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# Exploring the Correlation between Visual Analog Scale (VAS) and WOMAC Pain Scores with Microrna-122-5p Expression in Knee Osteoarthritis and Post-Menopausal Osteoporosis

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**ABSTRACT:**

**Background:** Musculoskeletal disorders such as Knee Osteoarthritis (KOA) and Osteoporosis (OP) present significant global health challenges. While the Visual Analog Scale (VAS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) are reliable tools for assessing pain intensity and functional impairment, their molecular underpinnings remain unclear. MicroRNAs (miRNAs), particularly miR-122-5p, have emerged as potential regulators in KOA and OP, with implications for pain perception.
**Methods:** Serum samples from 268 participants were analyzed for miR-122-5p expression, and its correlation with the clinical-radiological parameters was statistically analysed.
**Results:** Despite previous associations between miRNAs and musculoskeletal disorders, miR-122-5p did not exhibit significant correlations with pain/functionality in this study. The findings highlight the complex nature of pain mechanisms and suggest the need for further research to elucidate additional factors influencing pain perception in KOA andpost-menopausal OP.
**Conclusion:** While miR-122-5p did not emerge as a significant molecular player in pain severity/functionality in KOA and post-menopausal OP, this study underscores the need for comprehensive investigations integrating genetic, environmental, and molecular factors to advance personalized interventions for musculoskeletal disorders.

**Keywords:** Knee Osteoarthritis, Post-menopausal Osteoporosis, miRNA, Kellgren- Lawrence (KL) Grade, Visual Analog Scale (VAS), Western Ontario and McMaster University Arthritis Index (WOMAC).



## 1. Introduction

Musculoskeletal disorders mainly Knee Osteoarthritis (KOA) and Osteoporosis (OP), encompassing a diverse range of conditions affecting the musculoskeletal system, present a significant global health challenge. KOA is linked to obesity, trauma, strain, pain, inflammation, and other related factors [1,2]. OP is characterized as a silent ailment prevalent among ageing individuals and primarily manifests through pain. Subjects afflicted with OP commonly suffer from disability and reduced quality of life as a consequence of fractures, leading to escalated financial expenses [3]. Among the various assessment tools employed to evaluate pain intensity and functional impairment associated with these disorders, the Visual Analog Scale (VAS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain scores stand out as reliable measures. While these tools have greatly contributed to our understanding of pain severity and functional limitations, the molecular mechanisms underlying these subjective assessments remain an area of active investigation.
In recent years, as molecular biology continues to advance, there has been a gradual increase in studies focusing on noncoding ribonucleic acids (RNAs) [4]. Notably, research on micro-RNAs (miRNAs), a specific type of short-chain noncoding RNAs, is advancing significantly.

MiRNAs are increasingly recognized for their ability to regulate gene expression by inhibiting gene translation into proteins or facilitating gene degradation through binding interactions [5]. MicroRNAs (miRNAs), small non-coding RNA molecules, have emerged as crucial regulators of gene expression and have been implicated in various pathological processes, including KOA and OP. It has been proven in studies that abnormally expressed miRNAs can predict the occurrence and development of disease in organisms, and they are closely involved in regulating vital biological behaviours of cells [6,7]. Among these, microRNA-122-5p (miR-122-5p) has gained attention for its involvement in inflammatory and degenerative processes. Recent studies have suggested a potential link between miR-122-5p expression and pain perception in musculoskeletal disorders, opening avenues for exploring its role as a molecular marker associated with pain severity and functional impairment.

This study aims to investigate the correlation between VAS and WOMAC pain scores and miR-122-5p expression in individuals with subjects of KOA, post-menopausal OP, and subjects having both KOA and post-menopausal OP. By delving into the molecular underpinnings of pain perception, this study aims to provide a deeper understanding of the biological processes contributing to pain severity and functional limitations in these conditions.

