Estimation Of Creatine Kinase activity In Colorectal Cancer, Polyps, And Ulcerative Colitis Patients.

Zahra`A Salim Muhsin Chemistry dep-College of science-Al-Mustansyria univ.

Abstract

The aim of this study is to evaluate the usefulness of serum creatine kinase enzyme (Ck) in the diagnosis of some cancers and diseases. Serum Ck activity prospectively measured in patients undergoing colon cancer (pre and post operation, n=30), rectum cancer (pre and post operation n=16), polyps(n=10) ,and another 9 patients with ulcerative colitis(U.C).The total activities of CK in 20 healthy individuals and in all the patients were assayed by enzymatic colorimetric method with knowledge of the clinical
The mean diagnosis.
(SD) activity of Ck did not differ significantly between controls 125.17(37.85) and :
a) colorectal cancer (CRC) preoperation (PrO) 121.66(43.67) and post operation (PoO) 132.11(32.95) , respectively p> 0.3].
b) U.C 156.56(65.55) ,p>0.1) , but it was significantly higher in colorectal polyps CRP 223.08(27.39), p<0.01.
The results suggest that there is no evidence of an association between knowledge the level of CK and the appearance of CRC and U.C.
However, such a knowledge is useful in diagnosing colorectal polyps.

Introduction
Creatine kinase (Ck , 2.7.3.2) is a cytoplasmic and mitochondrial enzyme with a wide tissue distribution and catalyzes the reversible transfer of the phosphate group of phosphocreatine (PCr) to ADP, to yield ATP and creatine (Cr) [1,2] :
Ck (pH =9.0)
Creatine + ATP ADP + Creatine phosphate
(pH = 6.8)
The Ck / PCr/ Cr system is present primarily in tissues with high and fluctuating energy demands such as brain, heart and skeletal muscle, and serves as a temporal and spatial " energy buffer " that helps to maintain a high intracellular phosphorylation potential in situations of increased metabolic demand [3,4].
The molecule is a dimeric enzyme composed of either M or B type subunits. The subunits combine to form 3 isoenzymes : Ck1(BB), Ck2 (MB) and Ck3(MM) . These isoenzymes are expressed at different levels in various tissues in humans : Ck-BB (brain tissues) , Ck-MB (heart muscle) , and CK-MM in skeletal & heart muscle [5, 6] . It is used clinically in the diagnosis of acute myocardial infarction [7, 8].
As a typical isoenzyme, macro-Ck 2 which is not found in the sera of healthy individuals, has been reported in patients with gastrointestinal tract malignancies and in particular of colorectal cancer [9]. Other reports showed the apparently high concentration of Ck-MB isoenzyme in the sera of lung, prostate cancer, metastatic ovarian tumor [10, 11,12], while Al- Mustansiriya J. Sci Vol. 17, No 1, 2006
the enzyme activity was lower than the normal in colon and lung adenocarcinomas, and squamous cell carcinomas [13].
Because of the above conflicting data of Ck level in carcinoma parts, the goal of the present study was to analyse the Ck enzyme in CRC, colorectal polyps, and in (U.C)disease and the change of its activitys were discussed. People undergoing CRC have an increasing risk for it starting at the age of 40. People over the age of 50 account for 93% of colorectal cancer
The exact cause is unknown, but most cases begin as polyps, which are small growths inside the colon or rectum[14] while the U.C (disease of dysregulation of the immune system of the colon) is associated with a major increase in the risk of colon cancer [16, 17]. The highest risk groups for CRC include ulcerative colitis, previous history of polyps or cancer, family history of colon cancer, certain genetic conditions and a personal history of female cancer. Smoking, drinking and physical inactivity are additional risk factors for CRC [16].

Materials and method

Patients:
The present project was approved by the ethics commission of Baghdad Teaching and AL-Shaheed Adnan Khir Alla hospital.
In total, 43 colorectal cancer samples were analysed. Nineteen samples were obtained from PrO colonic cancer (11 males cases and 8 females cases), 8 males cases from PoO colonic cancer, 11 male sample was obtained from PrO rectal cancer and 5 males samples from PoO rectal cancer. The diagnosis was based on clinical and biological exam. Reports by Dr. Falih AL-Aubaidy, Dr. Abd AL-salam AL-Taie, and Dr. Saaeb AL-Gailaney.
Ten male patients with colorectal polyps, where chosen from the same Hospitals to study the comparison of the Ck level in malignant and benign tumors. Nine males with U.C were also enrolled in this study, the diagnosis was based on Endoscopies tests reports by Dr. Azaam Agah and Dr. Helmy AL-Kazaz.
Twenty adjacent control (10 males, 10 females) were included to define the normal Ck values.
Blood samples were collected between 9.00 and 11.00 am, sera were separated at 4500 xg for 20 min and directly analyzed. Creatine kinase

Estimation Of Creatine Kinase activity

Zahra’A Salim Muhsin

activity was measured in sera of patients and normal donors by colorimetric method.

