The Role of Serum Level of Col2a1 and Hyaluoronic Acid in Diagnosis of Knee Osteoarthritis

Abbas A Rudini[1], Prof. Dr. Basil O Saleh[2], Prof. Dr. Fiaq Isho Gorial[3]

[1]MSc, Ph.D. Clinical Biochemistry, Biochemistry Department, College of Medicine, University of Baghdad
[2]MSc, Ph.D. candidate, Clinical biochemistry, Biochemistry Department, College of Medicine, University of Baghdad
[3]FICMS College of Medicine, University of Baghdad

KEYWORDS

Knee osteoarthritis , Coll2a1, HA.

ABSTRACT

Osteoarthritis (OA) is a complex condition in which affected tissues experience changes in metabolism, structure, biochemistry, and function. This condition arises when chondrocytes fail to balance the synthesis and degradation of extracellular components like proteoglycans and collagens, leading to inflammation of the synovial membrane and joint capsule.

Aim of the study: This study was designed to evaluate the serum levels of Type II Collagen alpha 1 (coll2a1) and Hyaluronic Acid (HA) in diagnosis of early stage of knee osteoarthritis in Iraqi patients

Subjects and Methods: This case- control study was carried out at Department of Biochemistry, College of Medicine, University of Baghdad and at Rheumatology Department
, Baghdad Hospital / Medical City, during the period from September 2022 to March 2023. It included ninety volunteer males and females subdivided into subgroups: Group 1consisted of 50 patients who have had early stages of knee osteoarthritis (KOA) and group 2 involved 40 apparently healthy subjects who served as control group. The diagnosis of KOA was made by a consultant rheumatologist using American College of Rheumatology (ACR) criteria. Moreover, patients were sub- classified according to their BMI (normal weight, overweight and obese) and KOA severity (grad1 and grad 2). Investigations included serum measurements of Human serum coll2a1(type II collagen alpha I) and Human serum HA (Hyaluronic Acid) in all patients and controls by ELISA technique.

Results: The mean (±SD) values of Coll2a1, HA, of KOA patients were significantly higher than those of controls (for all, $p < 0.01$). There was significant positive correlation between. The mean value of serum hyaluronic acid was significantly increased in grad 2 KOA when compared with grad 1 KOA patients ($p < 0.004$). The results found that fibulin-3 has the highest receiver operator characteristic (ROC) and area under curve (AUC=89.2%) at cutoff (7.50 ng/ml) with sensitivity 80.0 and specificity 80.0 in diagnosis of KOA. Also, serum Coll2a1 has high diagnostic utility (AUC=88.6%) at cutoff (286.00 pg/ml) with sensitivity 82.0 and specificity 80.0 .

Conclusion: Assessing non-invasive serum levels of coll2a1 (with a cutoff of 286.00 pg/ml) offers a new biochemical marker for diagnosing the early stages of knee osteoarthritis (KOA) potentially before changes become visible on X-rays. Additionally, measuring serum hyaluronic acid levels could serve as an indicator of disease severity, helping to differentiate between various grades of KOA.

1. Introduction

Osteoarthritis

Osteoarthritis is a complex condition where affected tissues undergo changes in metabolism, structure, biochemistry, and function [Semenistaja S et al., 2023]. It results from chondrocytes' inability to maintain a balance between the production and degradation of extracellular components like proteoglycans and collagens, leading to inflammation in the synovial membrane and joint capsule [Baccarin RY et al., 2022]. The specific factors and processes that trigger this imbalance remain unclear. As cartilage breaks down, these products are released into the synovial space, and their types (such as type II collagen markers and COMP) are being studied as potential biomarkers for the development of osteoarthritis. [Kumavat R et al., 2021].

Knee osteoarthritis

The three compartments of the knee joint—the medial, lateral, and patellofemoral joints—are affected by knee osteoarthritis (OA), which usually worsens over the course of 10 to 15 years and interferes with day-to-day activities. It was formerly thought to be exclusively a "wear-and-tear" ailment of the articular cartilage brought on by ageing, unrelated to inflammation. Although the

exact aetiology is still unknown and under investigation, knee OA is now known to have a complex aetiology. The condition is mostly caused by biomechanical and inflammatory mechanisms. Family history, age, obesity, diabetes, synovitis, innate immunity, systemic inflammatory mediators, lower limb alignment (such as genu valgum and genu varum), joint form and dysplasia, trauma, and inflammation associated with metabolic disorders are among the factors that might cause knee OA.

. [Espandar R et al., 2010].

Diagnosis of knee osteoarthritis

The diagnosis was traditionally based on the patient's medical history, physical examination, and results of conventional radiography, which included joint space constriction, osteophyte formation, subchondral sclerosis, and subchondral cysts. (Gress *et al.*, 2020). Additionally, arthroscopy, magnetic resonance imaging (MRI), and high-frequency colour ultrasonography evaluation can be used to diagnose KOA. (Xu *et al.*, 2019). According to the American College of Rheumatology (ACR), knee osteoarthritis can be diagnosed using radiologic and clinical criteria. (Di cesare *et al.*, 2017) as follow:

Clinical ;

1. Knee pain for the majority of the previous month
2. Active joint motion coupled with crepitus
3. Stiffness in the morning that lasts for at least thirty minutes
4. Examination-related bony growth of the knee
5. Be over 38 years old.

