

The Role of Chloroquine Phosphate on Acute Phase Reactant Proteins in Patients with Knee Osteoarthritis[#]

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Abstract

The acute phase response is a major pathophysiologic phenomenon that accompanies inflammation whether acute or chronic. Complement (C3 and C4) and C - reactive protein (CRP) are positive acute phase proteins (+ ve APPs). Their production takes place in hepatocyte and the blood concentration of these parameters are increased in osteoarthritis (OA). Chloroquine (CQ) is a diprotic weak base traditionally used to treat malaria. Recently the phosphate salt of CQ is used to decrease this type of (+ve APPs) . In this study, patients who suffered from knee osteoarthritis (KOA) are treated with oral dosage form of chloroquine phosphate (CQP) for one month, twice daily. Our results demonstrate that CQP improves the patient status by decreasing complement and C-reactive protein in blood.

Key words: Chloroquine , knee osteoarthritis , acute phase proteins , complement , C-reactive protein.

الخلاصة

لقد تمت دراسة دور عقار فوسفات الكلوراكوين على مستوى البروتينات التفاعلية C3, C4, CRP لدى المرضى الذين يعانون من الفصال العظمي للركبة. أجريت تجربة عشوائية على 50 شخصاً من الإصحاء (30 أنثى, 20 ذكر) و 74 مريضاً (45 أنثى و 29 ذكر) في مستشفى مدينة الطب العيادة الاستشارية من (شهر كانون الثاني إلى أيلول 2008) تم تشخيصهم من قبل الطبيب الاختصاصي حسب طريقة الكليه الأمريكيه لمبحث الرثيه حيث تم تحريز الأعراض بواسطة مؤشر (كليكزين ولورنس) للفصال العظمي ، أخذت صور شعاعيه أماميه خلفيه لمفصل الركبه وحدد مستوى البروتين التفاعلي- C مع مستوى كل من أمتهمين C3 و C4. وصف لهم حبوب فوسفات الكلوراكوين بتركيز (250 ملغم) مرتين باليوم ولمدة شهر. سحبت عينة من دم الإصحاء والمرضى أولاً ثم عينة من دم المرضى الذين استعملوا هذا العقار بعد شهر لغرض قياس نسب البروتينات التفاعلية في المصل. لوحظ انخفاض مستويات هذه العوامل بصورة واضحة ومعنوية عند استعمال العلاج حيث زيادتها تلعب دوراً مهماً في تسريع تآكل الغضاريف وتكوين التواءات العظمية.

Introduction:

OA is the most common disease in the world and a major cause of pain and disability which usually develops in distal inter phalangeal (DIP) joints of finger, the weight bearing joints of leg and the movable portion of spine⁽¹⁾. It is associated with a breakdown of cartilage in any joint in the body⁽²⁾. Pathologically, OA was defined as a gradual loss of articular cartilage combined with thickening of subchondral bone/ bony out growth (osteophyte) at joint margins with mild to chronic non specific synovial inflammation⁽³⁾. CQ is 4 -aminoquinoline approved for treatment and prophylaxis of malaria. Recently CQP is used by some authors in the OA as disease modifying anti -rheumatic drug (DMARD)⁽⁴⁾, claimed to cause lowering of blood level of proinflammatory mediators⁽⁵⁾. Our study shows the effect of CQP on the

serum concentration of CRP, C3 and C4 in patients with KOA. C-reactive protein is a laboratory marker that is important in the assessment of inflammation, serve as a predictor and indicator of response to therapy and overall outcome in various disorders⁽⁶⁾. The major function of CRP is to bind phosphocholine, this is by permitting recognition of foreign pathogens and phospholipid constituents of damaged cells⁽⁷⁾, so the activation of complement system and / or binding to phagocytic cells will take place, and to initiate elimination of targeted cells by interaction with both humoral and cellular affecter system of inflammation, as a result CRP is a component of innate immune response⁽⁸⁾, and useful in early detection of low grade inflammation⁽⁹⁾.

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C3 and C4 serve proinflammatory roles, including chemotaxis, plasma protein exudation at the site of inflammation and opsonization of infectious agents and damaged cells⁽¹⁰⁾. C3 is beta - 2 protein 180 KDa , cleave by C3 convertase into C3a and C3b. C3b reacts with factor B to produce more C3 convertase and activate C5, so the serum level of C3 is increased in acute inflammation⁽¹¹⁾. C4 is beta -1 protein, 210 KDa cleave by C1s to produce C4a and C4b, C4b interact with C2b to activate classical pathway C3 convertase.⁽¹²⁾

Patients and method

Fifty healthy people (30 female and 20 male) as control and seventy - four patients (45 female and 29 male) are selected randomly from the Out Patient Clinic in Baghdad Teaching Hospital / Medical City / Baghdad from January to September 2008 with inflammatory KOA diagnosed according to American College of Rheumatology⁽¹³⁾. All patients were assessed by Kellegren and Lawrence grading criteria for radiographic severity of knee osteoarthritis with different signs and symptoms such as joint pain, stiffness, bony enlargement, bony tenderness and crepitus⁽¹⁴⁾. Their ages are ranged from (45 to 78) years with mean value \pm standard error of mean (55.07 \pm 6.18). Chloroquine phosphate tablet (Medoquine 250mg/

