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Uzma parveen
MD Scholar, Department of
Moalajat, School of Unani
medicine, Jamia Hamdard, New
Delhi, India

Mohd maaz
Assistant Professor, Department
of Moalajat, School of Unani
medicine, Jamia Hamdard, New
Delhi, India

Umar Jahangir
Assistant Professor, Department
of Moalajat, School of Unani
medicine, Jamia Hamdard, New
Delhi, India

Roohi azam
Assistant Professor, Department
of Moalajat, School of Unani
medicine, Jamia Hamdard, New
Delhi, India

Bibhu Prasad panda
Assistant Professor, Department
of Pharmacognosy, School of
Pharmaceutical Education and
Research, Jamia Hamdard
New Delhi, India

Pyridinoline: A narrative biomarker for osteoarthritis

Uzma parveen, Mohd maaz, Umar Jahangir, Roohi azam and Bibhu Prasad panda

Abstract

Osteoarthritis OA is the most common form of arthritis. Prevalence of osteoarthritis is expected to increase with aging of the population. In OA, the earliest changes in cartilage appear at the joint surface in areas where mechanical forces, in particular shear stress, are greatest particularly in knee joint. Due to its high prevalence, Knee OA generally accounts for disability of lower extremity much more than due to any other disease. It is a major cause of mobility impairment, particularly among females.

Knee OA is important not only due to its high prevalence rate compared with other types of OA but also for its appearance at earlier age mainly in younger age groups of obese women. The incidence of knee OA increases by age and additionally increases with longer lifetime and elevated average weight of the population.

Current diagnosis for osteoarthritis is based on time consuming and cost-intensive haematological and radiological parameters. There is need to find some alternative to identify the disease at early stage of development and to establish new diagnostic markers for OA.

As pyridinoline is much more prevalent in cartilage, assessment of this compound may provide a key for monitoring the increased joint destruction that occurs in arthritic disease at very early stages. In this review we have identified the role of urinary pyridinoline as a promising, time- and cost-effective biomarker of osteoarthritis.

Keywords: Diagnostic markers; osteoarthritis; urinary pyridinoline; urinary markers

Introduction

Osteoarthritis (OA) causes degenerative changes in the knee joint as a result of abrasion of these structures [1]. Generally molecules or molecular fragments present in cartilage, bone and synovium, are the best candidates and may be specific to one type of joint tissue. Pathogenesis of OA is still poorly understood but some factors are identified as the reason of OA like inflammation and the altered biochemical conditions as they contribute to the development and progression of OA [2] As the diagnosis of osteoarthritis is formally based on radiographic criteria like joint space width, osteophytes, etc and clinical symptoms like pain and loss of function etc. as per the ACR criteria [3] For the evaluation of new disease modifying osteoarthritis drugs (DMOADs) and therapies, evidence to correlate the impact of OA on radiographic findings and clinical symptoms is on anvil [4, 5] Presently certain problems related to radiography (manual error, technical issues, and radiation sensitivity) [6] and magnetic resonance (high cost, lack of availability and validated international score) are general predicaments for clinicians treating OA [6, 4, 7]. Apart from this, in most cases till the definitive diagnosis of disease is made, the irreversible changes have already taken place. Hence it is important to find out some alternative to diagnose osteoarthritis at earlier stages and perhaps biomarkers will serve as the best contender for the purpose.

Biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic and pathogenic processes or pharmacologic responses to a therapeutic intervention [5, 7]. Structural molecules or fragments linked to cartilage, bone or synovium are the best candidates for biomarkers in osteoarthritis. They may be specific to one kind of joint tissue or common to all of them. They may represent tissue degradation or tissue synthesis and can be measured in blood, urine or synovial fluid samples, which shows the dynamic and quantitative changes in joint remodeling and disease progression [8]. Urinary pyridinoline is found to be a biochemical marker for cartilage destruction or metabolism as well as bone metabolism [9]. Robins *et al.* observed that total urinary pyridinoline excretions were significantly higher in OA and suggested that a major source of pyridinoline is liable to be from a breakdown of cartilage collagen [10]. Others have found that the ratio of the concentrations of pyridinoline: deoxypyridinoline was 50: 1 in cartilage, 3: 1 in bone and 25: 1

Correspondence
Umar Jahangir
Assistant Professor, Department
of Moalajat, School of Unani
medicine, Jamia Hamdard, New
Delhi, India

in synovium. These findings specify that pyridinoline is most abundant in cartilage and moderately abundant in synovium. Therefore, as pyridinoline reflects both cartilage metabolism and synovitis (joint inflammation), this makes it a much more suitable marker for arthritis [11].

(BIPED) classification scheme

Osteoarthritis Biomarkers Network developed BIPED (Burden of disease, Investigative, Prognostic, Efficacy of intervention and Diagnostic) classification scheme, with an aim to provide a common framework for communication in the field of biomarkers in osteoarthritis [12]. They have categorized biomarkers into five parts viz. based on Burden of disease, Investigative, Prognostic, Efficacy of intervention and Diagnostic. The aim of the classification, planned specifically for osteoarthritis but also relevant to other diseases, was to confine information in the early stages of development of the disease, to plan the design of future clinical trials and research in osteoarthritis [13]. Pyridinoline is kept under the category of diagnostic biomarkers as urinary GlcGal Pyd were found to be increased in patients with osteoarthritis and considered a potentially useful biomarker for the presence of osteoarthritis since it is correlated with joint surface area [14]. Diagnostic biomarker can be used to distinguish between individuals with and without osteoarthritis.

Importance of osteoarthritis biomarkers

OA is associated with loss of the normal equibilirium between synthesis and degradation of the structural components of the extracellular matrix. These components are essential to provide articular cartilage, menisci, ligaments and bone with their normal biomechanical and functional properties. Further with these changes, synovitis develops resulting in the destruction of joint cartilage, menisci and ligaments, with widespread remodeling of subchondral bone. The active processes in the joint, includingng changes in both synthesis and degradation, results in altered release of matrix molecules, proteolytic molecular fragments, and other molecules. They are involved in their altered metabolism such as proteases, cytokines, chemokines, growth factors, etc. For the synovial joint, synovial compartment is probably the first and most proximal compartment where these potential biomarkers may be present. Products released into it may be removed by capillary and lymphatic, sometimes they may endure metabolism and appear in the urine after advance processing by the kidneys.

Although Knee OA affects all aspects of the joint, but the hallmark feature is degeneration of articular cartilage [15], which can be assessed with urinary excretion of biomarkers [16]

Urinary analysis plays major role for disease diagnosis since long decades. It is most suitable as sample from both control as well as from a comparable control group can be obtained easily on very low cost, compared to other fluids in the body eg. Synovial fluid, peritoneal fluid. Urine concentration do not only signifies the local environment of the joint but it can also symbolize entire body input. It may also reflect cartilage activity at the bone cartilage edge [16].

Significance of Urinary Pyridinoline

Urine contains pyridinium crosslink's i.e. two major molecules: pyridinoline and its analogue deoxypyridinoline. Measurement of these pyridinium crosslink's in urine

provides information regarding the stage, activity, level of bone involvement and efficacy of drug therapy in arthritic diseases. These crosslinks are also related to growth velocity, as observed in practice over healthy children [17].

Although both of the crosslinks are to be found in numerous tissues, deoxypyridinoline particularly located in bone, and used clinically as a marker of bone metabolism [18]. Level of DPD was found to be increased significantly in OA patients suggesting accelerated bone degradation [19].