Understanding the molecular basis of pain and functional impairment is crucial for developing targeted therapeutic interventions and advancing personalized medicine approaches. The identification of miR-122-5p as a potential molecular player in the context of VAS and WOMAC pain scores could pave the way for the development of novel diagnostic and therapeutic strategies, ultimately improving the management of musculoskeletal disorders. Through this study, we aim to contribute to the growing body of knowledge bridging the gap between subjective pain assessments and molecular mechanisms, bringing us closer to more effective and tailored interventions for individuals grappling with KOA, post-menopausal OP, and common subjects of KOA and post-menopausal OP.

## 2. Material and Methods

**Study Design:**

A total of 268 participants were included in the study, with 67 individuals assigned to each of the four groups: Knee Osteoarthritis (KOA), post-menopausal Osteoporosis, combined KOA and post-menopausal OP (common), and the Control group. The inclusion criteria for KOA consisted of individuals meeting the following conditions:

1. Diagnosis of knee osteoarthritis (OA) according to the American College of Rheumatology (ACR) guidelines, characterized by:
1. Knee pain accompanied by an osteophyte visible on X-ray.
2. At least one of the following:
3. Crepitus during knee range of motion.
4. Age 45 years or older.
5. Morning stiffness lasting less than 30 minutes.

Confirmed knee OA based on anteroposterior (AP) standing and lateral knee radiographs, with severity equivalent to a Kellgren and Lawrence grade (KL Grade) of at least two.
Willingness to provide informed consent for participation in the study.
Eligibility criteria for the Knee Osteoarthritis (KOA) group excluded individuals who had:

1. Secondary Osteoarthritis of the knee.

2. Hypercalcemia (total serum calcium exceeding 10.5 mg/dL).
3. Hyperparathyroidism (PTH surpassing 65 pg/mL).
4. Significant medical conditions or impairments that could hinder study participation.
5. Plans for permanent relocation from the region during the trial period.

On the other hand, individuals included in the post-menopausal OP group met the following criteria:
1. Both sexes (Male/Female).
2. Age >50 years.
3. Chronic back pain.

Exclusions for the post-menopausal OP group consisted of:
1. Uncontrolled diabetes.
2. Elevated levels in random blood urea creatinine, HBA1C.
3. Chronic renal failure, elevated serum creatinine.
4. Thyroid disorder.
5. Rheumatoid arthritis.
6. Gout and Pseudogout.
7. Malignancy.
8. Prolonged steroid intake.

The inclusion and exclusion criteria for common subjects in the KOA and post-menopausal OP groups were the same as those for the individual KOA and OP groups, respectively.

**Sample acquisition and handling:** After collecting 5 ml of peripheral blood, allow it to clot undisturbed at room temperature. After 15–30 minutes, remove the clot by centrifuging the sample at 1,000–2,000 x g for 10 minutes using a refrigerated centrifuge. The liquid remaining above the sedimented material is the serum. Store this final supernatant at -80°C.

**MiRNA analysis:** The miRNeasy serum/plasma kit by QIAGEN was utilized for the purification of cell-free total RNA. For each 200µL of briefly thawed (on ice) serum samples, 1mL of lysis buffer was added. To generate cDNA from the isolated RNA, the miRCURY LNA RT kit (QIAGEN) was employed. This involved the addition of 5x miRCURY RT reaction buffer and 10x miRCURY RT enzyme to the isolated RNA. Subsequently, 2µL of the eluent was used for 200µL of the serum plasma to adjust each RNA template to 5ng/µL. For cDNA preparation, 1µL of cDNA was mixed with 5µL of Applied Biosystems™ TaqMan reagent. The reaction mixture also included 0.5µL of a primer specific for miR-122-5p, followed by the addition of 3.5µL of nuclease-free water, resulting in a total sample volume of 10µL. RT-PCR was then conducted with the following conditions:
1. Hold stage: 50℃ for 2 minutes; 95℃ for 10 minutes.
2. PCR stage: 95℃ for 15 seconds; 60℃ for 1 minute.

