Faecal Tumour M2-Pyruvate Kinase™ (ScheBo Test) for Colorectal Cancer (CRC) Detection in Adult Egyptians.

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Abstract
This cross-sectional case-control study was carried out on 46 consecutive treatment-naïve patients with sporadic CRC proved by colonoscopy, histopathology and abdominal computed tomography. All cases and 20 matching controls had tumour staging with TNM and Duke's staging systems. Faecal tumour test was assessed in all patients 24 males, 22 females, whose age 50% of them above 50 yrs. Results showed that rectal lesions found during colonoscopy in 47.8% while colonic lesions in 52.2%. Masses were encountered (in 67.4%, 31 cases) and adenocarcinoma was the commonest type (76.1%, 35 cases) followed by mucinous adenocarcinoma (15.2%, 7 cases). The faecal levels of tumour M2-PK™ were significantly higher (P<0.001) in cases compared to controls (35.66 ± 17.32 and 2.74 ± 4.36 U/ml, respectively). Its levels showed a significant positive correlation with both TNM and Duke's stages (p>0.05). Applying ROC curves, at a cut-off value of 3.9 U/ml, faecal tumour M2-PK™ was 97.8% sensitive and 85% specific for detection of CRC with AUROC = 0.96.

In conclusions, faecal tumour M2-PK™ was a sensitive and specific marker for detection of CRC at values ≥ 3.9 U/ml and positively correlated with the tumour grade and stage.

Introduction:
Colorectal cancer (CRC) represents the third most common tumour worldwide and is still burdened by significant morbidity and mortality despite several therapeutic improvements (Gellad and Provenzale, 2010). CRC incidence rates are rapidly increasing due to the effect of many risk factors, including smoking, physical inactivity, obesity, red and processed meat consumption, as well as excessive alcohol intake (Jemal et al., 2011).

Key Words: colorectal cancer (CRC), ScheBo Test, M2-PK™.
In Egypt, CRC is one of the most common malignant neoplasms. Its incidence ranges between 2-6 % of the total number of cancer cases reported annually and it ranks as the sixth most common cancer in both males and females (Zalata et al., 2000 and ZeenEledin et al., 2012). The median age of CRC cases in Egypt is 48 years for both males and females (El-Attar, 2005).

Most cases of CRC develop from polyps. Three types of polyps occur: hamartoma (junior polyp), hyperplastic mucosal proliferation (hyperplastic polyp) and adenomatous polyp. Adenomatous is the only premalignant type, it may be tubular, villous, or tubulovillous, with villous adenomas the most likely to be cancerous. Adenomatous polyps may be either sessile (flat) or pedunculated (stalked), with sessile types being more likely to progress to cancer. Finally, polyps greater than 2.5 cm are five times more likely to be cancerous than those less than 1.5 cm. Overall, once an adenomatous polyp forms, it takes at least 5 years of growth to reach clinical significance, suggesting the need to initiate screening and perform routine follow-up evaluation to identify polyps that are of concern before they become cancerous (Steinberg, 2012).

CRC mortality rates can be decreased by early diagnosis through screening. However, the present CRC screening techniques (colonoscopy, faecal occult blood test (FOBT) and serum carcinoembryonic antigen (CEA) testing are limited by their difficulties and costs beside uncertain or delayed results (Foo et al., 2012). Hence, the identification of biomarkers that are simple, non-invasive, cost-efficient and reasonably sensitive/specific is urgently needed (Xi et al., 2014).

A gold standard for early non-invasive detection of CRC in adult Egyptians is to assess faecal tumour M2-PK™ (ScheBo test) which is a key enzyme within glycolysis, a process that catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate. Depending upon the metabolic functions of the tissues, different isoenzymes of pyruvate kinase are expressed. During tumour formation, the tissue-specific isoenzymes disappear and the pyruvate kinase isoenzyme type M2 is expressed (Gupta and Bamezai, 2010).

The aim of the present study, therefore, was to assess faecal tumour M2-PK™ (ScheBo test) as a marker for CRC detection in adult Egyptian patients.

