SERUM SELENIUM CORRELATIONS WITH C-REACTIVE PROTEIN, SERUM CALPROTECTIN AND DISEASE ACTIVITY IN IBD PATIENTS TREATED WITH INFLIXIMAB

Manal M. Younus*1, Mohammed A. Taher2, Basim A. Askar3, Jaffar M. Kurmanji4

1Ministry of Health, Iraqi Pharmacovigilance Center. Baghdad, Iraq, Master Degree in Clinical Pharmacy.

2Baghdad University, College of Pharmacy, Head of Biochemistry Department, Ass. Prof.

3Medical City Directorate, Gastroenterology and Hepatology Specialized Hospital, Consultant Gastroenterologist.

4AL-Isra University College- Pharmacy Department-Ass. Lecturer.

ABSTRACT

Crohn's disease and ulcerative colitis affect both small intestine and colon. Diagnosis require clinical, laboratory, endoscopy and imaging techniques. Nowadays infliximab as an anti TNF alpha is successfully used in the treatment of both CD and UC. Additionally, there is an increasing interest in using complementary and alternative medicines (CAM) in IBD such as micronutrients including selenium. Many biomarkers have been studied extensively specially c-reactive protein and fecal calprotectin and correlations were suggested with disease activity and endoscopy activity but there is a lack of information regarding the correlations between serum selenium and disease activity, CRP and serum calprotectin in IBD patients receiving infliximab which is why it was studied in this study. Patients and methods: The study was designed as an interventional prospective study conducted in the Gastroenterology and Hepatology specialized hospital, a tertiary center in Medical City Directorate in Baghdad, Iraq. A total of 46 IBD patients treated with infliximab were enrolled and they were systematically randomized into 2 groups; one received infliximab with no selenium supplementation and the other group received infliximab plus selenium supplementation and followed up for 3 consecutive visits every 8 weeks for 24 weeks after the baseline visit. At baseline and study end patients provided blood samples for selenium, CRP and calprotectin.
and clinical activity for crohn's disease was measured by CDAI and mayo scores for ulcerative colitis. **Results:** Selenium supplemented patients showed a significant decrease in CRP and serum calprotectin levels (p – value 0.001 and 0.008) while selenium level showed a significant increase (p – value 0.001) and disease activity was not affected by the addition of selenium supplementation. Both CRP and serum calprotectin correlated significantly with serum selenium (Spearman’s r - 0.531 and - 0.387, P 0.001 and 0.002). No correlation was found between serum selenium and CDAI and mayo scores. **Conclusions:** selenium supplementation significantly decreased CRP and serum calprotectin and serum selenium correlate negatively with c-reactive protein and serum calprotectin in IBD patients treated with infliximab.

**KEYWORD:** (CD) Crohn's disease, (IBD) inflammatory bowel disease, (IFX) infliximab, (TNF) tumor necrosis factor,(UC) ulcerative colitis, (Se) selenium, (CRP) C-reactive protein, TNBS, (hsCRP).

**OBJECTIVES OF THE STUDY**

1- Investigate the effect of selenium supplementation on CRP and serum calprotectin in IBD patients receiving infliximab.

2- Find out the correlations between serum selenium and (CRP, serum calprotectin and disease activity).

**INTRODUCTION**

In spite of all the efforts in unraveling the etiology of IBD, the real cause is still to be known. Many factors are involved and four of them have a strong debate basis; a genetic predisposition plus environmental factors that trigger an imbalanced immune response (and subsequently inflammatory response) to gut microbiota\[1-4\] in which proinflammatory cytokines, especially tumor necrosis factor (TNF), are produced mainly by activated immune cells in inflamed mucosa during the process of IBD, and those proinflammatory cytokines further activate immune cells, as the feedback, to produce toxic molecules including super oxygen products, chemokines, proteinases, and cytokines which result in tissue damage and inflammation development.\[5\]

The diagnosis of IBD is based on the combination of clinical features , laboratory abnormalities, imaging studies and endoscopic findings.\[6\] Clinical assessment of disease activity in CD patients is most widely assessed by the Crohn's disease activity index (CDAI)
and it is the most commonly used assessment tool worldwide.[7] The Mayo score (12 scores) and a non-invasive 9-point partial Mayo score are used for assessing therapy for ulcerative colitis and to identify clinical response.[8]

