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Association Between Visual Analogue Scale (VAS) and Inflammatory Markers (ESR & CRP) in Patients with Knee Osteoarthritis Before and After Injection with Platelets –Rich Plasma(PRP)

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Abstract
Osteoarthritis (OA) are defined as a progressive architecture destruction of the joints compared to slow healing of these joints which leads to reduce protection against degeneration and the movement of the joint is associated with roughness and accompanied by pain. Often this disease affect the knee joint, it may affect any other joints in the body. It is considered the most common disease among people over age 60. The aim of this study is to evaluate the efficacy of platelets-rich plasma injection in treatment of knee osteoarthritis and estimation of the biological and immunological markers associated with the treatment. This study involved 50 patients suffering from inflammation of the knee joint, most of these patients attended Al-Shaheed Firooz hospital in wasit Governorate and private Clinics of Orthopedic and Rheumatology in the district during the period from April 2015 until October 2015. Diagnosis of OA was done according to kellgren and Lawrance system.The age range of patients was 35 - 65 years; 31 females and 19 males. All patient groups were injected in the knee joint by a specialist physician with two injections (Two week apart) during one month with platelets-rich plasma (PRP) after its preparation from the same patient's blood in a sterile condition. This was done after the signing of the patient or one of his relatives on the written consent to conduct the injection process after explaining and clarifying the principle of injection and the purpose.The level of pain to patients group was measured by Visual Analogue Scale (VAS). The enzyme linked immunnosorbant assay (ELISA) was applied to estimate the concentration of C.Reactive protein in serum of patients group as well as healthy control the rate of Erythrocyte Sedimentation Rate (ESR) was also estimated .All these tests were done to all patients groups before and after injections with PRP. Statistical analysis showed no significant differences between males and females ages (54.5±1.2 males, 53.2±2.1 females).The study showed significant decrease in the mean of the Visual Analogue Scale (VAS) of patients before and after injections with (PRP) 5.98 ± 0.129,8.46 ± 0.104, respectively, with highly significant differences (P=000.0) , also the study showed that there was a differences with no significant differences in VAS between age groups (30′s, 40′s, 50′s , 60′s) before and after injections with PRP (7.5,8.0 ,8.38 ,9.05), (5.0,5.55,5.69,6.7) respectively.The study also revealed a difference in the mean for inflammatory marker C. reactive protein concentration before and after injections with PRP (7.156± 0.328 µg/ml, 5.384± 0.196) respectively, with highly significant differences (P=0.000).The study also revealed a difference in the mean of ESR before and after injections with PRP (35.66± 0.879 mm/1hr, 23.7±0.856) (respectively), with highly significant differences (P=0.000). Positive correlation were obtained between VAS score and CRP as well as ESR after treatment.
Keywords: Osteoarthritis, PRP, VAS score, ESR, CRP

Introduction

Osteoarthritis (OA) is a chronic degenerative disorder of the joint associated with a progressive architecture destruction opposed by healing process (Bijlsma, et al.,2011).OA invades all joints of the body, but the knee is the most frequently affected associated with pain, reduce physical activities with stiffness and swelling. Epidemiological death data, reported in USA that about 27 million above 25 years of age are affected by OA (Lawerance et al.,2008).OA affects more than 100 million globally, mostly more than 65 years of age. The total amount national cost estimate of 15.5 – 28.6 billion dollars each year. So it is crucial to develop an effective treatment strategy(pharmacological and non pharmacological) to reduce pain, improve physical functions to reduce functional dependency (Bhatia et al.,2013). Since surgical regenerative treatment are not satisfactory as stated by Filardo et al.,(2013),research projects have been redirected towards the application of other research strategies with the minimally massive procedure, low cost, low side effect, by changing joint homeostasis(Civinini et al.,2013).Till now different non-invasive methods and pharmacological management have been used with limited improvement of OA pain and physical functions. Platelets-rich plasma is another suggestion for OA treatment .It is obtained from the same patient(autologous),easy to prepare with minimal invasive and low cost with a good source of growth factors and cytokines (Dhillon et al .,2012).Autologous platelets-rich plasma injections were reported for the first time in 1987during an open heart surgery. Ferrari et al.,

