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Potential Use of Synovial Fluid Peptidase Activity as A Biomarker to Anticipate How Egyptian Patients with Knee Osteoarthritis Will Fare Clinically: A Cross-Sectional Study

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ABSTRACT

Background: Osteoarthritis (OA) is a chronic protracted inflammatory joint disorder that frequently coexists with other comorbidities. Previous studies analyzed peptidase activity in synovial fluid in knee OA (KOA) patients, but they did not investigate whether type of therapy influences enzyme activity. Moreover, it is yet unknown if the activity of any particular enzyme can be taken into account as a reliable prognostic marker for the severity and progression of KOA.

Objective: The aim of the current study is to assess synovial fluid peptidase's potential role as a biomarker for KOA in Egyptian patients as well as to determine whether it can predict the clinical progression of such disease.

Patients and methods: A cross-sectional study was estimated on 75 KOA patients (Ahlback grade 3 or higher and indicated for arthrocentesis), who were subjected to sociodemographic and clinical characteristics, as well as Functional and Physical Health Status assessed every month. Puromycin sensitive aminopeptidase (PSA), neutral aminopeptidase (NAP), prolyl endopeptidase (PEP), aminopeptidase B (APB) glutamyl aminopeptidase (GLU), aspartate aminopeptidase (ASP), and pyro glutamyl aminopeptidase (PGAP) are among the peptidases shortlisted for their activity as biomarkers in synovial fluid.

Results: A significant association between NAP (P=0.038) and APB (P=0.05) peptidases and pain generation on movement in patients with KOA was noticed. Only ASP peptidase showed significant correlation with range of motion (P=0.049). Another clinical condition of knee failure significantly influenced by the presence of ABP (P=0.013) and PEP (P=0.033) peptidase presence. When diagnosing knee effusion, PEP (P=0.050) and PGAP (P=0.018) differ significantly depending on the clinical symptom. PSA was protective (P=0.003), whereas GLU and PEP were risk factors, according to a binary logistic regression (P values 0.001 and 0.019, respectively).

Conclusion: Peptidase activity is considered an important synovial fluid biomarker to determine disease progression in osteoarthritic patients, which could aid in early disease detection and the development of better treatment protocols.

Keywords: Synovial fluid peptidase, Knee osteoarthritis, Osteoarthritis progression, Peptidase enzymes activity, Cross sectional study, Tanta University.

INTRODUCTION

Osteoarthritis (OA) is a chronic protracted inflammatory joint disorder that frequently coexists with other comorbidities. It restricts joint motion, causes discomfort and pain, with functional limitations, that places a heavy financial burden on patients [(1,2)]. Although the presence of a variety of moderately effective strategies [(3)], it is well recognized that medications do not totally halt the disease's progression, which is related to joint inflammation and cartilage breakdown causing severe impairment and disability [(1)].

There are numerous OA phenotypes, including metabolic, biochemical, inflammatory, and osteoporotic aspects of the disease. MRI and biochemical indicators are used to further characterize these phenotypes [(3)]. New aberrant gene expression mediators and prospective therapeutic targets for OA have been identified as JUN, JUND, MYC, RELA, EGR-1, and other dysregulated transcription factors [(4)]. Additionally, Leptin, IL-1, IK-6, and IL-18 levels as well as changes in messenger RNA, circular RNA, and long non-coding RNA in OA-affected cartilages are considered post-traumatic osteoarthritis biomarkers that could be used to determine clinical progression of such disease [(5)].

Additionally, one of local renin-angiotensin systems has been previously characterized and is found in the synovial fluid and synovium. It seems to contribute to knee osteoarthritis (KOA) and worsens periarticular osteopenia in animal models by boosting bone resorption and reducing bone formation [(6)]. Also, some studies on humans suggest that the development of rheumatoid arthritis may be influenced by intra-articular injection of renin and angiotensin-converting enzyme. Hence, the use of renin inhibitor or Angiotensin receptor blockers have been demonstrated to help rats with OA and osteoporosis resulting in reducing the inflammation in arthritic animal models [(7,8)].

