Role of Innate Immune Response Components in the Osteoarthritis

Eman Amer (MSc)\textsuperscript{1}, Ammar N Abood ((MBChB, MSc))\textsuperscript{2}, Walaa Najm Abood(PhD)\textsuperscript{3} and Abass Abood Frahan(PhD)\textsuperscript{4}

Abstract

Background: Osteoarthritis is a progressive joint disease is mainly worrying on weight bearing in the body, the joints, particularly the knee and hips. Osteoarthritis as an inflammation in the joints must there is an immune defense in the body against this inflammation. This disease that appears primarily in the elderly characterized by erosion articular cartilage, osteophyte, subchondral bone stiffness, synovitis inflammation, many causes of the disease such as age, sex, and obesity, the location of the joint injury and various other factors.

Objective: To understand the role of innate immune response components in the osteoarthritis, this study was investigated the relation between the innate immune response and osteoarthritis.

Patients and Methods: Fifty osteoarthritis patients and fifty healthy persons were participate in this case-control study. The total WBC count, neutrophil percent, lymphocyte percent, monocyte percent, eosinophil percent, basophile percent, platelet number, level of ESR, Level of CRP, and level of C3 complement were investigate in serum and synovial fluid in the osteoarthritis patients and control healthy persons.

Results: The results were shown significant elevation in the WBC count, platelet number, level of ESR, level of CRP and level of C3 complement in the serum of osteoarthritis patients compared with control group. A significant positive correlation was shown with weight of patient and the severity of osteoarthritis. The elevation level of C3 complement in the synovial fluid was a significant positive correlation with elevation of level of C3, level of ESR, level of CRP, and WBC count in the serum of patients related to the severity of osteoarthritis status.

Conclusion: The results were given an evidence for the crucial role of innate immune response in the defense against osteoarthritis inflammation and any defect in the innate immune component lead to increase the severity of osteoarthritis.

Key words: Knee osteoarthritis, innate immune, synovial fluid.

Corresponding Author: Eman90amer@gmail.com

Received: 17\textsuperscript{th} August 2017
Accepted: 24\textsuperscript{th} September 2017

\textsuperscript{1,2}Department of Biology-College of Pure Sciences- Diyala University - Iraq.\textsuperscript{3,4} College of Medicine- Diyala University - Iraq.

Introduction

Osteoarthritis (OA) is a degenerative joint disease, occurring primarily in older persons (Jordan ,et al 2003). OA characterized by erosion of the articular cartilage, hypertrophy of bone at the margins (osteophytes), subchondral sclerosis, and a range of biochemical and morphological alterations of the synovial membrane and joint capsule (Brandt et al ,2009). Despite its prevalence, the precise etiology, pathogenesis, and progression of OA remain beyond our understanding, primarily due to confounding
factors in human epidemiologic; including individual variation in physical activity, diet, and medical history (Firestein et al, 2013). The poor correlation between symptoms of OA and radiographic lesions and our inability to detect early disease (Aaboe et al, 2011) immune cells and their cytokines play an important role in the pathogenesis of OA and because a better understanding of the biological mechanisms involved in this process may lead to better therapies for OA patients. (Golgring, 2009). A number of risk factors have lately been identified mechanical factors, among others, are likely to play a very important role in the initiation of the disease process. Endogenous factors such as type II collagen mutation or dysplastic conditions are also known to be involved in initiating the OA process (Thorpe et al, 2013). There is now strong evidence that the structural changes globally observed in OA are due to a combination of factors ranging from the mechanical to the biochemical (Krenn et al, 2006). The disease process affects not only the cartilage, but also the entire joint structure, including the synovial membrane, subchondral bone, ligaments, and periarticular muscles (Benito et al, 2005). In OA synovium, the inflammatory changes that take place include synovial hypertrophy and hyperplasia with an increased number of lining cells. In patients with severe disease, the extent of inflammation can sometimes reach that observed in rheumatoid arthritis (RA) patients at the clinical stage (Calder, 2003). Some degree of synovitis has also been reported in even the early stages of the disease, Synovial inflammation is clearly reflected in many of the signs and symptoms of OA, including joint swelling and effusion, stiffness (Vander, 2014). The aim of this study to understand the role of innate immune response components in the osteoarthritis. this study was investigated the relation between the innate immune response and osteoarthritis by detection The total and differential WBC count, platelet numbers, level of ESR, Level of CRP, and level of C3 in both serum and synovial fluid in patients and healthy persons.