(1987) have been suggested the use of autologous platelets-rich plasma (PRP) as a good source of agents in regeneration of damaged tissue due to the presence of many bioactive molecules, these molecules provide an active role in cell migration, angiogenesis and metabolism and anabolism of the joint cartilage (Andia, et al, 2012, Andia and Maffuli, 2013).Sampasm et al.,(2010) also reported significant linear improvement in knee injury and osteoarthritis outcome scores by applying VAS scale and showed many improvements, including in moving pain at different times and significant reduction in resting, moving and bent knee pain. Because OA has a multi-factorial etiology and the interplay between the main branches of immunity; the local and systemic, so it is necessary to study the factors which have a determining role in OA(Haseeb A and Haqqi TM.,2013).There is a shortage of information can carry the changes in the biological and immunological parameters also there is contradictory data before and after treatment with PRP especially in the circulation.

Aim of the study

Assessment the impact of PRP injection in OA patients, by using VAS pain, and the changes in certain markers (ESR & CRP) in patients before and after treatment with PRP.

VISUAL ANALOGUE SCALE (VAS) FOR PAIN

Pain VAS is used to measure intensity of pain and it is considered as a uni-dimensional measure for adult populations, even those rheumatic diseases (McCormack et al., 1988). The pain VAS comprised of two scales the first called horizontal (HVAS) and the second called vertical (VVAS) (Scott and Huskisson .,1979).

Material and Method

This study was conducted in the period between April/ 2015 and the mid of October / 2015. One hundred and eighty patients suffered from knee joint pain and inflammation in the knee. Fifty patients who suffer from Osteoarthritis (OA) in the knee were selected out of the one hundred and eighty, according to the exclusion criteria used, as listed below.

Patients Study Group

This group includes fifty patients with knee Osteoarthritis (OA), who fulfilled the 1987 American College of Rheumatology (ACR) criteria. These patients attended the Rheumatology Department of Al-Karama Teaching Hospital, Al-Shaheed Fairooz hospital and a specialist private clinic in Al-Hay city during the study period. The diagnosis and injections of platelet- rich plasma (PRP) to those patients has been performed under the supervision of orthopedic specialist. The patients age range was between 35-65 years with (19) males and (31) females. From each individual, 20 ml of blood has been harvested, divided in to two portions, one used for serum sample separation and the second portion for hematological investigations .Serum was used to determine antinuclear antibody(ANA) and anti double stranded-DNA(Anti ds-DNA) by Immuno-fluorescent Technique (IFT), Anti cyclic-citrullinated protein (Anti CCP antibody) by ELISA technique to exclude SLE and RA. Estimation of uric acid level was used to exclude gout. Whole blood (WB) was used for platelet count to exclude thrombocytopenia in addition to determination of ESR. All patients were informed about the study procedure and they all signed an informed consent.

VISUAL ANALOGUE SCALE (VAS) FOR PAIN

It is a 10 centimeters and for each symptom there is a two verbal descriptors anchors (Huskisson.,1974).The intention of using the pain scale each literature has a different reporting style including, instructions, reporting time and finally the most important verbal descriptors anchors. After surgery such as knee replacement pain VAS scores are described as follows out of 100 mm scale no pain (0–4 mm), mild pain (5–44 mm), moderate pain (45–74 mm), and finally severe pain (75– 100 mm) (Jensen e al.,2003). Pain score related to activities undertaken by the patients such as pain after walking using stairs, lying and pain at standing are not considered and normative. To get specific data participants should ask to record their pain at rest and at the movement on the numerical rating scale of 0 – 100 mm scale. The pain VAS should be completed by the patients by placing a line perpendicular to the VAS line at a distance equivalent to the pain intensity(Scott and Huskisson .,1979).The VAS takes less than 1 minute to achieve by the patient (Burfkhard and Jonsen,2003).The limitations of VAS use include the following patients:due to cognitive impairment of the old patients it is difficult to achieve also it can not be reported by telephone and scoring is complicated compared to the Numeric Rating Scale for pain (NRS) (Hawker et al.,2011).

Preparation of PRP

Principle of PRP Preparation

PRP was prepared by a method known as differential centrifugation. In differential centrifugation, acceleration force is adjusted to sediment some cellular constituents, based on different specific gravities (Perez et al.

