Biochemical role of bone morphogenetic Protein in the inflammatory arthritis

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Abstract

Back-ground: “Rheumatoid arthritis (RA)” is a chronic-autoimmune illness marked by synovial membrane inflammation, which can result in joint deformity and physical disability. The biomarkers that studied in RA were vary and they have significant role in the diagnosis, prognosis of treatment, monitorization of the disease activity, and prediction of the response to biologic therapy. Proteoproteins involved in bone morphogenesis BMPs are “transforming growth factor beta (TGF-β)” super-family members, that promote the production of cartilage and other connective tissues. The aims of this study were to examine the role of “BMP2” in rheumatoid arthritis patients and to correlate with the standard disease markers.

Material and method: The present work included a case control study for a group of (100) samples: (60) samples of rheumatoid arthritis patient, (40) healthy control samples. patients with Rheumatoid arthritis were selected from the biological treatment centre at marjan medical city in Babylon. Measurement of serum human bone morphological protein 2, MDA level and SOD activity were performed using ELISA Technique.
Results: Hypernatremia, hypokalemia and hypomagnesemia were presented in RA patients. Decrease level of BMP2 was associated with RA. The median level of BMP2 in patients group was (0.15 ng/ml while in control was 2.95 ng/ml). Furthermore, results were indicated high levels of oxidative stress marker (MDA) and low antioxidant activity. ROC analysis of BMP2 was also shown a specificity 57% and high sensitivity 95% towards rheumatoid arthritis with AUP (0.824) at an optimal threshold >0.12 ng/ml.

Conclusion: The study concluded that BMP2 and MDA/SOD ratio correlated with RA. It may shed light on the complex phenomenon represented by major disease outcome contributors, and increased awareness should be credited to these biomarkers.

Key words: bone morphogenetic Protein (BMPs), BMP-2, Rheumatoid arthritis (RA), inflammatory arthritis, osteogenic cells.

Introduction:

Rheumatoid arthritis (RA) is a chronic gradual inflammatory autoimmune disease, It is accomplished by articular, extra-articular, and systemic influences. In developed regions, RA nearly affects 0.5-1% of adult population (1). Some individuals with RA have a moderate self-limited illness, and many others suffer from severe physical impairment, joint destruction, and various co-morbidities (2). Patients with RA have mortality rates that are more than twice as high as the general population and this disparity seems to be expanding (3). In the pathogenesis of RA, T cells, B cells, and the coordinated interaction of pro-inflammatory cytokines play significant roles (4). TNF-α and IL-6 are the cytokines most directly involved in this mechanism and IL-1 and IL-17 may also play significant roles (4). The assessing of disease severity in terms of structural injury found the percentages of men and women is similar in this erosive disease (5) (6). It has been
reported that the predictor of inability is female sex and that the developed of inability is three times quicker in female (7). The disease progresses in a variety of ways, but the majority of patients develop a chronic, progressive disease causing pain, joint destruction, and inability (8) (9). A 70% of patients with RA have developed erosive joint damage (10). Bone Morphogenetic Protein (BMPs) were initially discovered in the pioneering work of Urist in 1965, who discovered that their activity caused ectopic bone formation (11). Although Urist was able to separate the bone-inducing proteins subsequently (12), another two decades were taken for these proteins to be separately cloned and characterized (13). The transforming growth factor beta (TGF-β) superfamily of secreted growth and differentiation factors has members known as bone morphogenetic proteins (BMPs). This unique ability of bone tissue led to the detection of bone morphogenetic proteins (BMPs), the growth agents that enhanced bone’s distinctive inductive capability (14). BMPs maintain the normal balance between bone formation and bone resorption by the paracrine and autocrine growth factors (15). Accordingly, the osteoclastic BMPs might participate to the anabolic pathway of bone remodeling.

Rheumatoid arthritis provides a unique opportunity to explore the involvement of BMPs in inflammatory processes in such system where they are already present. Immune-mediated inflammatory mechanisms are involved in the pathophysiology of RA. Synovial inflammation, increasing joint degeneration, and bone loss which are characteristics signs of RA. In the synovium of RA cases, BMP-2 and BMP-6 are expressed and their expression in patient-derived fibroblast-like synoviocytes in vitro which is response to pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-17 (16). The pro-inflammatory cytokines TNF-a and IL-17 promote a pro-inflammatory phenotype within synoviocytes which demonstrated by elevated
expression of pro-inflammatory cytokines GM-CSF and IL-6, formation of
metalloproteinases MMP2 and MMP3, and elevated expression of the chemokine
IL-8. The activity and expression of these products are significantly involved in the
development of RA. Therefore reviewing the previous studies regarding the BMPs
could bring the light to a critical contribution for a crucial improvement in bone
regeneration. That could be a part of the knowledge gap which significantly affect
some applications in nanomaterial that involve bone tissue engineering
(osteoblastic differentiation and bone formation) with multiple proposed functions
such as these reported recently about using Nanosilicates (17).