Patients and Methods:
This cross-sectional, case-control study was carried out on 66 subjects. The cases group comprised 46 consecutive patients with CRC who were attending the Departments of Hepatology, Gastroenterology and Infectious Diseases and Internal Medicine at Benha University Hospital and Surgical Oncology at National Cancer Institute - Cairo, within the period between January and September 2015. Another 20 apparently healthy subjects (without CRC and with normal colonoscopy) served as the control group (age and sex matching the cases group). The indication for colonoscopy in these subjects was either long history of unexplained abdominal pain and/or altered bowel habits. The study protocol was approved by the Ethical Committee of Benha Faculty of Medicine, University of Benha.
Patients with the following criteria were excluded from the study namely: those refusing the written medical consent, pregnant females, patients with familial adenomatous polyposis or hereditary nonpolyposis CRC, those who were undergoing or received chemotherapy and/or radiotherapy, those with inflammatory bowel diseases, primary sclerosing cholangitis and other malignancies. All the studied cases had given an informed written medical consent. They were subjected to thorough history taking, general and abdominal examination as well as per rectal examination, routine laboratory investigations; namely: (stool analysis, complete blood count (CBC), erythrocyte sedimentation rate (ESR), random blood sugar (RBS), glycated haemoglobin (Hb A1C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum albumin, serum bilirubin (total and direct), prothrombin time (PT) and concentration, serum creatinine and blood urea) and carcino-embryonic antigen (CEA) and CA 19-9. Sandwich ELISA with two monoclonal antibodies highly specific for human tumour M2-PK™ (ScheBo®, Biotech Aktiengesellschaft, Germany) was applied for quantitative assessment of faecal tumour M2-PK™. Abdomino-pelvic ultrasonography, computed tomography (CT scan) and complete colonoscopy followed by histopathological examination of the biopsied specimens. Finally, TNM- and Duke's staging systems were applied.

Statistical analysis: was done using SPSS 20. Quantitative data was expressed in Mean ± Standard Deviation, Qualitative data was expressed in number and percentage. Comparison between groups was done using Mann-Whitney U and Kruss-Kal Wallis test. The sensitivity and specificity were examined at different cutoff points using ROC curve analysis to determine the best cutoff point as well as the diagnostic power of each test. A P value <0.05 was considered statistically significant (S).

Results:

This study evaluated 46 patients with CRC (24 males and 22 females) with mean age of 50.6 yrs and range 22-81 yrs. The 20 healthy controls were 13 males and 7 females with mean age of 54.1 yrs and range of 27-80 yrs. The mean age of male patients (50.3 yrs) was relatively (non significantly) younger than that of female patients (51.1 yrs). Distribution of age among the studied cases showed 26% below 40 yrs and 50% above 50 yrs.

Results are shown in tables 1 - 3 and figures 1 - 8.

Fig. (1): Age distribution within the studied cases.
Bleeding per rectum was the main presenting symptom followed by recent onset constipation in the studied cases. Positive family history (1st or 2nd degree relatives) was found in 21.7% of the studied cases while anemia leading to blood transfusion was found in 17.4% of them.

The faecal levels of tumour M2-PK™ were significantly higher (P<0.001) in the studied cases compared to the control group (35.66 ± 17.32 and 2.74 ± 4.36 U/ml respectively). The median faecal tumour M2-PK™ level in the control group was 0.7 U/ml and ranged from 0.02 U/ml to 17.4 U/ml. The median faecal tumour M2-PK™ level in cases was 36 U/ml and ranged from 0.3 U/ml to 81 U/ml.
Table (1): Faecal M2PK in Diabetes and Smoking.

<table>
<thead>
<tr>
<th>Diabetes</th>
<th>No</th>
<th>Mean</th>
<th>S.D</th>
<th>Mann- Whitney U</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>22</td>
<td>29.06</td>
<td>16.44</td>
<td>2.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>24</td>
<td>41.13</td>
<td>16.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>26</td>
<td>34.79</td>
<td>20.04</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>20</td>
<td>37.09</td>
<td>13.15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was a statistically significant difference in faecal M2PK level between diabetics which is significantly lower (29.06 ± 16.44 U/ml) than nondiabetic (41.13 ± 16.69 U/ml) (p= 0.01) while a non significant difference was found between smokers (34.79 ± 20.04 U/ml) and non-smokers (37.09± 13.15 U/ml) (p-value 0.6).

There was a statistically significant positive correlation between faecal M2PK level and BMI in the studied cases (p-value = 0.004).

There was no statistically significant difference in faecal Tumour M2-PK™ levels as regards site, morphology and pathological types of CRC, while there was a statistically significant rise in faecal Tumour M2-PK™ levels as regards pathological grades. The mean value of grade I was (6.25 U/ml), grade II (33.79 U/ml) and grade III (47.9U/ml) (P< 0.05). The higher the pathological grade of CRC the higher the mean value of Tumour M2-PK™.

