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ORIGINAL ARTICLE

Effect of Proteolytic Enzymes and Insulin Sensitiser in Treatment of Joint Osteoarthritis in Diabetic Patients

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Abstract

Background/Aim: Knee osteoarthritis is a frequently crippling chronic condition. Numerous pharmacological medications have been successfully utilised to treat knee osteoarthritis. This research aimed to compare the efficiency of metformin and serratiopeptidase in treating and preventing osteoarthritis development *via* distinct mechanisms.
Methods: Between 1 January and 30 May 2019, a randomised-clinical-trial was done at Al-Kindy Hospital on 80 osteoarthritis patients, divided in two groups. Group I was given metformin 850 mg orally, whereas Group II was given serratiopeptidase 20 mg and metformin 850 mg orally. Parameters in these groups were compared with forty healthy normal controls.
Results: Following treatment, patients in Group II have shown a significant decrease in pain levels ($p = 0.001$). Interleukin 8 (IL-8), tumour necrosis factor-alpha (TNF-α) and interleukin 1 beta (IL-1ß) levels were significantly decreased in Group II ($p = 0.001$).
Conclusion: The combination of serratiopeptidase and metformin was effective and safe in treating knee osteoarthritis.

Key words: Metformin; Inflammatory parameters; Knee osteoarthritis; Serratiopeptidase.

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Introduction

Osteoarthritis (OA) of knee is a prevalent global chronic debilitating disease.[1] It is a synovial joint condition that is pathologically defined by cartilage damage, increased load, capsule thickness, osteophytosis and subchondral bone alterations.[2] Obesity is a global epidemic strongly associated with OA and has a multifactorial effect on OA.[3] Similarly, the influence of metabolic factors such as humoral and lipids mediators, obesity initiates the mechanical effects that result in knee joint injury due to excessive load, muscle weakness and biomechanical alterations.[4, 5]

Knee OA therapy aims to alleviate pain, improve the overall quality of life, limit joint cartilage degradation and maintain movement. The disease is managed in three distinct ways: non-pharmacologically, pharmacologically and surgically. Non-pharmacological approaches frequently entail lifestyle modifications, for instance, increased physical activity, weight loss and programmed dieting.[6]

Weight loss reduction has been shown to improve movement in people with knee OA. Nu-

merous pharmaceutical treatments, including systemic non-steroidal-anti-inflammatory drugs (NSAIDs), metformin, platelet-rich plasma, diacerein, glucosamine, topical creams and serratiopeptidase, have been utilised successfully in the treatment of knee OA.[7,8]

Metformin (biguanide) is a medication that is commonly used to treat type II diabetes mellitus (T2DM). Metformin affects glucose production and may influence insulin production in people with diabetes. Additionally, it aided in weight loss.[9] Along with its anti-diabetic impact, metformin has been proven to alleviate the inflammation and pain intensity associated with OA with no documented adverse effects, making it a viable therapy option for individuals with OA of knee and a prospective medication for inflammation-related problems. Although, the mechanism of metformin anti-inflammatory effect is uncertain, numerous researches have demonstrated that it affects oxidative stress and reduces inflammatory markers.[10]

Serratiopeptidase, is proteolytic enzyme that exhibits anti-inflammatory properties. Nowadays, serratiopeptidase enzymes are widely employed as the preferred inflammatory and anti-pain treatment in Japan and Europe.[11]

The prevalence of obesity and overweight has increased significantly over the last two decades in Iraq, resulting in improved knee OA cases, particularly between the elderly, poor life quality, resulting in impaired function and significant burden on the health services. Several medications were introduced for the treatment of knee OA, showing little evidence of efficacy and numerous bad effects. Aim of this study was to determine the efficacy of metformin and serratiopeptidase in treating and reversing OA development *via* various mechanisms.

