



The Effect of Calcium Phosphate Nanoparticles on Alkaline Phosphatase Activity (ALP) in the Sera of Patients with Rheumatoid Arthritis (RA)

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Abstract

For the last decades, one of the most developed sciences is nanotechnology. Similar with their respective particles at higher levels, the nanoparticles show biological properties, unique chemical and physical at nanoscale. Calcium phosphates nanoparticles (CaP Nps) are used as a bio nanomaterial in the fields of nanomedicine. One of the human's body most important basic mineral elements in bones and teeth is calcium phosphate. The aim of this study was to determine the relationship between the rheumatoid arthritis (RA) and Alkaline Phosphatase (ALP) level, and study the effect of CaP NPs and CaP NPs particles coated with casein on ALP level in the sera of rheumatoid arthritis patient. Seventy Rheumatoid arthritis patient (Male and female) with age range (20-55year) and 30 healthy subjects with sex and sex-matched as a control group was included in the present study. The ALP activity, calcium, and phosphate were measured by using a Colorimetric method. The result showed that serum ALP level in the RA group was highly (108.79 ± 30.07 U/L) than the control group (66.74 ± 19.08 U/L) ($p \leq 0.01$). While in sera of RA patients a non-significant difference in mean values ($p > 0.05$) of calcium and phosphate level was stated in contrast with that rate in the control group. Anon competitive inhibition effect of both calcium phosphates nanoparticles and calcium phosphates nanoparticles coated with casein were demonstrated. We conclude that CaP NPs has a higher inhibition effect than CaP NPs coated casein on ALP in sera of rheumatoid arthritis patients.

Keywords: ALP, Calcium Phosphate, Casein, Arthritis and Nanoparticle.

1. Introduction

For the last decades, one of the most developed sciences is nanotechnology. Nanotechnology involves the development and use of materials ranging in length from 1 to 100 nanometer (nm) [1]. Nanoparticles display unusual physical, chemical, and biological properties at the nanoscale compared with their respective particles at higher scales. These phenomena are due to a relatively larger volume surface area, increased stability enhanced mechanical strength and chemical process reactivity. [2]. Such nanoparticles characteristics have resulted in their use in different applications. The nanoparticles, a part from their material differs in sizes and shapes from different dimensions. [3]. Nanotechnology technologies have been extremely useful in biomedicine, and have helped to develop a new field called nanobiotechnology. [4]. Nanomaterials have noteworthy applications in nanobiotechnology, especially in drug delivery systems and diagnosis. [5]. Nanomedicine is expected to lead to the development of better devices, drugs, and

other early diagnosis or treatment applications for a wide range of high-specific and effective diseases. [6] [7]. One of the major mineral constituents of natural teeth and bones which is non-immunogenic, non-toxic, biodegradable and biocompatible. Nano-sized calcium phosphate materials are expected to have better bioactivity than conventional materials. [8]. The bone is the most common human calcified tissue, formed in all kinds of shapes and sizes in order to provide the body with mechanical support and various protective functions. [9]. Swelling and pain in joints is a fundamental feature of Rheumatoid Arthritis (RA), it is a chronic multisystem disease of unknown cause [10]. The presence of ALP may provide a specific chemical biomarker of osteoblastic activity [11]. Alkaline phosphatase (ALP) is an enzyme that is effective in the process of bone formation, where it catalyzes a chemical reaction that removes a phosphorus molecule and allows calcium to be incorporated into the newly formed bone. Since it is found to be located in the osteoblastic cells' plasma membrane, its position in bone mineralization is

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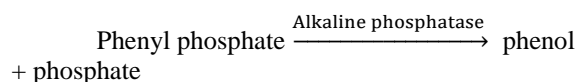
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justified, increased serum concentration of ALP is a common feature in RA [12] [13]. In fact, the level of serum ALP is increased in disorders characterized by accelerated bone turnover. Longtime ago, the diagnosis of RA is mainly based on clinical evidence. Furthermore, in the very early phase of the disease it is often difficult to diagnose RA and, in many cases, irreversible damage is verified by the time of diagnosis. It is also important to enable intervention and earlier diagnosis in laboratory tests that are specific early and sensitive in the course of the disease. [14]. The objective of this study was to determine the relation between the level of rheumatoid arthritis (RA) and Alkaline Phosphatase (ALP). And then research the effect of casein-coated Cape NPs and Cape NPs on ALP rates in the sera of rheumatoid arthritis patients.

2. MATERIALS AND METHODS

For this study, 70 patients with rheumatoid arthritis with age range (20-55) year were collected at the Baghdad Teaching Hospital / Medical City. Thirty samples of healthy people with age and sex-matched were collected from various places. General information was recorded for patients and healthy people included (Name - Age - Sex - Weight - Period of illness Type of treatment).

