**VALUE OF CARTILAGE OLIGOMERIC MATRIX PROTEIN AND YKL-40 AS BIOCHEMICAL MARKERS IN PATIENTS WITH KNEE OSTEOARTHRITIS**

By

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**ABSTRACT:**

**Aim:** This investigation intends to assess the potential utility of COMP and YKL 40 as biochemical indicators of osteoarthritic condition in persons with knee osteoarthritis (OA).

**Methods:** This study included as controls 40 patients with symptomatic knee OA who met ACR criteria for idiopathic knee OA and 15 healthy volunteers matched for age and gender. Using ELISA, COMP and YKL-40 serum levels were determined. Among our OA patients, we associated the levels of these markers with changes in Kellgren Lawrence (K-L.) grade, joint space width (JSW), age, disease duration, gender, body mass index (BMI), and western Ontario MacMaster (WOMAC) index.

**Results:** There were significant elevations of the serum COMP and YKL-40 in OA patients in comparison to the control group (P<0.001), more in bilateral than unilateral cases. There were highly significant positive correlations of both parameters with K-L. Radiological severity of the disease, but an inverse significant correlation with JSW (P-value<0.001). As regard synovitis, there was significant increase of serum YKL-40 (P-value <0.05) while serum COMP level showed a slight but insignificant increase. There were highly significant positive correlations of serum COMP and YKL-40 levels with WOMAC index and age (P-value <0.001) while there were no significant correlations with disease duration and BMI, CRP was significantly higher in OA patients than in controls and showed positive significant correlation with serum level of YKL-40 (P-value <0.001). There was a positive highly significant correlation between both parameters (P-value <0.05).

**Conclusion:** Elevated levels of serum COMP and YKL-40 can suggest disease severity and multiple joint involvement based on these findings. YKL-40 may be an effective indicator of synovitis in OA. The combination use of both markers can aid in early diagnosis and enhance illness prognosis.

**INTRODUCTION:**

Osteoarthritis (OA) is a physically complicated illness with several dimensions that affects the entire joint. A molecular imbalance in the production of cytokines, metalloproteinases, and their natural inhibitors characterizes OA. These alterations ultimately result in articular cartilage degeneration, as well as subchondral bone and synovial membrane abnormalities (Iannone and Lapadula, 2003).

Attaching to type II collagen fibers, cartilage oligomeric matrix protein (COMP) is a non-collagenous protein that maintains the collagen fiber network in articular cartilage. This protein's concentration rises in response to cytokines and growth hormones. Multiple diseases that deteriorate cartilage result in the release of matrix protein fragments into the synovial fluid and, eventually, the blood (Wislowska and Jablonska, 2005).

YKL-40, also known as human cartilage glycoprotein 39 (HC gp-39) and chondrex, is produced by degenerative articular cartilage, inflammatory or hyperplastic synovium, activated macrophages, neutrophils, and chondrocytes (Kirkpatrick et al., 1997). Multiple studies indicate that YKL-40 may be a valuable new marker for rheumatoid arthritis (RA) and OA patients (Johansen et al., 2001).

OA is diagnosed based on radiographic and clinical changes that come very late in the development of the illness and have a limited sensitivity for tracking disease progression; therefore, measuring these markers would appear to be a promising strategy for improving the disease progression prediction at individual level (Taka hashi et al., 2004).

The use of many variables concurrently in OA patients looks crucial for improving disease severity prediction (Jung et al., 2006).

**AIM of the WORK:**

This study evaluated the potential use of blood levels of COMP and YKL 40 as biochemical indicators of osteoarthritic state in knee OA individuals.

**PATIENTS and METHODS:**

This study was done on 40 patients with knee OA selected from the outpatient rheumatology and rehabilitation clinic at Benha University Hospitals, and 15 healthy volunteers of the same age and gender who serve as controls. The American College of Rheumatology (ACR) criteria for idiopathic knee OA, were met by all patients (Altman et al., 1986).

