

## Relation of Interleukin-21 with Primary Knee Osteoarthritis Severity and Functional Disability

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**Abstract:** *Aim:* It was to assess the relation between serum interleukin-21 (IL-21) and primary knee osteoarthritis (OA) severity regarding pain, stiffness, functional disability and radiological structural damage. *Materials and Methods:* The study included 50 patients with primary knee OA. The control group included 40 apparently healthy volunteers. A clinical examination was done. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Health Assessment Questionnaire-Disability Index (HAQ-DI) were used to assess knee OA severity. Kellgren-Laurence (K/L) grading scale was used to assess the knee joint radiologically. The Serum level of IL-21 was measured in both patients and control subjects. *Results:* Serum IL-21 level was statistically significantly higher among the patients group in comparison to the control group. Serum IL-21 level was statistically significantly higher among patients group with knee joint line tenderness versus those without tenderness. Serum IL-21 level was statistically significantly higher among patients group with knee joint effusion versus those without effusion. There were statistically significantly positive correlations between IL-21 serum level and WOMAC stiffness subscale and HAQ-DI score. *Conclusions:* Serum IL-21 was significantly higher in patients with primary knee OA. Serum IL-21 was positively correlated with WOMAC stiffness subscale and HAQ-DI. Knee pain, stiffness, joint tenderness, effusion and functional disabilities were related to IL-21.

**Key words:** Health Assessment Questionnaire • Interleukin-21 • Kellgren-Laurence Grading Scale • Primary Knee Osteoarthritis • Western Ontario and McMaster Universities Osteoarthritis Index.

### INTRODUCTION

Primary osteoarthritis (OA) is the most common arthritis in the community [1]. It could be severe enough to cause mobility impairment, joint deformities and compromising quality of life [2]. Knee OA is the most common form of primary OA. The etiology of primary knee OA is multifactorial which include aging, obesity and genetic susceptibility [1, 3, 4]. The pathogenesis of primary OA is not well understood up till now and it is complex [5, 6]. However, inflammatory reaction was found to have a major role in the pathogenesis of primary knee

OA. There are a large group of proinflammatory cytokines as tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-1 (IL-1), IL-15 and IL-18 that are contributed in OA pathogenesis [7-11]. This makes OA to be considered an inflammatory arthritis rather than a pure degenerative arthritis [12, 13].

Interleukin-21 is a proinflammatory cytokine [14]. It has many physiological actions. It modulates the proliferation and function of T-cells, B-cells and natural killer (NK) cells responses [15, 16]. IL-21 is a candidate to be a potential biological marker for primary knee OA [17]. There are scanty studies that assessed the relation

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between serum IL-21 level and primary knee OA severity regarding pain, stiffness, functional disability and structural damage, as well as, OA progression [12, 18, 19].

The aim of the study was to assess the relation between serum IL-21 level and primary knee OA severity regarding pain, stiffness, functional disability and radiological structural damage.

## MATERIALS AND METHODS

This cross-sectional study included 50 patients with primary knee OA. Knee OA was diagnosed according to the clinical and radiographic criteria of the American College of Rheumatology criteria for the classification of primary knee OA [20]. Patients were recruited from those attending the Physical Medicine, Rheumatology and Rehabilitation outpatient clinic, Alexandria Main University Hospital, Faculty of Medicine, Alexandria University, Egypt. Exclusion criteria included patients with endocrine disorders, overlap with other rheumatologic diseases, traumatic arthritis, secondary OA and patients with diseases in which serum IL-21 level increased as autoimmune bleeding disorders and skin psoriasis [14]. The study included a control group of 40 apparently healthy volunteers. The researchers explained the study to the participants. All participants gave a written informed consent. The study was approved by the Ethics Committee of Faculty of Medicine, Alexandria University, Egypt.

All the participated patients were subjected to demographic data collection, anthropometric measurements [height, weight and body mass index (BMI)] and clinical musculoskeletal examination stressing on both knees [21]. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used to assess primary knee OA severity [22]. The WOMAC has three different subscales for assessment of pain, stiffness and function. The patients were rated against each question on a 5-point Likert scale (0, none; 1, slight difficulty; 2, moderate difficulty; 3, severe difficulty; and 4, extreme difficulty). The scores are summed for items in each subscale and then for the three subscales to calculate the total WOMAC score. The total WOMAC score was graded into: mild (from 0-24), moderate (from 25-48), severe (from 49-72) and extreme (from 73-96)-[22].

