Determination Total Creatine Kinase Activity and Creatine Kinase Isoenzymes Activities in Patients with Ulcerative Colitis and Crohn's Disease under Infliximab Therapy

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Abstract

Creatine kinase (CK) catalyzes the transfer of a phosphate group from creatine phosphate to adenosine diphosphate, and the CK has three isoenzymes CK-MM, CK-MB and CK-BB. Ulcerative colitis (UC) and Crohn's disease (CD) they are both conditions characterized by long life inflammation of the gastrointestinal system. The Purpose of this study was to determine the total CK activity and CK isoenzymes in patients with Crohn's Disease and Ulcerative Colitis under Infliximab therapy. For the UC patients, CD patients and controls the CK isoenzyme activities and total CK activity was measured, the three CK isoenzymes separate by suitable ion-exchanger small columns (DEAE-Sephadex A-50 exchanger). The results display Creatine kinase isoenzyme chromatograms of all studied groups (UC, CD patients and controls), with variable activities of (CK-BB) and (CK-MB) isoenzymes with noted an elevation of (CK-MM) isoenzyme activities and significantly elevation total CK activity in serum of patient groups after therapy when compared with before therapy patient groups.

Key Words: *Ulcerative Colitis, Crohn's Disease, Infliximab therapy, Creatine kinase isoenzymes.*

Introduction

Crohn's disease (CD) and Ulcerative colitis (UC) are both conditions characterized by long life inflammation of the digestive tract [(1).]

Multiple possible aspects have been considered, including genetic aspects, infectious factors and autoimmune, environmental agents, psychosomatic and neuromotor agents[(2).] The barrier function disorders of Inflammatory bowel disease involve: decrease in barrier and antimicrobial secretions, increased permeability, incapacitated tight junctions through to substantial decrease and even total loss of the epithelium where ulceration happens [(3).]

One of the important differences between Ulcerative colitis (UC) and Crohn's disease (CD), where Ulcerative colitis is an inflammatory mucosal disorder that mostly affects the rectum and spreads proximally, and may affect the entire colon., while, Crohn's Disease is a transmural inflammation of any component of the gastrointestinal (GI) mucosa from the oral cavity to the anus [(4).]

In recent decades, a similar pattern has also been observed among Asian countries, incidences for both UC and CD have risen rapidly in Asia, with CD having a steeper rise in more developed countries, resulting in a decrease in UC: CD ratio over time [(5).] The highest and lowest incidence rates were 3.2 and 0.06 for CD and 4.6 and 0.42 for UC per 100 000 person-years in East Asia [(6).] However, a recent study implicated measuring some oxidative stress parameters of both CD and UC patients, and in a research study shows that many cytokines were substantially upregulated in both CD and UC in contrast with Control group or the non-IBD [(7, 8).]

Biological drugs block the action of a protein in the body called TNF-α (tumor necrosis factor) that is made by the body's immune system. People with Ulcerative Colitis and Crohn's Disease may produce too much tumor necrosis factor which can cause inflammation [(9).] Among the biological drugs the Infliximab (IFX) is important monoclonal antibodies used specific against TNF-α, cytokine related to the establishment of Ulcerative Colitis and Crohn's Disease [(10).]

Infliximab therapy actually offer a significant improvement in inflammatory bowel diseases (UC and CD), as outcome of their use and the longer follow-up treatments periods, there are an increasing amount of skin side effects occur in patients with inflammatory bowel diseases during anti-TNF therapy [11].

Creatine kinase (CK) is creatine N-phosphotransferase enzyme catalyzes the production of high-energy adenosine triphosphate by moving phosphate from creatine phosphate to adenosine diphosphate (which is the main energy storage reservoir during muscle rest) [12].

Creatine kinase (CK) presents in serum as highest concentrations in response to the muscle injury and it is the most widely used enzyme to diagnosing and following disease of muscle, and CK isoenzymes are dimeric molecules that two dissociable subunits, identified as subunit M or subunit B, the three cytoplasmic CK isoenzymes readily designated in tissues of human [13, 14].

The aim of this study was to estimation serum total CK and isoenzymes activities in Ulcerative Colitis and Crohn's Disease patients as well as, investigate the effect of Infliximab therapy on Creatine kinase isoenzymes.