Laboratory assessments involves CRP measurement which is a good marker for disease and treatment follow up[9] and the second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis in 2010 states that "serum levels of CRP are useful for assessing a patient's risk of relapse and that high CRP levels are indicative of active disease or a bacterial complication".[10] Studies found that elevated CRP levels was associated with clinical disease severity and endoscopical and histological active disease.[11] Calprotectin as a marker of neutrophil activation, has been reported to be elevated in the blood and feces of patients with active IBD[12] and has important features represented by stability and resistance to degradation and is detectable in body fluids by means of ELISA.[13] Fecal calprotectin is widely used nowadays as a biomarker in IBD patients and it can differentiate inflammatory bowel disease (IBD) from irritable bowel syndrome[14] and differentiate inactive disease from mild, moderate and highly active diseases.[15] The most important issue to solve, requiring further studies, is the non specific variation of fecal calprotectin over time[16] and the lack of predictive value of uniform thresholds at an individual level.[17] The sensitivity and specificity vary greatly based on chosen cutoff values.[18] In practice, measuring fecal calprotectin is not always practical and it is unpleasant for some patients: therefore the search of a more reliable way to measure calprotectin is through measuring the serum calprotectin and recently researches were directed to study serum calprotectin and its value in IBD, in this regards, a study demonstrated that TNBS-induced colitis results in elevations in serum calprotectin that correlate closely to stool output, biochemical, endoscopic and histological measures of colitis reflecting the state of bowel mucosa.[12] Serum calprotectin was found to discriminates well between active and inactive Crohn's disease.[19] It is also complementary to fecal calprotectin and high sensitivity CRP (hsCRP) for prediction of relapse after infliximab withdrawal in CD patients[20] also comparable with serum CRP in predicting outcome in acute severe ulcerative colitis.[21]

Infliximab is a purified, recombinant DNA-derived chimeric human mouse IgG monoclonal antibody, can quickly form stable complexes with the human soluble or the membrane form of TNF and terminate the biological activity and signals of TNF. Its serum half-life 9.5 days.
and still detectable in serum of IBD patients 8 weeks after infusion treatment, infliximab provides a useful strategy to neutralize TNF and to inhibit immune responses of IBD.\textsuperscript{[5]} Indications for anti-TNFα therapy include induction of response, remission, and maintenance for patients with moderate or severely active Crohn’s disease\textsuperscript{[22]} particularly Patients with fistulas or penetrating disease and extraintestinal manifestations may benefit more from IFX therapy. In UC the indications include moderate to severe disease.\textsuperscript{[23]} Additionally there is an increasing interest in using complementary and alternative medicines (CAM) in IBD such as micronutrients including selenium.\textsuperscript{[24]} Selenium is important for the activity of the enzyme glutathione peroxidase which has an important role in managing the oxidative stress in IBD patients.\textsuperscript{[25]} A recent review regarding selenium and selenoprotien showed that Se status affects gene expression, signaling pathways, and cellular functions in the small and large intestine as well as the gut microbiome composition. This data, specially from animal experiments, give hope that adequate dietary Se supply may counteract chronic intestinal inflammation in humans.\textsuperscript{[26]}

