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STUDY HISTOPATHOLOGICAL CHANGES IN THE ANTERIOR AND POSTERIOR CRUCIATE LIGAMENT AFTER KNEE REPLACEMENT: CORRELATIONS WITH VITAMIN D, CALCIUM AND C- REACTIVE PROTEIN IN IRAQI PATIENTS WITH OSTEOARTHRITIS

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ABSTRACT

Osteoarthritis (OA) is a chronic disease and the most common arthritis forms which involving deterioration of articular cartilage. The present study is a case control study aimed to examine some histopathological change with knee OA patients and biochemical test in both patients and controls. Tissue samples anterior cruciate ligament and Posterior cruciate ligament (ACL, PCL) obtained from 50 knee OA patients (35 females and 15 males) during total knee replacement for histological study. Blood sample were obtained from 50 knee OA patients and 25 controls. The tissue sample and whole blood were collected during the period from October 2015 to June 2016.

In the current study, there are many histopathological changes in knee OA tissues (ACL and PCL) such as congestion of blood vessels, edema, fibrocystic proliferation and infiltration of inflammatory cells. Likewise, the assessment of vitamin D revealed that there was significant difference ($p < 0.05$) between (Healthy or Patients). Although vitamin D concentration was lower in patients than controls but the difference was not statistically significant, but there is a significant difference in serum vitamin D concentration between male OA patient and female OA patient. Also, there was a significant decrease ($p < 0.05$) in serum Calcium concentration of males in patients group when compared with males in controls group. Calcium concentration showed no-significant difference between male and female in patients group. On the other hand, there was no significant difference in concentration of C-reactive protein (CRP) among all groups.

Key words: Osteoarthritis, ACL, PCL, vitamin D, knee joint; calcium con. And CRP

1-INTRODUCTION:

The knee joint is the largest and most heavily synovial joint in the musculoskeletal system of human body, in which it supports the body weight and facilitates locomotion (Donlagic, *et al.*, 2008).

The cruciate ligaments are a main part of the knee's structure and can be found within the joint cavity, these paired ligaments act as joint stabilizers and movement restrainers. The anterior cruciate ligament (ACL) together with the posterior cruciate ligament (PCL) plays an essential role in the physiological kinematics of the knee joint (Kweon, *et al.*, 2013).

The OA is a progressive degenerative multifactorial disease where both environmental and genetic factors play major roles in its etiology. The OA can affect all joints in the human body, but the knees, hips and hands are most commonly affected. OA of the knees has the greatest impact on disability and the most common parts to be affected are the tibiofemoral and lateral patellofemoral compartments. It affects every tissue present in the synovial joint, slowly destroys the articular cartelage that lines the knee joints and the subchondral bone surfaces and synovium in weight bearing joints and frequently used upper extremity joints (Lane 2007).

Symptomatically, patients with OA are characterized by joint pain, loss of motion, weakness, and joint instability and stiffness all causing disability and functional limitation (Martel-Pelletier and Pelletier 2010). Pain is the most notable symptom of OA, resulting in reduced involvement in activities and bad effects on sleep, mood and quality of life (Shimura, *et al.*, 2013).

Vitamin D is the "sunshine vitamin" which is converted in the body to a hormone (1, 25-dihydroxyvitamin D3) by the photolytic action of ultraviolet light on the skin. Normal bone and cartilage metabolism depends on adequate levels of vitamin D, also it plays an important role with the essential minerals calcium and phosphorus in the maintenance of healthy bones and teeth. Vitamin D deficiency is a common problem affecting approximately 1 billion people worldwide(Adams and Hewison 2010).

Calcium is important for bone enhancement. So an insufficient intake when bones are growing and developing, they may never reach full strength or peak bone mass (Ermawati and Wibisono 2017; Klaewklad, *et al.*, 2017). Calcium supplementation has also been involved in the prevention of OA. It is Believes OA is inversely related to bone mineral density (osteoporosis). As bone loses mineral content and becomes softer it may become more deformed leading to pain and inflammation (Power and Scheffer 1999). The objectives of this study were

to identify histological changes in the ACL and PCL from human knee joints and to determine the correlation of Vitamin D, Calcium and C- reactive protein changes in OA patients.

2-PATIENTS AND METHODS

The OA patient subjects recruited to this study from the Nursing Home Hospital and Ghazy Al_ hariri Hospital for surgical specialties/Medical City. The histopathological study was conducted at Al_ Mustansiriya University in the laboratories of Biological Sciences Department during the period from October 2015 to June 2016. Subjects were randomly divided into two groups, control group: 25 healthy subjects and Patients group: which involved 50 patients with OA (35 females and 15 males). The age range was 37-80 years old. Is the OA patients were diagnosed by a specialized doctor?

