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Original Article

Alterations of biomarkers in assessing the prognosis of osteoarthritis in patients with total knee replacement: A cross-sectional study

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Abstract

Introduction: Osteoarthritis (OA) is a chronic disorder characterized by inflammation, cartilage degeneration and bone remodelling with molecular derangements followed by anatomic and physiologic elements. The aim of the study was to assess the alterations of biomarkers in patients who underwent total knee replacement (TKR) for OA.

Methods: A cross-sectional retrospective analysis was carried out on 206 participants who underwent either unilateral or bilateral TKR for knee OA from January 2017 to December 2020. Data regarding medical and surgical history, demographic characteristics, X-ray findings, complete blood count (CBC), plasma glucose and high-sensitive C-reactive protein (hsCRP) were collected and analysed. Ethics approval was obtained. Based on the normality of distribution appropriate statistical tools were used. P value ≤ 0.05 was considered statistically significant.

Results: Body mass index was significantly higher in participants who underwent bilateral TKR rather than unilateral TKR. Females were affected at a younger age compared to males. Hypothyroid participants had unilateral than bilateral TKR. Neutrophil lymphocyte ratio was higher in bilateral rather than unilateral TKR ($P = 0.038$). Males had higher monocyte lymphocyte ratio ($P \leq 0.001$), especially in individuals 61-80 years of age as well as in obese individuals.

Conclusion: The use of markers such as total leukocyte count, differential count, neutrophil to lymphocyte (NLR), monocyte to lymphocyte ratios (MLR) and hsCRP are cost-effective and could predict the severity of the disease. These markers could be used to screen individuals at risk for developing OA of the knee and help in assessing the prognosis; thus allowing surgeons to decide on the appropriate management of the disease.

Introduction

Osteoarthritis (OA) is one of the most common chronic progressive degenerative diseases of the joints, which leads to functional disability and decreased quality of life. It is a heterogeneous disorder with genetic, biochemical, endocrine and inflammatory components. The disease is characterized by the progressive degeneration of chondrocytes of the joints, and the most commonly affected joint is the knee. A study by Cui et al, has given the global prevalence of 16% in individuals aged 15 to 39 years and 22.9% in individuals aged more than 40 years.[1] Risk factors of OA are based on two fundamental mechanisms which could either be the consequence of trauma on a normal joint or of normal loading on a maligned joint.[2] Investigations performed include complete blood count (CBC), X-ray and magnetic resonance imaging (MRI). The disease is graded depending upon the disease severity as shown by the Kellgren-Lawrence (KL) classification in Table 1.[3]

Total knee replacement (TKR) is the management of choice for severe OA. In the recent times, various studies have been conducted to establish the role of biomarkers such as cytokines and cartilage degradation products in the diagnosis of OA. We aimed to investigate the alterations in biomarkers in patients with OA who underwent TKR.

Methods

A retrospective cross-sectional study was done in the Departments of Orthopaedic Surgery and Medical Records, Sri Ramachandra Institute of Higher Education and Research (SRIHER), Chennai. The study included 206 patients who had undergone TKR. The data were

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Table 1. Grading of knee osteoarthritis based on KL classification[3]

Grade name	Definition
Grade I	Doubtful narrowing of joint space and possible osteophytic lining
Grade II	Definite osteophytes and possible joint space narrowing
Grade III	Moderate multiple osteophytes with joint space reduction
Grade IV	Joint space greatly reduced with subchondral sclerosis

collected between January 2017 and December 2020. Waiver of informed consent was obtained since this was a retrospective study and all the participants were treated and discharged from the hospital.

Inclusion and exclusion criteria

Participants of both sexes, aged between 30 and 80 years, were included in the study. They were diagnosed with primary OA and had undergone TKR surgery in either unilateral or bilateral knee joints. Participants with known autoimmune disorders, tumours or any other joint problems like osteomyelitis of bone within the joint, patients on anti-inflammatory drugs and antineoplastic drugs were excluded. Patients with chronic liver, renal, heart and lung diseases were also excluded.

Sample collection

The data were obtained from the Departments of Orthopaedic Surgery and Medical Records from patients who had TKR in SRIHER. Collected data included medical and surgical history, demographic characteristics, X-ray findings, CBC, plasma glucose and high-sensitive C-reactive protein (hsCRP). Neutrophil to lymphocyte (NLR) and monocyte to lymphocyte ratios (MLR) were calculated.