2014).In the PRP method, an initial centrifugation to separate red blood corpuscles (RBCs) was followed by a second centrifugation to concentrate platelets, that were suspended in the smallest final volume of plasma. In Figure 1, flow chart describes a double centrifugation process of PRP. Whole blood(WB) was firstly collected in tubes that contain anticoagulants. The first spin step was performed at constant speed to separate RBCs from the remaining WB volume. After the first spin step, the WB was isolated into 3 layers(an upper layer that contains mostly platelets and white blood cells, a middle thin layer that is known as the buffy coat and that is rich in leukocytes, and a lower layer that consists mostly of RBCs. For the production of pure PRP (P-PRP), upper layer and buffy coat were transferred to a sterile tube. For the production of white blood cells rich PRP (WBC-PRP), the layer of buffy coat and few RBCs are transferred. The second turn step was then performed. 'g' for second spin should be just enough to formation of soft pellets (erythrocyte-platelet) at the bottom of the tube. The upper part of the volume that is composed mostly of PPP (platelet-poor plasma) was removed. Pellets were homogenized in lower third (5 ml of plasma) to create the PRP (Platelet-Rich Plasma).(Mazzocca et al.2012).

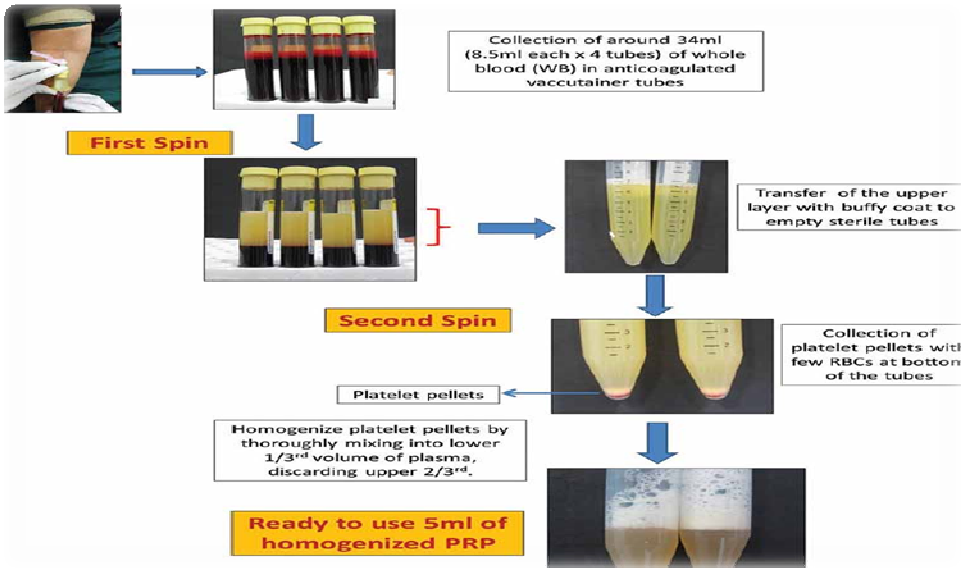

Figure 1:Summary of PRP preparation(Dhurat and Sukesh., 2014)

In the buffy coat method, whole blood (WB) was centrifuged at a 'high speed' with multiple collection of the buffy coat. A buffy coat contains a high concentration of white blood cells. From small volume of WB (30-35 ml) a very thin layer of a buffy coat can be produced. The trouble lies in isolating this dainty layer of buffy coat that contains primarily WBCs and platelets, from the underlying red blood cell layer.

PRP Preparation (Mazzocca et al.2012)

1. Whole blood was obtained by venipuncture in Anticoagulant Citrate Dextrose (ACD) tubes.
(The blood should not chill at any time before or during platelet separation).
2. The blood was centrifuged using a 'Low' spin (1500 RPM for 5 min).
3. The supernatant plasma that containing platelets was transported into another sterile tube (without anticoagulant).
4. The tube was centrifuged at a higher speed (6300 rpm for 20 min)to obtain a platelet concentrate.
5. The lower 1/3 rd. of the tube is PRP and upper 2/3 rd. is platelets-poor plasma (PPP). At the bottom of the tube, platelet pellets were formed.
6. Platelets-poor plasma was removed and platelet pellets suspended in a base amount of plasma (2-4 ml) by delicately shaking the tube.

