Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 12, 2023

Original research article

PLASMA OSTEOPONTIN AND RADIOGRAPHIC GRADING CORRELATE IN PATIENTS WITH KNEE OSTEOARTHRITIS

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Abstract

Background and Objectives: Studying the relationship between plasma osteopontin levels and radiological grade in patients with knee osteoarthritis and serum hyaluronic acid in patients with knee osteoarthritis will help determine whether osteopontin plays a role in the etiology of OA degeneration by activating matrix metalloproteinase 13 and elevating serum hyaluronic acid levels.

Materials and methods: This study was conducted at the Department of General Medicine, GMC/GGH Jayashankar, Bhupalpally, Telangana, India, between the September 2022 to August 2023. The study included 50 patients with different degrees of radiological indications of osteoarthritis in the knee joint, as well as 25 healthy individuals as controls. Anteroposterior knee radiographs were obtained while the patient was standing in order to assess the severity of the disease in the affected knee joint. The Kellgren-Lawrence grading system was used to assess the severity of osteoarthritis in the knee joint using radiographic evaluation. Plasma osteopontin levels and serum hyaluronic acid levels were quantified using enzyme-linked immunosorbent assay and subsequently compared.

Results: In comparison to healthy controls, individuals with knee OA had a substantially higher mean plasma osteopontin concentration. There was a strong correlation between disease severity and plasma osteopontin levels. Patients with osteoarthritis of the knee had much greater mean serum hyaluronic acid concentrations than healthy controls. A strong correlation was found between the serum hyaluronic acid level and the K/L grades. Statistical analysis revealed that there was a statistically significant rise in concentrations of serum hyaluronic acid and plasma osteopontin when the radiological K/L grade rose in cases with grades 2, 3, and 4.

Conclusion: The levels of osteopontin and hyaluronic acid in plasma and serum, respectively, are associated with the worsening of knee OA joint degeneration. Therefore, the severity of the disease can be assessed using a combination of osteopontin and hyaluronic acid as biomarkers.

Keywords: Phospholipids, hyaluronic acid, and osteoarthritis grading system

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 12, 2023

Introduction

Osteoarthritis (OA) is a prevalent condition that affects individuals of all genders and ethnicities, making it a widespread joint disease. Osteoarthritis (OA) is a condition that primarily affects individuals of advanced age. Particular joint cartilage injury, an overactive bone growth response just under the cartilage, and the formation of new bone around the joint's periphery are all symptoms of this condition. Synovial membrane inflammation that is neither localized nor short-lived is a hallmark of this illness. Osteoarthritis is a long-term degenerative condition affecting the joints, where the articular cartilage gradually breaks down over time, leading to different levels of damage within a specific joint [1-3].

In order to identify patients who are at a high risk for destructive osteoarthritis and to monitor the effectiveness of drugs, it is necessary to use more sensitive techniques than just "plain x-rays". Therefore, the utilization of specific and sensitive biochemical markers that indicate anomalies in the bone, cartilage, and synovial tissue turnover could be beneficial for the examination and surveillance of patients with OA [4].

Osteopontin is a significant noncollagenous protein found in the bone matrix. It is produced by different types of cells, including activated T cells, macrophages, osteoblasts, and chondrocytes. Osteopontin potentially plays a role in the development of osteoarthritis by contributing to the gradual breakdown of articular cartilage at the molecular level [5]. Osteopontin induces the activation of matrix metalloproteinase 13, leading to the degradation of the primary constituent of the bone matrix and subsequent release of hyaluronic acid into the bloodstream, ultimately resulting in bone loss. The objective of this study is to determine the significance of plasma osteopontin levels as a biomarker for osteoarthritis and to establish a correlation between the plasma concentration of osteopontin in patients with primary knee OA and the radiographic grading. To comprehend the role of osteopontin in expediting the development of osteoarthritis, which is expected to increase the amounts of hyaluronic acid in the bloodstream [6, 7].

The aim and objectives of the study were to assess if plasma osteopontin can serve as a biomarker for monitoring the degree of knee osteoarthritis. To evaluate the contribution of osteopontin in the development of osteoarthritis by activating MMP13, which leads to an elevation in hyaluronic acid concentrations in the bloodstream [8, 9]. The objective is to establish a correlation between the plasma level of osteopontin and the radiographic grade in patients diagnosed with knee osteoarthritis. The objective is to establish a correlation between the plasma level of osteopontin and the serum level of hyaluronic acid in patients diagnosed with knee osteoarthritis.

Materials and Methods

This study was conducted at the Department of General Medicine, GMC/GGH Jayashankar, Bhupalpally, Telangana, India**,** between the September 2022 to August 2023. The study included 50 patients with different degrees of radiological indications of osteoarthritis in the knee joint, as well as 25 healthy individuals as controls. Anteroposterior knee radiographs were obtained while the patient was standing in order to assess the severity of the disease in the affected knee joint. Plasma osteopontin levels and serum hyaluronic acid levels were quantified using enzyme-linked immunosorbent assay and subsequently compared.