Alkaline phosphatase has been determined using a Colorimetric method the following reaction scheme:



Free phenol produced by hydrolysis of substrates then interacts with 4-amino-antipyrin in the presence of alkaline potassium ferricyanide to create a red-colored complex that is directly proportional to the ALP behavior in the sample of sodium arsenate integrated in the reagent abolition. in the reagent, more enzyme action is reduced and color dilution found through earlier methods is stopped.

Nanoparticles effect was studied by using calcium phosphate nanoparticles which previously prepared with size (55.01 nm) and (CaP NPs coated with casein) with size equal to (111.79nm), by using a chemical method.

The same experiment was repeated, under the same circumstances but different concentrations of the substrate were used (500 μL , 400 μL +100 μL (DW), 300 μL + 200 μL (DW), 200 μL + 300 μL (DW), 100 μL + 400 μL (DW)) in order to study the inhibitor type of nanoparticles on ALP activity.

The same experiment was repeated, under the same circumstances but using different concentrations of the (Cap NPs) and (Cap NPs+ protein) record (0.01, 0.008, 0.006, 0.004, 0.002), where 25 μL of serums and 25 μL of NPs were added instead of 50 μL of sera, to study of the effect of different concentrations of (Cap NPs) and (Cap NPs binding with casein) at the level of ALP.

3. RESULTS AND DISCUSSION

In this current study, the levels of (alkaline phosphatase, calcium, phosphate) were measured in the sera of rheumatoid arthritis patients and compared with those of the control group Table (1).

Table 1: Mean values of phosphate, Calcium and Alkaline phosphatase, in sera of controls and RA patients.

parameter	patient			control			p-value
	mean	$\pm\text{SD}$	SE	mean	$\pm\text{SD}$	SE	
Alkaline (U/L) phosphatase	108.79	30.0729	3.59	66.74	19.08	3.48	0.0001**
Calcium (mg/dL)	8.75	0.4892	0.050	8.81	0.392	0.071	0.457
phosphorus (mg/dL)	4.31	0.61846	0.73	4.25	0.967	0.186	0.695

The findings shown in Table (1) indicate a very substantial increase in the alkaline phosphatase rate (p-value > 0.01) in patients with sera of RA compared to that level in the control group as shown in Figure (1). These results were agreement with Nanke Y. et al found that ALP rates in both RA patient synovial fluids and sera are elevated, which is consistent with

disease activity as measured by erythrocyte sedimentation frequency and serum CRP levels. They also illustrated that synovial tissue from RA develops ALP bone type. Such results may enable us to understand ALP's function in RA pathogenesis. [15]. Also, Pervaind K. et al who's demonstrated that increased bone formation is associated with Rheumatoid arthritis together with bone resorption

which affects the bone mineral density. Thus it can be concluded that estimation of serum alkaline phosphatase levels may be used as a biomarker for diagnosis of Rheumatoid arthritis and may provide a useful tool for its management [11]. The findings of this study indicate a substantial increase in serum alkaline phosphatase levels relative to controls that support serum alkaline phosphatase association with rheumatoid arthritis. The elevation of alkaline phosphatase in rheumatoid arthritis patients is probably due to degenerative changes in the wrist joint and other small joints leading to auto-immune erosive changes in bone. A compensatory mechanism, in turn, comes into action to restore the destroyed bone in the joints leading to the proliferation of osteoblasts hence the elevated alkaline phosphatase activity [16].

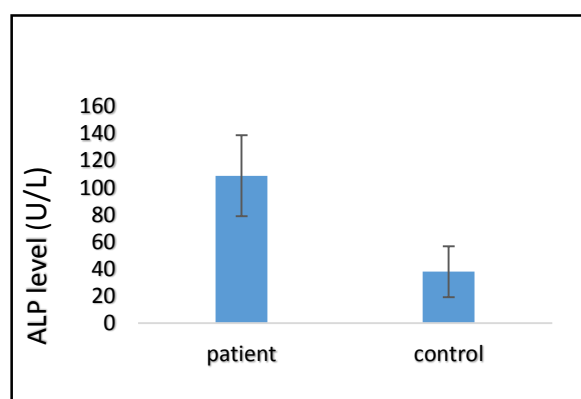


Figure (1) Show the mean value \pm SD of ALP levels in sera of RA patients and control.

The result shown in Table (1) indicates a non-significant difference in mean value ($p > 0.05$) of calcium rate in patients with sera of RA relative to that amount in control group as shown in Figure (2).