Furthermore, Peptidases may break down bioactive peptides, altering their physiological functions, and so controlling signal transmission, cell proliferation, and differentiation [(8)].

De Silveira *et al*. [(9)] demonstrated in an animal model of arthritis that activation of the angiotensin-converting enzyme-2/Ang-(1-7)/Mas receptor pathway reduced joint inflammation whereas stimulation of the

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renin-angiotensin system increased it. Additionally, any research studies have revealed that there are preoperative predictive indicators for knee joint infection [10].

Also**, Seco-Calvo and Colleagues** [11] analyzed peptidase activity in synovial fluid in KOA patients, but they did not investigate whether type of therapy influences enzyme activity , Moreover, it is yet unknown if the activity of any particular enzyme can be taken into account as a reliable prognostic marker for the severity and progression of KOA. Therefore, the aim of the current study is to assess synovial fluid peptidase's potential role as a biomarker for KOA in Egyptian patients as well as to determine whether it can predict the clinical progression of such disease.

PATIENTS AND METHODS

Study design and participants:

A cross sectional study was conducted at the Department of Rheumatology, Rehabilitation & Physical Medicine, Faculty of Medicine, Tanta University, from September 2022 to November 2022.

Inclusion criteria:

All patients aged 25 to 80 years old, of both genders, with primary KOA (Ahlback grade 3 or higher) [12] with clinical indication for KOA and its related medical (NSAIDs) or interventional (PRP intra-articular injection) therapy were included in this research.

Exclusion Criteria:

KOA patients who were undergoing chemotherapy or had cancer, blood coagulation disorders, infectious diseases, or any contraindications to arthrocentesis as well as pregnant females were excluded.

The study enrolled 75 eligible patients using a non-purposive sampling strategy, and the sample size was estimated using the Raosoft sample size calculator.

Methods:

All patients were subjected to the following:

1. Full history taking, general examination, and local rheumatologic examination (joint pain, tenderness, swelling, and limitation of joint motion).

2. Pain, Stiffness and Physical functional status assessment, using the modified knee society score [13] assessed by 2 independent researchers at baseline and every month till the end of study.

3. Laboratory evaluation: The peptidases that were chosen for their activity included the puromycin-sensitive aminopeptidase (PSA) (EC 3.4.11.14), neutral aminopeptidase (NAP) (EC 3.4.24.11), prolyl endopeptidase (PEP) (EC 3.4.21.26), aminopeptidase B (APB) (EC 3.4.11.6), glutamyl aminopeptidase (GLU) (EC 3.4.11.7), aspartate aminopeptidase (ASP) (EC 3.4.11.21) and pyro glutamyl aminopeptidase (PGAP) (EC 3.4.19.3).

Preparation of synovial fluid sampling for analysis: Through prescribed arthrocentesis, centrifugation, and freezing until blind analysis, synovial fluid samples were calculated for all enrolled individuals. The most recent intraarticular injection administration with duration of more than 6 months at the time of sample collection was one of the criteria for sample collection. Using a saturating (0.125 mM) concentration of β-naphthylamine substrates, samples were incubated to evaluate peptidase activity fluorometrically. These tests work by using the fluorescence of β-naphthylamine that is produced when the enzyme hydrolyzes the substrate [14].

Depending on the enzyme and substrate, reactions were started by adding 30–50 L of sample to 1 mL of the proper incubation mixture. 1 mL of 0.1 M sodium acetate buffer (pH 4.2) was added to the mixture to stop the reaction after the mixture had been incubated for 30 min at 37°C.

A JASCO model FP-6300 spectrofluorometer (Tokyo, Japan) was used to measure the change in fluorescence intensity of the released β-naphthylamine and record the results for 30 minutes. A standard curve produced with escalating doses of β-naphthylamine was used to translate relative fluorescence into nanomoles of product. One nanomole of β-naphthylamine produced per minute per milliliter of enzyme sample was used to represent one unit of enzyme activity. The Bradford method was used to detect protein concentrations [15].