- **Tissue collection:** The tissue specimens were obtained from 50 OA patients during surgical operations involving total knee replacement includes (ACL and PCL), then they were kept in the fixative solution (formalin10%). After the fixation, sections are processed, embedded in paraffin and 5µm thick glass mounted sections are prepared, which are routinely stained with Haematoxylin and Eosin (H & E) and with Van Gieson's stain according to Bancroft and Stevens.
- **Serum collection:** Whole blood samples were collected from all subjects (healthy controls and patients). By using peripheral vein punctures about 5 ml of blood were aspirate then was dispensed in a non-heparinized plain tube to taken serum. After that separated by centrifugation at 3000 rpm for 5 min, and then they were saved in -20ºC till the time for testing.

Biochemical test:

- **Vitamin D level test** was determined using cobas e-411 Roche which are fully Automated immunoassay analyzers.
- **Calcium level test** was determined by colorimetric method for the using commercially available kit Biomaghreb.
- **CRP** was determined using a rapid latex slide test for the detection of CRP in serum provided by AFCO Company kit.

Statistical Analysis: The Statistical Analysis System (SAS), version 9 (2012) program was used to test the difference factors in study parameters. T-Test was used to test significant difference between means in this study at the $p < 0.05$.

3-RESULT AND DISCUSSION:

3-1 Histopathological study

3-1-1 Anterior cruciate ligament (ACL): All the ACL obtained from the knee OA patients were stained with H & E. light microscope appearance histological changes and different proportions, these changes are:

- Congestion of blood vessels with edema (fig. A 3-1) and Fibrocystic proliferation with disorder emplacing collagen fibers (fig. B 3-1).
- Hemorrhage and mild fibrosis (Fig. 3-2). On the other hand, sections of ACL from the knee OA patients were stained with Van Giesons' shows many changes, these-change are:
- Deposition of fibrin in many areas of ACL section obtained from the knee OA patients with degenerative changes (Fig. A 3-3) and Increase of collagen fibers around blood vessel (Fig. B 3-3).

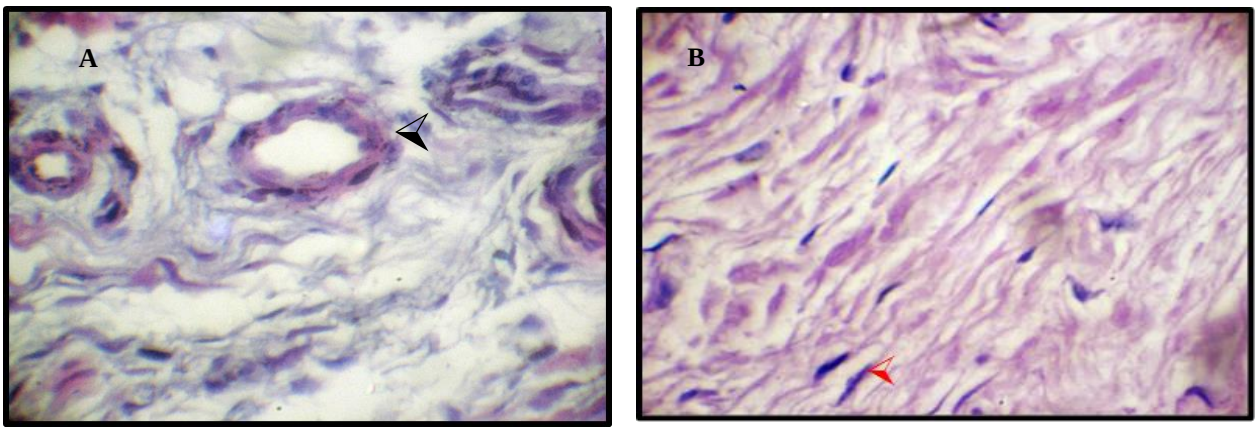

Figure (3-1): Anterior cruciate ligament section of knee OA patient demonstrate, **A:** congestion of blood vessels with edema (head arrow).**B:** disorder emplacing collagen fibers with fibrocystic proliferation (head arrow) (H& E, ×40).

