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Research article

Evaluation of circulating levels of Interleukin-10 and Interleukin-16 and dietary inflammatory index in Lebanese knee osteoarthritis patients

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ABSTRACT

Objectives: To investigate plasma concentrations of Interleukin-16 (IL-16) and Interleukin-10 (IL-10) in Lebanese knee osteoarthritis (KOA) patients and to examine the association between the diet-associated inflammation and increased risk for KOA.**Methods:** A total of 208 study participants were assigned to one of the 3 groups: Diagnosed Knee Osteoarthritis group (DKOA) (N = 78); Undiagnosed Knee Osteoarthritis group (UKOA) (N = 60) and controls matched on age, sex and sociodemographic characteristics (N = 70). UKOA represents KOA features before they are altered by therapeutic intervention and lifestyle modifications that follow the diagnosis. Energy-adjusted dietary inflammatory index (E-DII™) scores were calculated using 2-day 24-hour recalls. IL-10 and IL-16 were measured using commercially available sandwich enzyme-linked immunosorbent assay kits.**Results:** The UKOA group and controls did not show any significant difference in plasma IL-16 levels (p = 0.28), whereas significantly higher levels of IL-10 were observed in the UKOA group compared to controls (21 ± 41 vs 7.5 ± 12 pg/mL; p = 0.01). The UKOA group had significantly higher IL-16 levels compared to the DKOA group (177 ± 215 vs 80 ± 57 pg/ml; p = 0.001) and significantly higher IL-10 levels compared to the DKOA group (21 ± 41 vs 8 ± 14 pg/mL; p = 0.02). Significantly higher levels of IL-16 were observed in the control group compared to the DKOA group (140 ± 161 vs 80 ± 57 pg/ml; p = 0.009) whereas the DKOA group and controls did not show any significant difference in plasma IL-10 levels (p = 0.82). Additionally, we found significantly higher E-DII scores in the UKOA group compared to controls (0.53 ± 1.028 vs 0.04 ± 1.580; p = 0.04) and in the UKOA group compared to the DKOA group (0.53 ± 1.028 vs -0.37 ± 1.899; p = 0.001). However, there was significant difference in E-DII scores between the DKOA group and controls (p = 0.16).**Conclusions:** Our findings indicate an association between circulating levels of IL-10 and KOA in Lebanese population, and a potential role of pro-inflammatory diet in KOA pathology. We did not find an association between circulating levels of IL-16 and KOA.

1. Introduction

Osteoarthritis (OA) is the most common joint disease worldwide [1]. Knee osteoarthritis (KOA) is ranked as the 11th highest contributor to disability worldwide and the knee is among the most common sites of

osteoarthritis [2]. With its relatively high prevalence and debilitating symptoms, OA is expensive for both affected individuals and to society at large [3].

Initially considered as a “wear-and-tear” disease, OA is now recognized as a progressive multifactorial disease with inflammation playing a

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major role in its pathogenesis [1]. To date, studies have focused on the role of local inflammation in OA development [4]. However, recent evidence indicates that, systemic inflammation associated with changes in the circulatory network of pro and anti-inflammatory cytokines can increase the risk of OA [5, 6, 7].

Interleukin-16 (IL-16) is a pro-inflammatory cytokine associated with chemoattraction and modulation of T cell activation functions [8]. Its circulating levels have been linked to various inflammatory and autoimmune diseases [9]. Interest in IL-16 as a mediator of inflammation in KOA has been growing, with several studies yielding conflicting results [10, 11, 12].

Interleukin-10 (IL-10) is an important anti-inflammatory cytokine with immunomodulatory properties. Among its effects is the inhibition of the production of pro-inflammatory cytokines [13]. Some inflammatory diseases, such as rheumatoid arthritis are characterized by elevated serum and synovial fluid levels of IL-10, where it is thought to decrease disease-induced inflammation [14]. In OA, IL-10 levels were found to be elevated in joint tissues where it exerts anti-inflammatory effects [15]. Additionally, IL-10 and IL-4 expression were increased in the synovium of OA-induced rats performing moderate physical activity [16]. This increased expression is part of a protective mechanism triggered by moderate physical activity against the development of OA and its progression [17]. However, there are limited data on the association between circulating IL-10 levels and KOA [3].