Whereas pyridinoline is found both in cartilage as well as in bone [20]. And most abundantly in cartilage [21, 22] therefore it is considered to be more potent than deoxypyridinoline for estimation of bone and cartilage metabolism and index for monitoring the increased joint destruction related to osteoarthritis [18]. Pyridinoline has also been found to be related with every type of radiographic grading and exhibited a significant relationship to the Kellgren-Lawrence grade [23].

Role of Pyridinoline as an indicator of collagen degradation in joint disease

Basically Pyridinoline is a 3-hydroxypyridinium compound [24] formed by interaction of an intermediate bifunctional cross link [25, 26] to give a stable amino acid derivative linking three collagen chains [27] This compound is most prevalent in cartilage and only a minor cross linking constituent of bone. It remains totally absent in normal dermal collagen [28, 22]. Pyridinoline has been detected in human urine both immunochemically [27] and by conventional chromatographic procedures [29, 30].

Increased amounts of pyridinoline in the urine of patients with arthritic disease was found in a preliminary study [31] and it was also found that kidney dysfunction contributes to the increased pyridinoline excretion when the results were expressed relative to creatinine levels.

In a study it is seen that 8-week course of aerobic training led to reduced bone resorption activity and increase in bone formation, while anaerobic training resulted in with a less marked increase in bone formation and significant acceleration of bone turnover. Assessment after four weeks, significantly reduction in the urinary excretion of pyridinium cross-links was observed, a finding compatible with a state of low bone resorption [32].

In other placebo-controlled study of ibuprofen in 201 individuals with symptomatic knee OA in flare, urinary glucosyl- galactosyl- pyridinoline were assessed and it was found that ibuprofen prevents increased cartilage and synovium degradation associated with joint inflammation during flare. After 4 to 6 weeks, Glc-Gal-PYD were increased significantly from baseline in the placebo group whereas marginal or no increase was observed in the ibuprofen group suggesting that in patients with a flare of knee OA, increase in markers takes place [33].

In one more study, ibuprofen compared against nimesulide in 90 patients of knee or hip OA over 4 weeks and nimesulide was found to be associated with decreased urinary CTX-II and serum HA, serum matrix metalloproteinase (MMP)-3 and -13 suggesting that nimesulide possibly have impact on joint inflammation and cartilage collagen degradation [34].

Variation in urinary pyridinoline concentration has been contributed to the following factors
Diurnal variations
There is importance of regulating the time of the urine sample collection for measuring urinary pyridinium cross-links [35].

As marked diurnal rhythm observed in U-Pyr/Cr and U-D-Pyr/Cr, with the peak between 5AM-8 AM and the lowest point between 2 PM-11PM in study done over premenopausal women. Higher values of biomarkers found in the early morning hours signifying that internal mechanisms control the rates of bone résorption along the 24 hours. The sleep-wakefulness cycle directly or indirectly influences the urinary excretion of pyridinium cross-links, as urinary pyridinoline and deoxypyridinoline found to be increased during the sleep and decreased during the activity period [36]. In other study one nighttime and one daytime collection was done and it was observed a night/day difference in peptide-bound DPD but not in free DPD [37] The nadir/peak ratio seems to be constant [38, 39]; Daytime concentrations were found to be 78% of night concentrations [40].

Circadian pattern
a. Bed rest: The circadian pattern of both Pyr/Cr and D-Pyr/Cr at the end of 5 days of total bed rest was unchanged, which shows that the circadian pattern observed in bone resorption is not mediated by the change in gravity pattern (for example sleeping and walking,). The average 24 hr excretion of pyridinium crosslinks increased significantly at the end of the 5 days in bed, suggesting that the bed rest had an impact on the bones.

b. Hormones: Sex hormones do not influence the circadian variations in biochemical markers of bone resorption as lack of effect of age or menopausal status on the circadian variation reported by Eastell *et al.* [41] while several other hormones known to influence bone turnover exhibit a circadian rhythm eg. Parathyroid hormone and glucocorticoid shows a circadian rhythm with the peak at night. Nielsen *et al.* demonstrated that 10 mg prednisolone given in the evening is able to alter the circadian variation seen in osteocalcin [42].