Materials and Methods

Patients samples

Serum specimens were obtained from thirty-one patients of Ulcerative Colitis (UC) and, thirty-seven patients of Crohn's Disease (CD) and twenty-nine persons as control. Endoscopic and radiological criteria and routine laboratory clinical analyses were used for the diagnosis of CD and UC and affirmed by histological assessment. The disease diagnosis was by staff hospital doctors in the Teaching Hospital for Gastroenterology and Hepatology of Baghdad/Iraq, and the medication for UC, CD samples patients was Infliximab therapy. The mean age ± SD for (patients with Ulcerative Colitis, patients with Crohn's Disease and the Control group) was (30.916 ± 9.212), (33.64 ± 12.69) and (29.3 ± 9.09) years, respectively.

Creatine kinase activities Measurement

Total Creatine kinase activity of serum and column-eluted CK was tested via the Oliver method (15). For the test of CK activities, the working reagent was prepared in accordance with the directions of the manufacturer, 50 µl of sample was mixed with 1 mL of working reagent at 37 °C through kinetic procedure. The CK activity was determined from the rate of formation of NADH, that estimated by the absorbance at λ= 340 nm with using UV-Spectrophotometer.

Separation of Creatine kinase isoenzymes by using Column Chromatography technique

Serum of Creatine kinase isoenzymes prepared to separation by pooling an equal volumes of serum samples as final volume 1ml of serum. Sera were chromatographed by discontinuous elution from Diethylaminoethyl -Sephadex A-50, basically as demonstrated in Mercer's assay [16].

Three types of tris-buffer solution. have been prepared: Tris-HC1 buffer (50 mmol/liter, pH 8.0) contained sodium chloride (0.1M) and Tris-HC1 buffer (50 mmol/liter, pH 8.0) contained sodium chloride (0.2M) and Tris-HC1 buffer (50 mmol/liter, pH 7.0) contained sodium chloride (0.3M).

The stepwise gradient elution technique applied to five small columns (DEAE-Sephadex A-50 exchanger). After addition the one milliliter of prepared serum to columns (125 mm height and 9 mm diameter), ten fractions of separation filtrate were collected in ten test tubes any tube contained two ml of filtrate.

Under these conditions, Creatine kinase isoenzymes separated, when, MM isoenzyme quickly eluted through the ion-exchanger by Tris buffer (0.1M NaCl), while, the CK-MB and CK-BB isoenzymes remain attached until the concentration of chloride has increased (their ionic contact with the ion-exchanger has decreased) therefore, MB isoenzyme eluted by Tris buffer (0.2M NaCl) and BB isoenzyme eluted by Tris buffer (0.3M NaCl) [16].

Results

The total Creatine kinase activity was determined by Colorimetric method kit in serum of the studied groups (two types of inflammatory bowel diseases and control). In figure 1.A, the mean ± SD of CK activity in Ulcerative colitis before therapy and after therapy patients was (14.221 ± 5.521), (26.571 ± 10.885) U/L,

respectively. Creatine kinase activity significantly increased (P= 0.0212) of UC patients after therapy by Infliximab compared with before treated patients. This rising Creatine kinase levels near to CK levels of the control. In figure 1.B, Crohn's Disease patients after therapy by Infliximab had CK activity mean ± SD (31.241 ± 10.915) compared with before therapy Crohn's Disease patients that have CK activity mean ± SD (16.982 ± 5.69) this comparison presented significantly increase (P= 0.0024) of Creatine kinase levels.