Methods:

The present work included a case control study for a group of (100) samples:
(60) patient samples, (40) healthy control samples. The study was conducted from
October 2020 to March 2021. Patients with Rheumatoid arthritis were selected from
the biological treatment centre at Marjan medical city in Babylon. History of
family, smoking state, job, duration of disease also weights and heights were taken
from each subject. The sociodemographic aspects of the patients were collected
through the self-reported technique (questionnaire) including age, gender, BMI and
having any current chronic diseases. They were also exposed to medical
examination for signs and symptoms of rheumatoid arthritis by specialized doctor
based on the World Health Organization (WHO) criteria. Measurement of serum
human bone morphological protein 2, MDA level and SOD activity were
performed using ELISA Technique. The efficiency of the predicting value was
assessed using receiver operating characteristic (ROC) curve.
Results and discussion:

Demographic and clinical characteristics:

The clinical demographic characteristics and laboratory parameters of both patients groups and the healthy control group were to be as following: the mean age of participants which was within the age group of (20 – 75) years old. Gender distribution among the studied groups was: 23.3% male, 76.67% female for patients group, while 37.5% male and 62.5% female for control group. Locally, The study was included an examination of the prevalence for the most common site of rheumatoid arthritis, figure (1) showed that the highest number of patient having rheumatoid arthritis in Knee joint, wrist joint, metacarpal joint and ankle with mean number of (60, 55, 41 and 31 respectively) while the lowest prevalence type were in elbow joint with mean number of patients (10). The study indicated that the knee joint was the most site of the body could be affected by rheumatoid arthritis. knee is the largest joint in the body and one of the most complicated. However, in the case of rheumatoid arthritis, this inflammation in the joint is unnecessary and causes problems. When the inflammation goes down, the capsule around the synovium remains stretched and can’t hold the joint in its proper position. This can cause the joint to become unstable and move into unusual positions.
Figure (1) The most common site of rheumatoid arthritis in patients group

The distribution of serum BMP isoform levels in rheumatoid arthritis patients were decreased markedly compare to control group. The median level of BMP2 in RA patients was (0.15 ng/ml) while in control was 2.95 ng/ml as shown in figure (2).

Figure (2) Boxplot of the Distribution of serum level BMP2 ng/mL in rheumatoid arthritis group compared to control group
BMPs were negatively correlated with local or systemic parameters of inflammation as well as the duration of the disease. This discrepancy might depend on differences in the biological function and regulation of individual members of the BMP family.

In fact, Lories and colleagues (18) reported that BMP-2 and BMP-6, was not increased by stimulation with IL-1β or TNF-α. A similar distribution and predominant expression of different BMPs in fibroblastoid and macrophagocytic cells was also shown by Lories and colleagues (18). The loss of BMP signal might reduce the regenerative capacity of cartilage (19). Also, loss of BMP expression could be involved in chronic inflammatory and not only degenerative joint diseases (20). Moreover, recent studies suggested that other factors such as BMP-2 and BMP-4 might be involved as downstream mediators of the TGF-β effect and that these BMPs might be released by macrophages of the synovial lining layer (21). Previously reported that the peripheral blood expression profiles of BMPs may act as predictive markers for the development of arthritis, its disease activity, therapeutic responsiveness and overall prognosis (22). Previously suggested that BMPs are beneficial for the repair of joint destruction and tissue responses that may form the basis of chronic arthritis (23). BMP-2 is a member of the BMP family that contributes to bone formation, joint anti-inflammation and synovial repair (24). Previous research has suggested that recombinant BMP-2 may induce bone formation and osteoblastic differentiation by regulating endochondral ossification (25). In addition, abnormal expression of BMP-2 in mesenchymal cells has been investigated in association with rheumatoid arthritis (26). BMP-2 expression levels were significantly lower in synovial cells from the mouse model (27). Other studies have reported that BMP-2 may be used to reconstruct segmental mandibular defects and repair ischemic damage by inducing angiogenesis and osteogenesis,
and by decreasing osteoclast bone reabsorption activity (28). However, the half-life of BMP-2 is short in vivo, which limits its clinical application (29). The pro-inflammatory cytokines IL-17 and TNF-α induce a pro-inflammatory phenotype within synoviocytes marked by increased expression of pro-inflammatory cytokines IL-6 and GM-CSF, increased expression of the chemokine IL-8, and increased production of metalloproteinases MMP2 and MMP3. Expression and activity of these products are strongly implicated in the pathogenesis of RA (30)(31).