4. Radiological evaluation: Knee cartilage thickness was determined using MRI images. The enrolled patients then allocated in to two groups. Arthroplasty group included patients who needed knee arthroplasty due to cartilage or joint deterioration with or without deformity, knee locking, severe effusion , not responding well to / or failure of conservative therapy . While the conservative therapy group included patients reacted well to conservative care. Flow chart of enrolled patients was demonstrated in **Figure 1.**

Ethical Consideration:

This study was ethically approved by the Institutional Review Board of the Faculty of Medicine, Tanta University (Approval Code No: 35935/10/22).

Written informed consent was obtained from all participants. All Patients were informed about the research's goals and completed written informed consent forms before the trial began. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) and the STROBE reporting criteria for studies on humans.

Statistical Analysis

The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 21 for windows. Qualitative data were defined as numbers and percentages.

Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as mean and standard deviation (SD), and independent sample t-test/ Mann-Whitney U test was used for comparison between groups. The optimal model for examining biomarker activity in KOA was determined using a binary logistic model for the target variables (effusion, knee locking or failure).

Initial consideration was given to all enzymes as possibilities, and the optimum model was found using a backward stepwise process and the Wald statistic. Additionally, B coefficients, Exp(B) values, and related 95% confidence intervals for the peptidase activities were determined for the prediction equation. P value ≤ 0.05 was considered to be statistically significant.