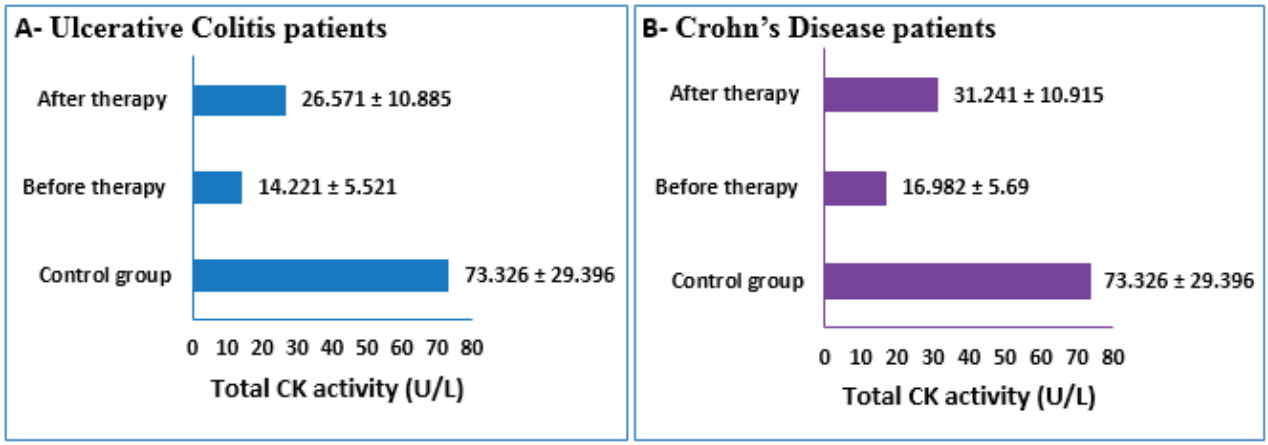

Figure 1: Elevation creatine kinase for after therapy patients group compared with patients before therapy, the patients under Infliximab with two diseases type: A- The Ulcerative Colitis patients (UC), B- Crohn's Disease patients (CD).

The five ion-exchange chromatographic column systems characterized by chromatographing serum of Creatine kinase highest activities. Figure 2.A and B illustrates the behavior of three CK isoenzymes on DEAE-Sephadex columns and the purification procedure of the Creatine kinase enzyme from human serum.

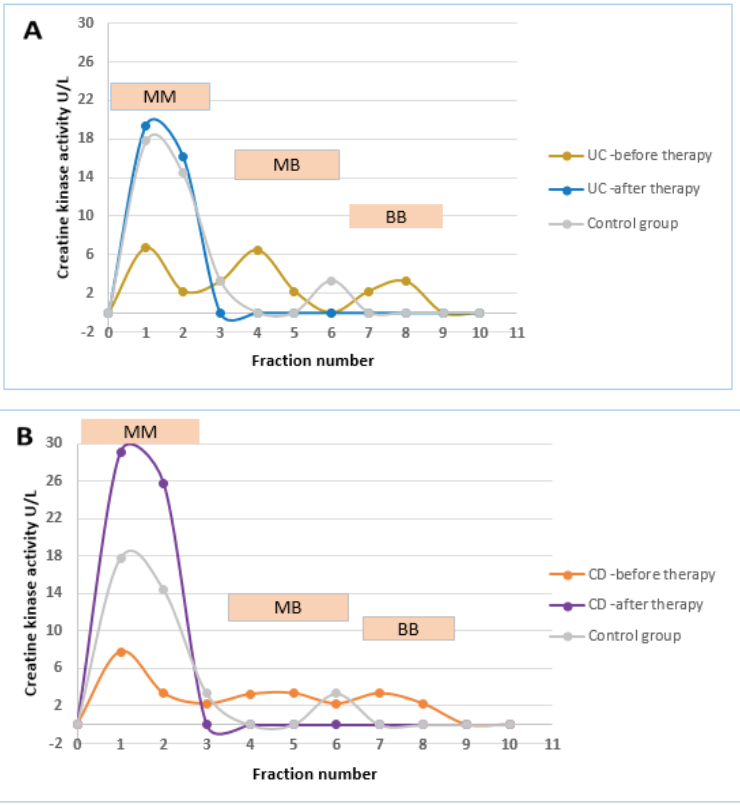

Figure2: Separation Creatine kinase isoenzymes from serum Control group and two patient groups: A:Ulcerative colitis disease (before and after) Infliximab therapy; B: Crohn's disease (before and after) Infliximab therapy.

Exhibited peak MM-CK activity in effluent fraction 1,2,3 for all five groups (before therapy UC, after therapy UC, before therapy CD, after therapy CD and Controls) chromatographic columns with differences of activities optimum in this studies groups. While, peak amounts of MB-CK activity manifested in effluent fractions 4,5,6 for (before therapy UC and before therapy CD and Control groups). While, the peak of BB-CK exhibited in effluent fractions 7,8,9 of (before therapy UC and before therapy CD groups) column and the BB-CK of after therapy UC, after therapy CD and Controls group does not have a BB-CK isoenzyme tops in their chromatographic column.