Statistical analysis

Data analysis was performed by SPSS version 16. The obtained data were checked for normality of distribution using Kolmogorov-Smirnov test, indicating that the data did not follow normality of distribution. Hence, continuous variables were expressed as median and interquartile range (IQR) and categorical variables were expressed as frequency and percentage. Mann-Whitney U test, Kruskal Wallis test, chi-square test and Fischer exact test were used to compare the data. Spearman correlation coefficient was used to test the correlation between the variables. Receiver operating characteristics (ROC) curve was carried out to find the area under the curve (AUC) and the cut-off values of biomarkers. P value ≤ 0.05 was considered statistically significant.

Results

The total number of participants who underwent TKR from January 2017 to December 2020 was 354. The following flow chart depicts the process for the recruitment of study participants into the study (Figure 1).

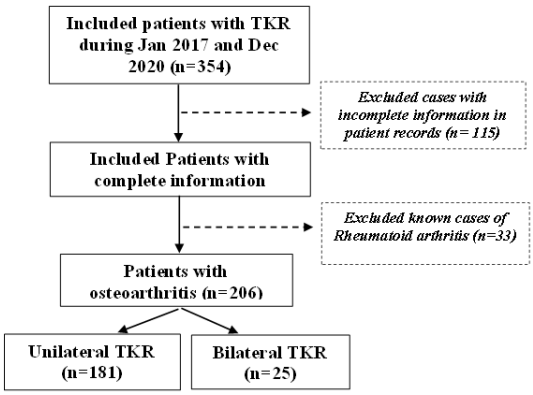

Figure 1. Recruitment of participants into the study

In the participants, grouped according to BMI as normal weight, overweight and obese, MLR was found to be statistically significant with P value of 0.05. Spearman correlation was done between MLR and other variables, however, the correlation coefficients and corresponding P values were not significant.

Receiver operating characteristics (ROC) curve was done for NLR and MLR. Youden index was used to arrive at the cut-off point. The AUC for NLR and MLR were 0.586 and 0.526 with 95% confidence interval of 0.494-0.678 and 0.435-0.617, respectively, and P values were 0.07 and 0.582, respectively, which were statistically not significant. The cut-off values of NLR and MLR were 2.72 and 0.27, respectively.

Discussion

Knee OA is one of the most common forms of OA. Initially, the disease was thought to be a disease of articular cartilage but recent research shows that the pathology involves the entire joint. It is a multifactorial disease with complex mechanisms involving interactions between the various joint tissues.[4] The early changes in the pathogenesis of the disease involve the mechanisms mediated by pro-and anti-inflammatory cytokines. It manifests as synovitis which promotes the release of inflammatory mediators and in turn the activation of the chondrocytes leading to metalloproteinase synthesis with subsequent cartilage degradation (Figure 2). Innate immunity also acts as a trigger of local inflammation in OA. These inflammatory events occurring within the joint tissues are reflected outside the joint in the systemic circulation in patients with OA.[5]

MRI, an imaging technique gives information on the extent of involvement of the structures within the joint due to the disease process. X-ray is the investigation of choice for patients with knee OA due to low cost and accessibility. This study was aimed to determine the levels of inflammatory markers such as hsCRP, total leucocyte count, neutrophils%, lymphocytes%, monocytes%, NLR, MLR and erythrocyte sedimentation rate (ESR) in OA, who underwent TKR.