Functional assessment of knee OA was done using Health Assessment Questionnaire-Disability Index (HAQ-DI) [23]. It has consisted of 20 questions. For each

question, there are four possible responses: without any difficulty, with some difficulty, with much difficulty and unable to do. The HAQ-DI score of 0 to 1 is considered to represent mild to moderate functional disability, >1 to 2 represent moderate to severe functional disability and >2 to 3 represent severe to very severe functional disability [23].

Kellgren-Laurence (K/L) grading scale was used for assessment of the radiological grading of knee OA severity. The K/L grading scale had four grades as the following: grade 1 (very mild), grade 2 (mild), grade 3 (moderate) and grade 4 (severe) [24]. Laboratory investigation consisted of measurement of serum IL-21 level in Unit per milliliter (U/ml) using enzyme-linked immunosorbent assay (ELISA) technique for quantitative detection of IL-21 using human IL-21 ELISA kit (SEB688HU, Cloud-Clone Corporation, 1304 Langham Creek Dr. Suite 226, Houston, Texas, USA) read on ELISA reader (Stat-Fax 2100; Awareness Technology Inc., Palm City, Florida, USA).

Statistical analysis of data was done by using the Statistical Package of Social Science (SPSS version 17) software (Statistical package for the social sciences, version 17. University of Cambridge Computing Service: London, United Kingdom; 2007). The descriptive measures included count, frequency, minimum, maximum, mean and standard deviation (SD). Analytic measures included: Mann Whitney test and Kruskal Wallis test were used to assess the quantitative variables. Chi-square test and Fisher's Exact test when required were used to assess the qualitative variables. The correlation was done using the Spearman correlation test. Statistical significance was assigned to any  $P$  value at  $\leq 0.05$ . Serum IL-21 level reference cut-off value was calculated by rounding the mean plus two SD of the control group to measure the upper limit of normal.

## RESULTS

The study included 50 patients with primary knee OA (44 females [88.0%] and 6 males [12%]). Their mean age was  $53.22 \pm 8.69$  years (ranged from 40 to 73 years). The control group included 40 apparently healthy volunteers (33 females [82.5%] and 7 males [17.5%]). Their mean age was  $49.67 \pm 6.89$  years (ranged from 40 to 66 years). There were no statistical significant differences between patients and control groups regarding gender ( $X^2=0.544$ ,  $P=0.552$ ) and age ( $Z=-1.822$ ,  $P=0.068$ ).

Table 1: Demographic data and anthropometric measures of the patients and control groups

Demographic data and anthropometric measures	Patients group (n=50 patients) mean±SD	Control group (n=40 subjects) mean±SD	Test of significance	P
Age (years)	53.22±8.69	49.67±6.89	Z=-1.822	0.068
Women†	44(88.0)	33(82.5)	X <sup>2</sup> =0.544	0.552‡
Anthropometric measures				
Height (cm)	161.42±8.31	164.44±6.57	Z=-1.671	0.095
Weight (Kg)	86.68±17.36	83.61±12.31	Z=-0.764	0.445
BMI (Kg/m <sup>2</sup> )	26.76±5.01	26.39±3.91	Z=-0.260	0.795
BMI categories				
Underweight†	2(4.0)	1(2.5)	X <sup>2</sup> =1.882	0.597
Normal weight†	18(36.0)	15(37.5)		
Overweight†	16(32.0)	17(42.5)		
Obesity†	14(28.0)	7(17.5)		

BMI, body mass index; n, number of patients or subjects; SD, standard deviation; Z, value of Mann Whitney test for comparing between the two groups; X<sup>2</sup>, value of Chi-square test for comparing between the two groups.

\* Statistically significant at P≤0.05.

† Data are reported as number (percentage).

‡ P value of Fisher's Exact test.