\textbf{PATIENTS AND METHODS}

The study was designed as an intervention clinical trial. The study was conducted in the Gastroenterology and Hepatology specialized hospital, a tertiary center in Medical City Directorate in Baghdad, to evaluate the effect of selenium supplementation on CRP and serum calprotectin in IBD patients receiving infliximab. Collection of data was conducted in the period between (April 2014 till February 2015). Systematic random sample was used to allocate patients into 2 groups (both groups consist of both CD and UC patients); the first group received selenium supplementation 200 mcg per day(Jamieson, France) plus intravenous infusions of infliximab (Remicade, Janseen, USA) at a dose of 5 mg per kilogram of body weight. The second group received infliximab without Selenium supplementation at the same dosing schedule. A bottle containing 100 selenium tables was supplied to each patient in the selenium group at 8 weeks intervals. Patients were enrolled in this study if : they were out patients aged between 18-60 years, previously diagnosed UC or CD (using endoscopic, histological and clinical criteria), scheduled to receive at least 3 doses of infliximab infusion and had received at least 3 doses of infliximab, with or without concomitant treatment (prednisolone and/or azathioprine or mesalamine), on stable medication for more than 4 weeks, and have the willingness to participate in the study. Patients were excluded if : age below 18 years, with the short bowel syndrome, an ostomy (fecal diversion), a recent history of abdominal surgery (within the previous 6 months), a
positive chest radiograph or tuberculin skin test, active infection with hepatitis B or C. On supplementations containing selenium or vitamin A, or other antioxidants, with a known hepatic dysfunction and/or renal insufficiency and unwillingness to participate at any time point. Patients were followed up for three visits for 24 weeks every 8 weeks and the first visit was considered as the baseline visit before any intervention was made. Data on disease extent and duration were extracted from hospital records, duration of symptoms, medication history, previous operation history, previous infliximab therapy and number of infliximab exposures was also recorded. Patients were hospitalized for at least 8 hours at each visit in order to receive infliximab treatment and the following were assessed for all the patients at each visit; measurement of body weight in kilogram (Kg), diseases activity for both CD and UC. At each visit and prior to infliximab administration, 3-5 ml of blood samples were collected (via vein puncture) in gel tubes, for laboratory investigation, allowing the specimen to clot for 30 minutes, then after centrifugation, separated samples(serum) were divided into two portions, both stored at –30°C. Blood samples were drawn from 20 sex and age match apparently healthy male and females at the start of the study, serum was separate and the resultant lab measurements of serum CRP, calprotectin and selenium to serve as controls. Disease activity was measured with Adults Crohn's disease activity index (CDAI) which incorporate eight weightified clinical and laboratory factors. Stool frequency, abdominal pain, well being, extra-intestinal manifestation, use of anti-diarrheal medication, abdominal mass, Hct, body weight. The sum of these factors produce a total score ranging from 0-600, with higher score indicating worse disease activity. CD clinical disease activity (CDAI) is grouped into mild, moderate and severe. Severe disease is defined as a score of more than 450, moderate activity 220-450, mild 150-220 and remission as a score of less than 150. The Mayo scoring system was used to assess the disease activity in UC patients. It has 4 components: stool frequency, rectal bleeding, findings at endoscopy, and a physician's global assessment. This has been used for US Food and Drug Administration (FDA) approval of delayed-release oral mesalamine and infliximab in the United States and in Europe as well. The Mayo score ranges from 0 to 12, with higher scores indicating more severe disease. This score can be used for both initial evaluation and monitoring treatment response and disease threshold can be divided into 4 categories, remission ≤2 with no subscore > 1,mild 3-5, moderate 6-10, severe ≥10. C-reactive protein was measured using ELISA kit (Demeditec, Germany, sensitivity level < 1μg/ml) and calprotectin was measured using ELISA kit (My Bio Source, USA. sensitivity level 1 ng/ml) and serum levels of selenium was determined by the hydride generation atomic absorption spectroscopy (HGAAS) at Ibn-
Sina research institute, were 10 μl of serum sample were injected into the spectroscopy, after the treatment of serum sample with 0.1% V/V nitric acid and 10% Triton X-100 and 20% of ammonium dihydrogen phosphate. The results were expressed as µg/L.

STATISTICAL ANALYSIS
Statistical package of SPSS-17 was used for the analysis of data.. the obtained data were expressed as means ± SD and percentages when needed. P value was considered to be significant when it is less than 0.05. Descriptive statistics were used to summarize differences in demographic and baseline characteristics among study groups. Differences between the two groups assessed using unpaired t- test. Spearman test was used to study the correlation between the groups. Diagrams were used to express the results when applicable.

ETHICAL CONSIDERATION
The study was approved by Iraqi MOH and college of pharmacy, Baghdad university ethical committees. All participated patients gave informed written consent.

RESULTS
Baseline clinical and lab. characteristic for the healthy controls and patients included in the study
Twenty (20) sex and age match healthy controls were enrolled in the study, to test the differences in the clinical, inflammatory and oxidative stress markers but it was unreliable to test for the disease activity and unethical to test for the endoscopy differences between the two groups, however, the results of the inflammatory and oxidative markers indicated a highly significant differences between the two groups as shown in (table 1).