Medochem Company is equivalent to 150mg CQ base), was prescribed by Rheumatologist as twice daily after meal for one month, because CQP is 4-aminoquinoline derivative(CQ+phosphate) ,it absorbed completely and rapidly from GIT ,its mean absorption half -life is four hours with a lag time slightly more than 30 minutes^(15,16), and its duration of action is extended from sixteen to forty five days⁽¹⁷⁾. The serum analysis of all patients and control was done in the General Health Laboratory Center / Baghdad, before using this drug and one month later in order to estimate the level of C3, C4 and CRP. C3 and C4 are determined by Radial Immune Diffusion⁽¹¹⁾ while CRP is assessed turbidimetrically by antigen antibody reaction technique⁽¹⁷⁾. Results were analysed by statistical package for social science (SPSS).

Results

The presented data in this study showed the comparisons between level of C3, C4 in microgram per milliliters ($\mu\text{g/ml}$) and CRP in milligram per liter (mg/ l) in healthy and patients before the treatment as well as after one month of using chloroquine phosphate are depicted in table (1), figures (1,2,3). There were a significant decrease in C3, C4 and CRP in male, female and total patients compared to their level at baseline and control.

Table (1): The serum level of C3, C4 and CRP before using CQP at baseline and after one month of the treatment.

		Control	Baseline	P value Control, Baseline	After one month	P value Pre-,post-treatment
C3 ($\mu\text{g/ml}$)	T	950.1 \pm 21.1	1794.4 \pm 34.2	S p>0.01	1504.5 \pm 31.1	S P<0.05
	M	877.3 \pm 17.3	1788.2 \pm 48.8		1503.4 \pm 40.9	
	F	987.4 \pm 20.2	1798.4 \pm 47.16		1505.3 \pm 41.7	
C4 ($\mu\text{g/ml}$)	T	320.23 \pm 12.2	396.08 \pm 14.6	S p>0.01	325.03 \pm 12.7	S P<0.05
	M	318.1 \pm 15.7	367.7 \pm 20.2		288.03 \pm 17.1	
	F	307.2 \pm 13.4	414.3 \pm 19.9		323.09 \pm 17.9	
CRP (MG/l)	T	1.08 \pm 0.1	4.3 \pm 0.3	S p>0.01	2.02 \pm 0.2	S P<0.05
	M	1.1 \pm 0.12	3.8 \pm 0.6		1.8 \pm 0.37	
	F	2.09 \pm 0.17	4.63 \pm 0.4		2.1 \pm 0.23	

SEM: Standard error of mean.

Significant P value : (P<0.05),(P>0.01).

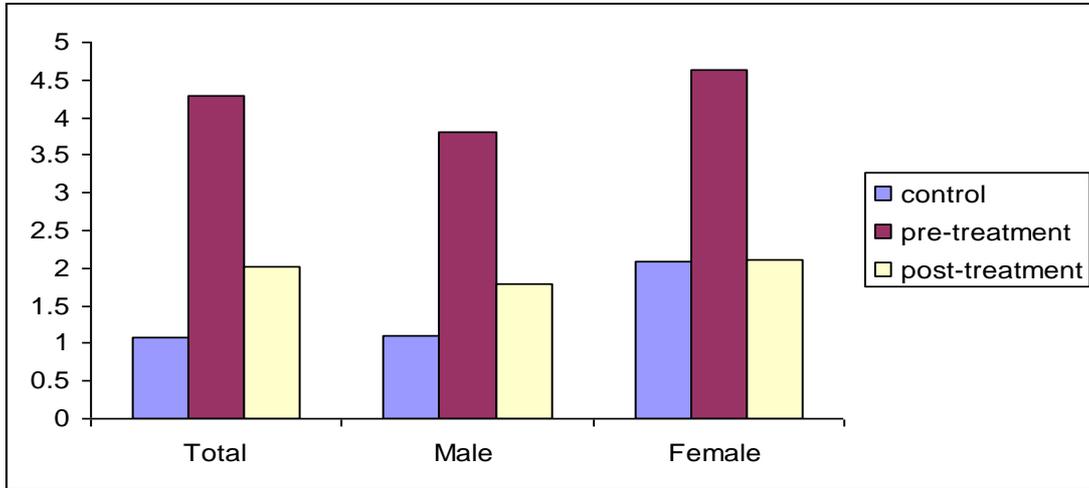


Figure (1): The level of serum C-reactive protein at baseline (pre-treatment), after one month of using chloroquine phosphate (post-treatment) and control.