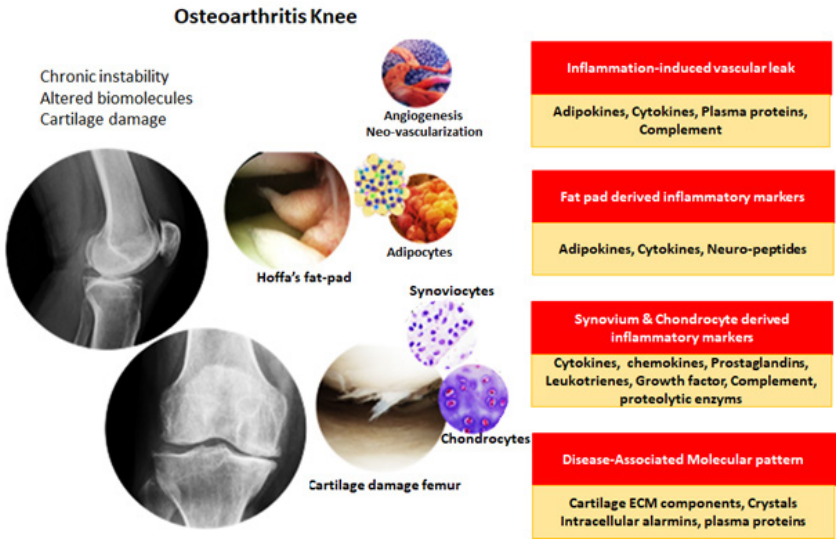

Figure 2. Pathogenesis of osteoarthritis of knee

In the present study, total number of participants were 206; of which 25 participants had bilateral TKR and 181 had unilateral TKR. The age was significantly higher in participants who underwent bilateral than unilateral TKR ($P=0.042$). Females (n = 163) outnumbered males (n = 43), but it was not statistically significant (Table 2). Females were found to be affected at an earlier age (60 years) compared to males (66 years), which was found to be significant ($P<0.001$; Table 3). There was no significant difference between males and females with regard to the number of joints operated for TKR. Males had higher height and weight compared to females which were statistically significant with $P<0.001$ and $P=0.0027$, respectively. But BMI was 27 in both males and females with no statistical significance (Table 3).

According to Asia-Pacific guidelines, human body weight is classified as normal weight, overweight and obese based on BMI of ≤ 22.9, 23-24.9 and ≥ 25 kg/m^2, respectively. In the present study, 62% (n = 129) of individuals were in the obese category, 15% (n = 32) were in overweight and 21% (n = 45) were in normal weight category (Table 4). BMI of patients with bilateral TKR belonged to the obese group with a median value of 32.2 kg/m^2 when compared to patients with unilateral TKR with a median value of 26.2 kg/m^2 and was statistically significant ($P=0.003$; Table 2). There were no significant differences with regard to age and gender among the groups when compared based on BMI. Patients with lower BMI had undergone unilateral TKR. Hence, it was inferred that increasing BMI poses burden on the joints predisposing them to OA. This was more common in females (n = 98) (Table 4). A meta-analysis illustrates the presence of sex differences in prevalence and incidence of OA indicating females are at a higher risk of developing the disease. And females tend to have more severe knee OA, particularly after menopause. These differences may be associated with the increased BMI, decreased physical activity, hormonal changes, and local bone factors.[6]

Table 2. Demographic characteristics of the study participants

Variables	All participants with TKR (n = 206)	Bilateral TKR (n = 25)	Unilateral TKR (n = 181)	*P* value
Age (y), Median (IQR)	61 (55, 67)	65 (60, 68)	61 (55, 67)	0.042*
Females, No. (%)	163 (79)	20 (80)	143 (79)	0.9
Males, No. (%)	43 (21)	5 (20)	38 (21)	
Height (cm),), Median (IQR)	155 (150, 160)	152 (148, 157)	155 (151, 160)	0.061
Weight (kg),), Median (IQR)	65 (55, 76)	70 (65, 88)	65 (55, 75)	0.035*
BMI (kg/m^2),), Median (IQR)	26 (23, 31)	32 (28, 35)	26 (23, 30)	0.003**
DM, No. (%)				
Absent	145 (70)	14 (56%)	131 (72)	0.093
Present	61 (30)	11 (44%)	50 (28)	
HT, No. (%)				
Absent	111 (54)	12 (48%)	99 (55)	0.5
Present	95 (46)	13 (52%)	82 (45)	
Hypothyroidism, No. (%)				
Absent	173 (84)	17 (68%)	156 (86)	0.036*
Present	33 (16)	8 (32%)	25 (14)	

TKR: total knee replacement; BMI: body mass index; DM: diabetes mellitus; HT: hypertension; IQR: interquartile range.
** *P* value highly significant; * *P* value significant.