**We excluded the following cases:**

* Secondary OA as joint injury, developmental deformities, osteonecrosis, RA, gout and seronegative arthritides.
* Patients who had a history of malignancy, myocardial infarction or elevated liver enzymes.
* Patients who had had intra-articular injections (chondroitin polysulphate, steroids, or hyaluronic acid) for a minimum of one month before this trial.

**All our patients were subjected to the following:**

* Full history taking with particular attention to onset, course, disease duration, pain, articular gelling, locking and instability.
* Thorough clinical examination with special emphasis on knee joints.
* Radiological investigation:
* Each patient had a weight bearing antero- posterior plain X-ray for both knees flexed at 30° (Schuss view) (Piperno et al., 1998).
* Knee radiographic grading was evaluated by a radiologist unaware of the patient’s status according to Kellgren-Lawrence Grading Scale (1957).
* The medial and lateral joint gap widths were measured in millimeters on anteroposterior knee radiographs. Altman et al. (1987) drew a line from the mid femoral medial and lateral condyles to the tibial plateau and utilized the lesser of the two measurements for the joint space width.
* The WOMAC index analyzes pain associated with knee OA (five items), stiffness (two items), and physical functional activities (17 items) (Bellamy et al., 1988). The visual analog scale (VAS) version of the WOMAC was determined. The range of WOMAC scores was as follows: function (0-170), pain (0-50), and stiffness (0-100). (0-170). (0-20).
* On the basis of the patients' clinical manifestations of knee joint inflammation (warmth, non-osseous swelling, and tenderness), synovitis was diagnosed.

-As regard the medical treatment, all the patients were receiving short courses of non-steroidal anti-inflammatory drugs (NSAIDs).

* Laboratory investigations:

-CBC.

Liver and kidney functions to exclude diseases interfering with the parameters investigated in this study.

-CRP was assayed by ELISA supplied from Abazyme company (USA) Catalogue Number: EL10022 (Comozier et al., 1998). Blood samples were collected for measur ing the biochemical markers.

**COMP immunoassay**

This test is based on an enzyme immunometric assay (ELISA) established for the quantitative detection of COMP in human blood or plasma and performed on microplates. The kit was supplied from Abnova corporation. These ELISA utilizes two antihuman COMP mouse monoclonal antibody and recombinant human COMP as a calibrator. Serum samples were collected and stored at -20°C, and they were diluted by the supplied dilution buffer.

* YKL-40:

Serum YKL-40 was assayed by ELISA kit, an YKL-40 (tm) (Metra bio-systems Inc, part number 8020, CA, USA). The biotin-conjugated Fab fragment of a monoclonal anti-YKL-40 antibody binds to streptavidin on the strip to capture YKL-40. A polyclonal anti-YKL- 40 antibody conjugated to alkaline phosphatase binds to the captured YKL-40. Bound enzyme activity is detected with p-nitrophenyl phosphate as substrate by reading the optical densities at 405°C.

**STATISTICAL ANALYSIS:**

Microstat, a 1985 product of ECOSOFT, was applied for statistical analysis on an IBM computer. Using Student's t-test to examine the difference between two groups Comparing three or more groups using a one-way analysis of variance (ANOVA). Spearman's correlation coefficient (r) was utilized to evaluate the degree of association between two continuous variables. P-values below 0.05 were considered statistically significant.