Discussion

Creatine kinase is by and large estimated in investigating patients presenting with myalgia, muscular weakness, suspected myopathy and/or developmental delay, and that more, in certain muscle conditions it might likewise help observing of illness progression and response to therapy. There are different conditions where an increase of CK levels may be seen without muscle involvement [17]. Information and knowledge of these conditions and the degree and pattern of increase in CK may be help distinguish between the different conditions, the clinical picture can guide the CK level interpretation [17].

In the ion-exchange chromatography technique, elution times usually associated with chromatographic column have been reduced with usage of mini columns and various concentrations of Sodium Chloride (gradiently elution technique) [18].

The researcher Graeber, et al performed to confirmation the assumption that CK was present in the intestinal wall and to assess its concentration and the distribution of CK isoenzymes (CK-MM, MB and BB) in the muscularis and mucosa, respectively [19].

From the results in figure 1,2 it is clear that Total Creatine kinase activity and the isoenzyme CK MM activities for patients after therapy was increase more than of patients before therapy.

The increase activities near to control group chromatogram while, the CK-BB and CK-MB activities after therapy patient showed a dropping activity when compared with activities of patient groups before therapy.

Ulcerative Colitis and Crohn's Disease are multi-factorial, chronic diseases which are characterized by active and quiet disease episodes. Fatigue is one of the most common symptoms of IBD and has been reported to be present in 44-86% of active disease patients, and up to 22–48% higher than the healthy population while, also fatigue is often attributed to active inflammation, even patients in remission show symptoms of fatigue [20].

In fast-twitch muscles, CK-MM dominates and it considered important for energy use at high-energy turnover sites. During periods of intense activity, CK is considered a key enzyme for maintaining a constant ratio (ATP/ADP). If muscle fatigue includes changes in high-energy phosphates, it may be postulated that fatigue in muscles without Creatine kinase will develop more rapidly [21].

Creatine kinase acting as a spatial energy buffer, can also be essential of the communication between intracellular sites for ATP production and consumption. Importantly, in clinical biopsies of IBD, intestinal expression of cytosolic CK enzymes has been shown to be decreased. Observed the levels of ATP in inflamed tissue from patients of inflammatory bowel disease have been reduced [22]. This agreed with the result in this study of total CK in figure (1- A, B) and the curves for UC and CD after infliximab therapy in figure (2- A, B) of CK-MM.

It is important to highlight the fact that in inflammatory bowel disease (IBD) patients, fatigue is a frequently reported symptom, even when the disease is in remission. When it has implications for the patient's work, daily life, and quality of life, it can be disabling. The previous study reported rapid decrease of fatigue scores after treatment with infliximab in daily practice. While, after infusion with placebo, fatigue scores rapidly decreased but returned to baseline values within two weeks. The reduction in fatigue scores after infliximab infusion, on the other hand, persisted until the end of that study, indicating a true pharmacological effect of infliximab (a chimeric antibody against TNF-α) [23].

From the results in figure (1- A, B), it is clear that significant elevation of total Creatine kinase activity for the CD and UC patients (comparison between after

and before Infliximab therapy). Also, elevation the CK-MM isoenzyme in CD and UC patients under infliximab therapy showed in figure (2- A, B).

However, in line with the ideas of previous study that reported TNF-alpha might be an alluring therapeutic objective, especially in traditional treatment-resistant myositis. Increase in muscle strength were associated with upgrades in serum levels of creatine kinase, electromyograms and the histological picture in post-treatment muscle biopsy samples. Creatine kinase activities in serum may guide the selection of patients for anti-TNF therapy, and patients with high levels of creatine kinase levels showed, were more likely to benefit monitoring the levels of creatine kinase also could help in evaluating the response to therapy and it probably indicating a more inflammatory type of disease (24).

Conclusion

Estimated an elevation in total CK activity of Ulcerative Colitis and Crohn's Disease patients under Infliximab (IFX) therapy and the CK isoenzyme had variation in their activities. CK-MM isoenzyme activity raised while, the CK-BB and CK-MB activities showed a dropping activity when compared with activities of patient groups before therapy.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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