C-Reactive protein (CRP) Determination:

Principle

C-reactive protein (CRP) is an acute-phase protein produced by the liver in conditions of inflammation, bacterial infection, and/or tissue trauma. Quantification of CRP is useful in determining inflammatory conditions difficult to diagnose, detect abnormalities in patients with chronic inflammatory conditions, and to monitor patients' response to treatment.

Serum sample should be diluted as (1: 100) incubated with the specific antigen (CRP) which coat micro titer plate's wells. Patient's antigen, if present in the specimen, it will combine with the anti-CRP in wells.

Wash un-bound serum proteins in the next step. Incubate anti-human immunoglobulin conjugated to HRP-conjugate with the Ag-Ab complex of the sample in the micro-wells. Wash off unbound conjugate.Add Tetra-Methyl Benzydine (TMB-substrate) to generate an enzymatically calorimetric (blue color) reaction. Add stopping solution (2N H2SO4) solution to yield yellow color. The degree of color formation from the chromogen is a function of the amount of conjugate-bound to Ag-Ab complex and this is corresponding to the initial concentration of the respective antibodies in the patient's sample.Read at Micro-titer plate reader at 450 nm with an optional reading at 620 nm

Procedure

1. One hundred µL of patient's diluted serum was pipetted to the designated micro wells.
2. One hundred µL of diluted calibrators , were pipetted into the designated wells then sealed the microtiter plate with adhesive strip and incubated for 30 minutes at room temperature (20-26^{o}C)
3. The microtiter plate was washed 5 times with 300 µL washing buffer (diluted 1:20).
4. The conjugate was pipetted in 100 µL volume into each well. The microtiter plate was sealed with adhesive strip and then incubated for 30 minutes at room temperature (20-26^{o}C), then washed 5 times and 100 µL of TMB was added to the wells.
5. The plate was incubated for 10 minutes at room temperature in the dark.
6. Fifty µL of stop solution was pipetted into every well, the substrate then incubated for 5 minutes minimum
7. The plate was agitated carefully for 5 second.
8. The absorbance was read at 450 nm (optionally 450/620 nm within 30 minutes).
9. The concentration was calculate by automated program in the instrument

Hematological Investigations

Platelets count were done by Sysmix Auto-Analyze (Japan) .Estimation of erythrocyte sedimentation rate (ESR) was measured by Westergreen's method in which the whole blood was diluted with sodium citrate and then the value of ESR was measured, expressed as mm /1hr.

Data analysis

Statistic of the readings distribution (mean, SD, SEM, minimum & maximum) were used to describe frequency distribution of the data

Differential statistics

These were used to accept or reject the statistical hypotheses, they include the followings:

A) Matched paired sample t. test used to compare mean in cases before and after injections with PRP.
B) P. Value (Less than 0.05) considers statically significant.
C) **Computer & programs**

All the statistical analysis was done by using cori -7 computer through the SPSS program (version-18) and Excel application.

Results

Fifty patient, knee osteoarthritis patients were treated by two intra-articular injections of autologous platelets-rich plasma (PRP) in one month interval with two weeks between each injection. Study of the changes in immunological parameters was done at the beginning of the treatment and two months later. C- Reactive protein and erythrocyte sedimentation rate also done for each patient. Twenty male and thirty female were involved in this study. The mean age of these patients was 54.5±1.2 years (mean±SE) ranging from 35 to 65 years, mean age of male was 55.3±1.6 and mean age of female was 53.2±2.1 years.

Visual Analogue Scale (VAS)

The mean of Visual Analogue Scale (VAS) result for both male and female was 8.46 ± 0.104 before injections it was declined to 5.98 ± 0.129 after injections of PRP with highly significant differences(P=0.000).(figure 2).

