

ORIGINAL ARTICLE

Validation of Levels of Decorin as A Reliable Biomarker of Osteoarthritis: Comparison of Serum and Synovial Fluid LevelsRIZWAN ANWAR¹, BILAL HABIB², YASIR MUSTAFA³, ASMA KAZI⁴, OMER FAROOQ⁵, MUFASSAR NISHAT⁶¹Assistant Professor, orthopedic surgery Islam medical college sialkot²Associate Professor, Department of Physiology Rai Medical College Sargodha³Consultant Orthopedic surgeon PSSHMC hospital Raiwind⁴Associate Professor, Department of Medicine Rashid Latif Medical College Lahore⁵Assistant Professor Orthopedic surgery. khawaja Muhammad safdar medical college sialkot⁶Associate professor plastic surgery University medical & dental college. Faisalabad

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ABSTRACT

Objective: The proteoglycan decorin, which plays an important part in the aetiology of osteoarthritis as well as a role in the binding of collagen, was the focus of this study's background information and objectives (OA). This investigation was carried out with the objectives of determining the levels of decorin in the blood and synovial fluid of patients who suffered from knee OA and determining whether or not these levels had a correlation with OA and the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) score.

Place of Study: Islam medical college Sialkot

Duration: December 2021 to May 2022

Methods: In this prospective study, a total of 60 persons were enrolled in the study and control groups: 30 people with knee osteoarthritis and 30 people with other knee joint problems. For the purpose of determining how well the knee functions, the WOMAC score was utilised. Both the levels of decorin in the blood and the levels of decorin in the synovial fluid were analysed by means of an enzyme-linked immunosorbent assay (ELISA). We used a technique known as binary logistic regression, which contained both a single and a multi-categorical predictor, in order to evaluate the potential risk factors for OA. This allowed us to determine which characteristics would increase the likelihood of developing OA.

Results: It was discovered that the levels of serum decorin in the group that was diagnosed with OA were statistically and considerably higher than the levels found in the group that acted as the control (P 0.001). There was not a significant difference in the amounts of decorin found in synovial fluid between those who had OA and the control group. WOMAC score (OR)=1.073, 95% confidence interval (CI): 1.032-1.116, P<0.001

This association between OA and high serum decorin levels was shown to be statistically significant (OR=1.114, 95%CI: 1.030–1.205, P=0.007). OA was demonstrated to be strongly linked with high serum decorin levels. There was shown to be a positive association between the levels of serum decorin and the WOMAC score in patients who had OA.

Conclusions: An increase in serum decorin levels may be indicative of changes in the structure of the extracellular matrix. The fact that there is a positive connection between the serum decorin level and the WOMAC score lends credence to the previous assertion that this conclusion is correct. It was found that having a higher WOMAC score and having higher serum decorin levels are both risk factors associated with osteoarthritis (OA). On the other hand, there was no link found between osteoarthritis and the amount of decorin found in the joint fluid.

INTRODUCTION

The structure of the extracellular matrix (ECM) of articular cartilage is composed of collagen, proteoglycans, and chondrocytes. Articular cartilage is a kind of hyaline cartilage. This ECM structure is responsible for supporting the body load in addition to providing flexibility, elasticity, and resistance against tensile forces and compressive forces, respectively. The collagen molecules that make up the extracellular matrix (ECM) are held together by proteoglycans, which act as a glue. In particular, short leucine-rich proteoglycans, commonly known as SLRPs, play a significant role in the process of generating, bonding, and determining the shape of the collagen fibril structure. This is because SLRPs are responsible for determining the shape of the collagen fibril structure. In the SLRP family of extracellular proteoglycans, decorin is regarded to be a member of class I, along with biglycan and asporin. This class is comprised of the extracellular proteoglycans. This provides evidence that decorin belongs to the SLRP family of proteins. The greatest number of SLRPs may be found in the cartilage of the body, and the quantity of these proteins continues to increase with age, notably decorin levels. In the process of fibrillogenesis, the purpose of decorin is to restrict the diameter of the fibril and to regulate its structure. This is accomplished by regulating the structure of the fibril. Decorin possesses the ability to function in a manner that is both anti-fibrotic and pro-inflammatory. The objective of this study was to determine the levels of decorin present in the serum and synovial fluid of people who had been diagnosed with osteoarthritis (OA), as well as to investigate whether or not the presence of decorin levels was related to OA and the osteoarthritis score developed by the Western Ontario and McMaster Universities.