Obesity, type 2 diabetes mellitus (T2DM), hypertension, and dyslipidaemia are interlinked factors leading to metabolic syndrome. These factors could be involved in the evolution of OA. In the present study, there were 61 (30%) diabetics, 95 (46%) hypertensives and 33 (16%) hypothyroid patients. There was no significant difference between the groups with regard to plasma glucose. HbA1c levels were higher in both bilateral and unilateral TKR

Table 3. Distribution of variables among study participants according to gender

Variables	All (n= 206)	Female (n = 163)	Male (n = 43)	*P* value
Age (y), No. (%)	61 (55, 67)	60 (54, 66)	66 (60, 70)	<0.001**
Bilateral TKR, No. (%)	25 (12%)	20 (12%)	5 (12%)	0.882
Unilateral TKR, No. (%)	181 (88%)	143 (88%)	38 (88%)	
Height (cm), median (IQR)	155 (150, 160)	154 (150, 158)	160 (156, 168)	<0.001**
Weight (kg), median (IQR)	65 (55, 79)	64 (51, 77)	69 (58, 90)	0.0027**
BMI (kg/m^2), median (IQR)	27 (23, 31)	27 (23, 31)	27 (24, 35)	0.6
hsCRP (mg/L), median (IQR)	1.3 (0.6, 2.65)	1.25 (0.6, 2.4)	1.4(0.4, 5)	0.866
TC (cells/ c.mm), median (IQR)	8500 (7025, 9875)	8600 (7100, 9900)	7800 (6950, 9450)	0.12
N%, median (IQR)	63 (57, 68)	63 (57, 68)	60 (54, 67)	0.13
L%, median (IQR)	26 (20, 31)	26 (20, 31)	26 (19, 31)	0.9
NLR, median (IQR)	2.41 (1.82, 3.45)	2.41 (1.89, 3.44)	2.41 (1.73, 3.40)	0.7
M%, median (IQR)	7.25 (6.23, 8.57)	7.10 (5.95, 8.40)	8.40 (7.45, 10.25)	<0.001**
MLR, median (IQR)	0.28 (0.22, 0.39)	0.26 (0.21, 0.36)	0.38 (0.26, 0.49)	0.001**
ESR (mm/hr), median (IQR)	38 (20, 61)	40 (21, 62)	29 (8, 48)	0.071

BMI: Body mass index; hsCRP: high sensitive C-reactive Protein; ESR: Erythrocyte Sedimentation rate; TKR: total knee replacement; TC: Total leukocyte count; N: Neutrophil; L: Lymphocyte; NLR: Neutrophil Lymphocyte ratio; M: Monocyte; MLR: Monocyte Lymphocyte ratio.
** *P* value highly significant; * *P* value significant.

Table 4. Distribution of data in three groups based on BMI

Variables	Overall, (n=206)	(BMI: <22.9 kg/m^2) (n=45)	(BMI: 23-24.9 kg/m^2) (n = 32)	(BMI: >25 kg/m^2) (n=129)	*P* value
Age (y)	61 (55, 67)	58 (53, 67)	60 (55, 67)	62 (58, 67)	0.2
Females, No. (%)	159 (78%)	38 (18%)	23 (12%)	98 (48%)	0.4
Males, No. (%)	47 (22%)	7 (3%)	9 (4%)	31 (15%)	
Bilateral TKR, No. (%)	25 (12%)	2 (1%)	4 (2%)	19 (9%)	0.2
Unilateral TKR, No. (%)	181 (88%)	43 (21%)	28 (13%)	110 (54%)	
RPG (mg/dL)	109 (93, 143)	109 (96, 130)	115 (99, 160)	101 (90, 144)	0.2
FPG (mg/dL)	116 (104, 158)	106 (100, 119)	134 (111, 162)	116 (102, 161)	0.2
PPPG (mg/dL)	167 (121, 245)	146 (112, 216)	253 (165, 357)	166 (115, 243)	0.2
HbA1c (%)	6.95 (6.27, 7.93)	6.50 (5.90, 6.75)	7.00 (5.75, 7.20)	7.20 (6.40, 8.52)	0.03*
hsCRP (mg/L), median (IQR)	0.6 (0.45, 2.4)	1.2 (0.4, 2.47)	2.3 (0.75, 6.27)	1.4 (0.5, 2.6)	0.06
TC (cells/c.mm), median (IQR)	8500 (7100, 9900)	8500 (7000, 9500)	7500 (6600, 9325)	8700 (7200, 10400)	0.08
N %, median (IQR)	62 (56, 68)	65 (59, 71)	62 (56, 67)	62 (54, 67)	0.13
L %, median (IQR)	26 (20, 32)	25 (18, 29)	25 (19, 31)	27 (22, 33)	0.05*
NLR, median (IQR)	2.40 (1.80, 3.43)	2.65 (1.99, 3.83)	2.34 (1.85, 3.48)	2.24 (1.71, 3.11)	0.07
M%, median (IQR)	7.40 (6.30, 8.60)	6.90 (5.70, 8.20)	8.45 (7.07, 10.22)	7.20 (6.18, 8.50)	0.01*
MLR, median (IQR)	0.28 (0.22, 0.39)	0.27 (0.24, 0.42)	0.34 (0.24, 0.50)	0.27 (0.21, 0.37)	0.05*
ESR (mm/h), median (IQR)	38 (20, 60)	40 (24, 60)	42 (22, 65)	32 (14, 52)	0.3