**Patients and Methods**

This study was conducted at Baquba Teaching Hospital and Specialized Clinics for Fractures and Joint Diseases in Diyala Governorate during the period from 2016/8/25 to 2017/5/1. A total of (100) persons (age range 30-79) years, fifty of OA patients and fifty healthy persons were participate in this study.

**Collection of the samples:** Intravenous blood samples about 3-5 ml were collected from patients and healthy persons. The sample was drawn and placed in special tubes for each test (ESR, CBC, Gel Tube for serum). The gel tube was placed at room temperature until the thrombosis. After the separation of the serum using a centrifuge device rate of 3000 r.p.m for 10 minutes. The serum was divided into 500 microliters per tube and kept at a temperature of -20C until used. The serum was used once to avoid freezing and melting of the sample. The synovial fluid was collected from
patients with 3-2 ml of the articular fluid, the synovial fluid was withdrawn for patients in this study by medical injection after placing the paws and sterilizing the withdrawal area with 70% ethylene fluid. Synovial fluid was placed in test tubes containing anticoagulants. Next, separate the synovial fluid in centrifuges at a rate of 3000 r.p.m for 10 minutes and split the hinge fluid into the test tubes. After placing the liquid separate in the test tubes and store them in temperature (-20°C) until the necessary test.

**Erythrocyte Sedimentation Rate**

The solution was prepared by adding 3.8 g of sodium citrate to 100 ml of distilled water. 500 μl of the work solution was taken and placed in a clean, dry tube and 2 ml of fresh blood was added to the work solution i.e [4:1] Mix well and put in Westergren rack and set the timer for a full hour and at the end of time the descent rate is recorded which means the sedimentation rate [Hunsley et al, 2010].

**Complete Blood Count**

The blood samples were examined in the Blood Count and Blood Indicators. The following blood parameters were obtained: WBC, BAS, EOS, MON, LYM, NEU, PLT using the EDTA tube blood sample In the Automated Blood Count System for Blood Analysis. The cell - DYN Ruby system is designed to measure a number of blood indicators and is used for diagnosis in clinical laboratories. (MAPSS) Multi-Angle polarized scatter separation technology.

**C-Reactive Protein:**

The effective protein level (CRP) was measured in the ichroma CRP, This test is based on the immune detection method (sandwich), where the antibody reagent in the buffer solution is linked to the antigen in the sample. Ag-Ab is migrated to the nitrocellulose matrix and held by the antibody installed on the test strip. More antigens in the sample (Ag-Ab complex) and lead to a strong intensity of the fluorescence signal on the antibody detector that is treated by an ichroma device. (ichroma was made in choria Boditech Med Inc).

**Complementary protein third C3**

The protein to be examined in the well of the agarose gel containing the specific antibodies is then formed. An immunosorbent complex will be formed in the form of a visible deposition loop around the hole after 72 hours of incubation in a moist chamber. Ring for the visual loop formed around the hole in proportion to the concentration of the sample. The plate is removed from its casing and left at room temperature for a few minutes, to condensed water evaporates in the drill holes of the plate. After that, fill the holes with 5 microliters of the sample or standard samples using a micro pipette and leave the dishes for 15 minutes at room temperature without stirring until sedimentation. The sample is in the inside of the hole in the plate where the condensed water evaporates before working on it. The plate is then covered and incubated in a humid chamber for 72 hours after which the concentration values are extracted by comparing the diameter of the sedimentation.
ring formed in the drill with the values fixed in the table attached to the dishes. (C3 Kit single Radial Immunodiffusion plate, LTA(Italia)).

**Statistical Analysis**

The statistical analysis was carried out using the Statistical Package for Social Sciences program No. 21 with respect to descriptive variables. It was described in the number and percentage formula and the comparison was done using the X² test. As for the variables with the numerical formula, they were described using the mean and standard deviation. T-test between two groups and ANOVA test during the comparison of more than two groups. Use correlation coefficient. Significant result at P value < 0.05.

