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# EVALUATION OF THE ANTI-INFLAMMATORY EFFECT OF METFORMIN AS ADJUVANT THERAPY TO NSAID (MELOXICAM) IN PATIENTS WITH KNEE OSTEOARTHRITIS

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**ABSTRACT**
The study was performed on 68 patients who have symptomatic and radiologic evidence of painful OA of the knee. Patients were allocated into two groups, group A: treated with meloxicam (15mg/day) alone, and group B: treated with metformin (500mg/12 hr.) + meloxicam (15mg/day). The treatment was followed for 12 weeks, and serum levels of inflammatory mediators including IL-1β, IL-8 and TNF-α were measured before, after 6, and 12 weeks of treatment. The results showed that metformin, when used in combination with NSAID resulted in significant reduction in the serum levels of inflammatory mediators and consequently the intensity of symptoms, higher than that produced by meloxicam when used alone. Study showed that administration of metformin as adjuvant therapy to NSAID, meloxicam, in OA patients produced very well characterized anti-inflammatory activities, and improves the therapeutic profile of meloxicam.

**KEY WORDS:** Metformin, Meloxicam, Osteoarthritis, Knee injury.

## INTRODUCTION

Osteoarthritis (OA) is a common disorder of synovial joints. It is characterized pathologically by focal areas of damage to the articular cartilage, centered on load-bearing areas, associated with new bone formation at the joint margins (osteophytosis), change in the subchondral bone, variable degrees of mild synovitis, and thickening of the joint capsule [(1)]. When this disease is advanced, it is visible on plain radiographs, which show narrowing of joint space (due to cartilage lose), osteophytes, and sometimes changes in the subchondral bone[(2)]. Gauging the severity of OAinvolves assessment of both joints and patients. This assessment may be done in the clinical setting in support of diagnosis, treatment decision, or evaluation of response to treatment. Clinical examination of the osteoarthritic joints can be helpful in assessing the extent of joint damage, such as deformity and instability, but the reproducibility of findings is low[(3)]. Many types of drugs, exemplified by non-steroidal anti-inflammatory agents (NSAIDs) currently being used to treat OA. However, NSAIDs elicit adverse effects particularly gastrointestinal ulcerations[(4)]. Moreover, some of these agents have been reported to disrupt extracellular matrix (ECM) metabolism, particularly proteoglycans synthesis [(5)]. Prolonged consumption of these drugs can result in severe adverse effects. Consequently, there is an urgent need for new strategies in OA therapy which can improve symptoms and are safe for clinical use over long periods of time[(6)]. The ability of metformin to reduce the intensity of pain and inflammation that contributed to the pathophysiology of OA, with no serious adverse effects, has been reported in many animal model studies. This may considered a new therapeutic approach added to the current NSAIDs treatment for pain, inflammation, and improve quality of life in patients with knee OA.It has been shown that metformin can serve as potential drug to treat inflammation-related disorders[(7,8)]. However, the specific anti-inflammatory mechanism of metformin is not clearly understood, several studies demonstrated that the pharmacological action of metformin goes beyond mere glycemic control, decreasing markers of inflammation and contributing to the reduction of oxidative stress [(9)]. Metformin dose-dependently reduced the production of nitric oxide (NO) and prostaglandin E2 (PGE2) and suppressed the mRNA and protein levels of inducible nitric oxide synthase (iNOS) and COX-2 in lipopolysaccharides-activated macrophages [(10)]. The discovery that many mediators such as cytokines or prostaglandins can increase the production of metalloproteinases (MMPs) by chondrocytes led to the first steps of an "inflammatory" theory [(11)]. Soluble inflammatory factors including cytokines, chemokines, adipokines, neuropeptides, and lipid inflammatory mediators have been implicated in OA pathogenesis. Following joint trauma or overuse, tissue damage results in the production of damage-associated molecular patterns (DAMPs), including cartilage ECM breakdown products and intracellular alarmins that signal through pattern recognition receptors on synovial macrophages, fibroblast-like synoviocytes (FLS), or chondrocytes to induce the local production of inflammatory mediators [(12)]. Several studies observed increased levels of Interleukin-6 (IL-6) and Interleukin-8 (IL-8) in OA serum and synovial fluid [(13)], and autocrine production of Interleukin-1 beta (IL-1β) and TNF-α as well as expression of other critical inflammatory and chrondrolytic mediators, including MMP-1, MMP-9, MMP-13, NO) and PGE2[(14)]. IL-1 and TNF-α produced by activated synoviocytes, or by articular cartilage itself significantly up-regulate MMPs gene expression. Cytokines also blunt chondrocyte

compensatory synthesis pathways required to restore the integrity of the degraded ECM [15].