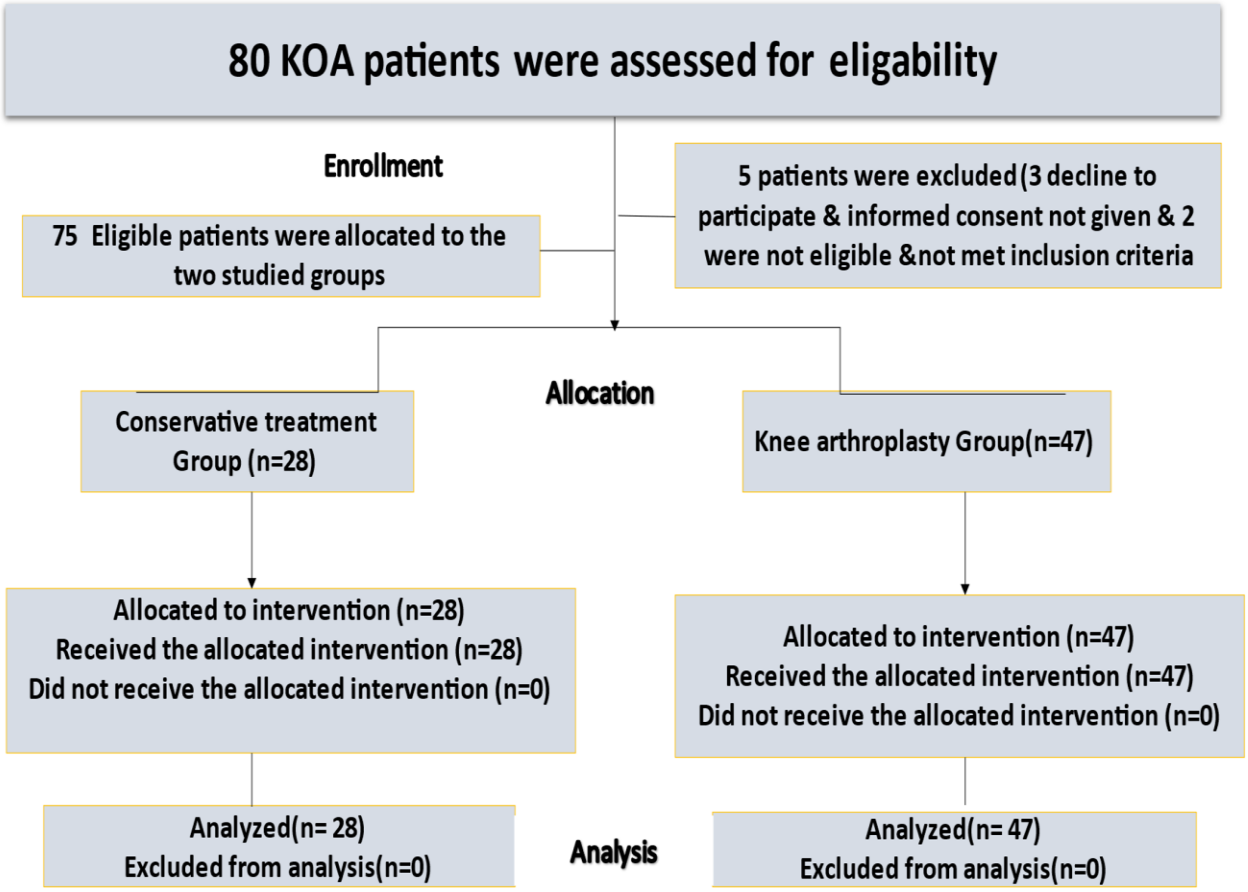

Figure 1: Flow chart of enrolled KOA patients.

RESULTS

A total of 75 patients with KOA (Ahlback grade 3 or above) were involved in the study, and they were allocated into two groups: Conventional care group and knee arthroplasty group.

Table 1 summarizes the demographic and clinical characteristics of all individuals involved in the study. Both groups had a significant association with laterality (P=0.016).

Table 1: Demographics and clinical characteristics of the participants.

Variables		Conventional treatment group (n=28)	Knee arthroplasty group (n=47)	P-value
Sex (No, %)	Female	18 (64.2)	20 (42.5)	0.069
	Male	10 (35.7)	27 (57.4)	
Risk of surgery dependent on physical status (ASA class)† (No, %)	No systemic disease	8 (28.5)	10 (21.2)	0.300
	Mild systemic disease	12 (42.8)	22 (46.8)	
	Severe systemic disease	8 (28.5)	15 (31.9)	
Laterality (No, %)	Right	14 (50)	23 (48.9)	0.016
	Left	14 (50)	24 (51.0)	
Contralateral pain (No, %)	No	10 (35.7)	22 (46.8)	0.062
	Yes	18 (64.2)	25 (53.1)	
State of the articular cartilage (No, %)	Ulceration depth (depth ≤50%)	5 (17.8)	10 (21.2)	0.092
	Ulceration depth (depth >50%)	10 (35.7)	15 (31.9)	
	Exposure of subchondral bone	13 (46.4)	22 (46.8)	

Both the conventional and knee arthroplasty groups had a strong statistically significant relationship in NAP activity as a possible synovial fluid biomarker (P=0.001), ABP (P=0.001), ASP (P=0.001), and GLU (P=0.001), respectively (Table 2).

Table 2: Comparison of Peptidase Activities in Both Groups.

Peptidase Activity	Conventional treatment group (n=28)	Knee arthroplasty group (n=47)	P-value
NAP	3 (10.71)	12 (25.5)	<0.001
PSA	4 (14.28)	11 (23.40)	NS
ABP	6 (21.42)	7 (14.89)	<0.001
PEP	2 (7.14)	4 (8.51)	NS
ASP	6 (21.42)	5 (10.63)	<0.001
GLU	4 (14.28)	2 (4.25)	<0.001
PGAP	3 (10.71)	6 (12.76)	NS

Our findings suggested a link between synovial peptidase enzymes and knee cartilage thickness using MRI images, although it was non-statistically significant. In individuals with KOA, we found a significant link between NAP (P=0.038) and APB (P=0.05) peptidases and painful active movement. Only ASP peptidase (P=0.049) had a significant relationship with range of motion (Table 3).

Table 3: Association between patient articular cartilage state, pain on movement and motion range variables with peptidase activity.