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 12, 2023

Inclusion Criteria

- The control group, which consisted of 25 persons who were matched for age and gender and included 7 males and 18 females.
- Did not exhibit any symptoms or signs of osteoarthritis (OA), and their knee joint X-ray showed no evidence of OA.

Exclusion Criteria

- Patients with malignancy.
- Patients with both hip and knee osteoarthritis.
- Patients with generalized osteoarthritis.

Sample collection and storage

A total of 5 milliliters of blood was obtained from the ante-cubital vein of both the patients and controls in the study population. The blood was divided into three aliquots, each placed in separate tubes. A single tube containing EDTA as an anticoagulant and 2mL of blood was transferred until it reached the designated spot on the tube. The tube was agitated delicately between the palms to ensure even blending. Another 2mL of blood was put into a separate serum tube with a red cover. The remaining 1mL of the blood was placed into a plain tube for subsequent sample processing. The specimen tubes were appropriately labeled and placed in a refrigerated container for transportation to the biochemistry laboratory. The collected samples were promptly centrifuged within a time frame of 45 minutes, and the resulting plasma/serum from each tube was divided into individual sample storage Eppendorf tubes. The samples were preserved in a deep freezer at a temperature of -20oC until they were ready for further analysis.

Results

Table 1: Patient age distribution in cases and controls

	Group	Number	Mean	Std. Dev.
Age in year	Control	25	54.44	7.478
	Cases	50	54.34	7.134

The current study included 50 patients with different levels of osteoarthritis, as validated by X-ray imaging of the knee joint, as well as 30 control subjects. The average and variability of patients with osteoarthritis and individuals without the condition. There was no significant disparity in the age between patients with osteoarthritis and the control group, as indicated by statistical analysis.

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 12, 2023

Table 2: Distribution of genders in OA knee patients and controls

Sr. No.	Gender	Controls	Cases
1.	Males	07	12
2.	Females	18	38

The gender distribution among patients with OA knee and controls indicates that females comprise a higher percentage than males in both groups. There is no discernible disparity in gender between the patients and controls.

Table 3: Calcium and phosphorus levels in patients and controls are compared

	Group	N	Mean	Std. Dev.
Calcium	Control	25	7.564	0.2324
	Cases	50	7.258	0.2127
Phosphorus	Control	25	3.786	0.2131
	Cases	50	2.113	0.1347

Table 3 displays the average, variability, and statistical significance of the substances being analyzed - calcium and phosphorus. There is no statistically significant disparity between the calcium and phosphorus levels in the patients and controls.

Table 4: OPN and HA concentrations in patients with OA knee compared to controls

	Group	Number	Mean	Std. Dev.
OPN	Control	25	601.020	311.3912
	Cases	50	889.883	401.0262
HA	Control	25	2.77451	0.120123
	Cases	50	2.38272	0.726574

The mean and standard deviation of the OPN values in patients with knee osteoarthritis and the control group are presented in Table 4. Table 4 displays the average and variability of the HA values across both the patients and controls. A ROC curve was constructed using data from 50 patients with different degrees of knee osteoarthritis and 25 healthy individuals as controls. Therefore, OPN can serve as a biomarker for evaluating the extent of knee osteoarthritis. The integral of the curve was 0.761 for the hypothesis alternative. Consequently, HA can serve as a biomarker for evaluating the extent of cartilage destruction in the knee affected by osteoarthritis.

Discussion

The objective of this study was to ascertain whether or not osteopontin plays a role in the aetiology of the degenerative process of osteoarthritis in individuals who suffer from osteoarthritis of the knee, and if it does and to what extent it does so. Therefore, in order to arrive at these conclusions, we examined the plasma amounts of osteopontin. Osteoarthritis (OA) of the knee is a degenerative disorder that manifests itself gradually over time. The process begins with the activation of chondrocytes and then proceeds to

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 12, 2023

the transcription factors that are further downstream. The final common mechanism is the activation of matrix metalloproteinase-13, which is responsible for cartilage erosion and the breakdown of type II collagen. One of the most important roles that OPN performs in the process of cartilage degradation and the release of HA is serving as an activator. An defective autophagy ultimately results in cell death, which in turn hinders the cartilage's ability to regenerate, as can be seen in the figure below [10, 11]. Conventional x-rays have been the only method available up until this point for determining the severity of osteoarthritis of the knee. Recent advances in proteomics and micro RNA have made it possible to uncover additional biomarkers for OA, and developing biomarkers that detect cartilage matrix synthesis and degradation are gaining therapeutic utility in the evaluation of the severity of osteoarthritis knee [12, 13].