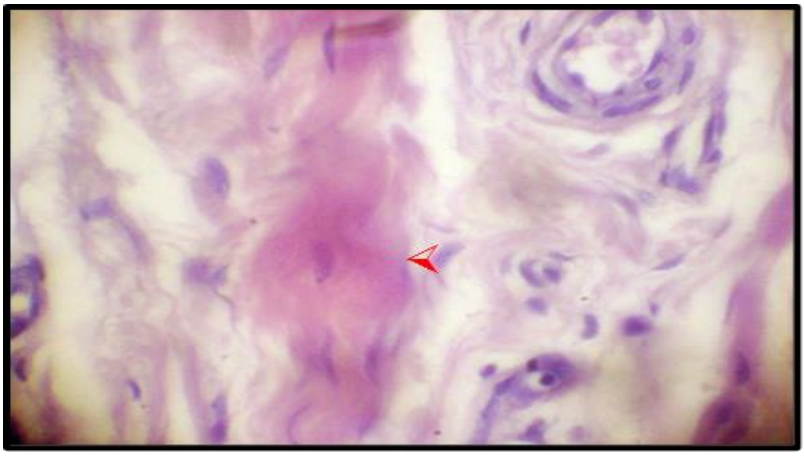

Figure (3-2): Anterior cruciate ligament section of knee OA patient illustrate hemorrhage (head arrow) and mild fibrosis (H&E, ×40).

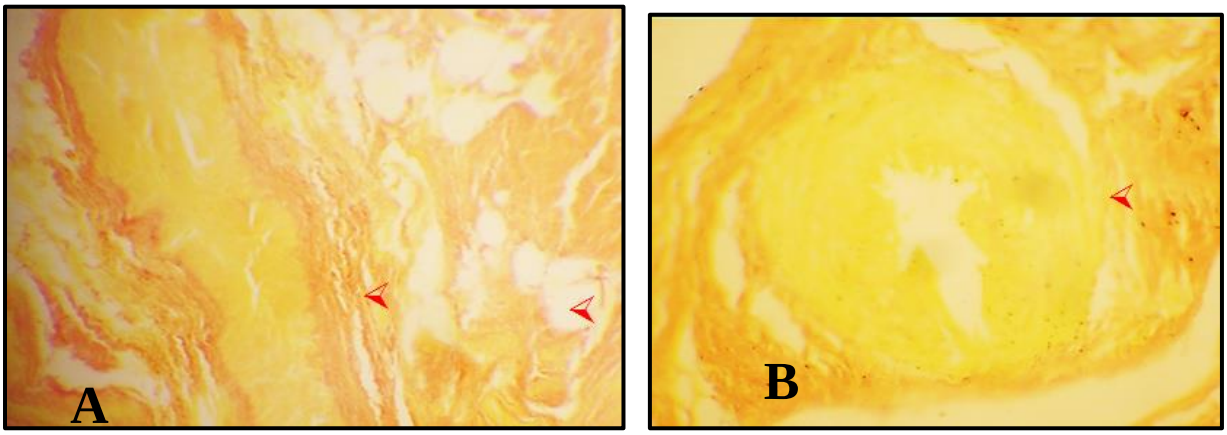

Figure (3-3): Anterior cruciate ligament section of knee OA patient show, **A:** deposition of fibrin in many areas with degenerative changes (orange color) (Van Giesons' staining, x10). **B:** deposition of fibrin around blood vessel (head arrow) (Van Giesons' staining, x40).

The ACL degeneration is observed in most knee joints affected with OA (Mullaji, *et al.*, 2008), ACL rupture considered a risk factor for cartilage degradation and development of knee OA.

This study showed several histological changes which might be related to several reasons such as sport activities, such as basketball or football (Siegel, *et al.*, 2012), ruptured of meniscus and the medial collateral ligament, cartilage lesions and ischemia (Dargel, *et al.*, 2007).

One of important histological changed in ACL of knee OA patient was hemorrhage which might be occur as response to injured, in the fact that; after tissue injury ACL trying to heal the injury through a specialized sequence of interference that done through three phases that occur over time: the acute inflammatory phase, the proliferative or phase, and the tissue-remodeling phase (Hauser, *et al.*, 2013). Also, hemorrhage which occurs due to acute inflammatory phase begins within minutes of injury and continues over the next 48 to 72 hours. During this phase, blood collects at the site of injury and platelet cells interact with certain matrix components, changing their shape and initiating clot formation, also new blood vessel formation, which increases vascularity in injured areas.