The altered levels of various circulating cytokines observed during OA can be attributed to various factors such as obesity and metabolic syndrome, as well as to the diet of individuals [18]. Diet represents a complex set of interactive factors that result in modification of inflammatory responses [19]. In this regard, the dietary inflammatory index (DII®) is a validated tool used to assess the potential inflammatory effect of the diet, and it has been shown to predict levels of inflammatory markers in over 30 studies throughout the world [20, 21, 22]. The DII has been associated with several serious diseases with underlying inflammatory pathogenesis [23, 24, 25]. To date, only few studies have been conducted to investigate the association between this index and risk of KOA [26, 27].

Basically, the management of OA focuses on palliation of symptoms using a combination of pharmacological agents which have narrow therapeutic safety margins [28]. Moreover, this pharmacological treatment does not modify the progressive course of the disease and often results in additional complications [28]. This explains the rising interest in safe, novel evidence-based nutritional interventions for the management of OA. Therefore, identification of the risk factors including diet and correlated serum biomarkers of the disease is of high importance in order to prevent the development and control the progression of OA, which is especially urgent in light of aging populations throughout the world. With this background, the aim of our study was to investigate plasma concentrations of IL-16 and IL-10 in Lebanese KOA patients and healthy controls and to examine the association between dietary components based on DII scores and increased risk of KOA. In the present study, we relied upon a group of undiagnosed KOA patients in order to study the characteristics of the disease. This group reflects KOA features before the implementation of any therapeutic and lifestyle interventions that may follow the diagnosis. This group also allowed us to examine the changes that may occur in the studied variables after diagnosis.

2. Materials and methods

2.1. Study design

A case-control study design was approved by the institutional review board at Beirut Arab University (2017H-0032-S-P-0216) and the study was conducted in accordance with relevant guidelines and regulations. A total of 208 study participants were assigned to one of 3 groups: Diagnosed Knee Osteoarthritis (DKOA) (N = 78); Undiagnosed Knee Osteoarthritis (UKOA) (N = 60) and controls matched on age, sex and

sociodemographic characteristics (N = 70). All study participants signed an informed consent before being enrolled in the study.

Patients diagnosed with primary KOA, by both clinical and radiological evaluation, were recruited from five health centers and hospitals in North Lebanon. Only patients who scored ≥ 2 points on Kellgren-Lawrence (K&L) radiological classification scale [29] were included in the DKOA group. Additionally, among the subjects visiting the health centers for regular check-ups, we recruited 130 volunteers who reported not being previously diagnosed with KOA and who presented with at least one of the following knee symptoms, even if they appear only after intense activity: pain, swelling, locking, clicking, catching and grinding sensation or morning stiffness that lasts less than 30 min; or presented clinical signs of OA revealed by physical examination (joint line tenderness, crepitus, limited range of motion, bone enlargement), or had varus or valgus deformity. An X-ray of both knees in weight-bearing anteroposterior and lateral view was performed for all volunteers, subjects who had scores ≥ 2 points on K&L scale were classified into the UKOA group and subjects with doubtful radiographic KOA (K&L score 1) or without radiological signs of KOA, were classified as a control.

Subjects with any other types of arthritis, any systemic inflammatory or autoimmune disorder, or previous traumatic knee injury were excluded from the study.

The sample size was based on previously published study [12].

2.2. General and socio-demographic characteristics

Data on demographic characteristics, medical history, current medications and supplement use and lifestyle behaviors were collected at baseline.

Instruments used to measure physical activity and dietary patterns were selected based on validated international standards.

2.3. Physical activity levels

Physical activity levels of the participants were classified as low, moderate or high using the short version of the international physical activity questionnaire (IPAQ), which is a validated international tool used to assess the physical activity level of individuals for research purposes [30]. The IPAQ questionnaire was administered to all participants and data were analyzed according to the IPAQ scoring protocol [31].