**RESULTS:**

Table (1): Characteristics of the 40 OA patients.

|  |  |
| --- | --- |
| Age, years, mean ± SD (range) | 40.3 ± (38-49) |
| Gender, male / female, number (%) | 10 / 30 (25% - 75%) |
| OA duration, years, mean ± SD | 3.8 ± 1.5 (1-8) |
| BMI, kg / m2, mean ± SD | 27.5 ± 1.6 |
| WOMAS index, mean ± SD | 144.8 ± 9.6 |
| Knee OA, unilateral / bilateral, number (%) | 8 / 32 (20% - 80%) |
| K-L grade I / I I / I I I, number (%) | 7 / 23 / 10 (17.5% - 57.5%-25%) |
| Knee synovitis, total / unilateral / bilateral, number (%) | 24 /20 /4(60% - 50% - 10%) |

Table (2): Comparison between the mean serum COMP and YKL-40 values in OA patients with the control group.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | N | Mean ± SD | t | P |
| COMP in OA (mg/ml) | 40 | 4.13±1.12 | 4.5 | <0.001\*\* |
| COMP in control (mg/ml) | 15 | 2.50±1.40 |
| YKL-40 in OA (ng/ml) | 40 | 126.5±28.81 | 4.83 | <0.001\*\* |
| YKL-40 in control (ng/ml) | 15 | 77.1±25.49 |
| CPR in OA (mg/ml) | 40 | 2.84±0.29 | 5.62 | <0.001\*\* |
| CPR in control (mg/ml) | 15 | 1.36±0.14 |

\*\* High significant

Table (3): Comparison between the mean serum COMP and YKL-40 values in unilateral and bi- lateral cases of OA knees.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Unilateral Knee OA | | Bilateral Knee OA | | t | Significant |
| N (%) | Mean±SD | N (%) | Mean±SD |
| COMP | 8 (20) | 3.20±1.92 | 32 (80) | 4.69±1.66 | 2.46 | S |
| YKL-40 | 8 (20) | 136.5±16.78 | 32 (80) | 107.8±33.02 | 2.61 | S |

Table (4): Comparison between the mean serum COMP and YKL-40 values in OA patients according to the presence of synovitis.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Unilateral | | Bilateral | |  | | F | Significant |
| N (%) | Mean±SD | N (%) | Mean±SD | N (%) | Mean±SD |
| COMP | 20 (50) | 4.32±2.11 | 4 (10) | 4.51±1.53 | 16 (40) | 3.88±0.61 | 0.5 | NS |
| YKL-40 | 20 (50) | 127.22±28.25 | 4 (10) | 146.00±16.78 | 16 (40) | 107.1±30.1 | 2.51 | S |

 Table (5): Comparison between the mean serum COMP and YKL-40 values in OA patients according to their sex.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Male | | Female | | T | Significant |
| N (%) | Mean±SD | N (%) | Mean±SD |
| COMP | 10 (25) | 4.2±1.01 | 30 (75) | 4.33±1.32 | 0.12 | NS |
| YKL-40 | 10 (25) | 120.0±1.14 | 30 (75) | 127.2±28.25 | 0.35 | NS |

Table (6): Correlation coefficient between serum COMP, YKL-40 with the age, disease duration, BMI, WOMAC index, K-L grading, JSW, CRP and between both parameters in OA patients.

|  |  |  |
| --- | --- | --- |
|  | COMP | YKL-40 |
| Age | 0.42\* | 0.51\* |
| Disease duration | 0.11 | 0.20 |
| BMI | 0.22 | 0.26 |
| WOMAC index | 0.52\* | 0.52\* |
| K-L grading | 0.75\* | 0.56\* |
| JSW | -0.42\* | -0.41 |
| CRP | 0.27 | 0.52\* |
| YLK-40 | 0.52\* |  |

\*P<0.001 highly significant, Critical value (r) = 0.29

**DISCUSSION:**

OA of the knee is the most common joint disease, affecting around 10% of the population over 50. In addition to cartilage deterioration, osteoarthritis is distinguished by metabolic changes in bone and synovial tissue. There are two identified cartilage degradation processes. First, chondrocytes degrade cartilage extracellular matrix (ECM), and then non-chondrocyte tissues and cells, including inflamed synovium, pannus tissue, and invading inflammatory cells, degrade cartilage ECM mostly via synovial fluid (SF) (Yoshihara et al., 2000).