3-1-2 Posterior cruciate ligament (PCL): Sections of all PCL obtained from the knee OA patients were prepared for light microscopy using H & E stain, different stages of histological changes revealed:

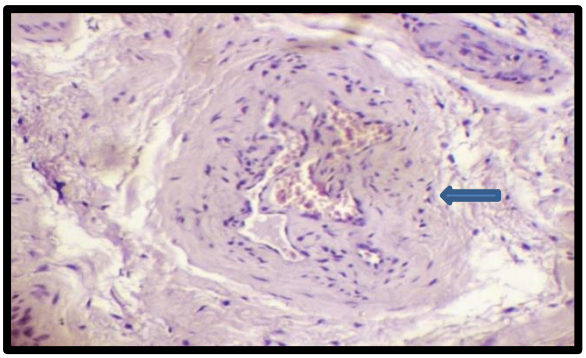

Figure (3-4): Posterior cruciate ligament section of knee OA patient show hypertrophy and hyperplasia of tunica media (arrow) (H & E, ×10).

- Different degree of infiltrated with macrophage and lymphocyte cells, also congestion of blood vessels with increase collagen fibers around blood vessel (Figure 3-4). Proliferation of fibrocystic with degeneration changes (Figure A3-5) and Disordered locating collagen fibers with mature adipo cytes (Figure B 3-5).

On the other hand, sections of PCL from the knee OA patients were stained with Van Giesons' shows many changes, these-change are Increased collagen fibers with degeneration changes (Figure A 3-6) and Increased collagen fibers around blood vessels and deposition of fibrin in different area of tissue (Figure B 3-6).

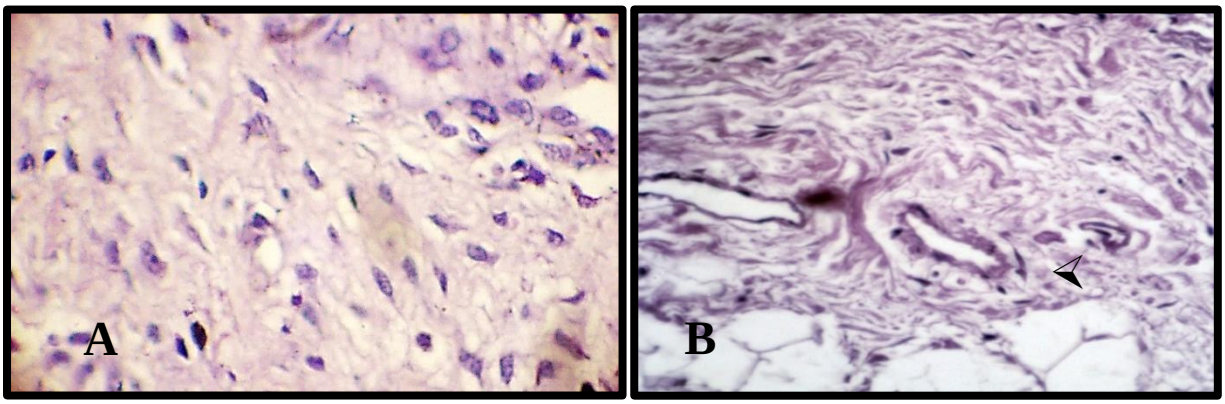

Figure (3-5): Posterior cruciate ligament section of knee OA patient illustrate, **A:** proliferation of fibrocystic (head arrow) with degeneration changes (arrow) (H & E, ×40). **B:** mature adipocytes (arrow) with disordered locating collagen fibers (head arrow) (H & E, ×10).

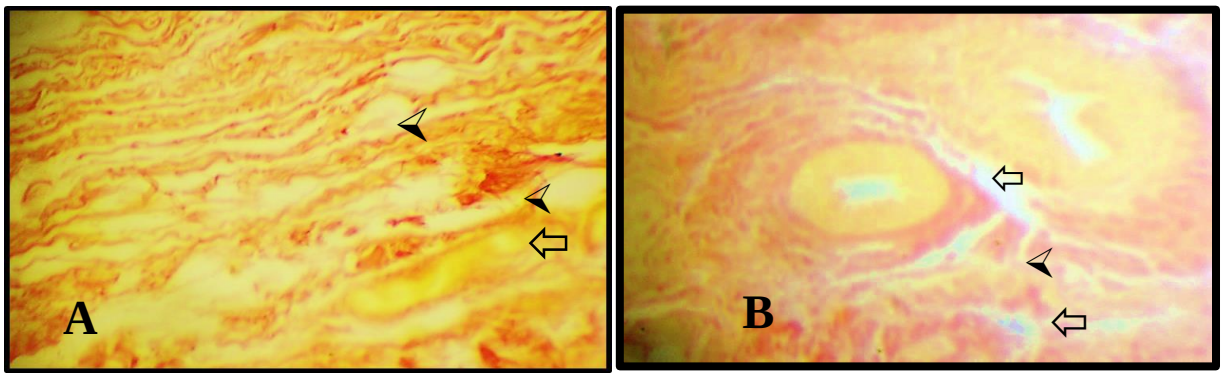

Figure (3-6): Posterior cruciate ligament section of knee OA patient reveal,**A:** increased collagen fibers (head arrow) with degeneration changes (arrow) (Van Giesons' staining, x40).**B:** increase collagen fibers around blood vessels (head arrow) and deposition of fibrin in different area of tissue (arrow) (Van Giesons' staining, x10).