Peptidase activity (U/mg protein)	Median (IQR)	Association with articular cartilage state (P-value)	Association with pain on movement (P-value)	Association with range of motion (P-value)
NAP	81.2895 (23.24 to 310.97)	0.316	0.038	0.280
PSA	110 (37.65 to 376.32)	0.132	0.325	0.097
APB	43.2981 (7 to 271.45)	0.569	0.050	0.478
PEP	11.2513 (2.24 to 56.65)	0.984	0.854	0.872
ASP	13.6344 (3.39 to 31.56)	0.606	0.512	0.049
GLU	11 (4.11 to 41.56)	0.629	0.297	0.146
PGAP	5 (2 to 19.42)	0.876	0.593	0.933

PEP was revealed to be a significant clinical biomarker linked with knee lock, knee effusion, and knee failure. PSA (P=0.021), PEP (P=0.011), ASP (P=0.043), GLU (P=0.007), and PGAP (P=0.004) were shown to have a significant relationship with knee locking. The presence of ABP (P=0.013) and PEP (P=0.033) peptidases had a substantial impact on another clinical state of knee failure. In assessing knee effusion, PEP (P=0.050) and PGAP (P=0.018) differ substantially as a function of clinical sign.

Table 4 shows peptidase activity in different clinical circumstances. Additionally, our findings demonstrated that, according to a binary logistic regression, PEP is a protective marker, but PSA and GLU are risky factors (Omnibus P=0.002, Nagelkerke R2 P=0.733, and Hosmer Lemeshow P=0.132). The results showed that knee locking action was unaffected by PSA, ASP, or GLU. However, there was a considerable influence of NAP on knee failure and a negligible impact of PGAP. None of the peptidases (NAP and PGAP) had a substantial impact on knee effusion.

Table 4: Association between patient knee lock state, knee failure and knee effusion conditions and clinical activity of peptidase.

Peptidase activity (U/mg protein)	Median (IQR)	Knee lock (P-value)	Knee failure (P-value)	Knee effusion (P-value)
NAP	81.2895 (23.24 to 310.97)	0.459	0.559	0.960
PSA	110 (37.65 to 376.32)	0.021	0.113	0.731
APB	43.2981 (7 to 271.45)	0.087	0.013	0.575
PEP	11.2513 (2.24 to 56.65)	0.011	0.033	0.050
ASP	13.6344 (3.39 to 31.56)	0.043	0.097	0.678
GLU	11 (4.11 to 41.56)	0.007	0.364	0.785
PGAP	5 (2 to 19.42)	0.004	0.539	0.018

The effect of peptidases on known knocking, knee failure, and effusion states was shown in **Table 5**.

Table 5: Binary logistic regression model for peptidase activity and clinical conditions.

Synovial fluid Peptidases		B	SD	Wald	P-value	Exp(B)	95% CI for Exp (B)
With knee locking	PSA	-0.014	0.667	2.025	0.034	0.974	0.941 to 0.995
	ASP	0.293	0.995	1.600	0.008	1.179	1.066 to 1.643
	GLU	0.185	0.237	2.388	0.078	1.161	0.965 to 1.441
	Constant	-2.146	1.162	2.378	0.111	0.129	N/A
With knee failure	NAP	0.689	0.176	3.694	0.056	0.981	0.854 to 1.001
	PGAP	0.122	0.043	6.759	0.087	2.551	1.345 to 4.912
	Constant	-3.638	1.150	4.873	0.052	0.082	N/A
With knee effusion	NAP	-0.023	0.116	3.154	0.056	0.987	0.951 to 1.043
	PGAP	0.777	0.558	6.690	0.006	2.619	1.311 to 4.011
	Constant	-3.270	1.078	3.087	0.031	0.035	N/A
Inclusion in the Knee Arthroplasty group	PSA	-0.089	0.185	8.647	0.004	0.929	0.917 to 0.985
	PEP	0.457	0.231	5.883	0.004	1.705	1.141 to 2.045
	GLU	0.618	0.077	4.114	0.022	1.552	1.091 to 2.145
	Constant	-3.817	2.117	3.802	0.085	0.024	N/A

Additionally, a statistically significant link between the need for knee arthroplasty and knee effusion, failure, and locking problems was detected (**Table 6**).