**Statistical Analysis:** Statistical analyses were conducted using SPSS version 21. Categorical variables were expressed as percentages (%), while continuous variables were reported as Mean±SD. The association of mean expression of biomarker with clinical and radiological parameters was assessed by paired t-tests and Mann-Whitney U test; one-way analysis of variance (ANOVA) (for more than two groups, as appropriate). To compare the changes in miRNA levels with clinical scores (VAS and WOMAC) and radiologic severity (KL grade of KOA and +/- of spine fracture in post-menopausal OP) correlation analysis using the Spearman rank test was performed Statistical significance was defined as a p-value $< 0.05$.

## 3. Result

**Correlation of miRNA with clinical-radiological findings in Knee Osteoarthritis cases:**
The correlation of miRNA values with the clinical-radiological parameters was analyzed. The KL grade was negatively correlated, WOMAC score and VAS score were positively correlated, and however, the correlation was not significant. The values are listed below in Table 1:

Table 1: Spearman rank correlation values with clinical and radiological parameters in Knee Osteoarthritis cases:

| Parameter | Correlation coefficient (r) | p Value | |
|---|---|---|---|
| **KL grade left** | -0.2276 (weak –ve correlation) | 0.064 | Spearman correlation for miRNA 122-5p |
| **KL grade right** | -0.01084 (weak –ve correlation) | 0.930 | |
| **WOMAC score total** | 0.05356 (weak +ve correlation) | 0.6669 | |
| **WOMAC score %** | 0.05193 (weak +ve correlation) | 0.676 | |
| **VAS score** | 0.08772 (weak +ve correlation) | 0.4803 | |

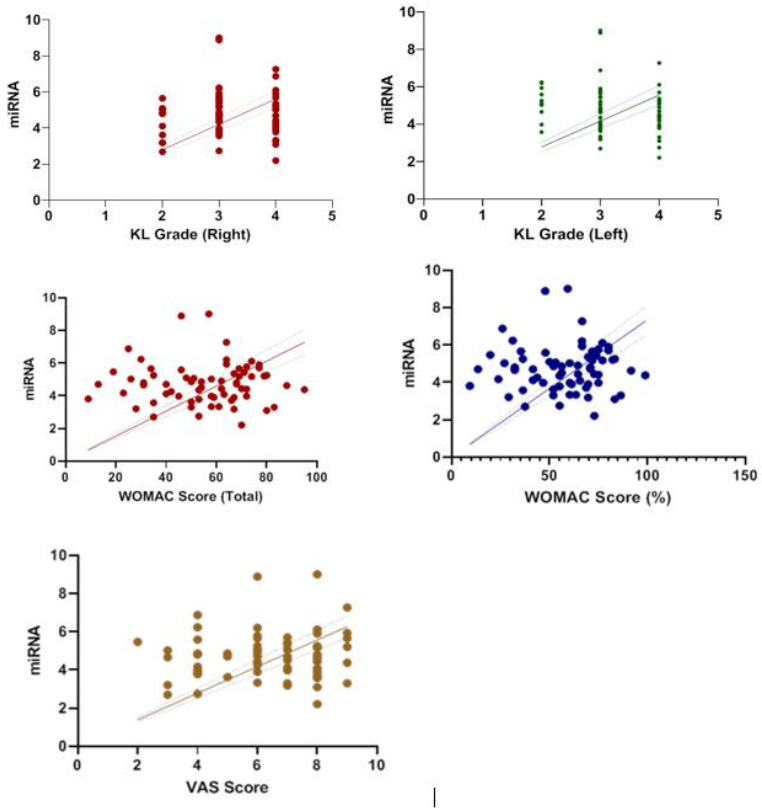


Figure 1: Spearman rank correlation graph for clinical and radiological parameters of Knee Osteoarthritis cases

In Figure 1, the dots represent the non-significant levels of miRNA 122-5p with the clinical and radiological parameters in KOA cases.