Fig (5): Mean value of Faecal Tumour M2-PK™ according to colonoscopic and histopathological findings.
Table (2) and Fig (6): Faecal Tumour M2-PK™ levels in different TNM and Dukes stages.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>Tumour M2-PK™ (U/ml)</th>
<th>KWT test</th>
<th>P</th>
<th>Sig pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Range</td>
<td>6.3</td>
<td>6.5-51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tis/T1</td>
<td>3</td>
<td>11.80 ±10.88</td>
<td>4.5-51</td>
<td>0.0</td>
<td>10.7</td>
</tr>
<tr>
<td>T2</td>
<td>18</td>
<td>31.87 ±19.72</td>
<td>4.5-51</td>
<td>0.0</td>
<td>10.7</td>
</tr>
<tr>
<td>T3</td>
<td>23</td>
<td>49.23 ± 17.64</td>
<td>0.5-91</td>
<td>0.0</td>
<td>10.7</td>
</tr>
<tr>
<td>T4</td>
<td>2</td>
<td>59.00 ± 38.80</td>
<td>4.5-69</td>
<td>0.0</td>
<td>10.7</td>
</tr>
<tr>
<td>N stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>26</td>
<td>30.40 ±14.03</td>
<td>0.3-54</td>
<td>0.0</td>
<td>10.7</td>
</tr>
<tr>
<td>N1</td>
<td>11</td>
<td>38.80 ±15.73</td>
<td>4.5-65</td>
<td>0.0</td>
<td>10.7</td>
</tr>
<tr>
<td>N2</td>
<td>0</td>
<td>52.32 ± 17.30</td>
<td>30.9-91</td>
<td>0.0</td>
<td>10.7</td>
</tr>
<tr>
<td>Duke A</td>
<td>3</td>
<td>12.70 ±19.48</td>
<td>4.5-51</td>
<td>0.0</td>
<td>10.7</td>
</tr>
<tr>
<td>Duke B</td>
<td>24</td>
<td>33.33 ±18.47</td>
<td>0.5-91</td>
<td>0.0</td>
<td>10.7</td>
</tr>
<tr>
<td>Duke C</td>
<td>18</td>
<td>49.23 ± 17.64</td>
<td>4.5-91</td>
<td>0.0</td>
<td>10.7</td>
</tr>
</tbody>
</table>

There was a statistically significant difference in faecal Tumour M2-PK™ levels in different TNM Stages. There was also a significant difference in faecal Tumour M2-PK™ levels in different Dukes stages. (P <0.05).

Table (3) and Fig. (7): ROC curve for the performance of Tumour M2-PK™ in detection of CRC.

<table>
<thead>
<tr>
<th>Tumour M2-PK™ (U/ml)</th>
<th>Sens %</th>
<th>Spec %</th>
<th>P</th>
<th>NPV %</th>
<th>AUC</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥3.9</td>
<td>97.8%</td>
<td>85%</td>
<td>0.0</td>
<td>94.4%</td>
<td>0.96</td>
<td>0.92</td>
<td>&lt;0.01 (HS)</td>
</tr>
</tbody>
</table>

At a cut-off value of 3.9 U/ml, faecal Tumour M2-PK™ was 97.8% sensitive and 85% specific for detection of CRC with AUROC = 0.96.

Fig (8) and Table (4): ROC curve for the performance of different markers in detection of CRC.

<table>
<thead>
<tr>
<th>Tumour markers</th>
<th>AUROC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA</td>
<td>0.871</td>
</tr>
<tr>
<td>CA19-9</td>
<td>0.543</td>
</tr>
<tr>
<td>ScheBo (Tumour M2-PK™) (U/ml)</td>
<td>0.971</td>
</tr>
</tbody>
</table>

ROC curve reveals that Tumour M2-PK™ was better than CEA and CA 19.9 in detection of CRC at any stage.
Discussion:

In this study, the age of the studied cases with CRC ranged between 22-81 yrs, with a mean of 50.6 ± 15.1 yrs. Fifty percent were > 50 yrs and 26% (more than 1/4 of patients) were ≤ 40 yrs. This finding comes in agreement with Said et al., (2013) and Gado et al., (2014) who found that the mean age of their assessed Egyptian patients was 51 yrs and 44.8 yrs respectively. El Attar, (2005) reported that the median age of CRC patients in Egypt was 48 yrs. On the other hand; Yi et al., (2013) indicated that the mean age of their assessed American Non-Hispanic white and American Asian patients was 70.3 yrs and 66.3 yrs respectively while Tonus et al., (2006) wrote that the median age of CRC cases was 70 years in Germany.

About 1/4 of the assessed cases in the current study were below the age of 40 yrs. This comes in agreement with Soliman et al., (1999), Abou-Zeid et al., (2002), Veruttipong et al., (2012) and Gado et al., (2014) who observed that CRC cases under the age of 40 yrs were 35.6%, 25%, 22%, 38% and one third respectively in their studied Egyptian patients. In Asia, Rojas-Puentes and his colleagues, (2014) reported that 19.5-28.6% of patients were less than 40 yrs and in Mexico, National Cancer Institute who reported 22.8%. On the contrary, Howlader et al., (2011) in USA, mentioned that CRC is rare before age 40 yrs in both men and women. Khuhaprema and Srivatanakul, (2008) suggested that CRC is rare before the age of 40 except for those with genetic predisposition or predisposing conditions and Rojas-Puentes et al., (2014) mentioned that the frequencies of CRC in patients less than 40 yrs in USA, France, Australia and Denmark were 6-7%, 3.1%, 5% and 2.5% respectively. The difference in mean ages could be attributed to different risk factors, dietary patterns, life style and life expectancy in different cultures and societies. This finding may enhance the need for early screening for CRC among the Egyptian population.