MATERIALS AND METHODS

Between the months of June and October of 2018, a prospective study was carried out in the orthopaedics department of the Konya Beysehir State Hospital in Konya, Turkey. The size of the sample was chosen so that it would be able to detect an expected effect size of 0.3 for the regression equation at a power level of 0.95 (=0.95) and a probability level of 0.05 (=0.05). This was accomplished by determining the size of the sample in such a way that it would be able to detect this effect size at both of these levels. The following formula was applied in order to achieve this result: power = power level + probability level. In total, sixty individuals expressed interest in contributing to the study and agreed to do so. It was determined that the patient had osteoarthritis of the knee based on the findings of the magnetic resonance imaging as well as the radiographic characteristics. The Kellgren–Lawrence scale was utilised to arrive at the conclusion that a total of forty-four patients were suffering from osteoarthritis of the knee. Consequently, it was necessary for there to be evidence of all five radiological criteria, which included osteophytes on the joint side, periarticular ossicles, joint area narrowing (JSN), and microscopic pseudocystic patches within the subchondral bone. Patients diagnosed with osteoarthritis were placed into one of five stages based on the severity of their condition: stage 0 (no changes in X-ray), stage 1 (osteophyte and no JSN), stage 2 (osteophyte and JSN), stage 3 (medium multiple osteophytes, JSN, minimal sclerosis, and deformity of bone ends), and stage 4 (severe deformity of bone ends) (giant osteophytes, evident JSN, severe sclerosis and deformity of bone ends). The patient had reached stage 0 at this point. For the purpose of serving as a comparison group, we recruited forty-four patients who suffered

from knee joint conditions other than osteoarthritis (arthroscopic anterior cruciate ligament reconstruction, meniscus angioplasty or arthroscopy, non-OA group). These patients had a body mass index (BMI) that was in line with their average age group. There was no evidence of osteophyte, JSN, sclerosis, or bone deformity on radiographs or in clinical examinations; the patient had not undergone knee surgery in the past; intra-articular local and/or systemic injections had not been administered; and the patient did not have septic arthritis. In addition to this, participants in the control group were required to fulfil the following inclusion criteria: Clinically, there was no evidence that they had knee pain in the previous month; there was no crepitus on active joint motion; there was no morning stiffness; there was no evidence that they had any other symptoms of knee discomfort. Every individual who took part in the research was graded when they were initially accepted into the programme for participation in the study. Following the completion of the clinical examination, the patient's anthropometric measurements, prior radiographic findings, and medical history were all noted. In order to assess how well the knee functions, the WOMAC was utilised. The WOMAC score is comprised of a total of twenty-four distinct criteria, some of which include pain (with a score range of 0–20), stiffness (with a score range of 0–8), and functional impairment (with a score range of 0–8). (with a score range of 0–4). (score range: 0–68). Patients who had any of the following conditions were not eligible to take part in the study: infectious diseases, a history of total knee arthroplasty or other types of knee surgery, septic arthritis, obesity, neurological or neuromuscular diseases, the use of antibiotics, bone tumours, osteoporosis- or trauma-related fractures, diabetes mellitus, Addison's disease, or an immune system disorder. Patients who did not have any of these conditions were eligible to take part in the study. An individual's history of therapy with systemic steroids, antibiotics, or injections of intra-articular hyaluronic acid was another factor that led to their exclusion from the study. The blood samples were obtained by venipuncture, and the separation of the serum from the blood took place within an hour of the blood being drawn from the vein. The amount of synovial fluid that is typically present in the knee joint can range anywhere from 0.5 to 4 millilitres on an average basis. Several different approaches were utilised in order to calculate the overall amount of joint fluids¹². In the research that we carried out, we employed a method known as the superolateral technique. An injection using a sterile syringe containing 5 millilitres was administered into the side of the patella after the positioning of a support below the knee and the flexion of the knee to a degree of thirty was completed. After that, the needle was moved downward and medially in the direction of the patella's posterolateral direction until the synovial space was reached. It was discovered that there was roughly 2 millilitres of synovial fluid that needed to be aspirated from the synovial space. The fluids that were removed from the joint were stored at a temperature of -80 degrees Celsius and did not undergo centrifugation before being stored. Before the day of the analysis, each of the serum samples were stored in a refrigerator at a temperature of 80 degrees Celsius. In the control group, joint fluid samples were collected prior to arthroscopy using a 22 gauge needle inserted into the dorsomedial synovial pouch of the knee joint with the carpus flexed at approximately 70 degrees in patients undergoing arthroscopy such as arthroscopic anterior cruciate ligament reconstruction, meniscus angioplasty, or arthroscopy. This was done in patients undergoing arthroscopy such as arthroscopic anterior cruciate ligament reconstruction, meniscus angioplasty Patients who were scheduled to have operations such as arthroscopic anterior cruciate ligament reconstruction and meniscus angioplasty were the ones who had this done to them. After obtaining a sample of the synovial fluid, the knee joint was subsequently inflated with 10 millilitres of sterile saline using the same needle that was used to get the synovial fluid sample. The superolateral method was utilised in order to collect synovial fluids from the individuals who did not have osteoarthritis but were a part of the non-arthroscopic control group. This was done in order to better understand the

relationship between osteoarthritis and the non-arthroscopic control group. Using a human decorin enzyme-linked immunosorbent assay (ELISA) kit paired with an immunoassay (ALISE) entirely automatic ELISA equipment, the levels of decorin in the serum and synovial fluid were determined. After then, the results were presented in the form of ng/ml.