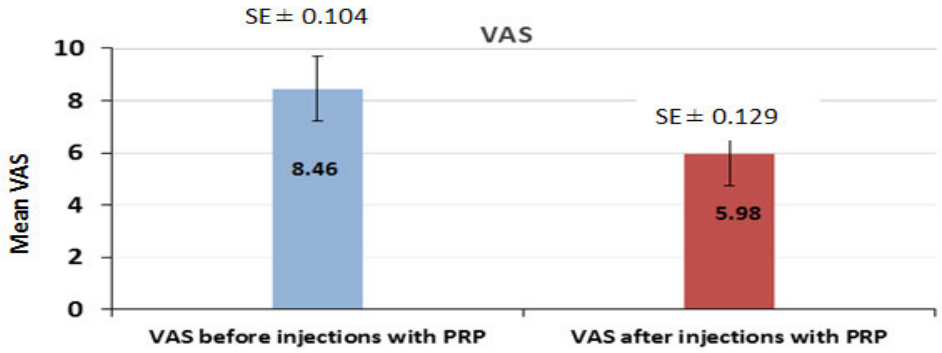

Figure:**2**: Mean of **VAS** for OA patients before and after PRP injections with p=0.000
Data are expressed as mean± standard error. Paired sample t. test was used for comparison with P value significant at ≤ 0.05.

Visual Analogue Scale VAS according to gender
Mean VAS scores in female and male was described in figure 3. The mean of VAS before injections with PRP in female and male was 8.5± 0.18, 8.43± 0.123, respectively, and the mean of VAS after injections with PRP in female and male 6.05± 0.234,5.93± 0.151 respectively with no significant differences between female and male in before and after injections (P=0.757 ,P=0.663) respectively

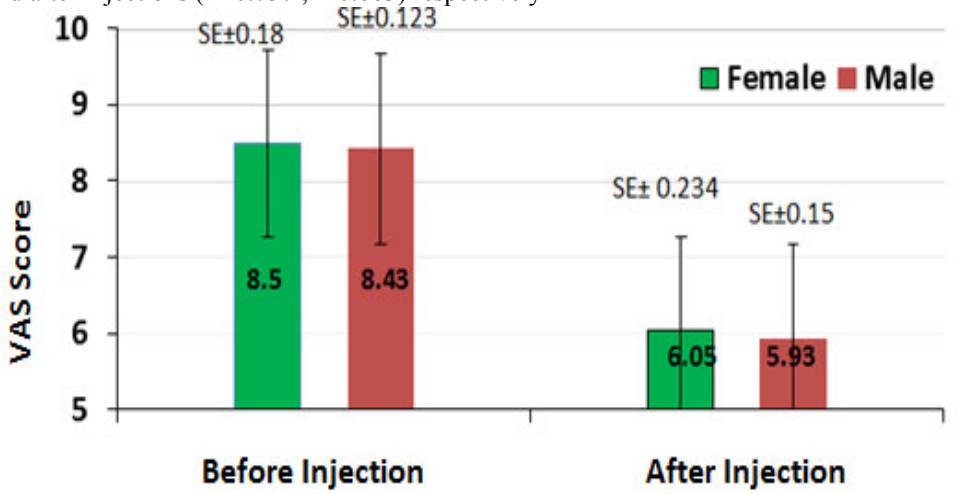

Figure 3: Mean of VAS between female and male (before and after injections with PRP).Data are expressed as mean± SE. Paired sample t. test was used for comparison with P value significant at **≤ 0.05**.

Visual Analogue Scale according to age group
It is clear from figure 4 that VAS results are an increasing order in patients before injections(7.5,8.0 ,8.38 ,9.05) according an increasing age groups (30′s, 40′s, 50′s , 60′s)respectively. All the VAS results after injections showed a decrease in all patients.