Table 6: Clinical indications for knee arthroplasty in the 2 studied groups.

Clinical variables	Conventional therapy group (n=28)	Arthroplasty group (n=47)
Knee locking	8 (28.57)	14 (29.78)
Knee failure	10 (35.71)	14 (29.78)
Knee effusion	10 (35.71)	19 (40.42)

DISCUSSION

To the best of our knowledge, our study is one of the first to demonstrate the utility of synovial fluid peptidase activity as a marker of disease severity and a clinical predictor in KOA. We noticed that in each patient, there were noticeable differences in the levels of certain biomarkers for pain and range of motion, with relatively unique patterns. Few researches have recently looked at how synovial fluid aminopeptidase activity is impacted by human intra-articular inflammatory processes. Angiotensin II receptors were claimed to be expressed in human articular chondrocytes taken from OA patients having arthroplasty some time ago [(16)].

Hence, Losartan has shown anti-inflammatory activity in human arthritis, supporting this theory [(17)]. According to **Cobankara *et al.***'s theory [(18)] which is validated by certain recent studies, the local articular renin-angiotensin system is implicated in joint degeneration in RA [(19)].

For this purpose, we conduct our study to assess synovial fluid peptidases' potential role as a biomarker for KOA in Egyptian patients receiving various conservative treatments as well as to determine whether it can predict the clinical progression in those patients. The enrolled patients were divided into two groups: (i) Conventional treatment group and (ii) Knee arthroplasty group.

Based on clinical manifestations such as knee locking, failure and effusion, we found significant variations in the levels of many peptidase biomarkers in synovial fluid, but not WOMAC (Pain, stiffness and physical functional status). Furthermore, synovial fluid peptidase analysis in patients with advanced KOA revealed differences between those who required total knee replacement surgery and those who were managed by conservative therapy, with the former one (patients with more functional impairment) having noticeably higher peptidase activity (i.e. APB, PEP, ASP, GLU, and PGAP) but not NAP or PSA. This is consistent with the elevated APB activity discovered in the synovial fluid obtained from swollen knees in a rat model of RA [(20)] and the synovial fluid NAP activity that triggers T-cell chemotaxis in a similar animal model [(21)]. Additionally, NAP seems to be involved in the pathogenesis of RA and maybe OA. While APN and PGLU had a significant impact on the prognosis for knee failure and effusion, PSA, ASP, and GLU had an impact on the prediction for knee locking (**Table 3**). Furthermore, we investigated the effect of PSA, PEP, and GLU, as predictors for the necessity of total knee arthroplasty and discovered that PSA was a protective while PEP and GLU were risky factors despite the fact that there were no statistically significant between-group differences for PSA. Hence, it was considered candidates for the identification of

clinical progression. However, our findings, suggested that the role was a predictor for PSA but not for NAP. Its possible importance is attributed to the expression of NAP by synoviocytes that may play a part in acute arthritis [22].

In our study, we might have focused on other cytokines of inflammations because they have been associated with clinical improvement in KOA, but instead we chose to focus on synovial soluble peptidases. **Yamasaki *et al*.** [23] claimed also in their research, the association between arthritis development and levels of NAP and DPPIV (dipeptidyl-IV) as susceptibility inducers. Although little is known about PSA, it has been suggested that it's endogenous enzyme inhibitor, Spinorphin, breaks down enkephalin and may be involved in pain and inflammation [24]. Scientific associations have underlined the relevance of pain in determining whether to recommend arthroplasty therefore the probable role of PSA in pain only serves to emphasize how crucial this clinical aspect is. This is why we chose to incorporate PSA into our research. Notably, serum enkephalin-degrading enzyme activity is low in fibromyalgia patients, suggesting that the condition may impair musculoskeletal pain and that PSA may be involved in pain neuro-modulatory pathways in OA [25]. Therefore, more research is needed to understand these difficulties.

