

The Diagnostic Value of Serum Fatty Acid Binding Protein-4 (FABP-4) Level and Angiotensin Like- 4 (ANGPTL-4) in the Diagnosis of Knee Joint Osteoarthritis: Case Control Study

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Abstract

Background: Osteoarthritis is one of the most common joint disorders worldwide and it is responsible for significant morbidity and deterioration of quality of life and in particular elderly population (Mora et al., 2018). The diagnosis is based on clinical as well as radiological assessment; however, biochemical investigation plays a little role in the diagnosis, but recent data had suggested a role for some biochemical markers in the pathogenesis as well as in the diagnosis and assessment of disease severity. Aim of the study: the aim of the present study was to evaluate the potential diagnostic role of FABP4 and ANGPTL-4 serum levels in the diagnosis of knee joint osteoarthritis in a sample of Iraqi patients. Patients and methods: This study is done in outpatient consultation clinic of Rheum& medical rehabilitation at Al-Imamain Al-kadhimain teaching hospital, Baghdad - Iraq. A case control study was included two groups, knee osteoarthritis group of patients and the control group. It was executed during the term from first of June 2021 to last of December 2021. Determination of Fatty acid binding protein-4 (FABP-4) level and Angiotensin like- 4 (ANGPTL-4) level was based on ELISA method. Results: Fatty acid binding protein-4 (FABP-4) level and Angiotensin like- 4 (ANGPTL-4) level were significantly higher in patients group in comparison with control group ($p < 0.001$). Based on these observations, the diagnostic potential of these markers was assessed using receiver operator characteristic (ROC) curve analysis in order to find the best cutoff value of these markers that can predict a diagnosis of osteoarthritis in terms of sensitivity and specificity and the results were shown in figures 1 and 2 and table 4. The cutoff value of FABP-4 was > 0.588 with 100 % sensitivity, 100 % specificity and 100 % accuracy. The cutoff value of ANGPTL-4 was > 0.522 with 96 % sensitivity, 100 % specificity and 99.4 % accuracy. Conclusion: Higher levels of Fatty acid binding protein-4 (FABP-4) and Angiotensin like- 4 (ANGPTL-4) in patients with knee OA suggest a diagnostic potential for these markers; however, the accuracies of these markers are variables in such a way that FABP-4 is the most accurate followed by ANGPTL-4.

Keywords: FABP-4, ANGPTL-4, knee joint osteoarthritis

1. Introduction

Osteoarthritis is one of the most common joint disorders worldwide and it is responsible for significant morbidity and deterioration of quality of life and in particular elderly population (Mora et al., 2018). The diagnosis is based on clinical as well as radiological assessment; however, biochemical investigation plays a little role in the diagnosis, but recent data had suggested a role for some biochemical markers in the pathogenesis as well as in the diagnosis and assessment of disease severity (Antony and Singh, 2021). FABP4 was first detected in adipose tissue and mature adipocytes in the 1980s (Hunt et al., 1986) and is mainly expressed in adipocytes and macrophages (Furuhashi et al., 2014). The exact role of FABP4 in the pathogenesis and progression of OA is not fully understood; however, in a recent experimental study it has been shown that FABP4 knockdown suppressed the inflammation, oxidative stress, apoptosis and

extracellular matrix degradation of IL-1 β -induced chondrocytes by activating PPAR γ to inhibit the NF- κ B signaling pathway (Mao et al., 2012). With respect to ANGPTL-4, its tissue overexpression has been linked to rheumatoid arthritis by; however, to the best of our knowledge, no previous study has raised its potential role as a serum marker for OA. Therefore, the aim of the present study was to evaluate the potential diagnostic role of FABP4 and ANGPTL-4 serum levels in the diagnosis of knee joint osteoarthritis in a sample of Iraqi patients.

2. Patients and Methods

This study is done in outpatient consultation clinic of Rheum& medical rehabilitation at Al-Imamain Al-kadhimain teaching hospital, Baghdad - Iraq. A case control study was included two groups, knee osteoarthritis group of patients and the control group. It was executed during the term from first of June 2021 to last of December 2021. Information was taken by a questionnaire paper after taking an informed consent

from all participants, the total number of 160 samples (80 patients & 80 controls) is chosen to participate in this study knee osteoarthritic patients aged between 40 to 70 years old is chosen after full examination & diagnosis by the consultant rheumatologist in the outpatient department during the period of the study. Regarding the control group, age and gender match's apparently healthy subjects was select from patients' relatives attending the teaching Laboratory. All the patients group are Iraqi subject came to departmental outpatient clinic complaining of knee pain and whose knee x-rays had confirmed of primary knee OA was prospectively include.