RPG: Random Plasma Glucose, FPG: Fasting Plasma glucose, PPPG: Post Prandial Plasma Glucose; hsCRP: high sensitive C-reactive Protein; HbA1c: Glycated haemoglobin: total knee replacement; TC: Total leukocyte count; N: Neutrophil; L: Lymphocyte; NLR: Neutrophil Lymphocyte ratio; M: Monocyte; MLR: Monocyte Lymphocyte ratio
The variables are expressed as median (IQR).
** *P* value highly significant; * *P* value significant.

and were 7.05 and 6.95%, respectively, which were not statistically significant. It was higher in bilateral TKR and showed a direct relationship between poor glycaemic control and OA (Table 2). HbA1c levels were found to be increasing with an increase in BMI, which was statistically significant ($P=0.03$; Table 4). Increased mechanical load on the weight-bearing knee joints increases the risk of OA. In obesity, adipocytes produce various cytokines, and hormones which induce systemic inflammation and insulin resistance.[7] A meta-analysis on BMI and the risk of OA by Zheng and Chen, concludes that obesity is a robust risk factor for knee OA and weight reduction is advisable in the management of knee OA.[8] According to Changulani et al, the mean age of obese patients who underwent knee replacement surgery was lesser than the individuals with normal BMI.[9] The present study observed

that the BMI was higher in both unilateral and bilateral TKR. Holliday et al showed that obese individuals showed an odds ratio of 2.68 in developing knee OA. Participants who were overweight in their earlier stages of life, showed higher risk of lower limb OA than the normal weight individuals.[10]

T2DM causes widespread oxidative stress and low-grade chronic inflammation. This is due to the increased formation of advanced glycation end products (AGEs), which activate chondrocytes and synoviocytes to produce pro-degradative and proinflammatory mediators. This provokes a low-grade systemic inflammation that induces local joint inflammation, as well as cause neuromuscular deficiencies which destabilize the joint.[11] GLUT-1 transporters present in the plasma membrane of the cells in the joint increase glucose uptake, which induces proinflammatory cytokines.[12] Insulin resistance plays a pivotal role in chronic inflammation. Alenazi et al showed that there is an association between localised OA and T2DM.[13] Hypothyroidism poses a higher risk of developing autoimmune diseases like rheumatoid arthritis. In the present study, there was a significant increase in the number of individuals with hypothyroidism compared to the euthyroid individuals, more so with bilateral TKR, which was statistically significant ($P=0.036$; Table 1). This is in contradiction to Hellevik et al, who stated that there was no association between thyroid dysfunction and TKR. The link between hypothyroidism and obesity may also be the cause of OA in hypothyroid patients.[14]

