5.0	2.0 – 4.0	1.0 – 3.0	1.0 – 2.0		
Mean ± SD.	4.0 ± 0.67	3.28 ± 0.57	2.47 ± 0.64	1.29 ± 0.49	32.535*	<0.001*
Median	4.0	3.0	3.0	1.0		

$KW\chi^2$: Chi square for Kruskal Wallis test
*: Statistically significant at $p \leq 0.05$

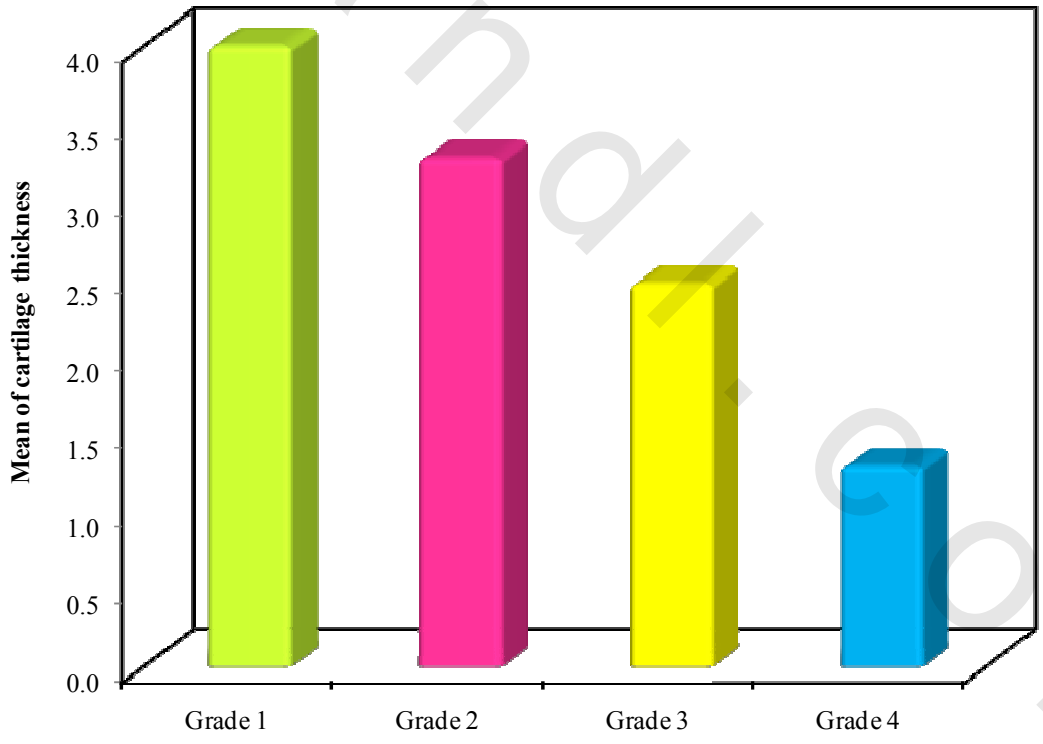

Figure (38): Relation between OA and cartilage thickness

Relation between OA grading and Ultrasound findings

In grade 1 OA : 6 patients had effusion, 3 patients had synovitis detected by ultrasound . In grade 2 OA ; 7 patients had effusion , 11 patients had synovitis 2 patients had Baker`s cyst while in grade 3 OA ; 10 patients had effusion .6 cases had synovitis , 4 patients had Baker`s cyst and in grade 4 OA ; 7 patients had effusion , 6 patients had synovitis and 4 patients had Baker`s cyst.