The PCL is an important knee structure which essential for knee kinematics and rotation. Articular cartilage disruption is considered a hallmark of knee OA, the disease process results in changes in other knee tissues such as ligaments, synovial membrane, subchondral bone and menisci (Pauli, *et al.*, 2011).

Our result showed many histopathological changed in PCL of patient with OA but also the histopathological changes in PCL were less severe than in the ACL and occur in later stages of OA.

This histopathological change may be related to cartilage, ACL damage, synovitis of the arthritic knee, severe knee trauma and Multi ligament injury (Nelissen and Hogendoorn 2001). In contrast to ACL, Spontaneously or in isolation PCL rupture was rarely occurs, also that the PCL is at lower risk for acute or chronic injury than the ACL (Mullaji, *et al.*, 2008).

The most important histopathological change in PCL was disorganization of the collagen fibers and it was also earliest and most common change in the extracellular matrix (Levy, *et al.*, 2013), these Changes in collagen fibrils affect the ligament biomechanical properties.

In our study, the result showed Proliferation of fibrocystic in PCL tissue, this result was corresponding with the result of Sargon *et al.*, (2004) and AL-Sharqi *et al.*, (2013) which reported increase in collagen fibril concentration with aging, with a

maximum increase in collagen fibril concentration in those aged 60–69 years and in contrast with Levy *et al.*, (2013) study which reported decrease in collagen fibrils in the PCL in individuals older than 60 years.

3-2 Biochemical test

3-2-1 Vitamin D level: Data presented in Table 1 and Fig. 3-7 showed that, the vitamin D concentration in serum of control male is (14.88 ± 1.55 Ng/ml) and for female is (10.18 ± 1.75 Ng/ml), While the vitamin D concentration in serum of patients with OA male is (11.27 ± 1.72 Ng/ml) and for female OA is (8.65 ± 0.94 Ng/ml).

The results show that the mean serum vitamin D concentration in patients was lower than controls but the difference was not statistically significant ($p > 0.05$). There is a significant difference ($p < 0.05$) in serum vitamin D concentration between male OA patient and female OA patient.

Table 1: Effect of study group and sex in Vitamin D level in serum of knee OA patient and control

Sex / Ng/ml	Mean ± SD		P-value
	Control	Patients	
Male	14.88 ± 1.55	11.27 ± 1.72	0.134
Female	10.18 ± 1.75	8.65 ± 0.94	0.434
P-value	---	0.0493 *	---

* significant difference P<0.05 between male and female
SD: Standard Deviation

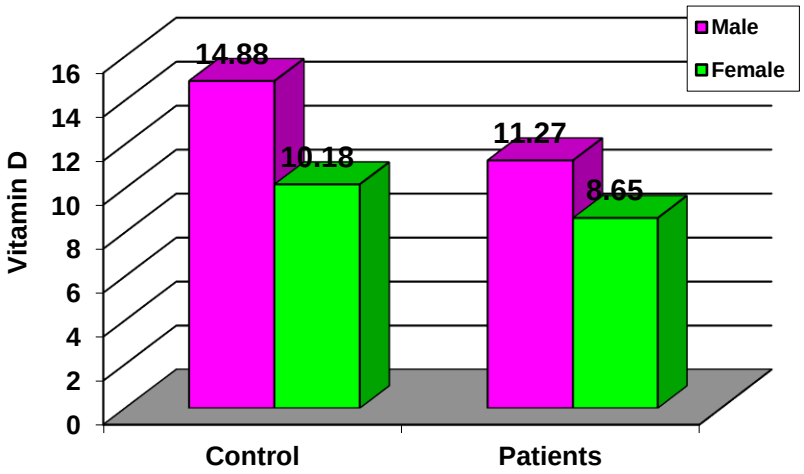

Figure 3-7: Effect of study group and sex in Vitamin D

The results of the current study were agreed with results of another previous study by Al-Jallili (2013) and El Sammak *et al.*, (2011). In contrast to our results Bischoff-Ferrari *et al.*, (2005) and Chaganti *et al.*, (2010).