Table 2: Clinical characteristics and results of radiological assessment of the patients group

Clinical characteristics and results of radiological assessment	Patients group (n= 50 patients) mean± SD
Duration of complaint (months)	38.83±33.89
WOMAC	
Pain subscale	10.74±2.31
Stiffness subscale	3.78±1.25
Function subscale	36.92±7.05
WOMAC total score	51.44±9.49
WOMAC total score interpretation	
Mild*	1(2.0)
Moderate*	16(32.0)
Severe*	32(64.0)
Extreme*	1(2.0)
HAQ-DI	
HAQ-DI score	1.08±0.49
HAQ-DI score interpretation	
Mild to moderate*	28(56.0)
Moderate to severe*	20(40.0)
Severe to very severe*	2(4.0)
Clinical examination	
Presence of knee joint line tenderness*	36(72.0)
Presence of knee joint effusion*	33(66.0)
Radiological assessment	
K/L scale of the right knees	
Grade 1*	2(4.0)
Grade 2*	21 (42.0)
Grade 3*	14(28.0)
Grade 4*	13(26.0)
K/L scale of the left knees	
Grade 1*	4(8.0)
Grade 2*	18(36.0)
Grade 3*	15(30.0)
Grade 4*	13(26.0)

WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; HAQ-DI, Health Assessment Questionnaire-Disability Index; K/L, Kellgren-Laurence grading scale; n, number of patients; SD, standard deviation.

\* Data are reported as number (percentage)

Table 3: Comparison between patients group and control group regarding interleukin-21 serum level

IL-21 serum level (U/ml)	Patients group (n=50 patients)	Control group (n=40 subjects)	Test of significance	P
Mean±SD	0.53±0.21	0.52±0.03	Z=-2.655	0.008*
Min. - Max.	0.42-1.96	0.47-0.61		

IL-21, Interleukin-21; U/ml, Unit per milliliter; SD, standard deviation; Min., minimal value; Max., maximal value; n, number of patients or subjects; Z, value of Mann Whitney test for comparing between the two groups.

\* Statistically significant at  $P \leq 0.05$ .

Table 4: Comparison between patients with knee joint line tenderness versus those without tenderness and control group regarding interleukin-21 serum level

IL-21 serum level (U/ml)	Patients group with knee joint line tenderness (n=36 patients)	Patients group without knee joint line tenderness (n=14 patients)	Control group (n=40 subjects)	Test of significance	P
Mean±SD	0.55±0.24	0.46±0.02	0.52±0.03	K=17.179	$\leq 0.0001^*$
Min. - Max.	0.44-1.96	0.42-0.52	0.47-0.61		
Test of significance	Z=-1.139†	Z=-4.294‡	Z=-2.982§		
P	0.255†	=0.0001*‡	0.003*§		

IL-21, Interleukin-21; U/ml, Unit per milliliter; SD, standard deviation; Min., minimal value; Max., maximal value; n, number of patients or subjects; Z, value of Mann Whitney test for comparing between two groups; K, value of Kruskal Wallis test for comparing between the three groups.

\* Statistically significant at  $P \leq 0.05$ .

† Value of Mann Whitney test for comparison between patients group with knee joint line tenderness versus control group and its P value.

‡ Value of Mann Whitney test for comparison between patients group without knee joint line tenderness versus control group and its P value.

§ Value of Mann Whitney test for comparison between patients group with knee joint line tenderness versus patients group without knee joint line tenderness and its P value.

Table 5: Comparison between patients with knee joint effusion versus those without joint effusion and control group regarding interleukin-21 serum level

IL-21 serum level (U/ml)	Patients group with knee joint effusion (n=33 patients)	Patients group without knee joint effusion (n=17 patients)	Control group (n=40 subjects)	Test of significance	P
Mean±SD	0.56±0.25	0.47±0.03	0.52±0.03	K=16.560	$\leq 0.0001^*$
Min. - Max.	0.43-1.96	0.42-0.58	0.47-0.61		
Test of significance	Z=-0.909†	Z=-4.274‡	Z=-2.785§		
P	0.363†	=0.0001*‡	0.005*§		

IL-21, Interleukin-21; U/ml, Unit per milliliter; SD, standard deviation; Min., minimal value; Max., maximal value; n, number of patients or subjects; Z, value of Mann Whitney test for comparing between two groups; K, value of Kruskal Wallis test for comparing between the three groups.

\* Statistically significant at  $P \leq 0.05$ .

† Value of Mann Whitney test for comparison between patients group with knee joint effusion versus control group and its P value.

‡ Value of Mann Whitney test for comparison between patients group without knee joint effusion versus control group and its P value.