Table 1: Baseline clinical and lab characteristic for the healthy controls and patients included in the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IBD patients (n=46)</th>
<th>Control (n= 20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease activity</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>2.96 ± 1.5</td>
<td>N/A*</td>
<td></td>
</tr>
<tr>
<td>Crohn's disease</td>
<td>259.26 ± 129.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (µg/ml)</td>
<td>24.26 ± 2.18</td>
<td>6.0 ±1.3</td>
<td>0.005</td>
</tr>
<tr>
<td>Calprotectin (ng/ml)</td>
<td>57.6± 37.44</td>
<td>17.2±2.4</td>
<td>0.017</td>
</tr>
<tr>
<td>Selenium (µg/L)</td>
<td>32.35± 9.48</td>
<td>63.43± 1.7</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* N/A = not applicable
Baseline clinical and lab. parameters of IBD patients included in the study

Baseline clinical and laboratory data of the two studied groups (infliximab group and infliximab plus selenium group) are presented in (table 2). Males represented 50% of the total studied groups and the age was in the thirties in both groups, also disease duration was around 5 years in both groups. Previous abdominal surgery difference between the two groups was also non significant. Regarding disease activity of patients in the infliximab group, the moderate disease activity was the predominant scores (43.5 %), while the mild disease activity shows the highest percentages in the infliximab plus selenium group (56.2%), in spite of that a statistically not significant difference was found between the two groups when comparing the disease activity of the two groups. The duration of IFX treatment was around 21 months. Patients received different medications, in addition to infliximab, including azathioprine (AZA),prednisolone, mesalazine ,but a non significant difference was found between the two studied groups. Finally, male also represented 50% of the studied healthy controls with a mean age of 31.21± 5.32 years.

Table 2: Baseline clinical and lab. parameters of IBD patients and healthy controls included in the study

<table>
<thead>
<tr>
<th></th>
<th>Infliximab group</th>
<th>IFX plus selenium group</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>23</td>
<td>23</td>
<td>46</td>
<td>NS†</td>
</tr>
<tr>
<td>Male % (no)</td>
<td>47.8 % (11)</td>
<td>52.2% (12)</td>
<td>50% (23)</td>
<td>NS</td>
</tr>
<tr>
<td>Age year, mean± SD</td>
<td>30.65 ± 6.09</td>
<td>36.43 ±11.52</td>
<td>33.54 ± 9.56</td>
<td>NS</td>
</tr>
<tr>
<td>Disease duration years, mean ± SD</td>
<td>5.826 ± 3.78</td>
<td>5.522 ± 3.95</td>
<td>5.67 ± 3.8</td>
<td>NS</td>
</tr>
<tr>
<td>Previous abdominal surgery (%) , no</td>
<td>8.7% (2)</td>
<td>13% (3)</td>
<td>10.8% (5)</td>
<td>NS</td>
</tr>
<tr>
<td>Disease activity score IBD % , no.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remission (inactive)</td>
<td>8.3 % (1)</td>
<td>8.7% (2)</td>
<td>6.5 % (3)</td>
<td>NS</td>
</tr>
<tr>
<td>Mild</td>
<td>39.1 % (9)</td>
<td>56.2% (13)</td>
<td>47.8 % (22)</td>
<td>NS</td>
</tr>
<tr>
<td>Moderate</td>
<td>43.5 % (10)</td>
<td>30.4 % (7)</td>
<td>36.9 % (17)</td>
<td>NS</td>
</tr>
<tr>
<td>Severe</td>
<td>8.3 % (3)</td>
<td>8.3 % (1)</td>
<td>8.8 % (4)</td>
<td>NS</td>
</tr>
<tr>
<td>Concomitant medication % ,no.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZA alone</td>
<td>52.2 % (12)</td>
<td>34.8 % (8)</td>
<td>43.5 % (20)</td>
<td>NS</td>
</tr>
<tr>
<td>Prednisolone alone</td>
<td>None</td>
<td>4.3 % (1)</td>
<td>2.2 % (1)</td>
<td>NS</td>
</tr>
<tr>
<td>Mesalazine alone</td>
<td>8.7 % (2)</td>
<td>4.3 % (1)</td>
<td>6.5 % (3)</td>
<td>NS</td>
</tr>
<tr>
<td>AZA plus Prednisolone</td>
<td>13 % (3)</td>
<td>13% (3)</td>
<td>13% (6)</td>
<td>NS</td>
</tr>
<tr>
<td>AZA plus Mesalazine</td>
<td>21.8 % (5)</td>
<td>26 % (6)</td>
<td>23.9 % (11)</td>
<td>NS</td>
</tr>
<tr>
<td>AZA, Mesalazine and prednisolone</td>
<td>None</td>
<td>13% (3)</td>
<td>6.5 % (3)</td>
<td>NS</td>
</tr>
<tr>
<td>Multivitamins</td>
<td>43.5 % (10)</td>
<td>26 % (6)</td>
<td>34.8 % (16)</td>
<td>NS</td>
</tr>
<tr>
<td>No medication</td>
<td>4.3% (1)</td>
<td>0</td>
<td>2.2 % (1)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of IFX treatment mean</td>
<td>20.4 months</td>
<td>21.6 months</td>
<td>21 months</td>
<td>NS</td>
</tr>
<tr>
<td>Healthy control information</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male %</td>
<td>50% (10)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Age (yr) median, mean  