Felson et al reported that the radiographic evidence of OA was increased with age and higher incidence of OA in women when compared to men.[15] The sources of age-related changes include both the peripheral sources such as adipose tissue that increase with age, local inflammatory production within the joint tissues and systemic inflammation due to the ageing. In the present study, the participants were divided into three groups based on the age such as early (young) adulthood (30-50 years), middle adulthood (51-60 years), and late (old) adulthood (61-80 years). Most of the participants were in late adulthood 53%, while 36% were in middle adulthood and 11% were in early adulthood. In the early adulthood, most of the patients were females (92%), and OA was increased in the males in the middle adulthood and late adulthood by 15% and 28%, respectively, which showed statistical significance ($P=0.029$; Table 5). During early adulthood, people are at their physical peak with good health. During middle adulthood, people start to show signs of ageing. The risks of health problems such as heart disease, cancer, and diabetes are more during this time. During late adulthood, people are not physically fit and usually have less muscle and slower reflexes. Their immune system doesn't function as it used to, henceforth are prone to infections.[16] In the present study, TKR was done in a single joint (100%) in the early adulthood group, and a slight increase in bilateral TKR as the age advanced to middle and late adulthood groups (11% and 16%, respectively) with no statistical significance ($P=0.079$). There was a significant increase in height and weight as the age advanced but there was no statistically significant change in BMI (Table 5).

OA is an inflammatory process with an imbalance between pro-inflammatory and anti-inflammatory markers. Inflammation is demonstrated to be present in the OA joints well before the radiographic OA. The inflammatory mediators include cytokines, adipokines,

Table 5. Distribution of data according to three age groups

Variables	All (30-80 years) (n= 206)	30-50 years (n = 24)	51-60 years (n = 74)	61-80 years (n = 108)	*P* value
Female, No. (%)	163 (79%)	22 (92%)	63 (85%)	78 (72%)	0.029*
Male, No. (%)	43 (21%)	2 (8%)	11 (15%)	30 (28%)	
Bilateral TKR, No. (%)	25 (12%)	0 (0%)	8 (11%)	17 (16%)	0.079
Unilateral TKR, No. (%)	181 (88%)	24 (100%)	66 (89%)	91 (84%)	
Height (cm), median (IQR)	155 (150, 160)	155 (150, 158)	154 (150,158)	156 (152, 160)	0.023*
Weight (kg), median (IQR)	65(55,76)	57(52,71)	65(55,74)	67(59,78)	0.021*
BMI (kg/m^2), median (IQR)	27 (23, 31)	24 (23, 27)	27 (23, 35)	27 (24, 31)	0.2
hsCRP (mg/L), median (IQR)	0.6 (0.45, 2.4)	0.9 (0.45, 2.1)	0.6(0.6, 2.4)	0.6 (0.32, 2.4)	0.841
TC (cells/c.mm), median (IQR)	8500 (7025, 9875)	8450 (7200, 10350)	8750 (7650, 10200)	8250 (6900, 9700)	0.3
N %, median (IQR)	63 (57, 68)	63 (56, 66)	63 (57, 70)	62 (56, 68)	0.6
L %, median (IQR)	26 (20, 31)	27 (22, 33)	26 (19, 31)	26 (20, 31)	0.3
NLR, median (IQR)	2.41 (1.82, 3.45)	2.30 (1.67, 2.95)	2.45 (1.85, 3.63)	2.30 (1.85, 3.44)	0.4
M%, median (IQR)	7.25 (6.23, 8.57)	6.55 (5.55, 8.43)	7.20 (5.93, 8.40)	7.75 (6.38, 8.93)	0.023*
MLR, median (IQR)	0.28 (0.22, 0.39)	0.23 (0.20, 0.25)	0.27 (0.22, 0.41)	0.31 (0.23, 0.42)	0.008**
ESR (mm/h), median (IQR)	38 (20, 61)	41 (29, 62)	40 (22, 55)	32 (13, 62)	0.6

TKR: total knee replacement; hsCRP: high sensitive C-reactive Protein; TC: Total leukocyte count; N: Neutrophil; L: Lymphocyte; NLR: Neutrophil Lymphocyte ratio; M: Monocyte; MLR: Monocyte Lymphocyte ratio; IQR: interquartile range.
** *P* value highly significant; * *P* value significant.

proteolytic enzymes etc. CRP is an acute-phase reactant released by the liver in response to proinflammatory cytokines during the inflammatory process. Measurement of serum CRP shows the extent of inflammation in an individual. In a meta-analysis by Zhang, higher CRP levels were strongly associated with the progression of knee OA and when used along with cartilage oligomeric matric protein (COMP) showed the increased chances of diagnosis of knee OA.[17] Shadyab et al suggested that the levels of hsCRP are not associated with TKR.[18] In the present study, there was no statistical differences in ESR and hsCRP between the groups. There were no significant differences in neutrophils and monocytes. The values of lymphocytes in patients with bilateral TKR and in patients with unilateral TKR were 24% and 27%, respectively which was statistically significant ($P=0.033$; Table 6). There was no significant change in hsCRP according to age groups ($P=0.841$; Table 5). HsCRP levels were higher in overweight group when compared to other groups ($P=0.06$; Table 4).