When examining the cases and controls for age, gender, height, systolic blood pressure, and diastolic blood pressure, p-values were found to be insignificant. If this is the case, then there was no mismatch between the cases and controls when it came to the confounding factors. When comparing the patients and controls for weight and body mass index, a statistically significant difference is seen. This provides more evidence that obesity is a major contributor to the development of OA knee. After ruling out post-traumatic stress disorder and calcium pyrophosphate deposition disease in the elderly by nutrient estimates, the two most prevalent secondary causes of osteoarthritis of the knee were examined further. Serum calcium and phosphorus levels were normal in the control and case groups, suggesting that the study has ruled out secondary causes of OA [14-16].

There was also a positive correlation between OPN and K/L grade, which suggests that the concentration of OPN rises as K/L grade progresses. This was discovered by the study. Therefore, there is a significant relationship between the severity of the disease and the amount of OPN that is present in the plasma. The results were also shown to be comparable. In addition to discovering that the levels of osteopontin were higher in cases compared to controls. The levels of osteopontin were higher in patients with radiological grades 3 and 4, in comparison to K/L grades. Plasma OPN concentrations were considerably greater in OA knee patients than in control subjects in this research. After patients were divided into three categories according to their radiological results, researchers found that their OPN amounts varied significantly. Patients with osteoarthritis of the knee showed an increasing concentration of plasma OPN as the radiographic grade progressed. In this investigation, there was no significant difference in OPN levels between the sexes in either group [17, 18, 19].

There is a school of thought that suggests that HA, which is produced by ECM cells, has a role in the process of cell signaling. During the process of extracellular matrix degradation and recycling, HA and its fragments are released into the bloodstream through endocytosis, which is facilitated by a specific HA receptor. After that, the lymphatic system and the liver eliminate them. Joint stiffness and edoema are symptoms that may be induced, at least in part, by an increase in the amount of HA that is present in the synovium that is inflamed. HA is released into the bloodstream as a result of synovial inflammation and cartilage deterioration, both of which cause an increase in serum HA levels. For this reason, serum HA levels could be utilised to determine the degree of cartilage breakdown in knees affected by osteoarthritis [20-22].

In the current study, it was discovered that people with osteoarthritis of the knee had

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 12, 2023

significantly greater serum HA concentrations as compared to the baseline control group. When the participants were divided into three groups based on the radiological data, there were discernible differences in the concentrations of HA that were found in each of the groups. Patients who were diagnosed with osteoarthritis of the knee had a rising concentration of serum HA as the radiographic grade increased [23]. There was a link that was statistically significant between the degree of radiological severity and the increase in the amount of HA. There was no discernible variation in the levels of HA between the sexes in any of the research groups that were taken into consideration. Researchers also discovered a significant correlation between HA and K/L grade, which suggests that HA concentrations increase in parallel with K/L grades according to the findings of the study. Because of this, there is a significant relationship between the levels of HA and the severity of the sickness as well as the degradation of cartilage. In addition, the results of another study indicated that there was a positive correlation between the levels of HA in the serum and the quantity of cartilage that was involved [24-27]. According to the findings of this study, OPN plays a significant role in osteoarthritis, which results in the degradation of articular cartilage and the release of HA into the circulation. This conclusion is reached as a result of the positive correlation that exists between OPN and K/L grade and HA and K/L grade, as well as the statistically significant increase in concentration of both OPN and HA with respect to radiological grade. This indicates that OPN and HA have the potential to collaborate as biomarkers in order to assess the severity of the disease [28-30].

The absence of a statistically significant correlation between OPN and HA in this study implies that OPN is not the sole factor that contributes to elevated HA levels. This is despite the fact that several factors contribute to elevate HA levels [31]. Inflammation can be triggered in a knee with osteoarthritis by a number of different pathways; but, in the end, these pathways come together to activate matrix metalloproteinase-13, which in turn causes cartilage degradation and the release of hydroxyapatite into the bloodstream. During the course of this experiment, a Receiver Operator Characteristic curve was utilised in order to evaluate the diagnostic value of plasma OPN and serum HA. As a result, elevated levels of plasma OPN and serum HA can be utilised as biomarkers for the purpose of determining the severity of osteoarthritis knee illness associated with cartilage breakdown [32-34].

Conclusion

One such degenerative joint disease that can cause impairment is osteoarthritis of the knee. This condition has been on the rise in recent years, becoming a serious concern for people's well-being. Possible solutions to this issue include earlier detection of OA. Researchers found that compared to healthy controls, people suffering from OA knee had much greater levels of OPN and HA. As radiological grade rises, OPN and HA concentrations rise as well, with no discernible effect from gender variations. You can use OPN as a biomarker to track the development of the disease and HA as a biomarker to evaluate cartilage damage. To determine whether markers are independent predictors of outcome, it may be necessary to conduct a comprehensive analysis of multiple sets of markers in a large number of patients with OA knee and to follow them prospectively. Potentially useful for future medication development targeting OA, these biomarkers could be a great place to start.

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 12, 2023

Funding: None.

Conflict of Interest: None.

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