| Median, mean | 31.21± 5.32 |

* the number of ulcerative colitis and Crohn’s disease patients were calculated as per 26 ,20 patient respectively.

† NS = non significant,
‡ Total may not equal to 100 since patients might receive multivitamins besides other medication.

Comparison of the inflammatory markers and selenium in the studied groups

As shown in (table 3), at the baseline, CRP level in the infliximab group was (26.51 ± 19 μg/ml) compared to (22.02 ± 25.19 μg/ml) in the infliximab plus selenium group, but at the study end it increased in the first group to (31.91 ± 18.21 μg/ml) and decreased in the second group to (10.57 ± 5.13 μg/ml) (p- value 0.001). On the other hand, calprotectin level at baseline in the infliximab group was (48.47± 27.89 ng/ml), while infliximab plus selenium group shows a higher level of (66.78 ± 43.75 ng/ml), but the difference was statistically not significant. At the study end infliximab group calprotectin level showed an increase to reach (68.39 ± 22.11 ng/ml) while it decreased in the infliximab plus selenium group to reach (47.68 ± 27.85 ng/ml) (p- value 0.008). Selenium level in the infliximab group and infliximab plus selenium group was (33.18 ± 8.58 μg/L) and (31.52 ± 10.42 μg/L) respectively at baseline and increased in both groups to reach (40.99 ± 13.45 μg/L) in the first group and (74.28 ± 25.98 μg/L) in the second group (p- value 0.001).

Table 3: Comparison of the inflammatory markers and selenium in the studied groups

<table>
<thead>
<tr>
<th>Variables*</th>
<th>Visits</th>
<th>CRP (μg/ml)</th>
<th>Mean ±SD</th>
<th>Mean ± SD</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>26.51 ±19.0</td>
<td>22.02 ± 25.19</td>
<td>0.499</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study end</td>
<td>31.91 ± 18.21</td>
<td>10.57 ± 5.13</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calprotectin (ng/ml)</td>
<td>48.47 ± 27.89</td>
<td>66.78 ± 43.75</td>
<td>0.099</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>68.39 ± 22.11</td>
<td>47.68 ± 27.85</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study end</td>
<td>33.18 ± 8.58</td>
<td>31.52 ± 10.42</td>
<td>0.558</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study end</td>
<td>40.99 ± 13.45</td>
<td>74.28 ± 25.98</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Disease activity

(Figure 1) shows that infliximab group moderate-severe scores went down from 52.2% to 13% and from 34.8% to 4.4% in the selenium group, while mild- inactive disease activity changed from 47.8% in the infliximab group to 87% and from 65.2% to 95.6% in the infliximab plus selenium group at the study end.
Study correlations

(Table- 4) shows that selenium had a negative correlation with CRP (p value 0.001), also selenium had had a negative correlation with serum calprotectin (p value 0.002), but no correlations with CDAI and mayo scores.

Table 4: Correlation coefficient (Spearman and associated p-value) between selenium and (CRP, calprotectin, disease scores)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>R</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se- CRP</td>
<td>-0.531</td>
<td>0.001</td>
</tr>
<tr>
<td>Se- calprotectin</td>
<td>-0.387</td>
<td>0.002</td>
</tr>
<tr>
<td>Se- CDAI</td>
<td>0.007</td>
<td>0.970</td>
</tr>
<tr>
<td>Se- Mayo score</td>
<td>-0.146</td>
<td>0.414</td>
</tr>
</tbody>
</table>