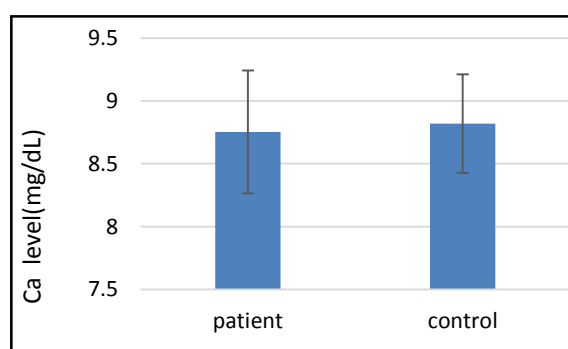


Figure (2) Show the mean value \pm SD of Calcium levels in sera of RA patients and control.

The results are contract with Scott *et al* who's demonstrated that serum calcium level in patients with RA was lower than in healthy but they did not find any evidence to prove that hypercalcemia is associated

with RA [17]. Also, Walwadkar *et al* showed that. In RA control, the calcium values were higher than in the hospital group and showed a reduced calcium / phosphorus ratio in RA patients compared to controls that clearly indicate that calcium and phosphorus metabolism were altered in RA [18][19].

The result shown in Table (1) indicates a non-significant difference in mean value ($p > 0.05$) of phosphate level in patients with sera of RA relative to that amount in the control group as shown in Figure (3). This result is agreement with T. A. Jambale *et al* who's demonstrated that the levels of phosphorus levels are increased while serum calcium and calcium/phosphorus ratio are decreased statistically in highly significant ($p < 0.0001$) [20]. Walwadkar *et al* study indicated that the phosphate levels in RA patients are lower than in the control group clearly indicate that calcium and phosphorus metabolism have been impaired in RA. [18]. This results in a dispute with Brance *et al*, who found that the phosphate values in control are higher than in the patient community The principal drawback of this research was the relatively small number of patients and the heterogeneity duration of the disease .However, this study involved only people with RA, so that the results can not be extrapolated to men [21]. Hypertrophy and hyperplasia establish hypoxic environment in synovial joints in RA patients. this hypoxia that induce degradation of ATP resulting in the release from cells of inorganic phosphorus. In RA patients' synovial joints, it is verified by reports of low glucose and high lactate levels. This acidosis should promote transfers of phosphate from the intracellular to the extracellular pool [20].

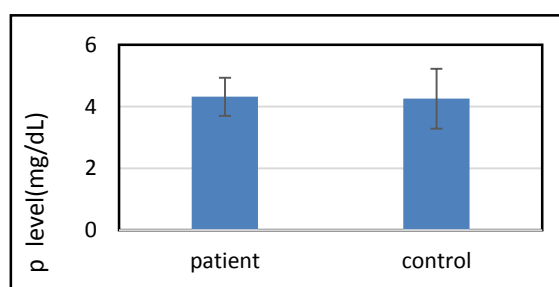


Figure (3) Show the mean value \pm SD of phosphorus levels in sera of RA patients and control.
Effect of (CaP NP) and (CaP NP coated casein) on ALP activity.

The results presented in Figure (4) show that the nanoparticles in sera of patients with rheumatoid arthritis have an inhibition effect on the enzyme activity of ALP. The present work is the first study to carry out the impact of (CaP NP) and (CaP NP coatings on casein) colloids on the behavior of the ALP, according to our understanding. effect on the enzyme activity in rheumatoid arthritis (RA) patients

shows that the CaP NPs have been more inhibition effect on ALP activity (43.3%) than CaP NP coated with casein (3.6%) as show in Figure (5). Calcium phosphate nanoparticles have been used because of their unique properties, such as the most important inorganic components of biological hard tissues, being light in weight, non-toxic chemically stable and one of the safe substances in the body. And is one of the components of the body and therefore can be used in the delivery of drug [22].

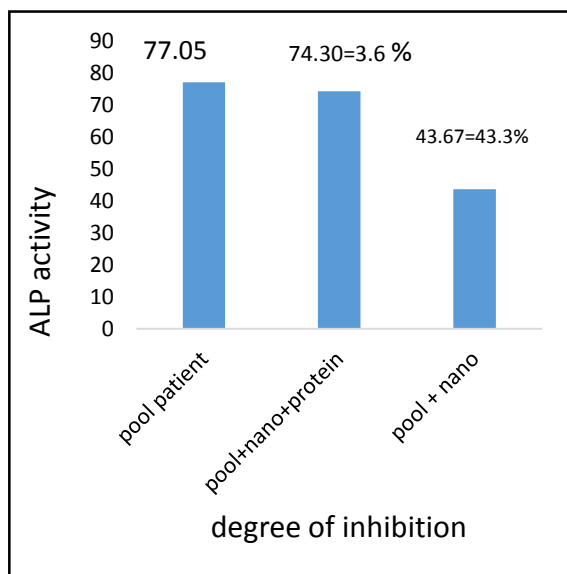


Figure (4) the effect of (CaP NPs) and (CaP NP coated with casein) on the ALP level and inhibitor ratios.