In the present study, NLR in bilateral and unilateral TKR groups were 2.72 and 2.32, respectively, which were statistically significant ($P=0.038$). MLR in bilateral and unilateral TKR groups were 0.31 and 0.27, respectively, and were not statistically significant ($P=0.086$; Table 6). Normal NLR in a healthy adult is reported to be 0.78-3.53.[19] In a cross-sectional study by Taşoğlu et al, NLR >2.1 had 50 % sensitivity and 77% specificity in predicting the severity of knee OA and it emerged as an independent predictor of severe knee OA.[20] The ratio has been reported to be different with age and sex groups.[21] Unlike, Chen et al stated a drop in neutrophil and an increase in the lymphocyte counts before 50 years. NLR in females and males were the same of 2.41 and was not statistically significant (Table 3). Oestrogen reduces the apoptosis of neutrophils and supresses the production of lymphocytes in bone marrow. As the age advances, oestrogen levels decrease, leading to increased neutrophil apoptosis and increased bone marrow lymphocyte production resulting in the decreased neutrophil to lymphocyte ratio.[22]

MLR constitutes the balance of immune disease progression. Monocytes are activated by inflammasome mediated pathways, and chronic low-grade inflammasome activation helps to drive OA progression.[22] A decrease in lymphocytes can be due to the accumulation at the site of inflammation.[23] Gao et al have suggested the diagnostic value of MLR in knee OA and its higher values in grade IV rather than in other grades of KL.[24] In the present study, MLR was found to be higher in males than in females and were 0.38 and 0.26, respectively, which was found to be highly statistically significant ($P<0.001$; Table 3). Buttle et al demonstrated the gender preference as the immune system in males is less effective than in females despite greater TLR2 and TLR4 expression, more Th17 cells and CD4 + cells, CD8 + T cells & NK cells.[25]

Inflammatory monocytes selectively travel to the sites of inflammation, produce cytokines and contribute to local and systemic inflammation.[26] Monocyte chemokines and cytokines are found at increased concentrations in the synovial fluid of OA joints. In humans, soluble monocyte and activated macrophages in serum and synovial fluid correlate with synovial macrophages, joint space narrowing and osteophytes. Low-grade inflammation in OA, activates circulating monocytes, increasing their surface expression of trafficking and activation markers and their production of proinflammatory cytokines. Loukov et al suggested that the activation of monocytes occurs prior to their entry into the synovium.[27] In the present study, monocytes increased from early to middle and late adulthood and were 6.55%, 7.20% and 7.75%, respectively, which were statistically significant ($P=0.023$). MLR were 0.23, 0.27 and 0.31 among early,

Table 6. Alterations in biochemical parameters among the study participants

Variables	All participants with TKR (n = 206)	Bilateral TKR (n = 25)	Unilateral TKR (n = 181)	*P* value
RPS (mg/dL)	109 (92, 142)	112 (98, 161)	109 (92, 139)	0.5
FPG (mg/dL)	116 (104, 158)	152 (119, 174)	114 (102, 153)	0.055
PPPG (mg/dL)	167 (121, 245)	228 (228, 228)	166 (119, 246)	0.5
HbA1c (%)	6.95 (6.27, 7.93)	7.05 (6.60, 7.32)	6.95 (6.27, 8.00)	0.8
hsCRP (mg/L)	0.6 (0.5, 2.4)	0.6 (0.4, 1.9)	0.6 (0.5, 2.4)	0.8
TC (cells/ c.mm)	8500 (7025, 9875)	8600 (7900, 9700)	8500 (7000, 9900)	0.8
N%	63 (57, 68)	66 (59, 77)	62 (56, 68)	0.058
L%	26 (20, 31)	24 (16, 26)	27 (20, 32)	0.033*
NLR	2.41 (1.82, 3.45)	2.72 (2.45, 4.60)	2.32 (1.80, 3.34)	0.038*
M%	7.25 (6.23, 8.57)	7.00 (6.00, 8.50)	7.30 (6.30, 8.60)	0.9
MLR	0.28 (0.22, 0.39)	0.31 (0.25, 0.53)	0.27 (0.22, 0.38)	0.086
ESR (mm/hr)	38 (20, 61)	51 (24, 62)	38 (20, 57)	0.6