Methods

The research was a randomised clinical trial undertaken at Al-Kindy Teaching Hospital, Baghdad, Iraq from 1 January to 30 May 2019. The study population consisted of individuals with knee OA who were offered to the Al-Kindy Teaching Hospital's Consultation Clinic. Adulthood, overweight or obesity and knee OA were all considered inclusion criteria. Pregnancy, coagulation, bleeding disorders, systemic conditions such as diabetes mellitus, hypertension and existing knee treatment OA with other prescription regimens were an non-inclusion/exclusion criteria.

Patients

Eighty patients with OA of the knee were divided into two groups using digital randomisation and non-randomisation: Group I was given metformin 850 mg orally, whereas Group II was given serratiopeptidase 20 mg and metformin 850 mg orally.

The rheumatologists of Al-Kindy Teaching Hospital have performed clinical and radiological diagnosis of patients with knee OA. A control group of 40 healthy volunteers were selected. Before inclusion in the trial, the height and weight of all research participants was analysed (n = 120). To determine their body mass index (BMI), they were measured on a calibrated scale. The BMI of all research patients was matched.

Patients were instructed to discontinue use of the medicine and to report to the researchers any instances of bruising or bleeding that occurred while they were taking it.

Pain scores (ranging from 1-10) were obtained from patients after being labelled and were input into specifically created questionnaire for each trial participant. Five mL blood sample was collected from each participant to evaluate their inflammatory markers serum level [tumour necrosis factor-α (TNF-α), resistin, interleukin 8 (IL-8), IL-1ß and adiponectin] using ELISA. The kits were as follows: *Human Tumour Necrosis Factor α (TNF-α) ELISA Kit*, product number: RAB1089, *Sigma-Aldrich*, Germany; *IL-1 beta Human ELISA Kit*, P01584, *Invitrogen,* MA, USA; *IL-8 Human ELISA Kit*, P10145, *Invitrogen,* MA, USA; *Resistin Human ELISA Kit,* Q9HD89, *Invitrogen,* MA, USA; *Adiponectin Human ELISA Kit*, Q15848, *Invitrogen,* MA, USA.

Rheumatologists divided the two treatment regimens into two research groups after collecting baseline data from patients and patients were monitored for 12 weeks. At the second visit, each patient's pain and BMI were recorded in a questionnaire and anti-inflammatory parameters were determined in the hospital's laboratory for three groups of research participants. Pain

scores, levels of inflammatory and BMI markers in knee joint OA patients were compared to those in the healthy group to determine the effectiveness of treatments. Throughout 12 weeks, the negative consequences of both research groups were observed.

Statistical analysis

Analysis was conducted using an SPSS application. Outcomes were classified using a contingency table. To compare two means before and after therapy, a paired t-test was performed. ANOVA was implemented to compare the means, while the Fisher exact test was utilised to examine categorical data.

Results

A total of 80 patients with knee OA were included in this study. Between study groups of patients, there were no significant variations in age or gender. In two study groups, female OA patients were substantially more common than male OA patients (Table 1).

BMI of OA patients for both study groups were slightly and non-significantly reduced (p = 0.10). Pain scores have shown significant decrease (p = 0.001) in Group II, but no significant change (p = 0.07) in Group I. Regarding IL-1ß and IL-8 levels they have shown significant decline in Group II (p = 0.001), but no significant change in Group I. Resistin serum didn't show significant changes after treatment of OA patients. TNF-α was significantly lowered in both research groups of OA patients following treatment (p = 0.001). Adiponectin serum didn't show significant changes after treatment for OA patients (Table 2).

Levels of IL-1ß, IL-8, TNF-α and adiponectin were significantly different between the study group and the healthy controls when inflammatory markers from OA patients in both study groups were compared to controls. There was a difference (p = 0.001), with Group II patients having lower parameter levels than Group I patients and healthy controls having lower levels. There was no significant difference in resistin levels (p = 0.2) (Table 3).