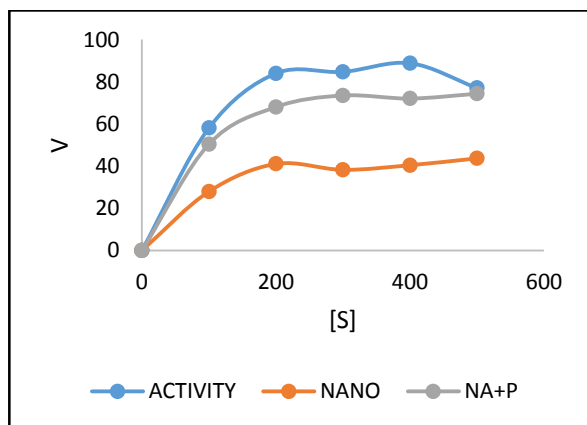


Figure (5) Show Effect of different concentrations of CaP NPs& CaP NP coated casein on the effective of ALP

line Weaver Burk was plotted in order to determine the inhibition type of CaP NPs and CaP NP coated with casein, as show in Figures (6) and (7), the result indicated that were non 6496competitive

inhibitor in both (CaP NP) and (CaP NP coatings on casein), the non 6496competitive inhibitor binds with enzyme and inversely in different positions of the Phenyl phosphate (S), Phenyl phosphate (S) binds to form (ES) with the enzyme and then the inhibitor binds to form (ESI) with complex (ES). The mechanism of non-competitive inhibition can be shown as follows: [23].

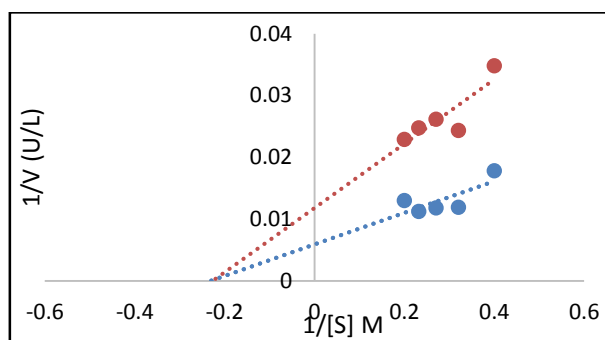
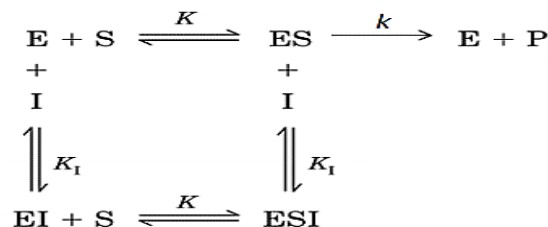


Figure (6) show inhibition type of CaP NPs effect on the ALP level in RA patient.

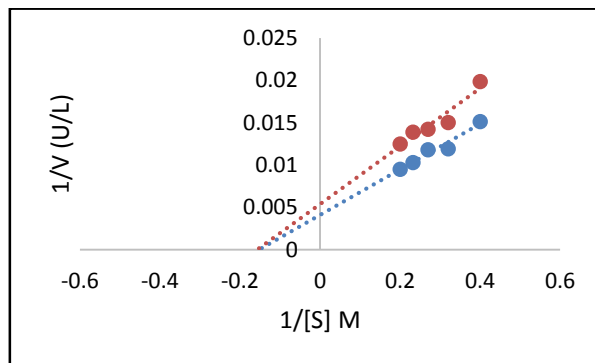


Figure (7) show inhibition type of CaP NP coated with casein effect on the ALP level in RA patient

4.CONCLUSIONS

The findings of this study indicate a substantial increase in serum alkaline phosphatase rates as opposed to controls that support the association of serum alkaline phosphatase with rheumatoid arthritis. It can therefore be assumed that serum alkaline phosphatase levels can be used as a biomarker for the diagnosis and prognosis of rheumatoid arthritis and can be a valuable resource for controlling it. CaP NPs

has a higher inhibition effect than CaP NPs coated casein on ALP in sera of rheumatoid arthritis patients. The type of inhibition of CaP NPs (and CaP NPs coated casein) on the ALP is a non-competitive inhibitor.

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