DISCUSSION

Inflammatory markers and selenium in the studied groups

CRP is used routinely to follow up patients with IBD as an indicator of the presence or absence of inflammation and its level reflects the inflammatory process\textsuperscript{[33]} and in this regards, the results of CRP in the infliximab plus selenium group decreased at the study end when compared to baseline (22.02 μg/ml ± 25.19 to (10.57 μg/ml ± 5.13) indicating a decrease in the inflammatory burden also levels of C-reactive protein are associated with response to infliximab therapy in patients with Crohn’s disease\textsuperscript{[34]} and UC patients ,accordingly the reason behind the increased IFX group CRP from(26.51 μg/ml ± 19 to 31.91 μg/ml ± 18.21) in this study could be an uncontrolled inflammatory process and such levels could be found in mild inflammatory conditions\textsuperscript{[35]}, the other reason could be unresponsiveness to IFX.
treatment, but this is need to be confirmed by measuring the levels of IFX and its antibodies in those patients which was not done in this study.

Calprotectin is a more specific marker of inflammatory bowel diseases. The level of calprotectin of IFX group, at this study end was significantly increased (from (48.47 ng/ml ± 27.89), to (66.78 ng/ml ± 43.75 ng/ml), as shown in (table 3), and it comes with the non significant increase of CRP level, this result could be interpreted by the finding of one study that followed up CD patients and their calprotectin levels for about one year, and concluded that only patients who achieved complete remission, did fecal calprotectin levels decrease and serum calprotectin is a novel biomarker that can predict outcome in acute severe UC, so it could be an indicator of uncompleted remission of this group of patients. Another explanation of the increased level of calprotectin in this group of patients in the present study could be found in the STORI trial when serum calprotectin was significantly higher for active disease (median=19,584 ng/mL) than for inactive disease (median=8353 ng/mL) (P<0.0001), also for UC, fast and sharp decrease in calprotectin predicts remission by infliximab in anti-TNF naïve patients with ulcerative colitis.

The results of low levels of selenium in IBD patients compared to controls in this study(32.35 μg/L ± 9.48 vs 63.43 μg/L ± 1.73 p 0.001) as shown in (table 1), was found in a Norwegian study in 1993 as well. However, in a recent study by Marco et al. trying to answer the question of decreased antioxidant capacity in CD patients, he measured the level of selenium in the whole blood and plasma but found no statistically significant differences between the CD serum selenium level (175.6 μg/L ± 11.65; 85.82 μg/L ± 3.86) and control groups (173.8 μg/L ± 12.00; 96.01 μg/L ± 3.182) (P= 0.916 and P= 0.051, respectively) which is inconsistent with the results of this study. Selenium level of IFX group, surprisingly, showed an increase from (33.18 μg/L ± 8.58) at baseline to (40.99 μg/L ± 13.45) at study end (table 3), this could be related to a high selenium food content which was not controlled during the study.

Disease activity
The addition of selenium supplementation to infliximab did not show any significant difference in the disease activity, in fact, the results of the infliximab group showed a better decrease in the moderate-severe disease activity with a percent decrease of 39.2 compared to 30.4 in the infliximab plus selenium group and this could be related to the differences in the internal integrity of the disease activity of indices of both CD and UC.
Study correlations
Correlation of selenium with other study parameters were studied and only two valuable correlation were found (table 4), one with CRP (Spearman r=-0.531 p 0.001), as well as with calprotectin (Spearman r=-0.387 p 0.002). These are important correlations connecting the inflammatory markers (CRP and calprotectin) with one of the oxidative markers (selenium) and up to our knowledge this is the first time to find such correlations.

Study limitations
1. The small number of patients participated in the study because the use of infliximab is conserved to patients who failed to respond to other treatment options.
2. The overall results were markedly influenced by the study population, which was comprised largely of patients whose disease was controlled at the time of enrolment.
3. Diet limitation was not achieved during the study period.

CONCLUSIONS
This study was designed to study the effect of selenium supplementation on CRP, calprotectin and disease activity and found the possible correlations of selenium with CRP, calprotectin and disease activity. The important results of this study represented by the finding that increased serum selenium after selenium supplementation accompanied by a decrease in the levels of CRP and calprotectin in IBD patients treated with infliximab, but not disease activity. In addition to that, correlations was found between (selenium and CRP r= -0.531, p value 0.001) and (selenium and calprotectin r= -0.387, p value 0.002).

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.

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