Role of Chloroquine phosphate on Acute phase reactant in patients with knee osteoarthritis

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Summary

Background: The acute phase response is a major pathophysiologic phenomena that accompanies inflammation whether acute or chronic. The complements 3 (C3), complement4 (C4) and C-reactive protein (CRP) are positive acute phase proteins (+ve APPs) their production is increased by hepatocyte in osteoarthritis (OA).

Chloroquine (CQ) which is a diprotic weak base traditionally used to treat malaria.Todate, the phosphate salt of CQ is used to decrease +ve APPs.

Objective: To evaluate the role of chloroquine phosphate on acute phase proteins C3, C4 and C-reactive protein in patients with knee OA.

Subjects and methods :A total of seventy four patients (45 female and 29male) were selected randomly from the outpatients clinic in Baghdad teaching Hospital, Medical City, Baghdad suffering from knee OA were treated with oral dosage form of CQP for one month, twice daily.

The levels of C3, C4 and C-reactive protein in serum of all patients were measured before and after treatment using ELISA technique.The serum level of C3, C4 and C-RP were calculated as mean ± standard error of mean (SEM); paired t-test was used; S= significant, P value < 0.**05**

Results : The levels of C3, C4 and C-RP were significantly reduced (P< 0.05) in serum of all patients after treatment with CQP for one month.

Conclusion :CQP is a disease modifying anti rheumatic drug (DMARD) used for patients suffering from KOA in order to reduce their C3, C4 and C-RP value and improves the disease status.

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

Introduction :

Osteoarthritis (OA) is the most common joint disease in the world, a major cause of pain and disability which usually develops in the distal interphalan ; gel joints of fingers , the weight bearing joints of the leg and the movable portion of spin [(1)] .

It is associated with a breakdown of cartilage in any joint in the body [(2)]. Pathologically can be defined as a gradual loss of articular cartilage combined with thickening of the sub chondral bone, bony outgrowth (osteophytes) at joint margins and mild, chronic non- specific synovial inflammation [(3)].

CQ is a 4-aminoquinoline approved for treatment and prophylaxis of malaria. Recently CQP is used by some authors in the treatment of OA as DMARD [(4)] claimed to cause decrease in a selected pro-inflammatory interleukins in serum of the patients.

The present study, discuss the effect of CQP on the serum concentration of CRP, C3 and C4 in KOA.

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Subjects and Methods:-

Sbjects:

Seventy four patients (45 female , 29 male) were selected randomly from the outpatient clinic in Baghdad Teaching Hospital Medical city , Baghdad; whom age ranged from (45 to 78) years . Their mean (55.07± 6.18 years).

All patients have symptomatic and radiologic evidence of OA in one or both knee joints with different signs and symptoms such as joint pain, stiffness, bony enlargement, bony tenderness and creptius.

CQP as tablet (medoquine 250 mg belong to Medochem Company) is dispeuced. Which equivalent to the net content of CQ base in dosage form of (150 mg). CQP tablet is prescribed by rheumatologist twice daily after meal, for one month.

Method:

The serum analysis of all patients was done in the general health laboratory center before using this drug and one month later in order to asses the level of C3 , C4 and CRP ;using ELISA technique. Serum samples were incubated in the micro plates coated with specific antigen (Ag).Patients antibodies (Ab) , if present in the specimen , bind to

Ag .The unbound fraction is washed off in the following step .After wards anti-human Ig conjugated to hoarse radish peroxidase (conjugate) are incubated and react with the Ag-Ab complex of the samples in the micro plates. Unbound conjugate is washed off in the following step. Addition of the tetramethyl benzidine (TMB) substrate generate an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color change to yellow). The rate of color formation from the chromogen is a function of the amount of the respective Ab in the patient sample.

AIDA, C3, C4 and CRP-check kit was purchased from Aida Gmba (autoimmune diagnostic assay).

Statistical analysis: The C3, C4 and CRP values in serum of all subjects were calculated as mean ± standard error of the mean (SEM). To compare the significant of the difference in the mean value of any two groups, student t-test was applied; **p< 0.05 was** considered statistically significant**.**

Results:

There were a significant reduction ($p<0.05$) of C3,C4 and CRP values in serum of patients with KOA treated with CQP for one month compared with the C3,C4 and CRP values in serum of the same patients before treatment.(Table 1)

Table (1): Mean, (M±SEM) of C3, C4 and CRP values (U/Ml) in serum of patients with KOA before (Baseline) and after treatment with CQP for one month.

Discussion:-

C3, C4 and CRP are components of +ve APPs, their production is increased by hepatocytes(5), [6]. The elevation of these proteins is detected in OA; it is due to the releasing of inflammatory molecules (cytokines) [7].

CRP is a laboratory marker that is important in the assessment of inflammation, serve as a predictor and indicator of response to therapy and over all outcomes in various disorders [8].

The major function of CRP is to bind phosphocholine; thereby permitting recognition of foreign pathogens and phospholipid constituents of damaged cells [9] 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 being a component of innate immune response [10]. CRP is useful in early detection of low-grade inflammation [11].

The presented data showed a significant decrease in serum level of CRP in KOA patients after one month of treating with CQP when compared with baseline ($p < 0.05$).

This result is in agreement with Jwad et al trial in 2004 when used CQP as DMARD for three months [4]

C3 and C4 serve proinflammatory roles, including chemotaxis , plasma protein exudation at the site of inflammation and opsonization of infections agents and damaged cell [12] .

C3 is of beta-2 protein, 180 KDa cleaved by C3 convertase into C3a and C3b. C3b react with factor B to produce more C3 convertase and activate C5, the serum level of C3 is increased in acute inflammation [13].

C4 is of beta-1 protein, 210 KDa cleaved by C15 to produce C4a and C4b. C4b interact with C2b to activate classical pathway C3 convertase [14].

CQP decreases serum level of CRP, C3 and C4 depending on its ability to enter lysosomes and all acidic compartment of the cell (lysosomotropic effect) [15]. It interferes with intracellular processing, receptor recycling and the secretion of proteins which lead to decrease the production of cytokines and other inflammatory mediators [16], decrease lymphocyte proliferation as an immune effect [17].

Non-lysosomotropic effect of CQP includes inhibition of phospholipases, antagonization of PG, and stabilization of lysosomal membrance in synoviocytes [18] [19] [20]. C3 and C4 are decreased in serum significantly ($p < 0.05$) at the end of this study; the result is in agreement with the mode of action of CQP .

	Mug / ml	Baseline	after1month	P valve
C3	T	1794.4 ± 34.2	1504.5 ± 31.1	S
	M	1788.2 ± 48.8	1503.4 ± 40.9	S
	F	1798.4 ± 47.16	1505.3 ± 41.7	S
C4	T	396.08 ± 14.6	325.03 ± 12.7	S
	M	367.7 ± 20.2	388.03 ± 17.1	S
	F	414.3 ± 19.9	323.09 ± 17.9	S
CRP	T	4.3 ± O.3	2.02 ± 0.2	S
	M	3.8 ± O.6	1.8 ± 0.37	S
	F	4.63 ± 0.4	2.1 ± 0.23	S

T=Total patients, M=Male and F=Female
S=Significant p value (p<0.05).

Conclusion:-

Chloroquine phosphate is used for many rheumatic as DMARD. Osteoarthritis is one of them.

Positive acute phase proteins (c-reactive , protein , complement 3 ,and complement 4) is decreased in serum after using chloroquine phosphate for one month . This drug acts through lysosomotropic and non- lysosomotropic effects. Further studies are needed to assess other parameters in serum and synovial fluid.

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