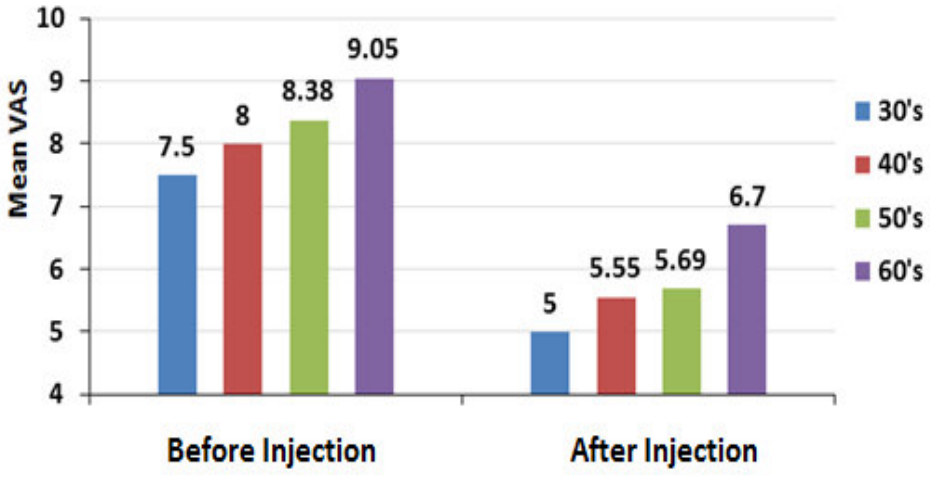

Figure 4: Mean of VAS according to age groups before and after injections with PRP. Data are expressed as mean± SE. Paired sample t. test was used for comparison with P value significant at **≤ 0.05.**

Serum C - reactive protein (CRP)

The mean CRP after injections with PRP was decreased to 5.384± 0.196 **µg**/ml compared with 7.156± 0.328 **µg**/ml before injections with highly significant differences (P=0.000).This test is used to monitor an inflammatory conditions and to monitor the effectiveness of treatment (Figure5).

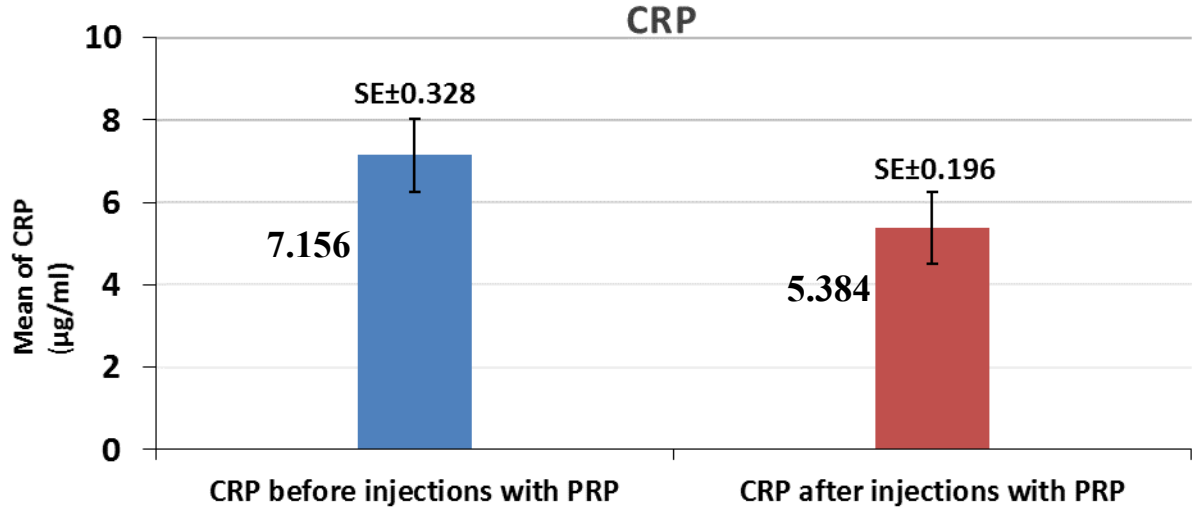

Figure 5: Mean of CRP concentration for cases before and after injections with PRP**.** Data are expressed as mean± SE. Paired sample t. test was used for comparison with P value significant at **≤ 0.05**.

Erythrocyte sedimentation rate (ESR)

The mean ESR of the cases before injections with PRP was 35.66± 0.879(mm/1hr.) and decreased after injections with PRP in to 23.7±0.856 mm/1hr.with a significant differences (P=0.000)(figure4.5).ESR is non specific test increase in many inflammatory diseases and used to help in diagnosis and monitor of different inflammatory conditions.