**The correlation of miRNA with clinical and radiological parameters ofpost-menopausal Osteoporosis cases**

The correlation of miRNA values with the clinical and radiological parameters was analyzed. The miRNA expression did not show a significant correlation with the clinical i.e. VAS score and radiological parameters in Osteoporosis cases. The T score was positively correlated with the miRNA values; however, the difference was not significant. The values are listed below in Table 2:

Table 2: Spearman rank correlation values with clinical and radiological parameters in post-menopausal Osteoporosis cases:

| Parameter | Correlation coefficient (r) | p Value | |
|---|---|---|---|
| **T score** | 0.2333 (+ve correlation) | 0.057 | Spearman correlation for miRNA 122-5p |
| **VAS score** | -0.098 (weak -ve correlation) | 0.427 | |

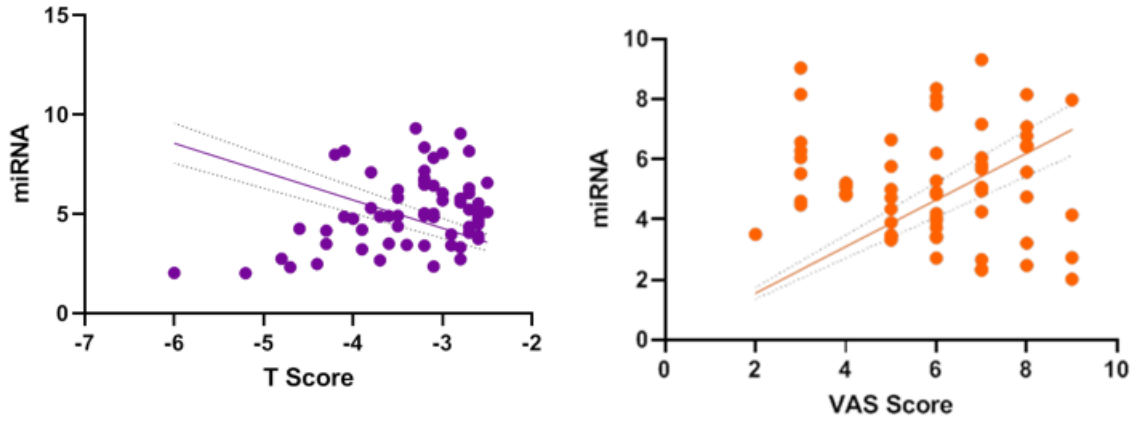


Figure 2: Spearman rank correlation graph for clinical and radiological parameters of Osteoporosis cases

In Figure 2, the dots represent the non-significant levels of miRNA 122-5p with the clinical-radiological parameters in post-menopausal OP cases.

**The correlation of miRNA with clinical and radiological parameters of Osteoporosis with Knee Osteoarthritis cases:**

The correlation of miRNA values with the clinical and radiological parameters was analyzed. The miRNA expression did not show a significant correlation with the clinical and radiological parameters in the post-menopausal OP with KOA cases. All the parameters were positively correlated with no significant difference. The values are listed below in Table 3:

Table 3: Spearman rank correlation values with clinical and radiological parameters in post-menopausal Osteoporosis with Knee Osteoarthritis cases:

| Parameter | Correlation coefficient (r) | p Value | |
|---|---|---|---|

| | | | |
|---|---|---|---|
| **KL grade left** | 0.0049<br>(very weak + correlation) | 0.968 | Spearman correlation for miRNA 122-5p |
| **KL grade right** | 0.001753<br>(very weak + correlation) | 0.988 | |
| **T score** | 0.1066<br>(+ve correlation) | 0.390 | |
| **WOMAC score total** | 0.1066<br>(weak +ve correlation) | 0.390 | |
| **WOMAC score %** | 0.1050<br>(weak +ve correlation) | 0.397 | |
| **VAS score** | 0.09985<br>(weak +ve correlation) | 0.421 | |