2.4. Dietary data

Dietary intake was measured using the 24-hour recall method [32]. During face-to-face interviews, participants were asked to recall foods and portion sizes consumed. Dietary intake was recorded for two days, one week day and one weekend day [33]. Food portion posters (2D Food Portion Visual; Nutrition Consulting Enterprises, Framingham, MA) were used to help participants accurately estimate portion sizes. FAO food-density tables were used to convert the portion sizes identified using the 2-dimensional shapes into weight in grams [34].

To calculate the average daily intake of energy and nutrients, Nutritionist Pro™ software version 7.3.0 (Axxya Systems, Woodinville, WA, USA) was used. Within the software, the USDA food database is used as the reference for the nutritional values of foods.

2.5. Dietary inflammatory index scores

Data derived from the analysis of the 2-day 24-hour recall was used to calculate the energy-adjusted dietary inflammatory index (E-DII™) scores according to a previously reported method [20, 35]. In the present study, 28 out of the possible 45 food parameters were available to calculate E-DII scores (carbohydrate, protein, fat, alcohol, total dietary fiber, cholesterol, saturated fat, monounsaturated fat, polyunsaturated fat, Linolenic acid, Linoleic acid, trans fatty acid, niacin, thiamin, riboflavin, cobalamin-vitaminB12, pyridoxine-vitaminB6, iron, magnesium,

zinc, selenium, vitamin A, vitamin C, vitamin D, vitamin E-alpha tocopherol, folate, beta carotene, caffeine). Total energy intake was used as a denominator for each of the specific food parameters used in computing the E-DII scores.

2.6. Anthropometric measurements

Weight was measured with light clothing on to the nearest 0.1 kg using calibrated electronic scales. Height was measured without shoes to the nearest 0.1 cm by using a standard stadiometer. Body mass index [BMI = weight (kg)/height(m)²] was calculated and classified according to the WHO criteria for overweight and obesity classification [36].

2.7. Blood samples and biomarker assessment

Venous blood samples were collected into EDTA vacutainer tubes. The collected blood samples were centrifuged and the supernatant plasma was aliquoted and stored at -20 °C until further use. The plasma levels of IL-10 and IL-16 were determined using commercially available sandwich enzyme-linked immunosorbent assay kits (ab100549 and ab100555, respectively) purchased from Abcam (Cambridge, UK) following manufacturer's instructions. The detection ranges for IL-10 and IL-16 ranged from 2.34 pg/mL to 150 pg/mL and from 4.12 pg/mL to 3000 pg/mL, respectively.

2.8. Statistical analysis

Statistical analysis was done using IBM SPSS Statistics version 25 (IBM, Armonk, NY, USA). Results were presented as frequencies (percentages) or means (SD) as appropriate. Univariate statistics were performed using T-tests and ANOVA to compare differences between groups

for unimportant determinants, with control for potentially confounding and effect modifying variables. The Chi-square test was used for comparison of categorical variables. A two-tailed p-value ≤ 0.05 was deemed statistically significant in all analyses.

3. Results

The characteristics of the DKOA, UKOA and control groups are summarized in Table 1. Individuals in the DKOA group tended to be less active (p-value ≤ 0.001) and were more likely to consume NSAIDs in comparison to the other two groups (p-value ≤ 0.001). The prevalence of obesity was lowest in the control group in comparison to the other two groups (p-value ≤ 0.001). There were no significant differences by sex, mean age, total energy intake, smoking, and presence of diabetes, hypertension and dyslipidemia between the three groups (p > 0.05). This suggests that group matching based on these variables was adequate.

There was no significant difference in plasma IL-16 levels between UDKOA group and controls (p-value = 0.28); whereas significantly higher levels of IL-16 were observed in UKOA group compared to the DKOA group and in controls compared to the DKOA group (p-value = 0.001 and 0.009 respectively; Figure 1). Moreover, among the three groups, plasma IL-10 levels were the highest in the UKOA group compared to the other two groups (p-value = 0.005; Figure 1) and significantly higher levels were observed in UKOA group compared to controls and in UKOA group compared to the DKOA group (p-value = 0.01 and 0.02, respectively; Figure 1). However, there was no significant difference in IL-10 levels between the DKOA group and controls (p-value = 0.82; Figure 1).