§ Value of Mann Whitney test for comparison between patients group with knee joint effusion versus patients group without knee joint effusion and its P value

Different demographic data and anthropometric measures of the patients and control groups are tabulated in Table 1. Clinical characteristics and results of radiological assessment of the patients are tabulated in Table 2. All the patients had bilateral knee OA.

Serum IL-21 level was statistically significantly higher among patients group in comparison to control group ( $P=0.008$ ) (Table 3). Serum IL-21 reference cut-off value obtained from the apparently healthy participants was  $\leq 0.58$  U/ml. Serum level of IL-21 was considered to be elevated if it was above its reference cut-off value. There were 7 patients (14.0%) had serum IL-21 level exceeded its reference cut-off value.

Comparison between patients with knee joint line tenderness versus those without tenderness and control group regarding IL-21 serum level was tabulated in Table 4. Serum IL-21 level was statistically significantly higher among patients group with knee joint line tenderness versus those without knee joint line tenderness ( $P=0.003$ ) (Table 4).

Comparison between patients with knee joint effusion versus those without effusion and control group regarding IL-21 serum level was tabulated in Table 5. Serum IL-21 level was statistically significantly higher among patients group with knee joint effusion versus those without knee joint effusion ( $P=0.005$ ) (Table 5).

Correlation between IL-21 serum level and demographic data, anthropometric measures, clinical characteristics and results of radiological assessment of the patients are tabulated in table 6. There were statistically significantly positive correlations between IL-21 serum level and WOMAC stiffness subscale ( $P=0.050$ ) and HAQ-DI score ( $P=0.031$ ) (Table 6).

Comparison of different demographic, anthropometric, clinical and radiological parameters between patients with serum IL-21 level exceeded its reference cut-off value versus those with serum IL-21 level within the reference cut-off value are tabulated in Tables 7 and 8. There were statistical significant differences between patients with serum IL-21 level

Table 6: Correlation between interleukin-21 serum level and demographic, anthropometric, clinical and radiological characteristics of patients group (50 patients)

Demographic, anthropometric, clinical and radiological characteristics	IL-21 serum level (U/ml)	
	$r_s$	<i>P</i>
Age (years)	-0.002	0.987
Duration of complaint (months)	0.068	0.640
Anthropometric measures		
BMI (Kg/m <sup>2</sup> )	0.244	0.088
WOMAC		
Pain subscale	0.068	0.641
Stiffness subscale	0.279	0.050*
Function subscale	0.213	0.137
WOMAC total score	0.220	0.124
WOMAC total score interpretation	0.219	0.127
HAQ-DI		
HAQ-DI score	0.305	0.031*
HAQ-DI score interpretation	0.109	0.451
Radiological assessment		
K/L scale of the right knee	-0.097	0.504
K/L scale of the left knee	0.066	0.649

BMI, body mass index; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; HAQ-DI, Health Assessment Questionnaire-Disability Index; K/L, Kellgren-Laurence grading scale; IL-21, Interleukin-21; U/ml, Unit per milliliter;  $r_s$ , Spearman correlation coefficient.

\* Statistically significant at  $P \leq 0.05$ .

Table 7: Comparison of demographic and anthropometric characteristics between patients with serum interleukin-21 level exceeded its reference cut-off value versus those with serum interleukin-21 level within the reference cut-off value

Demographic and anthropometric characteristics	Patients group with serum IL-21 level exceeded its reference cut-off value (> 0.58 U/ml)	Patients group with serum IL-21 level within the reference cut-off value ( $\leq$ 0.58 U/ml)	Test of significance	<i>P</i>
	(n=7 patients) mean $\pm$ SD	(n=43 patients) mean $\pm$ SD		
Age (years)	53.71 $\pm$ 5.73	53.13 $\pm$ 9.13	Z=-0.308	0.758
Women†	7(100)	37(86.0)	X <sup>2</sup> =1.110	0.384‡
Anthropometric measures				
Height (cm)	162.85 $\pm$ 8.57	161.18 $\pm$ 8.34	Z=-0.336	0.737
Weight (Kg)	95.78 $\pm$ 21.86	85.20 $\pm$ 16.35	Z=-1.636	0.102
BMI (Kg/m <sup>2</sup> )	29.37 $\pm$ 6.60	26.34 $\pm$ 4.67	Z=-1.468	0.142
BMI categories				
Underweight†	1(14.3)	1(2.3)	X <sup>2</sup> =11.306	0.010*
Normal weight†	1(14.3)	17(39.5)		
Overweight†	0(0)	16(37.2)		
Obesity†	5(71.4)	9(20.9)		

BMI, body mass index; IL-21, interleukin-21; U/ml, Unit per milliliter; n, number of patients; SD, standard deviation; Z, value of Mann Whitney test for comparing between the two groups; X<sup>2</sup>, value of Chi-square test for comparing between the two groups.