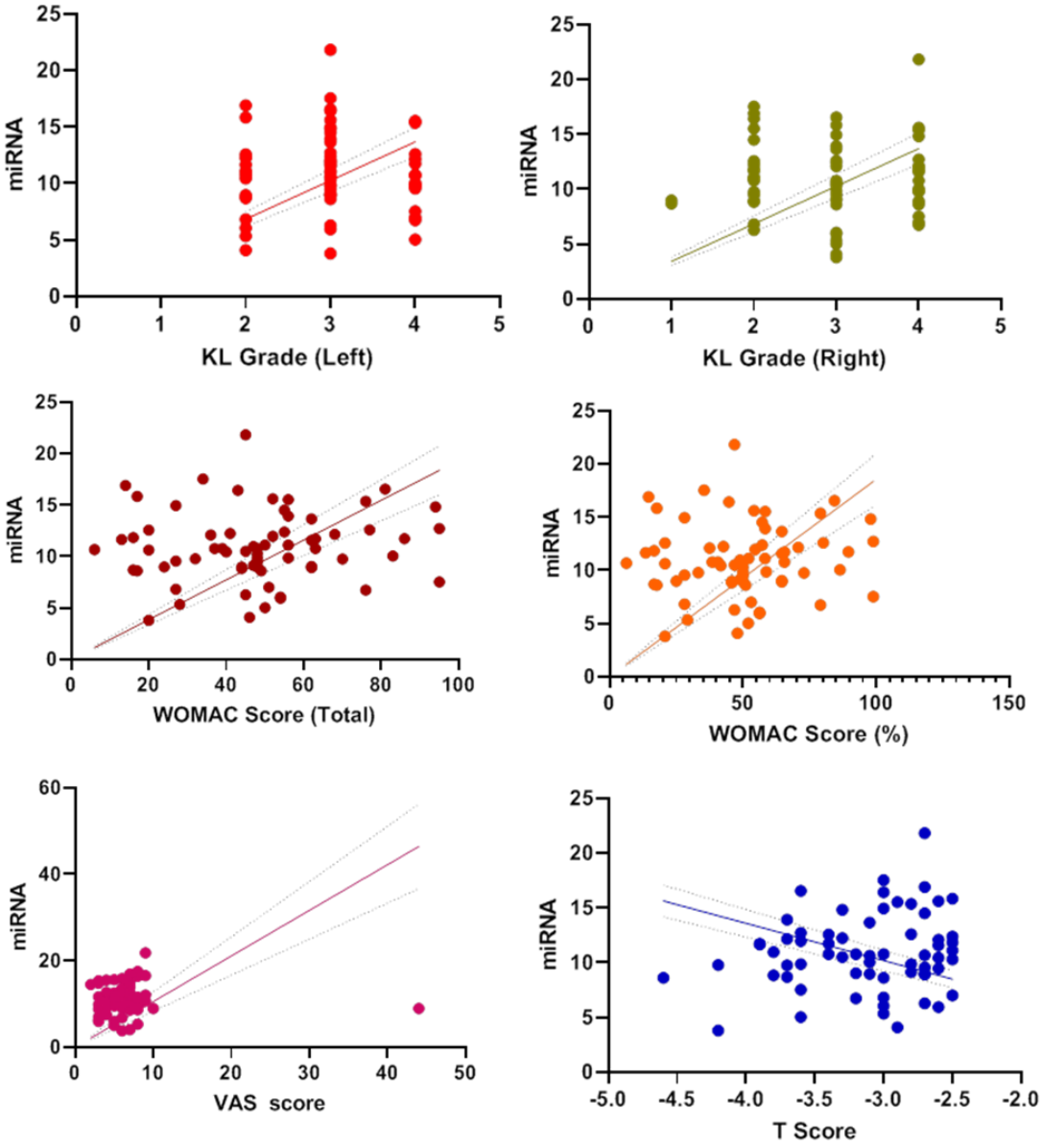


Figure 3: Spearman rank correlation graph for clinical and radiological parameters ofpost-menopausal Osteoporosis with Knee Osteoarthritis cases

In Figure 3, the dots represent the non-significant levels of miRNA 122-5p with the clinical and radiological parameters in the post-menopausal OP with KOA cases.

## 4. Discussion

KOA is a prevalent musculoskeletal condition characterized by joint degeneration, leading to pain and functional impairment. Concurrently, post-menopausal OsteoporosisOP, a disease marked by reduced bone density and strength, poses additional challenges. When these conditions coexist in a patient, they can exacerbate symptoms and complicate treatment strategies. Understanding the interplay between KOA and post-menopausal OsteoporosisOP is crucial for effective management, as interventions targeting one condition may impact the progression or management of the other. This comprehensive approach is essential for optimizing outcomes and enhancing the quality of life for patients facing this dual burden of musculoskeletal disorders.