**Results**

The results of the present study showed an increase in the total number of white blood cells in the serum of patients with knee osteoarthritis compared with the healthy persons (mean ± SD) (8119.9 ± 3946.61 and 7397.18 ± 1372.56) cell / mm3 respectively and significant difference P <0.05 , also the results of the present study showed an increase in the percentage of neutrophil cells and a decrease in the percentage of the other types of white blood cells( lymphocytes, monocyte) whereas eosinophil and basophil had relative ratios in the serum of patients with knee osteoarthritis compared to healthy persons with significant P value < 0.05 In Table (1).

**Table (1):** Measuring the total number of serum white blood cells (WBC), Neutrophil (NUT), Lymphocyte (LYM), Monocyte (MON), Eosinophil (EOS), Basophil (BAS) in patients and healthy people.

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC cell/mm³</th>
<th>NUT%</th>
<th>LYM%</th>
<th>MON%</th>
<th>EOS%</th>
<th>BAS%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7397.18±1372.56</td>
<td>57.5±7.8</td>
<td>40.2±6.3</td>
<td>6.2±1.4</td>
<td>1.2±0.6</td>
<td>0.1±0.16</td>
</tr>
<tr>
<td>Patients</td>
<td>(8119.9±3946.61)*</td>
<td>(62.8±8.2)*</td>
<td>30.6±7.2</td>
<td>5.3±1.8</td>
<td>1.2±0.4</td>
<td>0.06±0.17</td>
</tr>
</tbody>
</table>

*The data was presented as (Mean±SD) * Significant at P value < 0.05

The results of the present study showed a significant (P < 0.05) high platelet count, erythrocyte sedimentation rate, C-reactive protein level, and C3 complementary protein level in patients with knee osteoarthritis compared with healthy persons in Table (2).

**Table (2):** Measurement Platelet (PLT), Erythrocyte sedimentation rate (ESR), C-Reactive protein(C-RP), and C3 complementary protein (C3) in patients with osteoarthritis and healthy persons.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PLT1000/mm³</th>
<th>ESR h/mm</th>
<th>CRP mg/l</th>
<th>C3 in serum mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>288.7±64.1</td>
<td>14±3</td>
<td>4.3±1.6</td>
<td>115.5±24.5</td>
</tr>
<tr>
<td>Patients</td>
<td>(299320±83484.4)*</td>
<td>(39±24)*</td>
<td>(37±22)*</td>
<td>(142±42.5)*</td>
</tr>
</tbody>
</table>

*The data was presented as (Mean±SD) * Significant at P value <0.05
Table (3): Correlation between C3 in Synovial Fluid (SF), Platelet (PLT), C-reactive protein (C-RP) and the state of the knee osteoarthritis.

<table>
<thead>
<tr>
<th>Osteoarthritis status</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
<th>Change Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>.894</td>
<td>.800</td>
<td>.798</td>
<td>19.26</td>
<td>.800</td>
</tr>
<tr>
<td>PLT</td>
<td>.876</td>
<td>.767</td>
<td>.764</td>
<td>78335.99</td>
<td>.867</td>
</tr>
<tr>
<td>CRP</td>
<td>.662</td>
<td>.438</td>
<td>.433</td>
<td>16.99</td>
<td>.438</td>
</tr>
</tbody>
</table>

* Significant at P value <0.05

Table (4): Correlation between Erythrocyte sedimentation rate (ESR), Lymphocyte (LYM) and C3 complementary protein in serum (C3 in serum) in patients with knee osteoarthritis and healthy persons.

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
<th>Change Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoarthritis status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td>.525</td>
<td>.275</td>
<td>.268</td>
<td>18.08</td>
<td>.275</td>
</tr>
<tr>
<td>LYM</td>
<td>.499</td>
<td>.249</td>
<td>.242</td>
<td>7.26</td>
<td>.249</td>
</tr>
<tr>
<td>C3 in serum</td>
<td>.351</td>
<td>.123</td>
<td>.114</td>
<td>34.83</td>
<td>.123</td>
</tr>
</tbody>
</table>

* Significant at P value <0.05

Table (5): Correlation between the number total of White blood cells (WBC), Neutrophil (NUT), Monocyte (MON), Basophil (BAS) and Eosinophil (EOS) in patients with knee osteoarthritis and healthy persons.