There is a strong evidence for the involvement of oxidative stress in pathophysiology of OA, oxidative stress may induces chondrocyte instability and catabolic changes in cartilage matrix structure and composition, leading to chondrocyte senescence and cartilage ageing [16]. Oxidative stress may induce apoptosis (programmed cell death) in different joint compartments (cartilage, synovial tissue and subchondral bone). Radical oxygen species (ROS) involvement in inflammation, fibrosis and pain nociception; has been proven [17]. Another pathway involved in the pathophysiology of OA is the induction of NO synthesis in the cartilage, which appears to be enrolled in the initiation of apoptosis which may be one of the important steps in OA pathology [18]. Potential peripheral targets to control OA pain currently under investigation include inflammatory mediators and their key receptors such as prostanoids, kinins, cytokines, and chemokines; sodium and calcium ion channels; and growth factors [19]. This study designed to evaluate the clinical utility of using metformin as anti-inflammatory agents in combination with NSAID of selective type ofCOX-2 inhibitor, meloxicam, in the treatment of knee osteoarthritis (OA).

## PATIENTS & METHODS

This study was performed on (68) randomly selected patients (20 males and 48 females) with painful osteoarthritis (OA) of the knee, at the Out Patients Clinic in Baghdad Teaching Hospital with age range 36-71 years (59.2 ± 7.3). All patients have symptomatic and radiological evidence of OA in one or both knee joints; their clinical features were in accordance with the description of OA in UK and North American Clinical Guidelines. They also show no significant differences in their initial pain, morning stiffness or global assessment; all patients were informed about the nature and the aim of the study. During patient selection certain exclusion criteria were followed, based on the following: 1.patient with positive history of gastric ulcer, 2.patients with end-stage radiological events of joint destruction, 3. patients with positive history of allergic reactions to any one of the known NSAIDs, 4. any patient who miss one of the treatment assessment indicated in the present study and/or his medication for any reason, 5. pregnant or lactating patients, 6. patients with renal or hepatic damage and those who are on treatment with drugs that interfere with the assessment method. The selected patients were randomly allocated into two groups as follow: Group A, includes 32 (11 males and 21 females) patients with negative GIT risk factors, treated with meloxicam tablets (15mg/day) taken at night for 12 weeks (20 patients only completed the study). Group B, includes 36 (9 males and 27 females) patients with negative GIT risk factors, treated with meloxicam tablets (15mg/day) taken at night and metformin (500mg/12 hours)for 12 weeks(20 patients only completed the study).Effects of drug treatment were assessed by measuring the serum levels of inflammatory mediators including IL-1β, IL-8 and TNF-α (using ready-made ELISA kits), before, after 6, and 12 weeks of treatment.The results were expressed as mean ± SEM; paired t-test and ANOVA were used to examine the degree of significance; P values less than 0.05 were considered significantly different.

## RESULTS

The data presented in (table-1) clearly indicated that treatment with meloxicam and metformin combination in OA patients significantly reduce the serum levels of cytokines IL-1β and IL-8 after 6 weeks (37% and 14.96 % respectively) and after 12 weeks (49.59%, 39% respectively) compared to pre-treatment levels. However; in case of treatment of OA patients with meloxicam alone,serum levels of IL-1β is found to be non-significantly elevated after 6 weeks and 12 weeks treatment (2.85%, 1.53% respectively) compared to pre-treatment value, on the other hand; IL-8 levels are still with non-significantly changed after 6 weeks and 12 weeks treatment (0.14%, 0.17% respectively), (figure-1) and (figure-2). The effect of treatment on serum level of TNF-α was explained in (table-1) which showed that both treatment regimens resulted in significant reduction in TNF-α levels at the end of the study compared to pre-treatment levels, while only the combination treatment of meloxicam and metformin resulted in significant reduction in TNF-α level after 6 weeks treatment in respect to pre-treatment level (11.45%), (figure-3).Combination treatment of meloxicam and metformin showed higher reduction in TNF-α levels which is significantly different compared to that levels resulted by treatment with meloxicam alone, after 6 weeks treatment (11.45% and 2.71% respectively), and after 12 weeks treatment (21.76% and 10.6% respectively), (figure-3).