TKR: total knee replacement; RPG: random plasma glucose; FPG: fasting plasma glucose; PPPG: Postprandial plasma glucose; HbA1c: glycated haemoglobin; hsCRP: high sensitive C-reactive Protein; TC: Total leukocyte count; N: Neutrophil; L: Lymphocyte; NLR: Neutrophil Lymphocyte ratio; M: Monocyte; MLR: Monocyte Lymphocyte ratio; ESR: erythrocyte sedimentation rate
All the variables are expressed in median and interquartile range (IQR); * *P* value significant.

middle and late adulthood participants, respectively, with a statistical significant difference ($P = 0.008$). There were no statistically significant alterations with regard to age in total leukocyte count, neutrophils, lymphocytes, and NLR (Table 5). Seidler et al demonstrated the dynamic changes of circulating monocytes, showing the increasing levels as the age advances in humans. This could be due to the changes in the innate and adaptive immune system, which have been reported to contribute to the immune senescence observed in old age.[28]

Several studies suggest alterations in the levels of neutrophils, lymphocytes, monocytes, and platelet count during systemic inflammation. These mediators could scope for pharmacological intervention which may slow down the disease and help in prognosticating the disease. The utility of NLR during stress responses and inflammation has been studied. Inflammation is accompanied by the rapid influx of neutrophils to the inflammatory site. These neutrophils cause chemotaxis, release of reactive oxygen species (ROS), granular proteins, production and release of cytokines. Neutrophil subsets can suppress T cell activation and proliferation and their presence may provide novel therapeutic ideas in the case of inflammatory disorders.[29]

In the present study, there was a significant increase in lymphocytes in the obese group ($P = 0.05$). There were significant increases in the monocytes and MLR according to BMI, with $P = 0.01$ and $P = 0.05$, respectively. NLR showed a consistent decrease with an increase in BMI. NLR was found to be 2.65, 2.34 and 2.24 in normal, overweight and obese groups, respectively ($P = 0.07$). MLR in normal weight, overweight and obese groups were 0.27, 0.34 and 0.27, respectively with a significance ($P = 0.05$). ESR was found to be high in all the groups with no statistically significant difference (Table 4). A cross-sectional retrospective data showed a positive correlation between NLR with BMI.[30]

NLR and MLR did not yield statistically significant results in ROC probably due to small sample size. Spearman correlation was done between MLR and NLR with other variables. But, correlation coefficients and corresponding *P* values were not significant.

Limitations

The study is a retrospective study which involves analysis of data from the database from a single centre. Entire data sets for all patients were not available. Longitudinal studies could be performed to assess the disease progression. Inclusion of lipid profile, parathyroid hormone, vitamin D, calcium and phosphorus could give complete information about the biochemical alterations in OA. Information on diet intake and physical activity could add value to the study.

Conclusion

Knee OA is one of the most common diseases of middle and old ages irrespective of gender. In this study, monocytes, and MLR were significantly elevated. Many advanced biomarkers are in research in order to halt or slow the disease process at an earlier stage. Use of routine markers such as total leukocyte count, differential count, NLR, MLR and hsCRP are cost-effective and could predict the severity of the disease. These markers could be used for screening individuals who are at risk for developing OA of the knee.

Authors' Contribution

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Competing Interests

The authors declare that there was no conflict of interests while conducting the research as well as during the publication of the article.

Ethical Approval

All procedures are performed in studies involving human participants were in accordance with the ethical standards of the institutional and/ or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai (CSP-MED/21/JAN/65/03, dated 11.02.2021).

Study Highlights

What is current knowledge?

- X-ray of the knee has been the gold standard method of diagnosis of osteoarthritis.

What is new here?

- Biomarkers such as NLR and MLR could be early indicators of osteoarthritis; they are cost-effective as well as radiation hazards can be avoided

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