<table>
<thead>
<tr>
<th>Osteoarthritis status</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
<th>Change Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>.037</td>
<td>.001</td>
<td>.009</td>
<td>2975.03</td>
<td>.001</td>
</tr>
<tr>
<td>NUT</td>
<td>.236</td>
<td>.056</td>
<td>.046</td>
<td>8.27</td>
<td>.056</td>
</tr>
<tr>
<td>MON</td>
<td>.248</td>
<td>.062</td>
<td>.052</td>
<td>1.63</td>
<td>.062</td>
</tr>
<tr>
<td>BAS</td>
<td>.138</td>
<td>.019</td>
<td>.009</td>
<td>.16900</td>
<td>.019</td>
</tr>
<tr>
<td>EOS</td>
<td>.027</td>
<td>.001</td>
<td>.009</td>
<td>.54567</td>
<td>.001</td>
</tr>
</tbody>
</table>

* Significant at P value <0.05
Table (6): Correlation between C3 complementary protein in serum (C3 in serum) with C3 in Synovial Fluid(SF), C-reactive protein(C-RP), Platelet (PLT), Erythrocyte sedimentation rate (ESR), in patient, and correlation between Erythrocyte sedimentation rate (ESR) with C-reactive protein(C-RP) in patients.

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
<th>Change Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R Square Change</td>
</tr>
<tr>
<td>C3 in SF</td>
<td>.325</td>
<td>.105</td>
<td>.096</td>
<td>35.19</td>
<td>.105</td>
</tr>
<tr>
<td>CRP</td>
<td>.329</td>
<td>.108</td>
<td>.099</td>
<td>35.13</td>
<td>.108</td>
</tr>
<tr>
<td>ESR</td>
<td>.200</td>
<td>.040</td>
<td>.030</td>
<td>36.45</td>
<td>.040</td>
</tr>
<tr>
<td>PLT</td>
<td>.370</td>
<td>.137</td>
<td>.128</td>
<td>34.57</td>
<td>.137</td>
</tr>
<tr>
<td>ESR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.460</td>
</tr>
<tr>
<td>CRP</td>
<td>.679</td>
<td>.460</td>
<td>.455</td>
<td>15.60</td>
<td>83.624</td>
</tr>
</tbody>
</table>

* Significant at P value <0.05

**Discussion**

The results on the table (1) of the present study are agreement with the study of Zhang et al. (2015), an increasing in the total number of white blood cells of patients with knee osteoarthritis compared with healthy persons for both sexes. The researcher pointed the reasons of this increasing to immune disorders in the body. The results were not consistent with the results of (Abid 2005), which showed no significant difference in the total number of white blood cells between patients and healthy. (Haywood et al. 2003) reported increasing the total number of patients compared with healthy patients. In previous study showed the role of white blood cells in osteoarthritis by stimulating innate immune cells, repairing damage and inhibiting the inflammatory response. In a similar study, the number of white blood cells increased in the case of knee osteoarthritis, and the role of white blood cells in the preventing inflammation, removal of dead and harmful tissues in the articular tissues (Tanaka et al. 2006).

In the present study the result was showed an increase in the percentage of neutrophils cells and a decrease in the percentage of the other types of white blood cells (lymphocytes, monocytes) whereas eosinophils and basophils had relative ratios in the serum of patients with knee osteoarthritis compared to healthy persons with significant P value < 0.05, are consistent with (Bruce 2012), who reported a higher rate of neutrophils cells compared with other white blood cells significantly(P<0.05) in patients with osteoarthritis, Neutrophils are important part of innate immune cells which, involved in the development of knee osteoarthritis, its the beginning of the inflammatory response and develop the defense mechanics of the host. While, in another study (Benito et al., 2005) mention the high rates of neutrophil
Role of Innate Immune Response Components in the Osteoarthritis

Eman Amer

and lymphocytes in patients compared with healthy persons and other white blood cells were relative ratios. In this study the increasing of lymphocytes for their role in the production of cytokines (IL-1Beta and TNF-α) are plays a role in the breakdown of the cartilage joint (Caspi et al. 2006) reported an increasing in neutrophil ratios in patients with knee osteoarthritis compared with healthy persons, Lymphocytes were lower in patients with osteoarthritis than in patients with rheumatoid arthritis (RA) and healthy persons (Xiang et al., 2004).