A significant difference in the E-DII scores was observed between the three groups (p-value = 0.005) with significantly higher E-DII

Table 1. General characteristics of the study subjects, Evaluation of circulating levels of Interleukin-10 and Interleukin-16 and dietary inflammatory index in Lebanese knee osteoarthritis patients.

		DKOA ^a	UKOA ^b	Controls	P-value
Gender N = 208	Male N (%)	22 (28.2%)	20 (33.3%)	14 (20%)	0.22
	Female N (%)	56 (71.8%)	40 (66.7%)	56 (80%)	
Diabetes N = 208	Yes N (%)	21 (26.%)	16 (26.7%)	10 (14.3%)	0.12
	No N (%)	57 (73.1%)	44 (73.3%)	60 (85.7%)	
Hypertension N = 208	Yes N (%)	33 (42.3%)	28 (46.7%)	20 (28.6%)	0.08
	No N (%)	45 (57.7%)	32 (53.3%)	50 (71.4%)	
Dyslipidemia N = 208	Yes N (%)	22 (28.2%)	16 (26.7%)	12 (17.1%)	0.25
	No N (%)	56 (71.8%)	44 (73.3%)	58 (82.9%)	
NSAID ^c intake N = 207	Yes N (%)	37 (48.1%)	8 (13.3%)	10 (14.3%)	<0.0001*
	No N (%)	40 (51.9%)	52 (86.7%)	60 (85.7%)	
BMI ^d N = 208	Normal N (%)	10 (12.8%)	0 (0%)	10 (14.3%)	<0.0001*
	Overweight N (%)	19 (24.4%)	16 (26.7%)	35 (50%)	
	Obese N (%)	49 (62.8%)	44 (73.3%)	25 (35.7%)	
Smoking N = 207	Yes N (%)	30 (39%)	32 (53.3%)	38 (54.3%)	0.12
	No N (%)	47 (61%)	28 (46.7%)	32 (45.7%)	
Physical activity level N = 208	Low N (%)	61 (78.2%)	34 (56.7%)	26 (37.1%)	<0.0001*
	Moderate N (%)	17 (21.8%)	26 (43.3%)	43 (61.4%)	
	High N (%)	0 (0%)	0 (0%)	1 (1.4%)	
Age (mean±SD ^e)		61 ± 11.43	59 ± 8.44	58 ± 9.32	0.14
Total energy intake in Kcal ^f (mean±SD ^e)		1650 ± 529	1575 ± 449	1598 ± 547	0.68

* p-value ≤ 0.05 .

^a Diagnosed Knee Osteoarthritis.

^b Undiagnosed Knee Osteoarthritis.

^c Nonsteroidal anti-inflammatory drugs.

^d Body mass index.

^e Standard deviation.

^f Kilocalorie.