\* Statistically significant at  $P \leq 0.05$ .

† Data are reported as number (percentage).

‡ *P* value of Fisher's Exact test.

exceeded the reference cut-off value versus those with serum IL-21 level within the reference cut-off value regarding BMI categories, percentage of patients with knee joint effusion, WOMAC pain subscale and

WOMAC total score interpretation grades (Tables 7 and 8). All patients (100%) with serum IL-21 level exceeded its reference cut-off value had knee joint line tenderness and knee joint effusion (Table 8).

Table 8: Comparison of clinical and radiological characteristics between patients with serum interleukin-21 level exceeded its reference cut-off value versus those with serum interleukin-21 level within the reference cut-off value

Clinical and radiological characteristics	Patients group with serum IL-21 level exceeded its reference cut-off value (> 0.58 U/ml) (n=7 patients) mean±SD	Patients group with serum IL-21 level within the reference cut-off value (= 0.58 U/ml) (n=43 patients) mean±SD	Test of significance	P
Duration of complaint (months)	34.80±24.14	39.30±35.04	Z=-0.103	0.918
WOMAC				
Pain subscale	12.85±3.13	10.39±2.01	Z=-2.019	0.044*
Stiffness subscale	4.42±1.81	3.67±1.12	Z=-1.653	0.098
Function subscale	38.71±11.48	36.62±6.21	Z=-0.406	0.684
WOMAC total score	56.00±15.91	50.69±8.05	Z=-1.120	0.263
WOMAC total score interpretation				
Mild†	1(14.3)	0(0)	X <sup>2</sup> =13.144	0.004*
Moderate†	1(14.3)	15(34.9)		
Severe†	4(57.1)	28(65.1)		
Extreme†	1(14.3)	0(0)		
HAQ-DI				
HAQ-DI score	1.25±0.44	1.06±0.49	Z=-1.250	0.211
HAQ-DI score interpretation				
Mild to moderate†	3(42.9)	25(58.1)	X <sup>2</sup> =1.175	0.556
Moderate to severe†	4(57.1)	16(37.2)		
Severe to very severe†	0(0)	2(4.7)		
Clinical examination				
Presence of knee joint line tenderness†	7(100.0)	29(67.4)	X <sup>2</sup> =3.165	0.084‡
Presence of knee joint effusion†	7(100.0)	26(60.5)	X <sup>2</sup> =4.193	0.043*‡
Radiological assessment				
K/L scale of the right knees				
Grade 1†	1(14.3)	1(2.3)	X <sup>2</sup> =3.574	0.311
Grade 2†	2(28.6)	19(44.2)		
Grade 3†	3(42.9)	11(25.6)		
Grade 4†	1(14.3)	12(27.9)		
K/L scale of the left knees				
Grade 1†	0(0)	4(9.3)	X <sup>2</sup> =3.737	0.291
Grade 2†	1(14.3)	17(39.5)		
Grade 3†	4(57.1)	11(25.6)		
Grade 4†	2(28.6)	11(25.6)		

WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; HAQ-DI, Health Assessment Questionnaire-Disability Index; K/L, Kellgren-Laurence grading scale; IL-21, interleukin-21; U/ml, Unit per milliliter; n, number of patients; SD, standard deviation; Z, value of Mann Whitney test for comparing between the two groups; X<sup>2</sup>, value of Chi-square test for comparing between the two groups.

\* Statistically significant at P≤0.05

† Data are reported as number (percentage).

‡ P value of Fisher's Exact test

## DISCUSSION

Primary knee OA is a common medical problem. It is due to a complex of environmental, biomechanical and genetic factors [1, 3, 4]. Its main clinical complaint is pain with its consequences of functional disabilities. All these affect the patient's quality of life. Primary knee OA has a great economic burden on the community [3, 10, 25].