Table 1: Demographic characteristics of obese patients with osteoarthritis

Parameters	Group I N (%)	Group II N (%)	p-value
Age (years)			
< 40	4 (10.0 %)	4 (10.0 %)	**0.8***
40-49	10 (25.0 %)	11 (27.5 %)	
50-59	9 (22.5 %)	6 (15.0 %)	
≥ 60	17 (42.5 %)	19 (47.5 %)	
Gender			
Male	12 (30.0 %)	11 (27.5 %)	**0.6****
Female	28 (70.0 %)	29 (72.5 %)	

**Fishers exact test; **Chi-square test; Group I was given metformin 850 mg orally; Group II was given serratiopeptidase 20 mg and metformin 850 mg orally;*

Table 2: Body mass index (BMI) and inflammatory markers levels for two groups of treated knee osteoarthritis patients pre- and post-treatment

Parameters	Group I			Group II		
	Pre-treatment	Post-treatment	p-value	Pre-treatment	Post-treatment	p-value
BMI	34 ± 5.1	34.0 ± 4.6	0.70	33.7 ± 5.0	32.0 ± 4.2	0.10
Pain score	7.9 ± 2.0	6.9 ± 2.0	0.07	8.0 ± 2.0	4.0 ± 2.5	**< 0.01**
IL-1ß [pg/mL]	425.0 ± 22.1	419.0 ± 20.9	0.20	427.0 ± 20.3	412.0 ± 17.5	**< 0.01**
IL-8 [pg/mL]	370.0 ± 25.0	366.0 ± 28.5	0.50	368.0 ± 30.3	228.0 ± 21.4	**< 0.01**
Resistin [µg/mL]	0.018 ± 0.010	0.016 ± 0.008	0.20	0.024 ± 0.001	0.022 ± 0.009	0.30
TNF-α [pg/mL]	65.0 ± 1.5	60.0 ± 0.7	**< 0.01**	70.3 ± 1.7	58.4 ± 0.7	**< 0.001**
Adiponectin [µg/mL]	31.0 ± 4.2	29.0 ± 4.5	0.10	30.4 ± 5.0	29.4 ± 4.9	0.30

**Paired t-test; BMI: body mass index; IL-1ß: interleukin 1 beta; IL-8: Interleukin 8; TNF-α: tumour necrosis factor-α; Group I was given metformin 850 mg orally; Group II was given serratiopeptidase 20 mg and metformin 850 mg orally;*

Table 3: Inflammatory markers expression in study groups and healthy controls

Parameters	Group I	Group II	Control	p-value
IL-1ß [pg/mL]	419.0 ± 20.9	412.0 ± 17.5	3.0 ± 0.8	< 0.01
IL-8 [pg/mL]	366.0 ± 28.5	228.0 ± 21.4	33.0 ± 12.4	< 0.01
Resistin [µg/mL]	0.016 ± 0.007	0.020 ± 0.009	0.020 ± 0.010	0.20
TNF-α [pg/mL]	60.0 ± 0.7	58.0 ± 0.7	38.0 ± 2.9	< 0.01
Adiponectin [µg/mL]	29.0 ± 4.5	29.0 ± 4.9	17.0 ± 0.0	< 0.01

**One-way ANOVA analysis; BMI: body mass index; IL-1ß: interleukin 1 beta; IL-8: interleukin 8; TNF-α: tumour necrosis factor-α; Group I was given metformin 850 mg orally; Group II was given serratiopeptidase 20 mg and metformin 850 mg orally;*

Table 4: Adverse effects reported in patients treated for knee osteoarthritis

Adverse effect	Group I	Group II	p-value
Nausea and vomiting	4 (10.0 %)	1 (2.5 %)	0.30
Diarrhoea	1 (2.5 %)	0 (0.0 %)	1.00
Vertigo	2 (5.0 %)	1 (2.5 %)	0.60
Headache	2 (5.0 %)	0 (0.0 %)	0.20
Bleeding/bruising	0 (0.0 %)	0 (0.0 %)	-
Muscle weakness	1 (2.5 %)	0 (0.0 %)	1.00