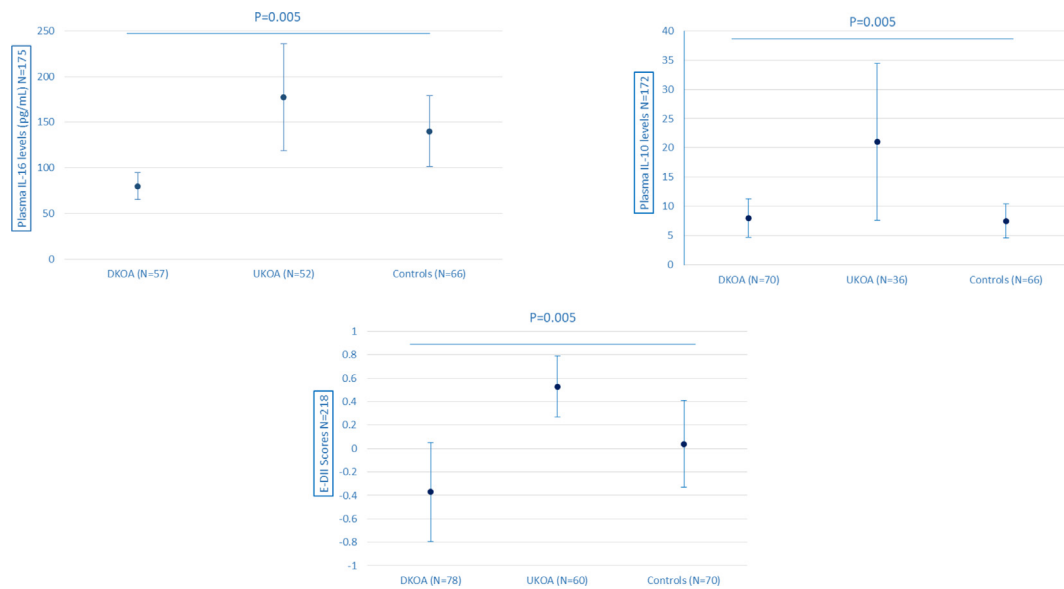


Figure 1. Plasma interleukin levels and Energy-adjusted dietary inflammatory index (E-DII scores) across the study groups. Points represent means, solid lines represent 95% confidence intervals. A. Plasma interleukin-16 (IL-16) levels across the study group. P-value DKOA/UKOA = 0.001; p-value UKOA/controls = 0.28 and p-value DKOA/controls = 0.009. B. Plasma interleukin-10 (IL-10) levels across the study group. P-value DKOA/UKOA = 0.02; p-value UKOA/controls = 0.01 and p-value DKOA/controls = 0.82. C. E-DII scores across the study groups. P-value DKOA/UKOA = 0.001; p-value UKOA/controls = 0.04 and p-value DKOA/controls = 0.16. DKOA: Diagnosed Knee Osteoarthritis; UKOA: Undiagnosed Knee Osteoarthritis.

scores in UKOA compared to the control group (p-value = 0.04) and significantly higher E-DII scores in UKOA group compared to DKOA (p-value = 0.001; Figure 1). However, there was no significant difference in E-DII scores between the DKOA group and controls (p-value = 0.16; Figure 1).

The bivariate test presented in Table 2 illustrates the variation in mean E-DII scores according to selected variables. No statistically

significant differences in the E-DII scores according to these variables were observed.

The intake of proteins, carbohydrates, total fat, alcohol, cholesterol, vitamin D, riboflavin, pyridoxine, cobalamin, zinc, selenium and caffeine were similar between groups stratified by E-DII median (p-value >0.05; Table 3). However, participants with a more pro-inflammatory diet had a significantly lower intake of monounsaturated fat, polyunsaturated fat,

Table 2. Variation in the E-DII^a scores according to selected variables, Evaluation of circulating levels of Interleukin-10 and Interleukin-16 and dietary inflammatory index in Lebanese knee osteoarthritis patients.

		Mean (SD) ^b	P-value
Gender N = 208	Male (N = 56)	0.34 (±1.519)	0.09
	Female (N = 152)	-0.09 (±1.638)	
Diabetes N = 208	Yes (N = 47)	0.01 (±1.360)	0.93
	No (N = 161)	0.03 (±1.685)	
Hypertension N = 208	Yes (N = 81)	0.21 (±1.361)	0.18
	No (N = 127)	-0.10 (±1.752)	
Dyslipidemia N = 208	Yes (N = 50)	-0.29 (±1.500)	0.12
	No (N = 158)	0.12 (±1.641)	
NSAID ^c N = 207	Yes (N = 55)	0.01 (±1.615)	0.96
	No (N = 152)	0.03 (±1.624)	
Smoking N = 207	Yes (N = 100)	0.21 (±1.477)	0.15
	No (N = 107)	0.11 (±1.687)	
Physical activity levels N = 208	Low (N = 121)	0.19 (±1.623)	0.13
	Moderate (N = 86)	-0.18 (±1.581)	
	High (N = 1)	-1.95	
BMI ^d N = 208	Normal (N = 20)	0.55 (±1.898)	0.2
	Overweight (N = 70)	-0.18 (±1.681)	
	Obese (N = 118)	0.06 (±1.512)	

^a Energy-adjusted dietary inflammatory index.

^b Standard deviation.

^c Nonsteroidal anti-inflammatory drugs.

^d Body mass index.