Diagnosis requires 1 + 2 + 4, or 1 + 2 + 3 + 5, or 1 + 4

Clinical and Radiographic;

- Knee pain for most days of prior month.
- Osteophytes at joint margins (radiological).
- Synovial fluid typical of osteoarthritis (laboratory).
- Age ≥40 years.
- Morning stiffness lasting ≤30 min.
- Crepitus with active joint motion.

Diagnosis requires 1 + 2, or 1 + 3 + 5 + 6, or 1 + 4 + 5 + 6

Type II collagen alpha 1

The majority type II collagen in cartilage is called Coll2-1 and Coll2-1NO2, and in the early stages of osteoarthritis (OA), these breakdown products of type II collagen are roughly twice as high as in more advanced cases. [Kumavat R et al., 2021]. Coll2-1 is a peptide that is released from collagen type II during the degradation of cartilage, and it is the native form, whereas Coll2-1NO2 is the deoxygenated version. Peptide nitration is triggered by the interaction of aromatic amino acids with peroxynitrite (ONOO-), a powerful oxidant derived from the combination of nitric oxide (NO) and superoxide (O2-), both of which are produced by macrophages and chondrocytes in osteoarthritis. The amino acid tyrosine in the alpha chain of type II collagen interacts with the nutrient salt nitrate to form Coll2-1NO2 [Kumavat R et al., 2021]. The Coll2A1 form that is nitrated indicates the presence of related cartilage damage and inflammation in OA. While the levels of Coll2-1 in the serum are stable throughout life, the levels of Coll2-1 in synovial fluid and the degradation of cartilage is increased in the serum and synovial fluid of patients with OA, this is attributed to the increased stressors in the serum and

synovial fluid. Coll2-1NO2 is a significant biomarker for the detection of oxidative-related damage to cartilage and the effects of anti-inflammatory and antioxidant therapies.. (Horcajada MN et al., 2021).

Hyaluronic acid (HA)

Hyaluronic acid (HA) is the primary component of synovial fluid (SF), it contributes to the joint's smoothness and viscosity, and the capacity of the cartilage to withstand pressure. It's also associated with the radiographic progression of osteoarthritis (OA) [Chen M et al., 2022]. In OA, the serum HA levels are elevated, but theSF levels decrease during the late stages of the disease. Elevated serum HA levels are associated with the severity of OA, as measured by the WOMAC score and the K&L score, respectively [46]. The K&L system categorizes the severity of OA on a five-point scale: grade 0 lacks radiographic evidence of OA, grade 1 has questionable joint space narrowing (JSN) and osteophytes, grade 2 is comprised of definite osteophytes and JSN, grade 3 has moderate osteophytes, sclerosis, and possible bone misform, and grade 4 is associated with large osteophytes and severe sclerosis with no visible bone misform. [Chahal J et al., 2019]. In clinical experiments,HA injections are employed to treat OA by increasing the production of chondrocytes of endogenous HA, this prevents the loss of cartilage, promotes the repair of cartilage, and decreases the stiffness of the joint through the production of anti-inflammatory mediators and metalloproteinases. (Bauer C et al.,2022).

Patients and Controls Groups

This case- control study was carried out at Department of Biochemistry, College of Medicine, University of Baghdad, at Rheumatology Department, Baghdad Hospital / Medical City, and at Rheumatology Unit of Al-Kindy Teaching Hospital, during the period from September 2022 to March 2023. It included 90 volunteers males and females subdivided into two groups:

Group 1: Fifty patients who have had early stage of knee osteoarthritis (KOA)

Group 2: Forty apparently healthy subjects who served as control group, they have had no history or clinical evidence of knee osteoarthritis**.**

Collection of Blood Sample:

Five milliliters of peripheral blood sample was collected from each participant (patient and control subject) and placed into a gelatin-based container that would allow the sample to clot for (15 minutes). After this, the sample was centrifuged at 3000 rpm for 10 minutes. The isolated serum samples were kept at -20ºC in Eppendorf tubes until the time of measuring serum biomarkers.

Statistical analysis

The analysis of data was conducted using SPSS-27 (Statistical Package for the Social Sciences, version 27). The information was presented through basic statistical analysis including the frequency, percentage, mean, standard deviation, and range. Differences between means (quantitative data) were evaluated using the Student's t-test for comparisons between two individual means and ANOVA for comparisons among more than two individual means. Differences in proportions (qualitative data) were assessed with the Pearson Chi-square test (χ^2-test). A significant difference was considered when the P value was smaller than or equal to 0.05.The correlation coefficient value (r):

0.3 - <0.5 indicates weak association, whereas values <0.3 indicate no correlation. A moderate correlation is 0.5 < -0.7, and a high correlation is >0.7. The Receiver Operating Characteristic "ROC" curve technique can be used to ascertain whether a given parameter can be utilised as a screening or diagnostic tool for disease, as well as to choose the cut-off value that has the best possible combination of sensitivity and specificity for disease diagnosis.