Table (XXVII): Relation between OA grading and Ultrasound findings

	OA grading								χ^2	^{MC}p
	Grade 1 (n =10)		Grade 2 (n =18)		Grade 3 (n =15)		Grade 4 (n=7)			
	No	%	No	%	No	%	No	%		
Effusion										
Absent	4	40.0	11	61.1	5	33.3	0	0.0	8.356*	0.038*
Present	6	60.0	7	38.9	10	66.7	7	100.0		
Synovitis										
Absent	7	70.0	7	38.9	9	60.0	1	14.3	6.339	0.083
Present	3	30.0	11	61.1	6	40.0	6	85.7		
Baker cyst										
Absent	10	100.0	16	88.9	11	73.3	3	42.9	8.545*	0.022*
Present	0	0.0	2	11.1	4	26.7	4	57.1		

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

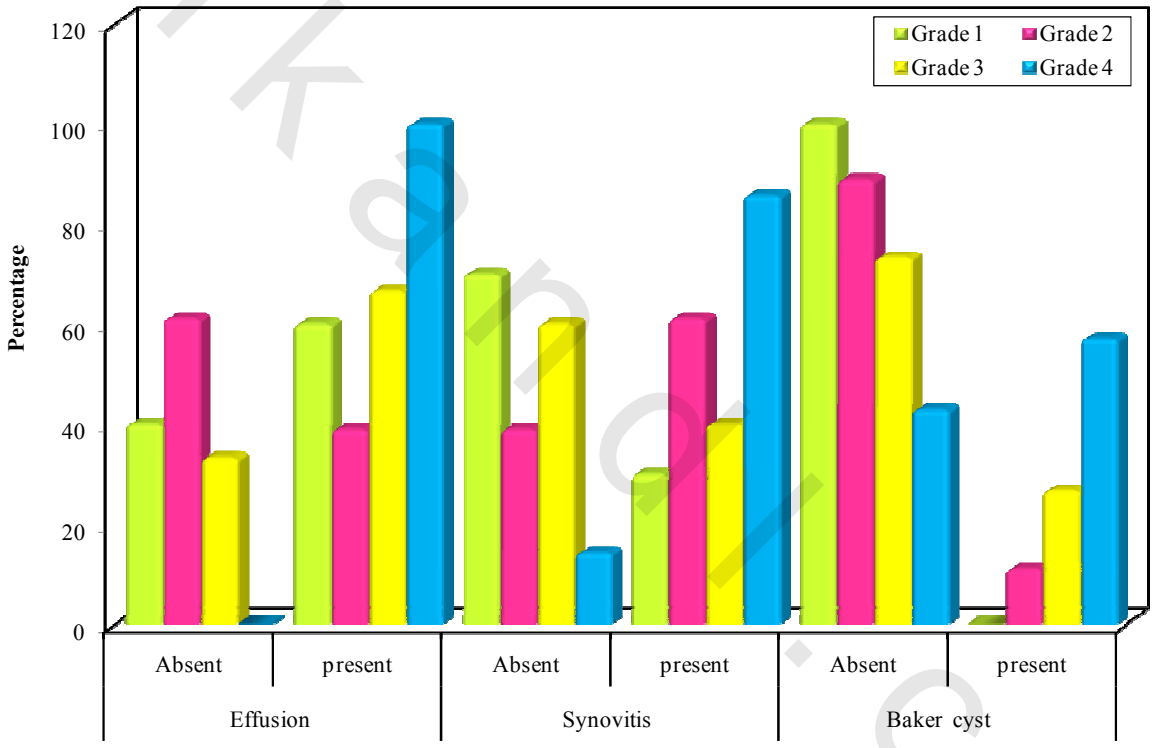

Figure (39):Relation between OA grading and Ultrasound findings.

Table (XXVIII): Relation between synovial CTX-II with effusion, Synovitis and baker cyst

	Synovial CTX		Z	p
	Absent	**Present**		
Effusion				
Min. – Max.	0 – 0.0	200.0 – 400.0		
Mean ± SD.	0 ± 0.0	238.67 ± 70.70	-	-
Median	0.0	200.0		
Synovitis	**(n=4)**	**(n=11)**		
Min. – Max.	200.0 300.0	200.0 – 400.0		
Mean ± SD.	225.0 ± 50.0	243.064 ± 78.39	0.252	0.801
Median	200.0	200.0		
Baker cyst	**(n=7)**	**(n=8)**		
Min. – Max.	200.0 – 400.0	200.0 – 380.0		
Mean ± SD.	228.57 ± 75.59	247.50 ± 70.05	0.744	0.457
Median	200.0	200.0		

Z: Z for Mann Whitney test