RESULTS

The demographic, clinical, and laboratory characteristics of both the study group and the control group at the beginning of the research are presented in Table I. Both groups served as the primary subjects of the investigation. When comparing the groups' mean ages and body mass indexes, there was no discernible statistically significant difference between them. When the OA group's WOMAC score was compared to that of the control group, the OA group's score was significantly and statistically higher (P 0.001). It was discovered that the levels of serum decorin in the group that was diagnosed with OA were statistically and considerably higher than the levels found in the group that acted as the control (P 0.001). Patients who had OA had decorin concentrations in their serum that were statistically significantly (P 0.001) greater than the concentrations of decorin in their synovial fluid.

A second round of ROC analysis was carried out in order to assess the WOMAC score as well as the serum decorin levels (Figure). Cut-off levels were determined, and AUC values were obtained. The area under the curve (AUC), as well as the ideal cut-off values, sensitivity, and specificity, are all provided in Table II for the purpose of deciding which group a particular parameter belongs to. It was shown that there was a significant relationship between a patient's WOMAC score and the amount of decorin found in their serum.

An investigation into whether or not there was a connection between the various groups and the predetermined threshold values for the laboratory parameters was carried out by means of a multivariate logistic regression analysis. The purpose of this investigation was to determine whether or not there was a correlation between the two. WOMAC score (odds ratio (OR) = 1.068, 95% confidence interval (CI): 1.031-1.116, P0.001) and high serum decorin levels (OR = 1.115, 95% confidence interval (CI): 1.031-1.202, P=0.006) were discovered to be significant in the determination of OA. WOMAC score was found to be significant in the determination of OA. In addition, we investigated whether or not there were any changes in the amounts of decorin found in synovial fluid as well as the ratio of decorin found in synovial fluid to decorin found in serum as a result of the treatment of OA. We found that there were no significant shifts in either of these variables. When the patients suffering from OA were examined once more, it was discovered that there had been no significant shifts in the condition that they were in.

Additional research was conducted to see whether or not there was a connection between the levels of serum decorin and the other factors that were discussed earlier in the sentence (age, BMI, WOMAC score and synovial fluid decorin levels). It was shown that there was a positive link between the levels of serum decorin and the WOMAC score in patients who were diagnosed with OA. It was discovered that there was no correlation between the levels of serum decorin and the other variables [age, BMI, synovial fluid decorin, and decorin (synovial fluid)/decorin (serum) levels] in the OA and control groups.

| Variable | OR (95% CI) |
|--|---------------------|
| Age (years) | 1.085 (0.998-1.176) |
| BMI (kg/m ²) | 1.032 (0.945-1.123) |
| WOMAC score | 1.068 (1.030-1.117) |
| Decorin (serum) (ng/ml) | 1.115 (1.031-1.202) |
| Decorin (synovial fluid) (ng/ml) | 1.112 (0.976-1.266) |
| Decorin (synovial fluid) (ng/ml)/Decorin (serum) (ng/ml) | 1.001 (0.455-2.284) |

^a P < 0.01, *** P < 0.001. Logistic regression model (Binary logistic regression) was used to determine the possible risk factors for OA. BMI, body mass index; WOMAC, Western Ontario and MacMaster Universities Osteoarthritis Index; CI, confidence interval; OR, odds ratio.

DISCUSSION

Pain, swelling, and limitations in range of motion are the clinical manifestations of osteoarthritis of the knee. Knee osteoarthritis (OA) is a progressive and chronic condition that is characterised by the degeneration of cartilage, the growth of subchondral bone, and changes in the synovium. The breakdown of cartilage is one of the most important factors that can lead to osteoarthritis (OA) and can also speed up the disease's progression. The fibril formation that results from the binding of different collagens to each other is the most important structure in the extracellular matrix (ECM), notably in terms of load-bearing and elasticity. Fibril formation is formed when diverse collagens are bound to each other. The production of fibrils occurs as a result of the binding of various collagens to one another. An increase in the levels of decorin is indicative of the presence of damage in circumstances that are characterised by elevated levels of production of pro-inflammatory cytokines and activity of proteases.