Day-to-day variability
When morning sample collections were done at the same time every day for each person, longer-term biologic variability contributes to variation. Day-to-day variation found to be 15.7% for PYD and 17.4% for DPD while weekly variation was same, averaging 15.8% for PYD and 17.1% for DPD. In other study it is seen that monthly variation in DPD assessed over 5 months in both males and females averaged 16.7%. Variations for DPD were every time higher than PYD [43].

Age
With increasing age there is increase in the pyridinoline content of certain tissues [40, 30, 35].

Diet
The influence of diet on urinary pyridinoline is almost certainly negligible [45] only a very low contribution in the output of hydroxylysine derivatives [46] No significant effect on pyridinium cross-links concentrations was found over 4 days of extreme fasting [47]. And increased excretion rates are found due to vitamin D-deficient diets or malabsorption [48]. Effects of calcium supplementation are found to be controversial while no association seen between smoking and pyridinium cross-links excretion [49] Apart with these so many other factors are also there which influence the pyridinoline excretion, they will be discussed later on in further issues.

Discussion and Conclusion
With increase in average life expectancy and advent of lifestyle disorders particularly related to joint affliction, OA biomarker research is the quite imperative. Using the biomarkers for drug discovery and development helps investigators in various ways for development of Osteoarthritis research related to pharmacological, non-pharmacological and surgical OA interventions. Biomarkers can be used at vital judgment points during the course of treatment. They can also help pharmaceutical companies' squirrel away costs during clinical research. In routine clinical practice, biomarkers can be used for diagnosis and prognostic purposes to recognize the patients who are in early stage of disease so as to check the progression, for monitoring disease development process and patient conformity with the suggested therapy and also to monitor the impact of an intervention and any undesirable events. At present although many biomarkers related to OA are being tested but it remains doubtful so as to which biomarker exhibit optimal prognostic strength and responsiveness for knee OA [7, 50]. If we are able to identify any one of them through evaluation of data from completed clinical trials or prospectively it will be a boon to medical fraternity.

Urinary excretion of pyridinoline serves as the biochemical marker for cartilage destruction or metabolism as well as bone metabolism [9]. Robins *et al.* observed significantly higher total urinary pyridinoline excretions in OA, and suggested that a major source of pyridinoline is liable to be from a breakdown of cartilage collagen [10]. Many researchers have found that the ratio of the concentrations of pyridinoline and deoxypyridinoline (pyridinoline: deoxypyridinoline) was 50: 1 in cartilage, 3: 1 in bone and 25: 1 in synovium. These findings indicates that pyridinoline is most abundant in cartilage and moderately abundant in synovium. Therefore, as pyridinoline reflects both cartilage metabolism and synovitis (joint inflammation), making it a suitable marker for arthritis [11].

Further, it has been concluded that the perhaps urinary pyridinoline may be affected by the synthesis of osteophytes, sclerosis of subchondral bone and synovial degeneration as well as cartilage degeneration in the joints of OA. As it has been that pyridinoline was related to every type of radiographic grading examined [23]. Knee joint being the largest joint in terms of articular surface and cartilaginous tissue cover, affected by OA will conceivably lead to higher levels of urinary pyridinoline excretion and if clubbed with clinical history and diagnosis will be an effective measure for diagnosis, disease progression and response to treatment.

In this review we have tried to summarize the importance and significance of urinary crosslinks specifically urinary pyridinoline [23]. This will aid in diagnosis osteoarthritis at early stage and to develop related therapies. It is found to be the one of the easiest identified biomarker as it can be seen easily in urine samples by HPTLC or Elisa [32, 51]. Furthermore if a disease is diagnosed in its initial grades then it is possible to plan its targeted and personalized treatment from the very beginning and proper precautions and safety measures related to it can be taken to check further progression of the disease.

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