The decrease in serum vitamin D concentration may be due to:

- Less exposure to sunlight due to cultural practices, very hot climate in several countries in Gulf area, decreased in outdoor activity, use of sun protection factors and darker skin (increase the time required for sun exposure to get the ideal vitamin D level (Kanan, *et al.*, 2013).
- Obesity (vitamin D is fat soluble and possible sequestration in body adipose tissue which leads to a decrease in serum vitamin D level (Holick, 2007).
- Poor in food fortification in general and in vitamin D in particular.
- Aging, due to a decrease in the amount of 7-dehydrocholesterol which is the precursor for vitamin D3 synthesis in the skin.

Current results showed decreased serum vitamin D levels in females than those of male, this significant difference are agreed with Verdoia *et al.*, (2015).

This significant decrease might be due to that most women worked mostly in indoor environments, so that men had higher exposure to sunlight compared to women because of their work activities, also women using sunscreen more than men. A study of Al-Mutairi *et al.*, (2012) reported higher vitamin D deficiency and insufficiency among participants who used sunscreen compa-red with non-sunscreen users. On the other hand, postmenopausal women had low estrogen levels that exacer-

bate the symptoms of vitamin D deficiency in the fact that estrogen increases the activity of the enzyme responsible for activating vitamin D (Buchanan, *et al.,* 1986).

3-2-2 Calcium Concentration: Table 2 and Fig. 3-8 showed calcium concentration in serum of control males were (8.09 ± 0.25 mg/dl) and for females were (7.54±0.16mg/dl). While the calcium concentration in serum of males and females within patients group were (7.03 ± 0.27 mg/dl) and (7.32 ± 0.15 mg/dl) respectively.

The results showed significant decrease ($p < 0.05$) in serum calcium concentration of males in patient group when compared with control males, but there is no-significant difference ($p < 0.05$) in serum calcium concentration of normal females compared with patient females. Also, there is no-significant difference in serum calcium concentration of males compared with females in patients group.

Table 2: Effect of study group and sex in Calcium concentration in serum of knee OA patients and control

Sex / Mg/dc	Mean ± SD		P-value
	Control	Patients	
Male	8.09 ± 0.25	7.03 ± 0.27*	0.0090
Female	7.54 ± 0.16	7.32 ± 0.15	0.446
P-value	---	0.268	---

* significant difference P<0.05 between male patients and control
SD: Standard Deviation.

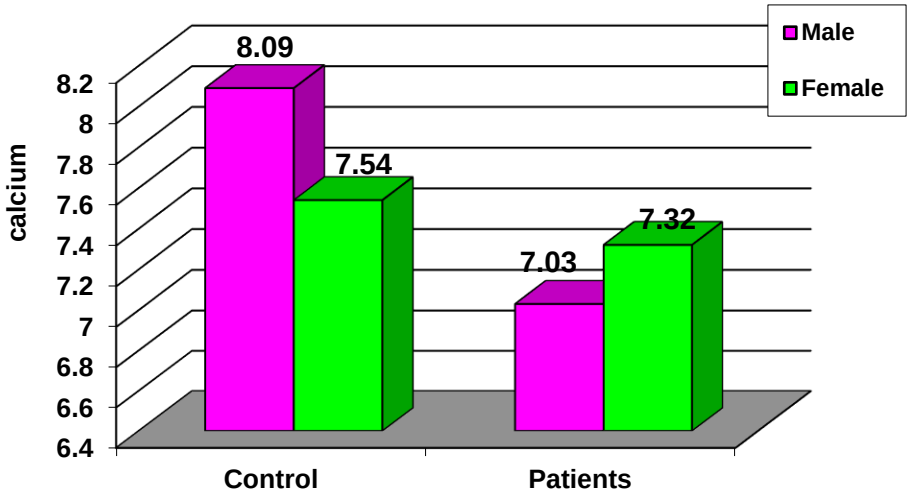

Figure 3-8: Effect of study group and sex in Calcium

The decrease in serum calcium concentration of the male OA patients may be due to:

- Deficiency of vitamin D in the OA patient in the fact that calcium absorption increase as serum 25-hydroxy vitamin D increase from 20 to 80 ng/ml (Abrams, *et al.,* 2005).
- Increase in phosphorus concentration (hyperphosphatemia). Phosphorus influence absorption through 1,25-dihydroxyvitamin D synthesis, as Phosphorus directly and independently determines the 1,25(OH)2D production rate, when Phosphate binds calcium avidly, causing acute hypocalcemia. In acute hyperphosphatemia, calcium is deposited mostly in the bone but also in the extra-skeletal tissue. In contrast, in chronic hyperphosphatemia, which is nearly always from chronic renal failure, calcium efflux from the bone is inhibited and the calcium absorption is low, because of reduced renal synthesis of 1,25-dihydroxyvitamin D (Yoshida, *et al.,* 2001).
- Menopause, body calcium maintenance through the activity of estrogen so that intestinal absorption and renal tubular reabsorption of calcium decrease after menopause(Kang, *et al.,* 2013).