Clearly, technologies more sensitive than X-rays are necessary for identifying individuals at high risk for severe OA and determining the efficacy of therapies. Alternately, biochemical markers suggesting abnormalities in bone, cartilage, and synovial tissue turnover may be useful for research and OA monitoring (Garnero et al., 2001).

In this study, there were significant elevations of serum COMP in knee OA patients compared to the control group, more so in bilateral than in unilateral cases, and there were positive highly significant correlations of serum COMP level with WOMAC index and age, whereas there were no significant correlations regarding disease duration, BMI, or sex.

These findings are consistent with those of Clark et al. (1999), who discovered that the serum COMP level of the OA group was much greater than that of the control group, which may represent the disease's severity and the involvement of many joints. In addition, similar to our findings, they discovered minor variations in serum COMP content based on gender or body fat, as well as a considerable increase with age.

In their investigation of 67 knee OA patients and 67 healthy controls, Garnero et al. (2001) discovered that OA patients had considerably higher serum COMP levels than controls. In addition, they found a significant association between serum COMP concentration and pain and physical function, as measured by the WOMAC score and quantitative radiography assessment of the joint space with the knees flexed 30 degrees.

Wislowsk and Jablonska (2005) found no association between COMP level in blood and patient age or illness duration, but a strong correlation between COMP level in serum and WOMAC Index.

The presence or absence of synovitis was associated with a small but negligible rise in serum COMP. This is consistent with the findings of Vilim et al. (2001), who reported that synovitis had a greater effect on COMP level than CRP level, duration of OA, and OA severity score.

In our investigation, a very significant positive link existed between serum COMP concentration and K-L grading of radiological severity, and a big negative correlation existed between serum COMP concentration and JSW. This finding is consistent with Vilim et al. (2002) and Garnero et al (2001).

Jung et al. (2006) discovered that individuals with knee ultrasonography-confirmed severe OA had greater amounts of hyaluronic acid and COMP in their blood. This demonstrates that the same clinical abnormalities in soft tissue and/or bone seen by ultrasonography in OA joints are reflected promptly in peripheral blood biochemical markers.

In the current work, patients with knee osteoarthritis had considerably greater YKL-40 levels than healthy controls; the difference was highly statistically significant (P<0.001), and bilateral cases were more severely impacted than unilateral cases. In addition, there were highly significant positive correlations between WOMAC and age (P<0.001), but no connections between sickness duration and BMI (P>0.05). According to the findings of Johansen et al. (2001), Volck et al. (2001), and Takahashi et al. (2004), YKL-40 may be a marker of severity for inflammatory and degenerative joint illnesses and may permit the treatment of arthritis prior to the onset of unpleasant symptoms.

Regarding this work findings, a significant positive association was found between serum YKL-40 concentration and K-L radiological severity grading, while a highly significant negative correlation existed between serum YKL-40 concentration and JSW. Connor et al. (2000) discovered that moderate to high levels of YKL-40 expression were confined to the superficial zone chondrocytes of patients with mild osteoarthritic cartilage degeneration. In advanced OA cartilage, cloned chondrocytes from the superficial, middle, and deep zones exhibited elevated levels of YKL-40.

In our study, OA patients had considerably higher CRP serum levels than healthy controls (P0.001). Conrozier et al. (1998) and Yoshihara et al (2000).

In addition, a significant association the connection between YKL-40 and CRP suggests that YKL-40 may serve as a measure of joint inflammation in OA. This was consistent with the findings of Takahashi et al. (2004) and Recklies et al. (2005), who discovered that the synthesis of YKL-40 is stimulated by the inflammatory cytokines interleukin 1 and TNF, indicating that its production is a component of the inflammatory response of articular chondorocytes.

Conclusions: The increased levels of serum COMP and YKL-40 can indicate disease severity and multiple joint involvement. YKL-40 may be a useful marker for synovitis in OA (COMP has a tendency to increase in knee synovitis presence). The combined use of both markers can facilitate early disease identification and improve prognosis.

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