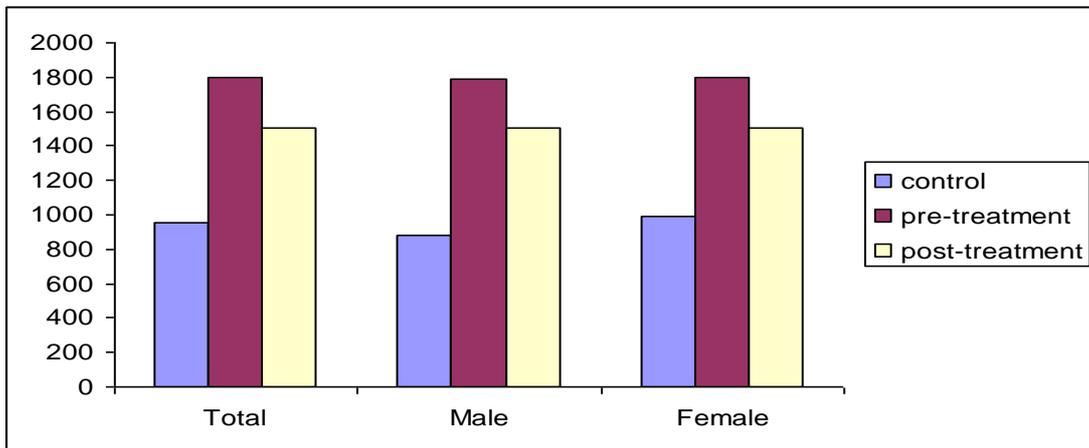


Figure (2): The level of serum complement three at baseline and one month later of using chloroquine phosphate.

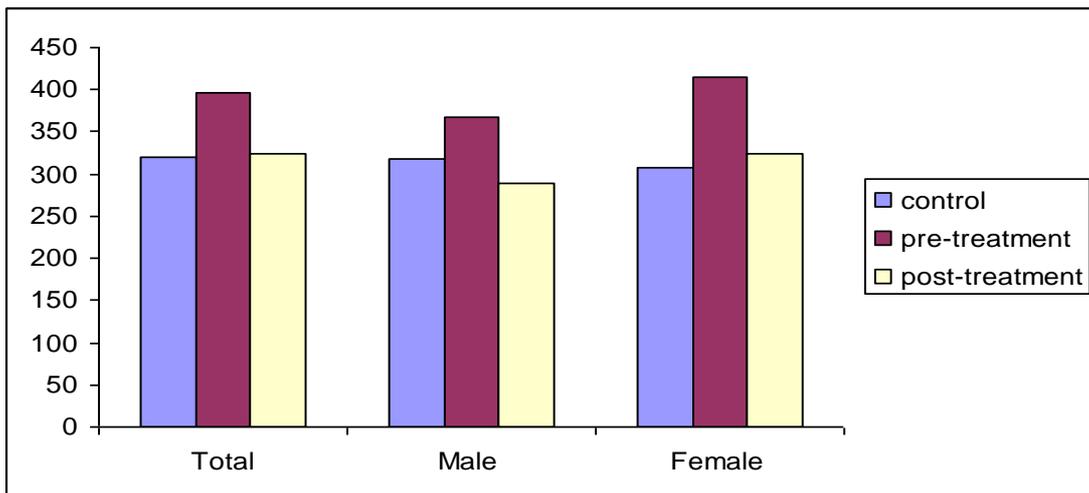


Figure (3): The level of serum complement four at baseline and one month later of using chloroquine phosphate.

Discussion

C3, C4 and CRP are components of +ve APPs , their production are increased by hepatocyte⁽¹⁸⁾. The elevation of these proteins are detected in patient with OA which is due to releasing of inflammatory molecules (cytokines)⁽¹⁹⁾. Jawad et al in 2004⁽⁴⁾ demonstrated that the using of CQP as DMARD for three months in patients with KOA, lead to decrease the serum CRP level. In our study the presented data shows a significant decrease in this parameter after one month of using CQP ($p < 0.05$), table (1), figure (1), so our result is in agreement with all explanation and findings. Chloroquine phosphate decreases serum level of CRP, C3 and C4 depending on it's ability to enter lysosomes and all acidic compartments of the cells (lysosomotropic effect)⁽²⁰⁾. It interferes with intracellular processing, receptor recycling⁽²¹⁾ and the secretion of proteins which lead to decrease the production of cytokines and other inflammatory mediators decreases lymphocyte proliferation as an immune effect⁽²²⁾. Non - lysosomotropic effect of CQP includes the inhibition of phospholipase, antagonization of prostaglandin stabilization of lysosomal membrane in synoviocytes^(23,24,25). In 2006 , Numman et al used silymarin to treat patients with KOA instead of CQP⁽²⁶⁾. Silymarin is a plant (mixture of flavolignans),isolated from the ripe seeds of *Silybum marianum* (Milk Thistle), it proved to have effective inhibitory effects on cyclooxygenase and 5-lipoxygenase in vitro and experimental animals⁽²⁷⁾. Their results showed that this drug when prescribed for two months in KOA, it decreases the serum level of C3 and C4 significantly. Our study shows a significant decrease in C3 and C4 at the end of trial ($p < 0.05$) table (1), figure (2) and (3). All findings, trials in addition to the mode of action of CQP are supported the results.

Conclusion:

CRP, C3 and C4 are decreased after using CQP for one month in patients with KOA.

Recommendation

Further study is needed to asses other parameters in serum and synovial fluid.

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