** Fisher Exact test; Group I was given metformin 850 mg orally; Group II was given serratiopeptidase 20 mg and metformin 850 mg orally;*

As demonstrated in Table 4, no significant changes in side effects were detected between the two study groups three months after medication. Nausea and vomiting (10 %), vertigo (5 %) and headache (5 %) were the most frequent adverse reactions associated with Group I regimen. Nausea and vomiting (2.5 %) and vertigo (2.5 %) were the most commonly reported adverse effects associated with Group II regimen.

Discussion

Numerous medication formulations, such as NSAIDs, have been utilised to treat knee OA and these drugs can disrupt extracellular matrix metabolism, particularly proteoglycan production.[12] Moreover, these pharmacological treatments were associated with several side events, including gastrointestinal ulcerations. As a result, novel medication formulations are required to alleviate symptoms safely and be long-lasting.[12] These data demonstrated the metformin and serratiopeptidase regimen's superior symptomatic efficacy and laboratory efficiency in the treatment of knee OA.

Metformin has been shown to have a synergistic impact with various medications and in a variety of disorders.[13] Mohammed et al[14] have shown that combining metformin with anti-inflammatory drugs like NSAIDs for knee OA has been demonstrated to improve outcome scores for knee OA. Metformin was reported to lower inflammatory indicators and contribute to reducing oxidative stress *via* an unknown mechanism.[15] These inflammatory indicators, such as cytokines and chemokines are increased following knee joint damage, indicating a significant role for oxidative stress in knee OA pathophysiology.[16]

Several authors validated metformin's osteogenic effect *in vitro*.[17] Study in China reported the beneficial effect of metformin in management of intervertebral disc herniation.[18] Although there was no significant association, after three months of treatment, patients BMI of both trial groups decreased. This outcome is consistent with the findings of study conducted in the United States of America, which concluded that there is insufficient evidence to support the use of metformin to treat overweight and obesity.[19]

In a study in India, both patients groups with knee OA used serratiopeptidase combined with various drugs and both modalities, including serratiopeptidase, had a better effect on knee treatment with mild side effects. The underlying mechanism of serratiopeptidase in knee OA is not fully understood but demonstrated to lyse dead, damaged tissue while sparing living tissue.[20] Indian studies have previously established that serratiopeptidase has anti-inflammatory properties after surgery.[21] When inflammatory indicators from both trial groups were compared to those from healthy controls, serum levels of IL-8, IL-1ß, adiponectin and TNF-α, were considerably lower in patients treated with serratiopeptidase and metformin than in patients treated with metformin alone. Such finding further established anti-inflammatory synergy between serratiopeptidase and metformin.

Furthermore, presented findings corroborate those of Bhagatet et al[22] and Nair et al,[8] both of which demonstrated the anti-inflammatory impact of serratiopeptidase enzyme and metformin. There was limited adverse effects in both groups, particularly for group II (serratiopeptidase and metformin), with no significant differences between groups. These results points to regimen's safety, proven in several prior studies.[14, 20]

Limitations of this trial were lack of follow-up, a single-centre design and a limited period for evaluating side effects; therefore, additional long-term follow-up studies are required to verify long-term effect of serratiopeptidase and metformin medication combination.

Conclusion

Serratiopeptidase and metformin in knee OA treatment are safe and effective. This regimen effectively reduces pain and inflammatory markers in people with knee OA.

Ethics

The study was approved by the Al-Kindy College of Medicine, University of Baghdad's Ethics and Scientific Committee, which gave it the registration No EAC-125603, dated November 2018. Written informed consent was obtained from patients prior to their participation in the study and for publishing of the anonymised patient data. The study was organised and implemented based on the adherence to the Ethical Principles for Medical Research Involving Human subjects (The Declaration of Helsinki, 8th Revision, 2013).

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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