**TABLE 1:** Effects of treatment with meloxicam aloneand combination of meloxicam + metformin on serum levels of IL-1β, IL-8 andTNF-α in osteoarthritic patients

| Group | Duration | IL-1β(pg/ml) | IL-8(pg/ml) | TNF-α(pg/ml) |
|---|---|---|---|---|
| *Meloxicam* | *Pre-treatment* | 427.31 ± 19.43 | 349.52 ± 19.49 | 69.83 ± 1.68 |
| | *6 weeks treatment* | 439.49 ± 22.17 a | 343.18 ± 20.58 | 67.94 ± 0.97 a |
| | *12 weeks treatment* | 433.83 ± 13.37 a | 347.26 ± 15.05 a | 62.43±0.77 *†a |
| *Meloxicam + Metformin* | *Pre-treatment* | 450.28 ± 21.82 | 372.35 ± 22.28 | 71.51 ± 1.27 |
| | *6 weeks treatment* | 283.66±12.38 **b | 316.63 ±17.13 ** | 63.32±0.91 *b |
| | *12 weeks treatment* | 226.99 ±8.49**†b | 227.12±5.59**†b | 55.95±1.1 **†b |

- Data are expressed as mean ± SEM.
- * P<0.05 significantly different compared to pre-treatment value within the same group.
- ** P<0.01 significantly different compared to pre-treatment value within the same group.
- † P<0.05 significantly different compared to 6 weeks-treatment value within the same group.
- Values with non-identical superscripts (a & b) within different groups are significantly different (P<0.05) at corresponding duration.

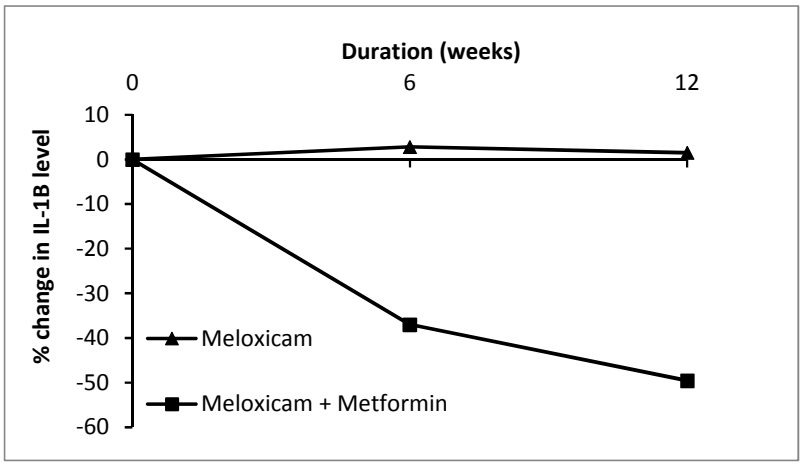


**FIGURE 1:** Percent changes in serum level of IL-1β produced by treatment with meloxicam alone and combination of meloxicam + Metformin in osteoarthritic patients.