Our study also showed significant association between NAP (P=0.038) and APB (P=0.050) peptidases and pain generation on movement in patients with KOA. Similarly, significant role of aminopeptidase N (CD13) activity is "Fibroblast Like Synoviocytes – FLS" leading to pathogenic hyperplasia was studied in RA patients and serves as knee osteoarthritis cartilage target in both genders [26,27,28]. Moreover, some investigations looked into the function of aminopeptidase activity in human synovial fluid and the inflammatory process, with data from osteoarthritic patients showing that arthroplasty improved the expression of angiotensin II receptors [29]. **Wu *et al*.** [21] looked at how varying amounts of renin angiotensin components affected joint degeneration in both RA and OA patients, with RA patients having a stronger impact. Our study observed significant difference in peptidase activity in different clinical conditions of knee locking, failure and effusion with significant association between knee locking and peptidase activity of PSA (P=0.021), PEP (P=0.011), ASP (P=0.043), GLU (P=0.007) and PGAP (P=0.004) was observed. Similarly, **Mendes *et al.*** [29] in their study on RA rat model demonstrated high activity of APB in synovial fluid of swollen knees. Additionally, a significant association of APB was found in knee failure in our research. However, reduced APB activity in RA models was reported through TNF synthesis inhibitors as a recent approach [29]. NAP is another synovial fluid biomarker that was involved in chemotaxis of T-cell in an animal model and its role is crucial in demonstrating heightened pro-inflammatory mediator expression and increasing circumference when injected into mice knee joints [22,26]. Knee locking was impacted by PSA, ASP, and GLU, according to the prediction model shown in Table 3. In line with this, an animal research model found PSA to be a potent proteotoxicity attenuator as well as a potential synaptic biomarker in the spinal muscular atrophy mouse model [24]. Our findings suggested that the requirement for total knee replacement was significantly correlated with NAP, PSA, PEP, and GLU, demonstrating that PEP is a protective factor while PSA and GLU are risky factors. The renin-angiotensin system also play important role in decision for knee arthroplasty as angiotensin is activated and converted to angiotensin II , III and IV through angiotensinases involvement, which are peptidases that generate active or inactive peptides including (APB, APA and APN) that may increase inflammation and pain [30]. Therefore, given the great specificity in the clinical characterization of KOA patients, our findings suggest the potential value of synovial fluid peptidase activity as an indicator of disease burden and a predictive biomarker of clinical progression. Orthopedic doctors see patients with severe KOA in outpatient settings every three to six months. Injections into the joints may also be given during these visits, and synovial fluid may be removed via an arthrocentesis. Such appointments permitted the quick and painless peptidase evaluation of a small volume of synovial fluid that could be acquired easily, inexpensively, and safely without causing bleeding and sent for peptidase analysis, which is a quick and affordable process.

Clinical decision-makers may be assisted by the use of certain peptidases (i.e. PSA, PEP, and Glu) activity as predictors of clinical progression. The risk of infection may also be decreased, saving patients from having to wait an additional year and potentially suffering functional decline, decreasing surgical reintervention and failure rates as well as the requirement for prosthetic arthroplasty. Despite all these promising results, our study has some limitations including: Failure to take into account the variations in the conservative treatment that was given, or other proteins and enzymes that are probably implicated in KOA. The single centered study and the fact that we only analyzed samples derived from arthrocentesis are further drawbacks. All of the enrolled patients had moderate to severe knee synovitis and effusion, therefore our findings might not appear to be true for joints with mild inflammation.

Future studies are therefore necessary to establish whether peptidase levels are actually accurate prognostic biomarkers for KOA progression and to ascertain whether the type of conservative therapy affects enzyme activity.

CONCLUSION

The study evaluated peptidase activity as a potential synovial fluid biomarker in determining disease progression in osteoarthritic patients which could help in early disease assessment and planning for future treatment protocols.

Conflict of Interest: We attest that no financial or business relationships existed during the course of the research that may be seen as having a possible conflict of interest.

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