In-silico And Proteomic-based Identification Of Angiotensinogen As An Early Diagnostic Marker For Knee Osteoarthritis

Bhavneet Kaur [1], Diksha Rana [1], Rinkle Sharma [1], Harvinder Singh [2], Uttam Saini [3], Jyotdeep Kaur [1], Bikash Medhi [2], Indu Verma [1], Sadhna Sharma [1]
[1] Department of Biochemistry, [2] Department of Pharmacology, [3] Department of Orthopaedics, Post Graduate Institute of Medical Education and Research, Chandigarh-160012, India.
Email of presenting author: bhavneetkaur522@gmail.com

DISCLOSURES: All the authors and co-authors have nothing to disclose.

INTRODUCTION: Osteoarthritis (OA) is the 11th most debilitating disease and, radiographically, is classified into different grades as per Kellgren Lawrence (KL) classification. Some limitations include challenges in early detection and no-disease modifying drugs, which can be practiced in clinical utility. Synovial fluid proteomics holds great promise being in direct proximity with joints and contributes towards a better understanding of disease aetiology/pathogenesis. Additionally, combining proteomics with *in-silico* drug discovery is the best strategy for candidate drug identification.

METHODS: Synovial fluid and serum were collected from subjects recruited from PGIMER, Chandigarh, after the institute ethics committee (IEC) approval. The recruited study subjects were categorized into different grades by KL Classification. Seven samples of each OA grade (case) were run for SWATH analysis on Triple Tof 6600 (AB Sciex, USA) MS instrument for the discovery phase. A mass dynamics tool was used for data analysis and visualization. Log FC ≥ 1 and p-value < 0.05 was used as the criterion for significant protein expression, and the shortlisted proteins were further validated by ELISA in collected synovial fluid (n=25 of each OA grade) and serum samples (n=10 of each OA grade) of paired OA patients. For protein targeting and drug discovery, an *in-silico* workflow (Figure. 1) was followed, including molecular dynamics (MD) simulations, energy decomposition analysis with MM/PBSA, following the high-throughput virtual screening of FDA databases of investigational, experimental, and approved small molecule library.

RESULTS: 323 proteins were found in comparative proteomic profiling of OA synovial fluid samples, among which 30 proteins showed altered expression upon grade-wise OA comparison. Among differentially expressed proteins, Angiotensinogen showed significant alteration within different grades of OA and hence, was shortlisted for validation by ELISA. With disease progression, a significant upregulation ($p < 0.0001$****) was observed in synovial fluid; however, serum protein levels showed significant downregulation ($p < 0.0005$***). Also, synovial fluid measurements of angiotensinogen levels showed good specificity (95.83%) and sensitivity (48%), implying discriminative ability for early and late grade OA detection. Additionally, synovial fluid and serum samples revealed a significant positive correlation ($r= 0.4688$, $R2= 0.141$, $P=0.009$**). By following the *in-silico* approach, drug target prediction for the corresponding receptor of Angiotensinogen, i.e., Angiotensin II receptor type 1 (AT1) receptor, was performed. Among the top 10 screened ligands, Beta-1,2,3,4,6-Penta-O-Galloyl-D-Glucopyranose (DB03208) showed the minimum binding free energy (-201.5 kJ/mol), implying more protein and ligand stability. The findings further provide insight to explore the role of Beta-1,2,3,4,6-Penta-O-Galloyl-D-Glucopyranose in OA by targeting the AT1R receptor and altering the downstream signalling cascade involved in OA pathogenesis.

DISCUSSION: In the present study, we observed significant differentially expressed protein profile in synovial fluid of early and late OA patients using proteomics-based approach. Angiotensinogen (an α2-globulin and precursor molecule for angiotensin II) showed a great diagnostic potential being significant differentially expressed among the different grades of OA. Noteworthy, angiotensinogen levels showed derangement locally at synovial fluid levels which is further supported by cited literature where local RAS activation is associated with cartilage degradation, which is one of the significant concerns in OA (1). Direct targeting of Angiotensinogen is not possible as it is a precursor for the angiotensin II protein, preferably which binds with the Angiotensin II receptor type 1 (AT1) receptor. Interestingly, its expression has also been documented on chondrocytes. Therefore, we utilized an *in-silico* approach to find possible drug candidates against AT1R, which can be explored in OA. Among the top 10 screened ligands, Beta-1,2,3,4,6-Penta-O-Galloyl-D-Glucopyranose (DB03208) showed the minimum binding free energy, and its anti-inflammatory role has been studied in inflammatory diseases like rheumatoid arthritis (2). This provides an insight to explore the potential of Penta-O-Galloyl-D-Glucopyranose in OA management.

SIGNIFICANCE/CLINICAL RELEVANCE:

1. Angiotensinogen showed an altered protein expression in synovial fluid of different grades of OA, highlighting its early diagnostic potential.
2. Targeting AT1R (corresponding receptor of angiotensin 2) with Beta-1,2,3,4,6-Penta-O-Galloyl-D-Glucopyranose might alleviate disease progression and eventually can be explored for therapeutic management of OA.

REFERENCES:

1. Wu et al., Renin-angiotensin system in osteoarthritis: A new potential therapy. Int Immunopharmacol. 2019 Oct; 75:105796.
2. Umar et al. Penta-o-galloyl-beta-d-Glucose (PGG) inhibits inflammation in human rheumatoid arthritis synovial fibroblasts and rat adjuvant-induced arthritis model. Front Immunol. 2022 Aug 10; 13:928436.

ACKNOWLEDGEMENTS: I extend my gratitude to my supervisor, Dr. Sadhna Sharma, and co-supervisors for their guidance; Dr Shantanu Sengupta, CSIR-IGIB, New Delhi, for immense contribution to the proteomics experiment; my colleagues and friends, Department of Biochemistry, PGIMER Chandigarh and Indian Council of Medical Research, New Delhi for the financial support (Project no.- 5/4-5/3/TF/2017-NCD-1).

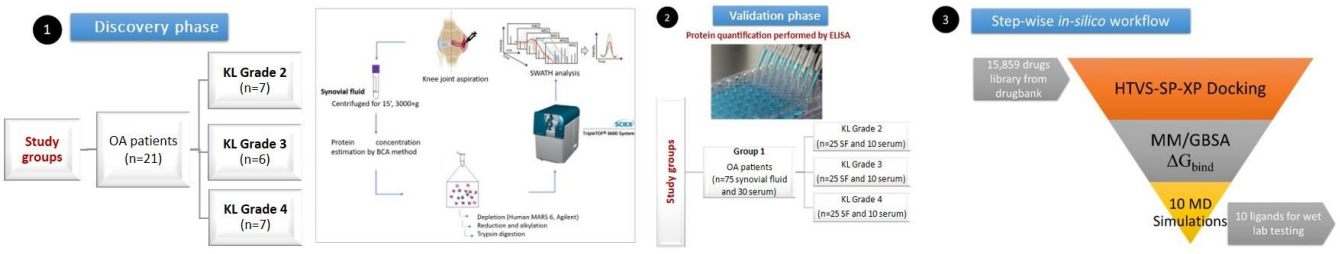

Figure 1: Stepwise basic workflow folllowed in present study including 1. Mass-spectrometry based discovery phase; 2. Validation phase by ELISA and 3. Computaional based in-silico screening and drug discovery.