3-2-3 Serum C reactive protein (CRP) Level: Data in Table (3-3) show the negative and positive value of CRP between patients and healthy control. The results showed no significant difference ($p > 0.05$) in concentration of CRP of total patients group compared with healthy control also between males and females groups.

Table 3-1: Distribution of sample study according to CRP results in knee OA patient and control

Sex	CRP	Control	patient	p-value
Male	V+	2 14.3%	4 26.7%	0.361
	V-	12 85.7%	11 73.3%	
	Total	14	15	15
Female	V+	1 9.1%	3 8.6%	0.679
	V-	10 90.9%	32 91.4%	
	Total	11	35	35
Total		25	50	

This result showed no association between CRP and OA were in agreement with the study of Sturmer *et al.,* (2004) and in contrast with Pelletier *et al.,* (2001). Elevation in CRP and ESR taken as indicator for primary OA inflammation.

CONCLUSION

According to the findings of this study, there was a histopathological change in ACL and PCL in knee OA patients occurred with the progression of OA disease. Vitamin D deficiency is prevalent among females with knee OA and their healthy controls than male which reflects females are more susceptible to OA. Significant decrease in calcium level in knee OA patients than normal control showed the role of daily nutrition as a risk factor for OA disease. Also, there is no significant change in level of CRP in knee OA patients.

REFERENCE

Abrams, S.A., I.J. Griffin, *et al.*, Relationships among vitamin D levels, parathyroid hormone, and calcium absorption in young adolescents. *The Journal of Clinical Endocrinology and Metabolism 90*: 5576-5581 (2005).

Adams, J.S. and M. Hewison, Update in vitamin D. *The Journal of Clinical Endocrinology and Metabolism 95*: 471-478 (2010).

Al-Jallili, Z.A., Association of vitamin (D) and serum Leptin levels with Knee Osteoarthritis in a sample of Iraqi females. College of Medicine. Baghdad, Baghdad University. Diploma thesis, 87 (2013).

Al-Mutairi, N., B.I. Issa, *et al.*, Photoprotection and vitamin D status: a study on awareness, knowledge and attitude towards sun protection in general population from Kuwait, and its relationship with vitamin D levels. *Indian Journal of Dermatology, Venereology and Leprology 78*: 342-350 (2012).

Al-Sharqi, S.A., M.S. Wahab, *et al.*, Histopathological study in posterior cruciate ligament of osteoarthritis and rheumatoid arthritis in Iraqi patients. *American Journal of Medicine and Medical Sciences 3*: 10-16. (2013).

Bischoff-Ferrari, H.A., Y. Zhang, *et al.*, Positive association between serum 25-hydroxyvitamin D level and bone density in osteoarthritis. *Arthritis Care & Research 53*: 821-826 (2005).

Buchanan, J.R., R. Santen, *et al.*, The effect of endogenous estrogen fluctuation on metabolism of 25-hydroxyvitamin D. *Calcified tissue international 39*: 139-144 (1986).

Chaganti, R.K., N. Parimi, *et al.*, Association of 25-hydroxyvitamin D with prevalent osteoarthritis of the hip in elderly men. *The osteoporotic fractures in men study. Arthritis and Rheumatism 62*: 511-514 (2010)

Dargel, J., M. Gotter, *et al.*, Biomechanics of the anterior cruciate ligament and implications for surgical reconstruction. *Strategies in Trauma and Limb Reconstruction 2*: 1-12 (2007)

Donlagic, D., B. Cigale, *et al.*, Patient-specific Knee Joint Computer Model Using MRI Data and'in vivo'Compressive Load from the Optical Force Measuring System. CIT. *Journal of Computing and Information Technology 16*: 209-222 (2008).

Elsammak, M. Y., A. A. Al-Wossaibi, *et al.*, High prevalence of vitamin D deficiency in the sunny Eastern region of Saudi Arabia: a hospital-based study/Prevalence elevee de carence en vitamine D dans la region ensoleillee de l'est de l'Arabie saoudite: une etude en milieu hospitalier. *Eastern Mediterranean Health Journal 17*: 317-324 (2011).