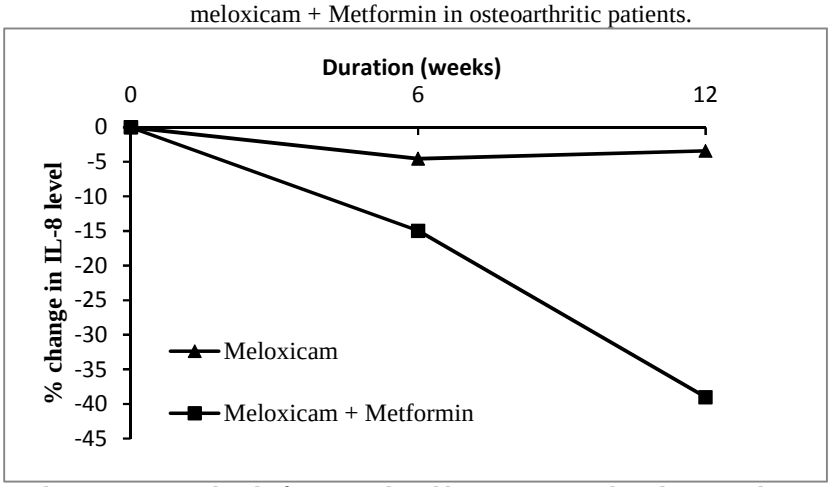


**FIGURE 2:** Percent changes in serum level of IL-8 produced by treatment with meloxicam alone and combination of meloxicam + metformin in osteoarthritic patients.

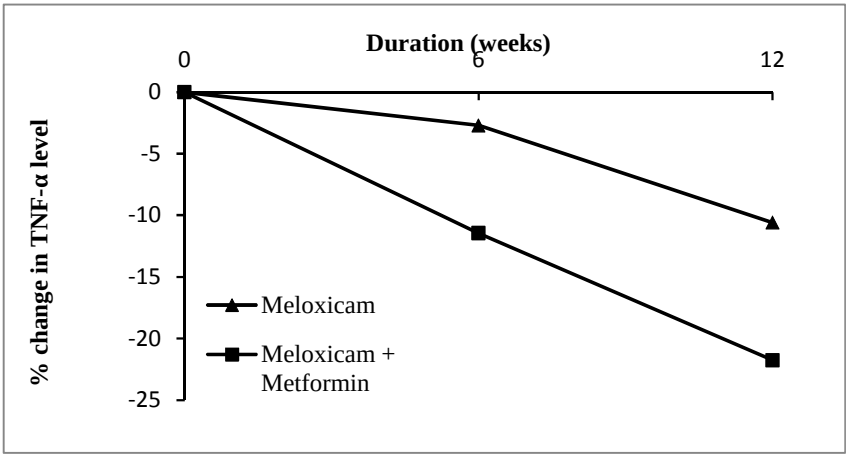


**FIGURE 3**: Percent changes in serum level of TNF-α produced by treatment with meloxicam alone, combination of meloxicam + metformin, and meloxicam + pioglitazone in osteoarthritic patients.

**DISCUSSION**

Osteoarthritis is associated with cartilage destruction, subchondral bone remodeling and inflammation of the synovial membrane. Secreted inflammatory molecules, such as pro-inflammatory cytokines, are among the critical mediators of the disturbed processes implicated in OA pathophysiology. IL-1β and TNF-α, in particular, control the degeneration of articular cartilage matrix, which makes them prime targets for therapeutic strategies[20], in addition to prostaglandins, chemokines, and specific enzymes including MMPs, collagenase and cyclooxygenases[21].

In OA synovium, a relative deficit in the production of natural antagonists of the IL-1 receptor (IL-1Ra) has been demonstrated, and could possibly be related to an excess production of NO in OA tissues [15]. Interleukin-1 also increased the expression of phospholipase A2 in rabbit chondrocytes, so that substrate availability for PG synthesis was increased in the joint [22]. Accordingly, the pro-inflammatory cytokines are believed to play a pivotal role in the initiation and development of OA process, among them IL-1β, IL-1α and TNF-α appear prominent. IL-1β is extremely important to cartilage destruction, while TNF-α appears to drive the inflammatory process. They can induce joint articular cells, such as chondrocytes and synovial cells to produce other cytokines such as IL-8, IL-6, as well as, stimulate proteases and PGE2 production. It has been demonstrated using cultured synovial fibroblasts, that blocking IL-1 activity with IL-1Ra reduced IL-6 and IL-8 production [23]. IL-8 can enhance the release of inflammatory cytokines in human mononuclear cells, including that of IL-1β, IL-6 and TNF-α which may further modulate the inflammatory reaction [24].