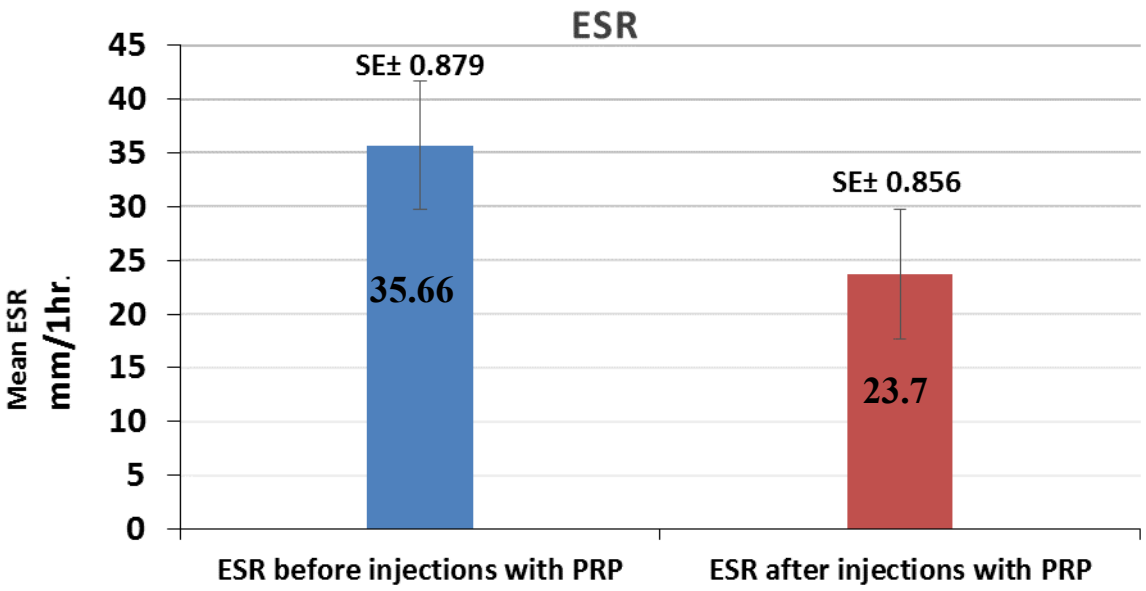

Figure 6: Mean of ESR for cases before and after injections with PRP. Data are expressed as mean± SE.Paired sample t. test was used for comparison with a P value significant at **≤ 0.05.**

Correlation between VAS score and CRP and ESR before and after injections with PRP

In the present study it was found that no significant correlation between VAS score (Before injections) and CRP (Before and after injections) but, there was highly significant correlation between VAS score (After injections) and CRP (Before and after injections). Highly significant correlation was found between VAS score before and after injections and ESR before and after injections in patients with OA as shown in table1 below.

Table 1: Correlation between VAS and CRP and ESR before and after injections with PRP.

Person correlation		VAS after	CRP before	CRP after	ESR before	ESR after
VAS before	r	.713**	.116	.260	375**	.422**
	P-value	**.000**	**.421**	**.068**	**.007**	**.002**
VAS after		R	.427**	.427**	.448**	.430**
		P-value	**.021**	**.002**	**.001**	**.002**

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Discussion

Osteoarthritis is mainly caused by degeneration of articular cartilage, which leads to pain and decrease function primarily in the knee and hips (Woolf and Pfleger, 2003).

All branches of the immune system seem to be involved in the disease process. The immune response is activated by many factors, including genetics, metabolic and mechanical factors, causing release of auto-antigens from cartilage injury with the result of an immune response induction, cellular immunity also involved by T-cell, B-cell and macrophages infiltration to the joint. Innate immunity represented by complement components which are activated by certain cytokines and some chemokines have also a role in joint degeneration (Haseeb and Haqqi, 2015).

Since surgical regenerative treatment are not satisfactory (Filardo et al.,2013),research efforts have been changed towards the application of other strategies of selecting a procedure with minimum invasion,economic,less side effect by influencing joint hemostasis (Civinini et al.,2013) .Platelets –rich plasma is another suggestion for OA treatment .Autologous PRP has been used in another part of the world but for our knowledge this technique is applied for the first time in Iraq.