Previous studies have demonstrated that decorin possesses features that are antagonistic to angiogenesis, fibrillogenesis, and pro-inflammatory. Inflammatory illnesses, such as osteoarthritis and osteoporosis, as well as other conditions, such as muscular dystrophy, Ehlers–Danlos syndrome, and ocular issues, have been associated to it as well. The presence of decorin is absolutely necessary for both the proliferation and differentiation of the cell, as well as the biological activity of the extracellular matrix (ECM). Extracellular matrix metalloproteinases (MMP) in particular are responsible for its cleavage. These MMPs include MMP-2, MMP-3, and MMP-7. In osteoarthritic patients, the levels of proteolytic levels of SLRPs such as decorin and biglycan were significantly higher when compared to the healthy group. These findings were discovered by Bock and colleagues. In addition, it was discovered that individuals with advanced OA had higher amounts of decorin, which led the investigators to the conclusion that decorin was responsible for the new structure of cartilage. Light microscopy allowed Poole and his colleagues to make the discovery that patients who had osteoarthritis (OA) had lower amounts of decorin and biglycan in their cartilage than healthy people. Comparing the levels of SLRP breakdown in normal and OA cartilage tissues was accomplished by Manfort and colleagues through the use of Western blotting. When the healthy cartilage tissue and the early-stage OA cartilage tissue were treated with MMP-13, the researchers found that there was no difference in the levels of decorin. The decorin, on the other hand, could not be found due to the advanced stage of OA cartilage's full breakdown, and the researchers confirmed their findings by sequence analysis. [Further citation is required] According to the findings of our study, those who were diagnosed with OA had higher levels of serum decorin in comparison to those who were in the control group; however, there was no significant difference in the levels of decorin that were identified in synovial fluid. Our findings were consistent with those of the previous research, which discovered considerably higher levels of decorin in patients suffering from OA. According to the research that was reviewed before, levels of decorin were discovered to be significantly elevated in OA patients.

Although a number of factors have been suggested as being involved in the pathogenesis of osteoarthritis (OA), the mechanical and inflammatory effects in the process that leads to the deterioration of the extracellular matrix (ECM) structure in cartilage and, as a result, decreased cartilage elasticity and regeneration are the most important factors. Although a number of factors have been proposed as being involved in the pathogenesis of osteoarthritis (OA), the mechanical and inflammatory effects in the process that leads to the Mechanical and inflammatory stimuli, respectively, are what cause the formation and release of proteases, which then leads to the development of proteoglycan and proinflammatory signals. Proteases are released as a result of the production of proteases. The findings of our study suggest that there is a connection between serum decorin levels and both levels of pain and functional impairment. However, we found that the amount of decorin present in synovial fluid was not related in

any way to the likelihood of developing osteoarthritis or the WOMAC score. It is widely established that there is a correlation between the WOMAC score and the severity of OA. Wardale and colleagues revealed indications of increased cell migration and matrix proteins, including decorin, in osteoarthritic human cartilages. These findings were published in the journal *Arthritis and Cartilage (ECM)*. The researchers Zhang and colleagues found that mice deficient in decorin had reduced tendon stretch properties. Dourte and colleagues discovered a lower viscoelasticity and collagen content in the tendons of patients with decorin heterozygosity. [Further citation is required] Even though cartilage oligomeric matrix protein and the WOMAC score are linked to the condition in some way, there is no research that links decorin levels in serum and synovial fluid to the severity of osteoarthritis (OA). This is the case despite the fact that decorin levels in serum and synovial fluid are measured. As a result of the research that we carried out, we discovered that a high level of serum decorin and a high WOMAC score were positively connected with one another. One of the limitations of our research was that it did not investigate the role of additional proteases and proteoglycans, both of which are associated with an increased risk of developing osteoarthritis. Another drawback was that only a small number of patients and controls had a history of joint damage and had undergone knee surgery for a variety of causes. This meant that the results of the study cannot be generalised to the general population.

According to the findings of our study, an elevated level of serum decorin as well as a higher WOMAC score were both independent risk factors that were associated to an increased likelihood of developing osteoarthritis. It has been demonstrated that there is a connection between the WOMAC score and the progression of OA. The presence of elevated levels of decorin in the serum may be an indicator of accelerated proteoglycan synthesis. Increased proteoglycan synthesis is important for patients suffering from osteoarthritis in order to provide for and regulate cartilage repair. The finding that there is a positive connection between the level of decorin in the serum and the WOMAC score lends credence to the previous assertion that this conclusion is correct.

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