The data presented in this study clearly demonstrated that treatment with combination of meloxicam and metformin significantly reduce the serum levels of IL-1β, IL-8 and TNF-α after 6 and 12 weeks compared to pre-treatment levels (table-1). These results can be attributed mainly to the anti-inflammatory action of metformin and seem to be compatible with those observed when metformin revealed significant inhibition of IL-1β, IL-6, IL-8, C-peptide and TNF-α in patient with coronary heart disease in the presence of metabolic syndrome and type 2 diabetes mellitus [25].Metformin, the well-known adenosine monophosphate-activated kinase (AMPK) activator, can suppress COX-2 and iNOS mRNA and protein expression dose dependently [26]. Metformin ability to reduce the intensity of pain, mainly associated with its effects on the profile of inflammatory cytokines (i.e., TNF a, IL-1β, IL-6, and IL-10) and adipokines[27], it significantly prevented the increased levels of pro-inflammatory mediators like TNF-α, IL-1β, IL-6, and IL-18 in many inflammatory disorders, Moreover; metformin prevented the expression of COX-2, iNOS, and decreased the levels of NO and PGE2 in cell culture media [28].In the cartilage of osteoarthritic patients, although cartilage tissues contain no pain receptors, sensation of pain likely results from the inflammatory mediators. Pro-inflammatory agents interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), as well as the growth factors have all been shown to induce COX-2 expression which produces measurable quantities of prostaglandins. On the other hand, the anti-inflammatory cytokines IL-4 and IL-13, as well as the immunosuppressive glucocorticoids, were shown to decrease COX-2 levels[29], an evidence which support the observed effect of metformin in reducing the consequence of pain in OA patients.

Meanwhile, treatment of OA with meloxicam for 12 weeks did not show significant changes in IL-1β and IL-8 levels, while TNF-α was reduced by 10.6%, a value which is found significantly different compared to pre-treatment values. Many experimental studies reported the anti-inflammatory effect of meloxicam to decrease the intensity of some diseases by suppressing the excessive TNF-α production [30, 31]. Moreover, meloxicam was found to effectively inhibit the production of MMPs from human synovial fibroblasts by TNF-α stimulation *in-vitro*. This suppressive effect of meloxicam on the production of MMPs and TNF-α may partly be involved in attenuation of the clinical conditions of osteoarthritis and rheumatoid arthritis [32]. In the synovium of patients with knee OA, catabolic cytokines induce target cells to produce more matrix-degrading products. They include IL-1α, IL-1β, IL-6, IL-8, IL-17, IL-18, and TNF-α. While anti-catabolic cytokines inhibit the activity of the catabolic cytokines, they include IL-4, IL-10, IL-1, and IL-1Ra [33].

Exposure to oxidative stress inducers, like hydrogen peroxide and cytokines, could result in perturbation of chondrocyte and cartilage homeostasis and could contribute to the pathophysiology of osteoarthritis [34]. Study by (Lee J, 2013), reported that regulation of MMP-13, IL-1β, IL-6, IL-15, iNOS in OA joints play an important role in ameliorating pain and cartilage degradation in a rat model of osteoarthritis[35].

Metformin treatment normalized the majority of the oxidative stress parameters that altered by diabetes. Metformin, besides its anti-hyperglycemic action, could induces a significant decrease inmalondialdehyde (MDA) level, glutathione peroxidase (GPx), glutathione reductase (GRed) activities and a significant increase in glutathione (GSH) level and superoxide dismutase (SOD) activity. These results indicated that metformin protects against diabetes-associated oxidative stress [36]. Metformin reduced the production of NO, PGE2 and pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) by inhibiting protein and mRNA expression in a dose-dependent manner. However, the protein expression of anti-inflammatory cytokines (IL-4 and IL 10) was up-regulated or maintained by metformin[10].

From the clinical perspective, current medical treatment of OA did not provide cure or completely eliminate arthritis-related pain and disability. Therefore, management of patients with OA should not aim only at decreasing pain, but remodeling of the pathophysiological processes to improve function of the affected part and enhancing quality of life [37].

## CONCLUSION:

Administration of metformin as adjuvant therapy to NSAID, meloxicam, in OA patients produced very well characterized anti-inflammatory activities, and consequently improves theprofile of pain, symptoms and quality of life, and improves the therapeutic profile of meloxicam when co-administered.

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