Table 3. Intake of individual dietary components stratified by E-DII^a median. Evaluation of circulating levels of Interleukin-10 and Interleukin-16 and dietary inflammatory index in Lebanese knee osteoarthritis patients.

	<Median E-DII ^a -4.6 to 0.0442	> Median E-DII ^a 0.0442 to 3.65	P-value
Protein (g)	70.67 (±26.96)	66.76 (±23.26)	0.26
Carbohydrates (g)	204.61 (±66.63)	199.14 (±79.02)	0.6
Total fat (g)	62.4 (±28.09)	60.47 (±23.82)	0.6
Alcohol (g)	0.31 (±2.21)	0.14 (±1.25)	0.5
Cholesterol (mg)	184.46 (±155.01)	206.48 (±131.12)	0.27
Saturated fat (g)	16.86 (±7.7)	21.98 (±10.37)	0.0001*
Monounsaturated fat (g)	24.85 (±12.79)	20.78 (±9.14)	0.009*
Polyunsaturated fat (g)	13.47 (±9.38)	9.39 (±6.39)	0.0001*
PUFA 18:2 (g)	11.77 (±8.88)	7.96 (±5.54)	0.0001*
PUFA 18:3 (g)	1.33 (±0.73)	0.99 (±0.59)	0.0001*
Trans fat (g)	0.43 (±0.5)	0.71 (±0.72)	0.001*
Vitamin A (RE)	870.78 (±971.33)	383.09 (±404.85)	0.0001*
Beta carotene (µg)	3954.84 (±5005.88)	968.48 (±1560.01)	0.0001*
Vitamin C (mg)	83 (±73.9)	44.64 (±49.28)	0.0001*
Iron (mg)	16.48 (±6.35)	12.81 (±4.78)	0.0001*
Vitamin D (µg)	0.95 (±1.56)	1.29 (±2.21)	0.2
Vitamin E (Alpha-Tocopherol) (mg)	7.61 (±6.39)	3.92 (±2.08)	0.0001*
Thiamin (mg)	1.44 (±0.53)	1.27 (±0.4)	0.012*
Riboflavin (mg)	1.34 (±0.68)	1.28 (±0.52)	0.46
Niacin (mg)	21.25 (±9.69)	18.67 (±6.96)	0.029*
Pyridoxin (mg)	1.52 (±0.66)	1.27 (±1.15)	0.053
Folate (µg)	486.33 (±244.74)	360.75 (±161.56)	0.0001*
cobalamin (µg)	2.7 (±4.53)	3.17 (±2.62)	0.375
Magnesium (mg)	310.89 (±118.61)	218.29 (±76.79)	0.0001*
zinc (mg)	9.37 (±4.73)	9 (±4.3)	0.55
selenium (µg)	96.67 (±43.88)	88.65 (±35.84)	0.15
dietary fiber (g)	25.09 (±10.34)	16.54 (±9.5)	0.0001*
caffeine (mg)	68.58 (±95.07)	78.1 (±112.67)	0.511

* p-value ≤ 0.05.

^a Energy-adjusted dietary inflammatory index.

linolenic acid, linoleic acid, vitamin A, beta carotene, vitamin C, vitamin E, thiamin, niacin, folate, magnesium and dietary fiber and a higher intake of trans fat and saturated fat than their counterparts with lower DII scores (p-value <0.05; Table 3).

4. Discussion

The present study is unique in that it is the only one, among other cross-sectional studies, that evaluated the same parameters across 3 groups including UKOA patients.

In this study, we found significantly higher circulating levels of IL-10 among UKOA patients compared to matched controls whereas we could not find any statistically significant difference in IL-16 levels between the two groups. This suggests an association between the circulating IL-10 levels and the presence of KOA in the Lebanese population. Moreover, we showed that the UKOA patients also had significantly higher E-DII scores compared to matched controls. Furthermore, the bivariate analysis revealed that there was no significant influence by potential confounding factors on the association between E-DII and KOA described above.