Method:
Colorimetric determination of Ck activity utilizes creatine phosphate as substrate to act as the initial catalyst for a series of reactions resulting in the formation of NADPH as outlined in the coupled enzyme assay:

CK
Creatine phosphate + ADP creatine +ATP
Hexo kinase
Glucose +ATP glucose-6-p + ADP
G6-p-DH
glucose-6-p + NADP+ gluconate-6-p + NADPH
Diaphorase
2NADPH + nitro blue tetrazolium diformazan + 2NADP
the reaction is stopped by the addition of HCl, the blue/violet colour of
diformazan has an absorption maximum around 560nm [18,19,20].

Statistical analysis:
Descriptive statistics were used in analyzing the patients, characteristics
and laboratory parameter for each group. In addition, unpaired Student's
T test was used to assess group differences when appropriate. Correlation
between groups were assayed by person test. A statistical significance
difference was accepted as p value < 0.05. All the statistical analysis in
this study were made using SPSS 10 for windows program.

Results
Comparison of serum Ck between healthy males and females:
The mean (SD) level of serum Ck in healthy males (n=10) and females
(n=10) was nearly closed 126.18 (40.91) vs 123.61 (34.10) U/l, p > 0.5,
(table -1, fig-1). 
Comparison of serum Ck between male & female in colon cancer:
Al- Mustansiriya J. Sci Vol. 17, No 1, 2006
57
The differences between Ck activity in male (n=11) and female (n=8)
colon cancer was non significant: 135.74 (37.92) vs 98.02 (18.11) U/l, p
> 0.05, (table-1, fig-2).

Patients characteristics:
The table(2) shows some of characteristics of the different groups.
Patients with CRC (30-60 years old) were older than either patients with
polyps (5-15 years old) or U.C (20-55), but they were in the same range
with healthy individuals (27-58 years old).

Serum Ck values in carcinoma and control cases:
The mean (SD) of serum Ck in control value was 125.17 (37.85) and in
patients with colon cancer PrO was 119.86 (35.95), colon cancer PoO
138.10 (34.08) U/l, rectum cancer PrO 132.47 (49.81), rectum cancer PoO
107.98 (48.93) U/l, CRC pre-130.819 (49.41) u/l, CRC post-132.11 (32.95)
U/l, polyps 223.08 (27.39) U/l, U.C 156.56 (65.56),
(table-2, fig-3).

Comparison of serum Ck in carcinoma patients and control:
The great activity of serum Ck was significantly shown in colorectal
polyps, this enzyme activities was significantly larger than these in
colorectal cancer (0.0001 < p < 0.003) or healthy control individuals
(p < 0.0002). Otherwise, the enzyme activity shows non significantly
increase than control in patients with U.C (p > 0.1), while it is still
significantly closed to control ranges in colorectal cancer (PrO & PoO)
when compared with normal adjacent values (p > 0.4).

Correlation of serum ALP between carcinoma types and disease:
There was a positive correlation between serum ck in rectum cancer pre and post operation \((r = 0.575)\). Likewise, correlation between serum Ck in polyps and U.C \((r = 0.517)\). On the other hand, a weak positive correlation between rectum cancer (PrO and PoO) with polyps \((r= 0.389\) and \(0.319\) respectively), thus the occurrence of polyps may lead to the development of benign rectum tumor to malignant.

In other hand, there was no correlation of serum Ck between colon cancer (PrO and PoO) with polyps \((r= -0.475\) and \(0.117\) respectively).

Estimation Of Creatine Kinase activity
Zahra’A Salim Muhsin

58

Table-1: The distribution of CK activity in males & females

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of cases</th>
<th>gender</th>
<th>Age (year)</th>
<th>CK mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon CA (PrO)</td>
<td>19</td>
<td>m 11</td>
<td>32 - 60</td>
<td>119.86 (35.95)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>f 8</td>
<td></td>
<td>n.s *</td>
</tr>
<tr>
<td>Colon CA (PoO)</td>
<td>8</td>
<td>m 8</td>
<td>30 - 60</td>
<td>138.10 (34.08)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>f 8</td>
<td></td>
<td>n.s *</td>
</tr>
<tr>
<td>Rectal CA (PrO)</td>
<td>11</td>
<td>m 11</td>
<td>30 - 65</td>
<td>132.47 (49.81)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>f 11</td>
<td></td>
<td>n.s *</td>
</tr>
<tr>
<td>Rectal CA (PoO)</td>
<td>5</td>
<td>m 5</td>
<td>25 - 65</td>
<td>107.98 (48.93)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>f 5</td>
<td></td>
<td>n.s *</td>
</tr>
<tr>
<td>Colorectal CA (PrO)</td>
<td>30</td>
<td>m 22</td>
<td>27 - 65</td>
<td>130.82 (49.41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>f 8</td>
<td></td>
<td>n.s *</td>
</tr>
<tr>
<td>Colorectal CA (PoO)</td>
<td>13</td>
<td>m 13</td>
<td>25 - 65</td>
<td>132.11 (32.85)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>f 13</td>
<td></td>
<td>n.s *</td>
</tr>
<tr>
<td>Colorectal polyps</td>
<td>10</td>
<td>m 10</td>
<td>5 - 15</td>
<td>223.08 (27.39)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>f 10</td>
<td></td>
<td>***s</td>
</tr>
</tbody>
</table>
### Ulcerative colitis