Primary knee OA is considered an inflammatory disease [7, 26, 27]. OA pathogenesis included a large variety of inflammatory mediators. These inflammatory mediators included inflammatory cytokines as interleukins and TNF-α [5, 11, 19]. Study of these inflammatory cytokines is essential to discover appropriate biomarkers

for primary knee OA [17]. These facilitate early diagnosis and assessment of severity and prognosis of OA. Subsequently, these reflect on early and tailored therapeutic management for primary knee OA. The detection of OA biomarkers helps in discovering effective therapeutic targets for OA pathogenesis [16]. There are a variety of inflammatory biomarkers for OA [8, 9, 28].

There are many studies conducted on Egyptian primary knee OA patients. They were targeted to detect and assess new biomarkers for OA pathogenesis and OA severity [29-32].

Serum level of IL-21 was significantly higher among patients group in comparison to the control group. This was in accordance with previous researches

[12, 18, 19, 33]. IL-21 is a cytokine that has proinflammatory effects. It has an essential role in primary knee OA pathogenesis. However, it has a role in the pathogenesis of other arthropathies as rheumatoid arthritis [15, 34].

Serum IL-21 level exceeded the reference cut-off value was percent in only 14% of the studied patients. This was not in agreement with previous studies [12, 18]. This could be due to differences between these researches and the present study regarding the demographic characteristics of the participated patients, patients' duration of OA complaint, racial differences between patients participated in these studies and the current study; and due to differences in the laboratory methods, procedures, techniques and measurement units used for the assessment of serum IL-21 level which included differences in the maneuvering and laboratory equipment used in different studies [12, 18, 35].

There was 72% of the included patients had knee joint line tenderness. There was 66% of the participated patients had knee joint effusion. These were in partial accordance with Mohamed *et al.* and Ibrahim *et al.* [31,32]. Mohamed *et al.* reported knee joint line tenderness and knee joint effusion were present in 57.5% and 15% of their studied patients respectively [31]. While, Ibrahim *et al.* reported that knee joint line tenderness and knee joint effusion were present in 80% and 45% of their studied patients respectively [32]. The differences between the current study and these studies could be due to differences in the demographic characteristics of the included patients, differences in disease duration and differences in percentage of bilaterality of knee OA affection in the included patients in these studies and the present study [31, 32].

Patients with knee joint line tenderness had a significantly higher serum IL-21 level in comparison to patients without tenderness. Also, patients with knee joint effusion had a significantly higher serum IL-21 level in comparison to patients without effusion. However, patients without knee joint line tenderness, as well as, patients without knee joint effusion had a significantly lower serum IL-21 level in comparison to control group. These findings indicated that serum IL-21 level was elevated in OA patients with acute synovitis. This was an indicator that IL-21 had a role in the acute inflammatory reaction in knee OA, with minimal or no role in the mild chronic inflammatory reaction in OA [12]. This was in accordance with previous studies [12, 18]. They reported that IL-21 increased in advanced forms of knee OA [8, 12, 18]. In acute exacerbation of chronic inflammation,

there are inflammatory cellular infiltration of more lymphocytes and macrophages with the release of a large group of inflammatory cytokines as TNF- $\alpha$  and IL-1 $\beta$  [14, 19, 26, 36].

There was a statistically significant positive correlation between IL-21 serum level and WOMAC stiffness subscale. This was in agreement with Shan *et al.* [12]. This indicated that IL-21 has an essential contribution in the pathogenesis of knee OA stiffness. Consequently, there was a statistically significant positive correlation between serum IL-21 serum level and HAQ-DI. This pointed to the consequences of the role of IL-21 on knee OA pathogenesis. This issue was not previously assessed in the literature. However, this was in accordance with the effect of other inflammatory mediators on knee OA [37].

Patients with serum IL-21 level exceeded the reference cut-off value were significantly more obese than those patients with serum IL-21 level within the reference cut-off value. This data was not previously assessed. However, obese patients had more severe and advanced knee OA associated with more severe inflammatory synovitis in comparison to less obese and slim patients [38, 39].

All patients with serum IL-21 level exceeded the reference cut-off value had knee joint line tenderness and knee joint effusion in comparison to those patients with serum IL-21 level within the reference cut-off value. This indicated that elevated serum IL-21 level was associated with more acute and severe synovitis [12, 37].