Visual analogue scale (VAS) for OA treatment by PRP:-

Visual analogue scale was applied beside other six methods as reported by Jenson et al.,(1986) and reviewed later by McComack et al.,(1988). This study involved 50 patients proved to be suffering from osteoarthritis. Two separate PRP injections two weeks apart were applied in their knees at one month interval. This study revealed that VAS was significantly decreased after OA patients treatment with PRP (8.46 ± 0.104 , 5.98 ± 0.129)

respectively, with P=0.0003,as shown in figure 2. These results suggested a significant clinical improvement in OA patients function, pain and duration of stiffness in activity. These results matched with the review conclusion carried out by Halpern et al.,(2012),Sampsen et al.,(2010) also found a significant and almost linear improvements in knee injury and osteoarthritis outcome scores, including pain and symptoms relief ,VAS showed many improvement including significant reduction in moving pain at different assessment times in relation to the baseline and reported a significant decline in the pain during knee bending also in moving and resting. The same results were documented by Wang-Saegusa (2011) who found a statistically significant difference after treatment with plasma-rich growth factor (PRGF) in many parameters specifically pain, functional capacity and stiffness according to VAS pain scores and they suggested that PRGF could be used as a proved therapy for OA.

A prospective cohort study involved 120 OA patients with three grades of disease were exposed to either PRP or Hyaluronic acid (HA) injection, showed high significant improvement to PRP compared to HA, without previous side effect (Spakova et al.,2012).

In case of patients who are not candidates for autologous PRP treatment , Bottegoni et al.,(2016) used international knee documentation committee (IKDC) and equal visual analogue scale (EQ VAS),to assess knee OA patients with 80 or more age with a developing degeneration. These patients showed a decreased potential for homologous PRP injection therapy.

To answer the question of whether gender has an impact on the response to PRP treatment, the present study showed that there were no difference in VAS scores for pain between male and female before and after treatment , VAS scores before treatment for female and male 8.5± 0.18,8.43± 0.123 respectively) and VAS after treatment was 6.05± 0.234,5.93± 0.151 respectively, with no significant difference between female and male in before and after treatment P=0.757,P=0.663 respectively (figure 4). The same results were reported by Rayegani et al (2014).

Figure 3 showed that as far as age increase there is an increase in VAS before and after treatment with PRP and the mean VAS before treatment was higher than after treatment.

Jang et al (2013)reported a significant improvement in patients with early OA treated with PRP compared to the patients with increasing age and with developing degeneration who showed decreasing efficacy of PRP injection treatment.

C. Reactive protein (CRP) and Erythrocytes Sedimentation Rate(ESR)

Study of C- reactive protein (CRP) and Erythrocyte sedimentation rate (ESR) used to monitor disease response to treatment with PRP.

It is clear from figure 5 that CRP concentration was decreased from 7.156± 0.328 µg/ml to 5.384± 0.196 µg/ml after injections with PRP with highly significant differences (P=0.000).

ESR mean value before injections was 35.66± 0.879 (mm/1hr.) and decreased after injections with PRP 23.7±0.856 mm/1hr.with significant differences P=0.000 (figure 4)

Previous studies showed that CRP was positively associated with functional disability, joint tenderness, global severity, fatigue, pain and depression, while, ESR didn't show this association, except weak association with functional disability and finally it is suggested that an inflammatory components can be detected in serum in cases of osteoarthritis (Wolfe, 1997).

CRP with – low level increase in serum of women with early OA while higher level appears in those whose disease will progress over 4 years, suggesting that this low level of CRP may be considered as an indication of OA and amendable to OA treatment and prevention.(Spector et al.,1997).

In a systematic review and meta- analysis carried out by Jin et al .,(2015), they concluded that serum CRP level were modestly elevated compared to the healthy controls, but significantly associated with pain and decreased physical function and this CRP levels have no significant association with radiographic OA. Several studies suggested a relationship between high sensitivity CRP(hsCRP) and knee OA ,CRP serves as a marker for systemic inflammation and these studies suggested that this local inflammation may be reflected systematically (Sharif et al., 1997,Spector et al.,1997, Wolfe 1997).

Conclusion and Recommendation

All patients involved have an improvement in pain after injections with platelets –rich plasma (PRP) depending upon the decrease VAS score. Serum CRP and ESR are significantly correlated with pain scores of osteoarthritis suggesting CRP can be considered as an inflammatory marker for OA. The positive correlation between CRP and ESR with VAS score was showed a significant association before and after injections with PRP. Platelet rich plasma contains many biological markers, to clarify the role of each marker it is necessary to do further research to focus on the contribution of each single marker especially growth factors in the treatment of OA.

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