The results of the present study showed a significant (P < 0.05) high platelet count, erythrocyte sedimentation rate, C-reactive protein level, and C3 complementary protein level in patients with knee osteoarthritis compared with healthy persons in table (2) of the current study were agreed with (William and Breana 2017), who showed that increased platelet counts in patients with knee osteoarthritis compared with healthy persons, Increase in collagen, which is from component of the articular tissue where the platelet activates the source of coagulation and healing of its adhesion ability the bonding between the tissue components of these plates. Releases factors have the potential to activate WBC and RBC for their role in curbing inflammation.

(Re델man et al, 2008) increased the number of platelets in patients with knee osteoarthritis have important role in the injury to begin the process of healing and stimulate stem cells, that begin to divide and multiply to give specialized types of cells including; cartilage cells and bone. Previous study was approved the important of platelet in the treatment of osteoarthritis by injection of plasma rich with platelets in the joints. Australia (Lee, 2013) showed increased platelet counts in patients with osteoarthritis of the knee. The results of the present study were consistent with the results of Saberi et al., 2016, which showed a high CRP level in patients' serum with osteoarthritis compared with healthy persons, CRP was associated with the risk of knee osteoarthritis. CRP associated With the clinical symptoms of the disease such as pain knee at night, kneeling and squatting and others (Stannus et al. 2013). rise of CRP in patients with knee osteoarthritis, especially in severe cases of the disease, while the index of ESR was considered classified from indicators that help in the detection of osteoarthritis in the serum of infected patients compared with healthy persons (James et al, 2016). (Hoffman et al. 2005) reported that ESR is an inflammatory marker that increased in all types of osteoarthritis and rheumatism.

The results of the present study were consistent with the results of Kuroki et al., 2012, which indicated a higher level of C3 in patients with knee osteoarthritis compared with healthy persons. In a similar study conducted at Baghdad Teaching Hospital on patients with knee osteoarthritis showed that C3 level was high in patients infected compared with healthy persons. reduction the level of C3 in the serum after treatment of patients with knee osteoarthritis, which is
higher in patients compared to healthy persons (Hussain, et al., 2009).

The current study showed correlation between knee osteoarthritis and the following biomarkers (C3 in SF, PLT, CRP) in table (3) were agreement with Birrell et al. (2011) that gave indicating a strong positive correlation between increased concentration of C3 in synovial fluid, C-reactive protein and platelets in the infected patients with the severity the disease , and the relationship between osteoarthritis of the knee and the following vital indicators (LYM, ESR, C3 in serum) in the table (4) were consistent with Dahaghin et al. (2009) which, showed that these indicators had a moderately positive relationship to predict the condition of osteoarthritis.

In the same time correlation between severity knee osteoarthritis and the following biomarkers (WBC, NUT, MON, BAS, EOS) in the table (5) concurred with the results of (Pearson et al., 2012) that showed present a low positive correlation between this markers and the severity of the disease compared to other indicators that are high positive.

For the correlation between biomarkers and their effect on other indicators, the results showed that there is low positive correlation between C3 in serum and C3 in SF, CRP, ESR, PLT, While the same study showed high positive correlation between ESR and CRP in the table (6). The results of this study were consistent with Chu, et al (2013).

**Conclusion**

The results were given an evidence for the crucial role of innate immune response in the defense against osteoarthritis inflammation and any defect in the innate immune component lead to increase the severity of osteoarthritis.

**References**


[23] Redelman, D.; Welnik, L. A.; Taub, D., Murphy, W. J. (2008) Neuroendocrine hormones such as growth hormone and prolactin are integral members of the immunological cytokine network. Cellular Immunology 252, 111-121.