This study observed the status of 268 patients (67 divided into 4 groups) diagnosed with osteoarthritis who received diagnosis and treatment at our hospital. Studies have exhibited that increased inflammatory factors can influence the knee joint function in OA patients to some extent and reflect the severity of the disease [8].

This work was the first to correlate the miR-122-5p in subjects with the coexistence of both KOA and post-menopausal OsteoporosisOP. The knee joint function in KOA patients was evaluated using the KL grading and WOMAC score. This study showed that the OA group's knee joint indexes of pain, stiffness, and physical function were higher than those of the control group, indicating that KOA patients have inflammatory responses and that these responses have a significant impact on daily living activities. In a study by Kong et al., miR-122-5p was found to be significantly upregulated in the subjects with KOA. During the validation stage, it was observed that miR-19b-3p, miR-122-5p, and miR-486-5p emerged as independent factors associated with the risk of KOA. Furthermore, miR-19b-3p and miR-486-5p exhibited positive correlations with comprehensive disease severity, encompassing pain VAS scores and WOMAC scores, while miR-122-5p was exclusively associated with negative correlations with pain VAS walking score only [9]. KL grade is an important index for the diagnosis and grading of osteoarthritis.Whereby in a study by Si et al., the relative expression level of miRNA-140 in both synovial fluid and chondrocytes wasnegatively correlated with the KL grade ($r$=−0.969, $P$<0.001; $r$=−0.970, $P$<0.001) [10]; in our study, the miRNA expression was upregulated but did not show a significant correlation with the clinical scoresi.e. VAS score and WOMAC and radiological parameters in KOA, OP and subjects with both diseases. In a study by Wang et al., the relationship of miR-145 was found to be positively correlated with KL grades [11]. In another study by Kelch et al., miR-122-5p was found to be significantly upregulated in the serum and osteoclasts of the OP subjects. Additionally, no significant association of this miRNA was found with the T-Scores (BMD), which was consistent with our studies [12].

In our investigation, miR-122-5p exhibited notable upregulation in individuals afflicted with KOA, post-menopausal OsteoporosisOP, as well as those presenting with both conditions concurrently. However, no statistically significant correlation was observed between the upregulated miRNA and the severity of either KOA or OP when considered independently or in combination.

## 5. Conclusion

The investigation aimed to explore the potential connection between microRNA-122-5p (miR-122-5p) expression and pain severity/functionality in knee osteoarthritis (KOA), post-

menopausal osteoporosis, and individuals with both conditions. However, the findings did not reveal significant associations between miR-122-5p expression and clinical/radiological parameters across the studied groups, despite the recognized roles of miRNAs in musculoskeletal disorders. While previous research suggested miRNAs' involvement in the inflammatory and degenerative processes of these disorders, this study uncovered a more intricate relationship, particularly regarding miR-122-5p. Although positive correlations between miR-122-5p expression and certain clinical parameters were observed, they did not reach statistical significance. These results highlight the complex molecular mechanisms governing pain perception and functional impairment in KOA and post-menopausal OP, suggesting that miR-122-5p might not be a primary driver of pain severity in these conditions, or its effects could be influenced by unexplored factors not addressed in the study. The study's outcomes provide valuable insights into the molecular landscape of musculoskeletal disorders, emphasizing the need for further investigations to delve deeper into the complex interplay between miRNAs and pain pathways. Future research should consider additional variables such as genetic variations, environmental influences, and comorbidities, and explore alternative miRNAs or molecular targets to uncover novel biomarkers or therapeutic avenues for managing pain and functional limitations in KOA and post-menopausal OP. The integration of multi-omics approaches and large-scale studies is recommended to achieve a more comprehensive understanding of the molecular underpinnings of musculoskeletal disorders, leading to tailored treatment strategies and improved patient outcomes.

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