Demographic Characteristics:

Gender disturbution and their percent (males versus males and females versus females) differences of subjects between knee osteoarthritis (KOA) patients and control groups revealed non- significant difference table 1. The gender distribution within KOA group showed comparable of males and females.

Table 1: Gender distribution of subjects between study groups

		Male	Female	p value
KOA group		25(50.0)	25(50.0)	0.15 NS
Control group	N (%)	26 (65.0)	0.15 NS	0.15 NS

Serum concentration of Col2a1 and HA

Table 2 reveals the (mean ±SD) values of serum levels of Coll2a1 and HA of KOA patients and controls. the mean value of Col2a1 of KOA group (367.40±106.88 pg/ml) was significantly increased when compared with that of control group (183.80±113.14 ng/ml, p< 0.01). However, the mean value of HA of KOA patients (42.43±13.40 ng/ml) was significantly elevated in comparison with that of controls (22.25±14.77 ng/ml, p < 0.01

Table 2: Mean ±(SD) values of serum concentration of Coll2a1 and HA of KOA patients and controls

Parameter	KOA (n= 50)	Control (n= 40)
Col2a1 (pg/ml)	367.40±106.88▲	183.80±113.14
HA (pg/ml)	42.43±13.40▲	22.25±14.77

▲ t-test revealed significant increase of Coll2a1 and HA in KOA than controls (for all, p < 0.01)

Serum COMP ,Col2a1, Fibulin-3 and HA concentrations and severity of knee osteoarthritis

Table 2 reveals the mean (±SD) value of serum Coll2a1 and Hyloronic acid levels in KOA patients with grad 1 & 2 according to K-L severity. The mean value of serum hyloronic acid of KOA grad 2 (47.97±7.38 pg/ml) was significantly increased compared with that of KOA grad 1 (37.55±15.50 pg/ml, p < 0.004). However, there were non- significant differences between grade I and grade 2 in serum col2a1.

Table 3: Mean (±SD) values of serum Col2a1 and Hyloronic acid in grad 1 & 2 knee osteoarthritis patients according to Kellegren Lawrence classification

Parameter	Grad 1 (n=28)	Grad 2 (n=22)
Col2a1(pg/ml)^{NS}	390.03±112.56	340.82±95.40
HA (ng/mL)	37.55±15.50	47.97±7.38•

t -test reveled ● siginificant increase in serum HA in grad 2 than in grad 1 (p< 0.004), NS: non-significnt differences in serum Coll2a1between grad 1 and grad2.

Discussion

Demographic and laboratory parameters of knee osteoarthritis patients and control:

The results of this study showed no significant difference in the mean age between primary knee osteoarthritis (OA) patients (47 years) and the healthy control group (50 years), which is important for ensuring age is matched and its effects are excluded (Table 3.2). In contrast, Jafari et al. (2019) reported a mean age of 57.60 years for their OA patients, while Akinmade et al. (2021) found a mean age of 65.53 years for their patients.

Additionally, the study found no significant gender difference between knee OA patients and control subjects, with both groups being matched for gender (Table 3.1). However, Jafari et al. (2019) noted that females with OA represented 78.30% of their sample, while males made up 21.70%. Similarly, Arellano et al. (2017) reported a higher percentage of female OA patients compared to males, with figures of 64.52% and 35.48%, respectively.

COMP, Fibulin 3, Coll2a1, and HA in Knee osteoarthritis:

The mean blood levels of human serum HA and human collagen type II alpha 1 (coll2a1) in patients with osteoarthritis of the knee showed a significant difference in this study, with both markers being greater than in the control group. This discovery aligns with the findings of Cai-xia Liu et al. (2019), who discovered that knee OA patients had considerably higher serum coll2a1 levels than controls. In a similar vein, patients with osteoarthritis in their knees showed significantly higher serum hyaluronic acid levels than the control group, according to Tatsuro Saruga et al. (2021).**Serum Coll2a1and HA concentrations in grad 1 and grad 2 of Knee osteoarthritis.**

When Grade I knee osteoarthritis was compared to Grade II, the study discovered a statistically significant rise in serum HA levels. This finding is consistent with Tatsuro Saruga's (2021) findings, which showed a substantial correlation between serum HA levels and the radiographic severity of osteoarthritis in the knee. This suggests that HA levels increase as the illness progresses. In a similar vein, Papaneophytou et al. (2021) discovered a correlation between increased blood HA levels and increased radiographic severity of knee OA. A link between blood HA levels and the radiographic course of knee OA was also documented by Sasaki et al. in a variety of populations, including healthy people and those with early or severe knee OA.

Limitations and Strengths of the Study:

A limitation of this study is its relatively small sample size, which may particularly impact results related to obesity. On the other hand, a notable strength of the study is the potential use of noninvasive blood biochemical markers for diagnosing early-stage knee osteoarthritis, which could complement and even precede the diagnostic value of clinical and radiological assessments.

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