Determination of Fatty acid binding protein-4 (FABP-4) level and Angiotensin like- 4 (ANGPTL-4) level was based on ELISA method.

Data were analyzed using statistical package for social sciences (SPSS) version 16 and Microsoft Office Excel 2007. Numeric variables were presented as range, standard deviation and mean, while, categorical variables were presented as count and percentage. Independent samples student t-test was used to study difference in means between two groups and chi-square test was used to study differences in proportions. The level of significance was set out at $p \leq 0.05$.

3. Results

The Demographic characteristics of patients and control subjects are shown in table 1. There was no significant difference in mean age between patients and control group, 52.04 ± 10.45 years versus 51.11 ± 6.99 years, respectively ($p = 0.309$). The patients group included 19 (23.8 %) males and 61 (76.2 %) females; whereas, control group 16 (20.0 %) males and 64 (80.0 %) females, respectively and there was no significant difference in the frequency distribution according to gender between patients and control group ($p = 0.566$). The mean body mass index (BMI) of patients with osteoarthritis was higher than that of control group, 32.55 ± 5.21 kg/m² versus 23.94 ± 3.14 kg/m², respectively ($p < 0.001$).

The mean duration of disease was 9.79 ± 12.20 months and the median was 6.0 months with a range of 0.5 to 60 months. Patients were classified according to

severity of disease into the following: 10 (12.5 %) having grade I, 38 (47.5 %) having grade II, 18 (22.5 %) having grade III and 14 (17.5 %) having grade IV.

Comparison of serum lipid profile between patients group and control group is shown in table 2. Total serum cholesterol, serum triglyceride, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels were all significantly higher in patients group in comparison with control group ($p < 0.01$); whereas, serum high density lipoprotein level (HDL) was significantly lower in patients group in comparison with control group ($p < 0.001$).

Comparison of Fatty acid binding protein-4 (FABP-4) and Angiotensin like- 4 (ANGPTL-4) is shown in table 3. Fatty acid binding protein-4 (FABP-4) level and Angiotensin like- 4 (ANGPTL-4) level were significantly higher in patients group in comparison with control group ($p < 0.001$).

Based on these observations, the diagnostic potential of these markers was assessed using receiver operator characteristic (ROC) curve analysis in order to find the best cutoff value of these markers that can predict a diagnosis of osteoarthritis in terms of sensitivity and specificity and the results were shown in figures 1 and 2 and table 4. The cutoff value of FABP-4 was > 0.588 with 100 % sensitivity, 100 % specificity and 100 % accuracy. The cutoff value of ANGPTL-4 was > 0.522 with 96 % sensitivity, 100 % specificity and 99.4 % accuracy.

Characteristic	Patients n=80	Control n=80	P value
Age (years)			
Mean \pm SD	52.04 ± 10.45	51.11 ± 6.99	0.309 I
Range	40-80	40-70	NS
Gender			
Male, n (%)	19 (23.8 %)	16 (20.0 %)	0.566 C
Female, n (%)	61 (76.2 %)	64 (80.0 %)	NS
BMI (kg/m ²)			
Mean \pm SD	32.55 ± 5.21	23.94 ± 3.14	< 0.001 I
Range	23-43	19.4-30.1	**
n: number of cases; SD: standard deviation; I: independent samples test; C: chi-square test; **: significant at $p \leq 0.01$; NS: not significant			

Characteristic	Patients n = 80	Control n = 80	P value
Total cholesterol (mg/dl)			
Mean \pm SD	181.44 ± 38.90	158.76 ± 27.77	0.008 I **
Range	76-256	105-209	
Triglyceride (mg/dl)			
Mean \pm SD	204.64 ± 74.52	144.48 ± 36.70	< 0.001 I **
Range	51.2-383	89.7-242	
HDL (mg/dl)			
Mean \pm SD	38.77 ± 12.52	57.02 ± 10.29	< 0.001 I **
Range	23.6-98.87	24.3-75.76	
LDL (mg/dl)			
Mean \pm SD	108.45 ± 29.94	86.17 ± 8.28	< 0.001 I **
Range	30.2-169.6	74-107	
VLDL (mg/dl)			
Mean \pm SD	39.90 ± 14.82	26.81 ± 7.88	< 0.001 I **
Range	6.9-76.55	11.14-48.5	
n: number of cases; SD: standard deviation; LDL: low density lipoprotein; HDL: high density lipoprotein; VLDL: very low density lipoprotein; I: independent samples test; **: significant at $p \leq 0.01$; NS: not significant			