**Examination the serum Oxidative stress / antioxidant levels in rheumatoid arthritis group:**

Since the pathogenesis of this disease is multifactorial, many Compelling evidence suggests that oxidative stress is involved in the onset of RA. The levels of oxidative stress markers and antioxidant enzyme activity in RA patients and the control group were also investigated in this study. In patients with RA, the data indicate high levels of oxidative stress marker (MDA) and low antioxidant activity as shown in figure (3), which is reflected by increased lipid peroxidation in peripheral blood of patients with RA. It has been reported by a mechanism in which MDA, the product of lipid peroxidation, reacts with lysine residues in protein to produce immunogenic molecules, which can exacerbate inflammation. The longer chain polyunsaturated fatty acids are especially potent at increasing lipid peroxidation and causing cell damage by oxidative stress (32). Articular cartilage and synovial fibroblasts have been found to synthesize substantial amounts of free radical suggesting joints as a potential source of these product. Affects physiological process within joints would included modulation of interleukin –1(IL-1) induced bone resorption and cartilage metabolism (33).
Formation of reactive oxygen species and lipid peroxides as a result of disease activity may play an important role in RA. While lowered concentrations of antioxidants in the blood considerably increase the probability of the occurrence of RA \(^{(34)}\). Many investigators have focussed on oxidative stress since last few years and suggest that RA patients are more prone to lipid peroxidation \(^{(35)}\). Generation of reactive oxygen species may be particularly important factor for bone resumption in inflammatory process \(^{(36)}\). Serum antioxidants are low because they have been used in reducing inflammatory products. SOD may affect the immunity of the organism by decreasing \(\text{H}_2\text{O}_2\) concentration and causing decrease in lymphocyte proliferation. It results in mild anti-inflammatory effect. It may play a role in controlling chronic inflammation of any cause \(^{(37)}\).
Figure (3) Boxplot of the Distribution of serum level of MDA mmol/L and serum activity of SOD U/L in rheumatoid arthritis group compared to control group

3.7. Receiver operating characteristics of biomarkers level in Rheumatoid arthritis patients:

Receiver operating characteristics (ROC) curve analysis of studied markers was performed. The area under the ROC curve (AUC) for the MDA/ SOD ratio was (AUC = 0.72 as shown in Figure(4). ROC analysis indicated that MDA/ SOD ratio >0.037 was predictive of increasing oxidative stress at the expense of antioxidant with 98% sensitivity, 94% specificity as shown in Table (1).

Table (1) AUC, optimal threshold, Sensitivity and specificity of MDA/ SOD ratio obtained by the ROC curves for prediction of Rheumatoid arthritis

<table>
<thead>
<tr>
<th>RA</th>
<th>AUP</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>P value</th>
<th>Cut-off</th>
<th>CI (95%)</th>
</tr>
</thead>
</table>

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| MDA/SOD Ratio | 0.72 | 0.98 | 0.94 | >0.001 | 0.037 | 0.604-0.833 |

**Figure (4) Receiver operating characteristics (ROC) curve analysis of MDA/SOD ratio in the Rheumatoid arthritis patients**

Results of the current study were focused on the potential biomarkers associated with an increased pro-inflammatory status and decreased antioxidant activity. Since the RA is an autoimmune disease, the pro-inflammatory status could be characterized by...
an overactivation of the inflammatory cascade which is simply reflected by the MDA/ SOD ratio. Recent investigation by (Mititelu et al; 2020) state that the major need to RA management is to discover noninvasive tests that can be used to monitor the immune status of the body in such patient (38). Roc analysis of MDA/SOD ratio indicated that these biomarkers might be a reliable result for this purpose. In spit of this ratio could be significantly altered in many other pathological conditions, but it might be a tool of inflammation in rheumatoid arthritis along with the previos reported markers such as RF, ESR, and CRP. the inflammasome signaling pathway and are actively involved in RA disease activity. Previous studies showed that the pro-inflammatory agents act as a trigger that switch on the inflammasome and contribute to RA disease progression (39). On the other hand, ROC analysis figure (5) of bmp2 was also shown a specificity 57% and high sensitivity 95% towards Rheumatoid arthritis with AUP 0.824 at an optimal threshold >0.12 ng/ml as shown in Table (2).

**Table (2) AUC, optimal threshold, Sensitivity and specificity of BMP2 level obtained by the ROC curves for prediction of Rheumatoid arthritis**

<table>
<thead>
<tr>
<th>RA</th>
<th>AUP</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Cut-off points</th>
<th>P value</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP2</td>
<td>0.824</td>
<td>0.95</td>
<td>0.57</td>
<td>0.12</td>
<td>&gt;0.001</td>
<td>0.721-0.927</td>
</tr>
</tbody>
</table>
Figure (5) Receiver operating characteristics (ROC) curve analysis of BMP2 level in the Rheumatoid arthritis patients

BMP2, 4 and 6 are all thought to play the most important roles in skeletogenesis. Many studies have suggested that BMP2 is a pivotal signal for the regulation of osteoblastogenesis\(^{40}\)\(^{41}\) also showed that BMP2 promotes and regulating bone formation and the expression of IHH which has a negative feedback loop regulating the onset of hypertrophic differentiation of chondrocytes\(^{42}\). These findings indicate that BMP6 is an important regulator of bone and cartilage cell proliferation and also could be used as a good sensitive biomarker of Rheumatoid arthritis.
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