The findings of the present study revealed that circulating levels of IL-10 were higher among UKOA patients compared to matched controls. Our results are in accordance with the findings of an earlier study that showed increased IL-10 levels among KOA patients compared to healthy controls [3]. Several studies conducted on rheumatoid arthritis patients also revealed increased levels of IL-10 in the serum and synovial fluid of the patients, where it is found to have two opposing actions: decreasing disease activity by decreasing pro-inflammatory cytokines levels and enhancing humoral autoimmune response, which warrants further research in the area [37]. Based on the current knowledge about IL-10 in

KOA, it is difficult to discern the exact function of IL-10 in the pathophysiology of the disease. Given that this was a cross sectional study, we cannot deduce whether those increased levels are the cause or the result of the disease. In this regard, a previous study found that a low innate *ex-vivo* production of IL-10 in response to lipopolysaccharide stimulation increases the risk of developing multiple site OA [38]. On the other hand, a later study included that an increased innate *ex-vivo* production of IL-10 in response to lipopolysaccharide stimulation increases the risk of KOA progression in KOA patients [39]. Further prospective studies are needed to elucidate the exact function of IL-10 in the pathophysiology of KOA.

Regarding circulating levels of IL-16, conflicting results have been reported [10, 12]. Our results are in line with the findings of a Malaysian study that found no association between IL-16 and KOA in a multi-ethnic sample of Malaysian population, whereas an earlier study reported a positive association between circulating levels of IL-16 and KOA in a Chinese population [10, 12]. These controversial findings may be related to racial differences. Several studies, have previously linked genetic polymorphisms in different genes coding for various interleukins, including IL-16, with their expression levels as well as with the risk of KOA [10, 11, 40, 41, 42, 43, 44]. This suggests that different populations, with different genetic backgrounds, may have different expression patterns and these, in turn, may influence the role of each interleukin in the complex interleukin network associated with KOA. In this regard, IL-16 levels were found to be significantly different between Indian, Malay and Chinese populations [12]. Therefore, it is important to study the association between circulating levels of IL-16 and KOA in different ethnic groups.

In our study, we were unable to find an association between circulating levels of IL-16 and KOA. However, we did find that circulating

levels of IL-10 are involved in KOA pathophysiology, which suggests the involvement of circulating cytokine network in OA development. Further studies are needed to identify all the other pro and anti-inflammatory cytokines involved in KOA as well as exact function of each interleukin in the development and progression of the disease.

Previous studies observed an association between various individual dietary components and OA [45]. Nevertheless, diet represents an opportunity for complex interaction between various nutrients. Hence, using tools such as E-DII/DII, which was designed to assess the inflammatory effect of the diet as whole, on the development of the disease is of high importance. Various studies suggested that diet-induced changes in circulating levels of inflammatory markers, assessed using DII scores, affect the bone remodeling process in a way that may lead to poor bone health [46, 47]. In this regard, a positive association between DII scores and KOA, which is also characterized by abnormal catabolic and anabolic processes in the cartilage, was previously reported and our findings corroborate their results [26, 27]. Our results suggest that diet has a role in the pathophysiology of the disease among the Lebanese population.

Plausible mechanisms may explain the association between pro-inflammatory diet and KOA. Interaction between various dietary components, assessed using DII/E-DII score, may increase circulating levels of inflammatory mediators including CRP, IL-1 β , IL-6 and TNF [20]. In addition to diet, other factors such as obesity or presence of other chronic diseases can create systemic inflammation [48]. Several previous studies suggest an association between low-grade systemic inflammation and OA, and increased levels of circulating inflammatory markers including CRP, IL-6 and TNF have been previously reported in KOA patients [5, 6, 7, 49, 50, 51]. In fact, it has been postulated that local joint inflammation can be promoted by systemic inflammation which may result in OA [48].