Table 3: Comparison of Fatty acid binding protein-4 (FABP-4), Angiopitin like- 4 (ANGPTL-4) and Paraoxonase (PON 1) enzyme activity between patients and control subjects

Characteristic	Patients n = 80	Control n = 80	P value
FABP-4			
Mean \pm SD	1.33 \pm 0.40	0.35 \pm 0.10	< 0.001 **
Range	0.7 -2.21	0.16 -0.59	
ANGPTL-4			
Mean \pm SD	0.93 \pm 0.28	0.35 \pm 0.08	< 0.001 **
Range	0.41 -1.7	0.2 -0.52	

n: number of cases; SD: standard deviation; FABP-4: Fatty acid binding protein-4; ANGPTL-4: Angiopitin like- 4; PON-1: Paraoxonase; I: independent samples test; **: significant at $p \leq 0.01$

Table 4: The characteristics of ROC analysis

Characteristics	FABP-4	ANGPTL-4
Cutoff	>0.588	>0.522
AUC	1.000	0.994
95% CI	0.954 to 1.000	0.942 to 1.000
p-value	< 0.01 **	< 0.01 **
Sensitivity %	100.0	96.0
Specificity %	100.0	100.0
Accuracy %	100.0	99.4

FABP-4: Fatty acid binding protein-4; ANGPTL-4: Angiopitin like- 4; PON-1: Paraoxonase; AUC: area under the curve; CI: confidence interval; **: significant at $p \leq 0.01$

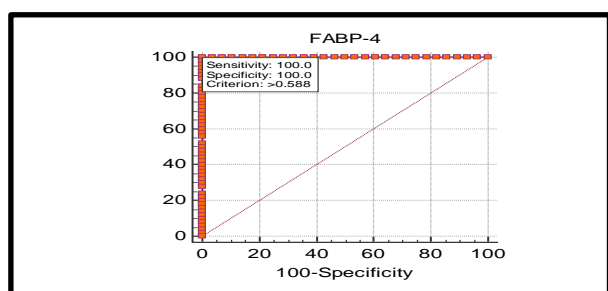


Figure 1: Receiver operator characteristics (ROC) curve analysis to find the best FABP-4 cutoff value that can predict a diagnosis of osteoarthritis in terms of sensitivity and specificity

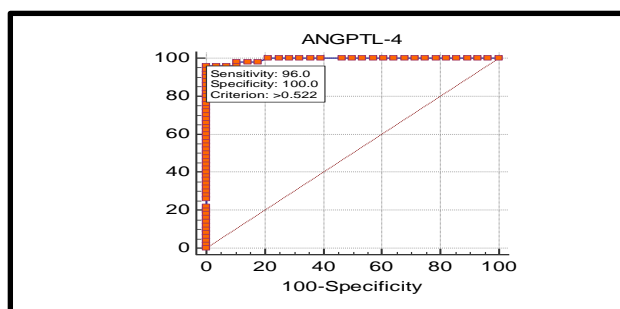


Figure 2: Receiver operator characteristics (ROC) curve analysis to find the best ANGPTL-4 cutoff value that can predict a diagnosis of osteoarthritis in terms of sensitivity and specificity

4. Discussion

In the current study, the duration of disease was ranging from 0.5 to 60 months. This wide range of duration of disease will affect the results in that shorter duration will be associated with fewer complications and less disability in comparison with longer duration of disease; therefore, we noticed various degree of severity grades and most of included patients were of grade II disease accounting for 48 % of cases. In the current study,

total serum cholesterol, serum triglyceride, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels were all significantly higher in patients group in comparison with control group; whereas, serum high density lipoprotein level (HDL) was significantly lower in patients group in comparison with control group.