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Gender</th>
<th>Mean (S.D) u/l</th>
<th>Significancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>10</td>
<td>126.18 (40.91)</td>
<td>p&gt;0.5</td>
</tr>
<tr>
<td>Colon CA(PrO)</td>
<td>8</td>
<td>135.74 (37.92)</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>

*n.s : non significant, ***s : p< 0.001

#### Estimation Of Creatine Kinase activity

Zahra` A Salim Muhsin

<table>
<thead>
<tr>
<th>Ck activity U/l (males)</th>
<th>female</th>
<th>male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>220</td>
<td>220</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ck activity U/l (females)</th>
<th>female</th>
<th>male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>220</td>
<td>220</td>
</tr>
</tbody>
</table>

**fig-1**: The level of creatine kinase in control males and females

**fig-2**: The level of Ck in males and females colon cancer (PrO) patients.
Fig. 3: The activity of CK in colorectal cancer (PrO & PoO), colorectal polyps, and ulcerative colitis.

**Discussion**

Creatine kinase is an enzyme still widely analyzed in clinical diagnostics. Although a wealth of CK measurements have been reported in the scientific literature, there still exist inconsistency and incomplete knowledge on such an apparently simple question as the CK enzyme content of mammalian colorectal in both health and disease.

In the present study, we detected the presence of total CK activity in normal control subjects, CRC (pre- and post operation), colorectal polyps, and UC. However, the total CK activity in normal and most colorectal cancer samples that was analyzed in serum, was significantly similarly (p > 0.5), this finding result is lower than data reported for a malignancy of gastrointestinal tract [9] where macro-CK2 isoenzyme was present before operation, and data reported for lung cancer or primary hepatocellular carcinoma [10,21] where CK-MB and mitochondrial CK isoenzymes were strongly increased. But our results were consistently higher than that reported for gastric cancer [22] and colon adenocarcinoma [12] where lower CK activity observed than the normal samples because there is a decrease in the expression of type B- and M-CK subunits.

We additionally observed that:

(i) The mean CK activity did not differ significantly in healthy males and females (p > 0.5).
(ii) The mean CK activity did not differ significantly in males and females (p > 0.05) CRC patients.
(iii) The CK activity in the colorectal polyps patients were significantly higher than that in adjacent control and colorectal cancer (p < 0.02). This maybe due to the predominant increscent of CK-BB fraction in polyps.
(ix) The good positive correlation between Ck in polyps and U.C may indicates that elevation of Ck in one part may be a prognostic signal of other disease cases appearance.

(x) A weak positive correlation between rectum cancer (PrO and PoO) with polyps means; the occurrence of polyps may lead to the development of benign rectum tumor to malignant [14, 16].

(xi) Ulcerative colitis samples revealed non significant increasing Ck activity than control (p>0.1). This result consist with that obtained by Perez C. et.al [24] where the total CK is not elevated, but MB isoenzyme activity was greater in U.C cases, and with that obtained in gastric ulcer patients [22] where total CK was significantly not elevated than control values.

**Conclusion**
The present findings shed light on some old enigmas and opened up fascinating avenues for future research in Iraqi patients. Our findings don’t support significant expression of serum CK in most CRC and U.C, but rather indicate that many of the previous misconceptions in this field can be explained by interference from Ck isoenzymes. On the other hand, give the need for improved the induction of Ck expression in colorectal polyps.

**Estimation Of Creatine Kinase activity**

**Zahra`A Salim Muhsin**

62

**References**


7. Yuu H; diagnostic significance of serum creatine kinase isoenzyme
22. AL_Taei W.A.; PhD thesis; Biochemical studies on Alpha-Fetoprotein (AFP) and some tumor markers in gastric cancer; college of Science; AL_Mustansirayh Univ.; 2000.

PROFLING OF LITHIUM AND POTASSIUM INTO SILICON
Ali M. MOUSA