In this study, the mean E-DII score of the DKOA group was significantly lower than that of the UKOA group. This suggests that the increased awareness about the disease that follows the diagnosis led to healthier food choices. Similarly to our results, a study conducted on Lebanese older adults found a negative association between Western diet and the presence of chronic diseases [52]. In addition, Lebanese adults suffering from chronic disease were reported to have reassuring level of adherence to dietary regimen and drug treatments, especially when compared to neighboring countries [53]. Moreover, we found that DKOA patients presented with lower levels of IL-10 and IL-16 than the UKOA group. This may be explained by the significantly higher intake of NSAID by this group compared to the UKOA and controls groups, as well as to the improved dietary patterns after the diagnosis. Additionally, it is important to point out that no significant differences in E-DII scores and IL-10 levels were observed in DKOA group compared to controls. These results suggest that the dietary patterns and IL-10 levels in the DKOA group resemble those of the control group rather than the UKOA group. As for IL-16, the levels were even lower in the DKOA group compared to controls, which may be explained by the intake of NSAID. Taken together, these findings highlight the importance of the presence of a UKOA group in our study as this group faithfully represents KOA features before they are altered by therapeutic intervention and lifestyle modification that follow the diagnosis.

The comparison of the individual intake of each dietary parameter used to calculate the DII scores between subjects with lower E-DII scores and subjects with higher DII scores revealed a higher intake of trans fat and saturated fat and a lower intake of monounsaturated fat, polyunsaturated fat, linolenic acid, linoleic acid, vitamin A, beta carotene, vitamin C, vitamin E, thiamin, niacin, folate, magnesium and dietary fiber among subjects with a more pro-inflammatory diet. Thus, improving the intake of these food components may lead to lower DII/E-DII scores and, potentially, to decreased risk of OA in Lebanese adults.

The strengths of the current work include the use of 3 groups; i.e. those with known knee osteoarthritis, those with undiagnosed knee osteoarthritis who had not been previously treated for KOA and disease-free controls. Additionally, the evaluation of the diet using 24-hour

recalls is considered state-of-the-art. Furthermore, the E-DII is a holistic measure of effect of diet on inflammation which has not yet been studied in Lebanese adults with osteoarthritis. On the other hand, the study limitations include the fact that, as in any case-control study, recall bias might have been present. Additionally, although we controlled for confounding factors, the possible presence of unknown confounders influencing our observation cannot be dismissed.

In conclusion, our findings suggest an association between circulating levels of IL-10 and KOA among the Lebanese population. Hence, our study suggests the involvement of circulating cytokine network in KOA development. Additionally, our results suggest a potential role of pro-inflammatory diet in KOA pathology. However, being cross sectional, our study couldn't investigate the clinical and biochemical changes, including any changes in circulating levels of interleukins, which may result after adoption of diets with lower inflammatory effect and whether these changes may slow down the progression of the disease. Thus, large prospective cohort studies are needed to elucidate all the inflammatory cytokines that are involved in KOA pathology and to discover the factors that can influence the circulating levels of these cytokines such as special dietary and physical activity patterns. These findings will contribute to the development of preventive strategies as well as nonpharmacological and nonsurgical treatments for KOA. Additionally, our study revealed a more pro-inflammatory diet and higher levels of IL-10 among UKOA patients which is to say unaware of their disease, therefore, future studies identifying the role of inflammatory cytokines as markers of KOA are very important to facilitate early detection of the disease and subsequently early implementation of therapeutic interventions in order to halt its progression. Meanwhile, there is a need for specially tailored nutrition educational programs for the general population, specially middle-aged and older adults, in order to change their dietary intake towards a healthier diet rich in dietary components that may decrease inflammation such as adequate intake of fiber, n-3 Fatty acids, β -Carotene, and flavonols and low in unhealthy dietary components that may promote inflammation such as saturated fat and trans fat.

Declarations

Author contribution statement

Zeina El Ali: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Germine El- Kassas and Nisrine Bissar: Conceived and designed the experiments; Analyzed and interpreted the data.

Fouad M. Ziade: Analyzed and interpreted the data.

James Hébert and Nitin Shipavva: Contributed reagents, materials, analysis tools or data.

Hassan Zmerly: Performed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare the following conflict of interests: James R. Hébert owns controlling interest in Connecting Health Innovations LLC (CHI), a company that has licensed the right to his invention of the dietary inflammatory index (DII®) from the University of South Carolina in order to develop computer and smart phone applications for patient

counseling and dietary intervention in clinical settings. Dr. Nitin Shivappa is an employee of CHI. The subject matter of this paper will not have any direct bearing on that work, nor has that activity exerted any influence on this project.

Additional information

No additional information is available for this paper.

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