The relationship between dyslipidemia and the risk of OA was investigated by three case-control studies (Frey et al., 2017; Inoue et al., 2011; Stürmer et al., 1998). Two case-control studies were population-based, and one case-control study was hospital based. According to Frey et al. (2017) and Sturmer et al. (1998), there exists an increase in the risk of knee OA among dyslipidemia patients. Two hospital-based, cross-sectional studies reported the influence of dyslipidemia on the risk of OA (Xie et al., 2017; Zhou et al., 2017). Both studies reported data on knee OA, and both reported an obvious connection of dyslipidemia with OA. A number of cohort studies have also highlighted the possible link between OA and dyslipidemia (Engstrom et al., 2009; Han et al., 2013; Monira Hussain et al., 2014; Garcia-Gil et al., 2017). All above studies were reviewed and meta-analyzed by Xiong et al in 2020 and they concluded that "Even though our meta-analysis of case-control and cross-sectional studies suggested a strong relationship between dyslipidemia and osteoarthritis; this relationship was not validated by our meta-analysis of only cohort studies. As a result, further investigation needs to be conducted on the relationship between dyslipidemia and osteoarthritis, considering the significant public health relevance of the topic".

In the current study, fatty acid binding protein-4 (FABP-4) level and Angiopietin like- 4 (ANGPTL-4) level were significantly higher in patients group in comparison with control group. We were able to find cutoff values for FABP-4 (> 0.588) with 100 % accuracy and for ANGPTL-4 (> 0.522) with 99.4 % to predict a diagnosis of knee OA.

In line with our study, Zhang et al (2018) reported that OA patients (n = 38) had significantly higher plasma FABP4 than control group patients (n = 29). In addition, Zhang et al (2018) stated that plasma FABP4 correlated with the clinical severity of knee OA. Indeed, the association between FABP4 concentration and severity of knee joint OA has been documented by (Schadler et al., 2021). FABP4 was first detected in adipose tissue and mature adipocytes in the 1980s (Hunt et al., 1986), and is

mainly expressed in adipocytes and macrophages (Furuhashi et al., 2014). However, if FABP4 plays roles in musculoskeletal diseases remains unknown. Cerezo et al (2013) compared the FABP4 concentrations of serum and SF in RA patients and OA patients. They found that the serum FABP4 was significantly higher in RA patients than OA patients, which suggest FABP4 as a biomarker for RA. However, in their study, the OA patients were treated as control cohort to RA patients. The exact role of FABP4 in the pathogenesis and progression of OA is not fully understood; however, in a recent experimental study it has been shown that FABP4 knockdown suppressed the inflammation, oxidative stress, apoptosis and extracellular matrix degradation of IL-1 β -induced chondrocytes by activating PPAR γ to inhibit the NF- κ B signaling pathway (Mao et al., 2012).

With respect to ANGPTL-4, its tissue overexpression has been linked to rheumatoid arthritis by (Swales et al., 2014); however, to the best of our knowledge, no previous study has raised its potential role as a serum marker for OA. Therefore, our result in that serum ANGPTL-4 is serum marker of knee OA is a point of originality. Hypoxia and the hypoxia-inducible factor (HIF) transcription factor drive pathological bone loss in conditions including rheumatoid arthritis (RA), osteoarthritis, osteoporosis, primary bone tumours, and bone metastatic cancer. There is therefore considerable interest in determining the function(s) of HIF-induced genes in these pathologies. Angiopoietin-like 4 (ANGPTL4) is an adipose-derived, HIF-1 α - and PPAR γ -induced gene that was originally discovered as an endocrine and autocrine/paracrine regulator of lipid metabolism. Given the inverse relationship between bone adiposity and fracture risk, ANGPTL4 might be considered a good candidate for mediating the downstream effects of HIF-1 α relevant to osteolytic disease (Knowles et al., 2017). The ANGPTL4 gene encodes angiopoietin-like 4, which is an important regulator of angiogenesis (Gealekman et al., 2008). Perdiguero observed that ANGPTL4-deficient mice have impaired angiogenesis and increased vascular leakage (Perdiguero et al., 2011). ANGPTL4 also stimulates endothelial cell growth and tubule formation, and prevents endothelial cell apoptosis (Gealekman et al., 2008).

5. Conclusion

Higher levels of Fatty acid binding protein-4 (FABP-4) and Angiopoietin like- 4 (ANGPTL-4) in patients with knee OA suggest a diagnostic potential for these markers; however, the accuracies of these markers are variables in such a way that FABP-4 is the most accurate followed by ANGPTL-4.

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