Ermawati, N. and Y. Wibisono, Early isolation of cell cycle-associated protein kinase (Oswee) gene in rice (Oryza Sativa L.). *Pak. J. Biotechnol.14*: 71-76 (2017).

Hauser, R.A., E.E. Dolan, *et al.*, Ligament injury and healing: a review of current clinical diagnostics and therapeutics. *The Open Rehabilitation Journal 6*: 16-23 (2013).

Holick, M.F., Vitamin D deficiency. *New England Journal of Medicine 357*: 266-281 (2007) .

Kanan, R.M., Y.M. Al Saleh, *et al.*, Year-round vitamin D deficiency among Saudi female outpatients. *Public health nutrition 16*: 544-548 (2013).

Kang, S.C., H.J. Kim, *et al.*, Effects of Astragalus membranaceus with supplemental calcium on bone mineral density and bone metabolism in calcium-deficient ovariectomized rats. *Biological trace element research 151*: 68-74 (2013).

Klaewklad, A., K. Nakkanong, *et al.*, Rubber elongation factor (REF) and small rubber particle protein (SRPP) gene expression responses to variation of seasonal change in four selected rubber clones. *Pak. J. Biotechnol.14*: 115-120 (2017).

Kweon, C., E.S. Lederman, *et al.*, Anatomy and biomechanics of the cruciate ligaments and their surgical implications. *In The multiple ligament injured knee, 18*: 17-27 (2013).

Lane, N.E., Osteoarthritis of the hip. *New England Journal of Medicine 357*: 1413-1421 (2007).

Levy, Y.D., A. Hasegawa, *et al.*, Histopathological changes in the human posterior cruciate ligament during aging and osteoarthritis: correlations with anterior cruciate ligament and cartilage changes. *Annals of the Rheumatic Diseases 72*: 271-277 (2013).

Martel-Pelletier, J. and J.P. Pelletier, Is osteoarthritis a disease involving only cartilage or other articular tissues. *Eklem Hastalik Cerrahisis 21*: 2-14 (2010).

Mullaji, A.B., S.V. Marawar, *et al.*, Cruciate ligaments in arthritic knees: a histologic study with radiologic correlation. *The Journal of Arthroplasty 23*: 567-572 (2008).

Nelissen, R.G.H.H. and P.C.W. Hogendoorn, Retain or sacrifice the posterior cruciate ligament in total knee arthroplasty? A histopathological study of the cruciate ligament in osteoarthritic and rheumatoid disease. *Journal of Clinical Pathology 54*: 381-384 (2001).

Pauli, C., S.P. Grogan, *et al.*, Macroscopic and histopathologic analysis of human knee menisci in aging and osteoarthritis. *Osteoarthritis and Cartilage 19*: 1132-1141 (2011).

Pelletier, J.P., J. Martel-Pelletier, *et al.*, Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. *Arthritis & Rheumatism 44*: 1237-1247 (2001).

Power, A. and D. Scheffer, The effect of nutritional supplementation and exercise on subjective pain and function in people with osteoarthritis of the knee. Physical Therapy Department, Grand Valley State University. M. Sc. theses 65 (1999).

Sargon, M.F., M.N. Doral, *et al.*, Age-related changes in human PCLs: a light and electron microscopic study. *Knee Surgery, Sports Traumatology, Arthroscopy 12*: 280-284 (2004).

Shimura, Y., H. Kurosawa, *et al.*, The factors associated with pain severity in patients with knee osteoarthritis vary according to the radiographic disease severity: a cross-sectional study. *Osteo-arthritis and Cartilage 21*: 1179-1184 (2013).

Siegel, L., C. Vandenakker-Albanese, *et al.*, Anterior cruciate ligament injuries: anatomy, physiology, biomechanics, and management. *Clinical Journal of Sport Medicine 22*: 349-355 (2012).

Stürmer, T., H. Brenner, *et al.*, Severity and extent of osteoarthritis and low grade systemic inflammation as assessed by high sensitivity C reactive protein. *Annals of the Rheumatic Diseases 63*: 200-205 (2004).

Verdoia, M., A. Schaffer, *et al.*, Impact of gender difference on vitamin D status and its relationship with the extent of coronary artery disease. *Nutrition, Metabolism and Cardiovascular Diseases 25*: 464-470 (2015).

Yoshida, T., N. Yoshida, *et al.*, Dietary Phosphorus Deprivation Induces 25-Hydroxyvitamin D3 1α-Hydroxylase Gene Expression 1. *Endocrinology 142*: 1720-1726 (2001).