Patients with serum IL-21 level exceeded the reference cut-off value had statistically significantly higher WOMAC pain subscale in comparison to those patients with serum IL-21 level within the reference cut-off value. Also, a significantly higher percentage of high grades of WOMAC was present among patients with serum IL-21 level exceeded the reference cut-off value. These data were not assessed previously in the literature. These findings indicated that IL-21 had a role in the inflammatory pathogenesis of knee OA. Inflammatory reaction increases pain sensitivity. This is through increasing the responses of the peripheral nociceptive fibers [26]. Subsequently, IL-21 affected pain and stiffness and consequently the functional abilities of knee OA patients.

There was no significant correlation between serum IL-21 level and K/L scale. Also, there was no significant difference between patients with serum IL-21 level exceeded the reference cut-off value versus those with serum IL-21 level within the reference cut-off value

regarding the percentage of different grades of K/L scale. IL-21 could have no direct role on knee joint structural damage. This was not mentioned previously in the literature.

Interleukin-21 is an autocrine proinflammatory cytokine. It is a multifunctional cytokine. It is a member of type I cytokine superfamily which also includes IL-15 [15]. It is produced mainly by follicular helper T cell and NK cells. It was reported that IL-1 $\beta$  and IL-15 stimulate the production and release of IL-21 [40]. It binds to a composite receptor to produce its physiological functions. This consists of a private receptor [IL-21 receptor (IL-21R)] and a common cytokine receptor gamma chain [34]. IL-21R is expressed on lymphocytes, NK cells, dendritic cells, macrophages, as well as, other non-lymphocytic cells as fibroblast-like synovial cells, epithelial cells and endothelial cells [34]. So, IL-21 has a variety of effects on both cellular and humoral immune responses. Also, IL-21 has a potent immunomodulatory effects on both innate and adaptive immune responses [15, 41, 42]. It has an essential role in the immune system. It leads to proliferation and differentiation of T helper cells, promote B cell generation and differentiation into plasma cells with enhancing the production of immunoglobulins. It stimulates activity of macrophages and NK cells. Consequently, these effects result in the release of many proinflammatory cytokines that share in the pathogenesis of primary knee OA [14, 15, 34, 36]. These include the release of TNF- $\alpha$  and IL-1 $\beta$  from the immune cells present in the synovial membrane. These cytokines contribute in the pathogenesis of primary knee OA [14, 15, 34, 36]. IL-21 enhances the production of matrix metalloproteinase (MMP) as MMP-1 and MMP-3 by synovial fibroblasts [15, 19]. It has a role in triggering and amplification of the inflammatory pathways which promote inflammation and joint damage [14, 15, 34, 36]. The proinflammatory effects of IL-21 explain all significant results found in the present study.

These pleiotropic effects of IL-21 suggest that it could be a therapeutic target for the treatment of primary knee OA. This is in the form of blocking IL-21 action [34].

The current study had some limitations. First one was the relatively small number of patients. This could be due to the wide range of exclusion criteria included in the study. Further studies on a larger scale of patients are recommended to verify the results of the current study. In the second one, only serum IL-21 level was assessed in primary knee OA patients. The level of IL-21 in the

synovial fluid aspirated from involved knee joints was not assessed. It could provide more data about its role in the OA pathogenesis. The third limitation, the study assessed serum IL-21 level and did not assess the level of other inflammatory cytokines as TNF- $\alpha$  and IL-1 $\beta$  to evaluate any relationship between serum IL-21 and other inflammatory cytokines in primary knee OA patients. The fourth limitation, the study was conducted in a single medical research center. So, the generalization of the resulted conclusions should be taken with caution.

In conclusion, serum IL-21 was significantly higher in patients with primary knee OA. Serum IL-21 was positively correlated with WOMAC stiffness subscale and HAQ-DI. Higher grades of WOMAC scores, joint line tenderness and joint effusion were associated with patients with serum IL-21 level exceeded the reference cut-off value. Knee pain, stiffness, joint tenderness, effusion and OA functional disabilities were related to IL-21. These findings suggest that IL-21 has a role in the pathogenesis of different aspect of primary knee OA. Serum IL-21 could be a potential biochemical marker for primary knee OA. It could reflect OA severity and disease progression. Resulting from these, researches on targeting IL-21 for the management of primary knee OA could be expected to have a therapeutic effect on different clinical aspects of primary knee OA.

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**Competing Interests:** The authors declare that they have no competing interests.

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