Males represented 52.2% (and females, 47.8%) of the studied cases in the present study, with no statistically significant difference (p > 0.05). This comes in agreement with El-Bolkainy et al., (2006), Zakaria et al., (2006) and Abotchie et al., (2012) who told that CRC affects men and women almost equally. In disagreement with this, Rim et al., (2009) and Murphy et al., (2011) emphasize that men have more incidence of CRC than women.

Here, The percentage of the studied cases with CRC with a positive family history was 21.7%. This comes in agreement with Chan et al., (2008) and Haggar and Boushey, (2009) who told a percentage of up to 20%. Fatemi et al., (2010) reported a higher figure (31.3%) in Iranian patients. These data make adherence to CRC screening programs mandatory in subjects with family history of CRC.

In the present study, bleeding per rectum was the commonest presenting symptom (39.1%) followed by recent onset constipation (34.8%) which is concomitant with the local reports of
Zakaria et al., (2010) who met bleeding per rectum as the main presenting symptom of their studied cases.

Mass was the commonest gross pathology (67.4 %) detected in the studied cases followed by ulcer 28.3 % and stricture 4.3 %. This comes in agreement with Zakaria et al., (2006) reported that fungating mass was the main colonoscopic picture in 86.2 % of cases.

In the present study, as regards the tumours site, rectal carcinoma (47.8 %) was nearly equal to colonic (52.2 %). This agrees with Kenawi et al., (1990) and Soliman et al., (1999) who stated that about half of their studied cases were rectal lesions. On the other hand, Yi et al., (2013) reported a lower incidence of rectal lesions (25.5 - 30.5 %) compared to colonic, while Khafagy and his colleagues, (2000) reported that rectal carcinoma was more common (68 %) than colonic ones.

Upon histopathological examination, most of the studied cases in the present study were adenocarcinoma (76.1 %), mucinous and signet ring adenocarcinomas constituted only 21.7 %. This comes in agreement with Khafagy et al., (2000) who wrote that histologic tumour type was predominantly adenocarcinoma (78.5 %) while the more aggressive mucinous and signet ring adenocarcinomas constituted only (21.5 %)of their studied cases. This was also in agreement with Hamilton et al., (2010), Veruttipong et al., (2012) and Said et al., (2013).

In the current study, the faecal levels of tumour M2-PK™ were significantly higher (P < 0.001) in the studied cases with CRC (35.66 U/ml) than in the control group (2.74 U/ml). This was in agreement with, Hardt et al., (2004), Tonus et al., (2006), Koss et al., (2008), and Parente et al., (2012). The median faecal tumour M2-PK™ level in the control group was 0.7 U/ml (range 0.02 - 17.4 U/ml) and in the studied cases was 36 U/ml (range 0.3 - 81U/ml). These findings come close to that of Shastri et al., (2006), Tonus et al., (2006) and Koss et al., (2008) who suggested that the median faecal tumour M2-PK™ level in the control group was 0.8, 2.10 and 1.75 U/ml, respectively; and the median level in CRC patients was 14.7, 22 and 11.72 U/ml, respectively.

In the present study, at a cut-off value of 3.9 U/ml, faecal tumour M2-PK™ was 97.5 % sensitive and 85 % specific for diagnosis of CRC with an AUROC = 0.96. Sithambaram et al., (2015) reported that M2PK had a sensitivity ranging from (73 – 97 %) and specificity from (78.6 – 100 %). Tonus et al., (2006) emphasize that the high sensitivity of the tumour M2-PK™ test is due to its ability to detect bleeding and non-bleeding tumours. From a practical point of view, the use of a single random formed stool sample for tumour M2-PK™ analysis, without requiring dietary restrictions, might be of greater patient convenience.

The present study found that faecal tumour M2PK ≥ 36.5 U/ml could significantly discriminate cases with grade III adenocarcinoma with 90 % sensitivity and 63.9 % specificity, with AUROC = 0.76, while at level ≥ 25.7 U/ml, it could significantly discriminate cases with Duke stage >
A with 83.7 % sensitivity and 100 % specificity, with AUROC = 0.89. At level ≥ 35.5 U/ml it could significantly discriminate cases > T2 with 68% sensitivity and 61.9% specificity, with AUROC = 0.71, and at level ≥ 50 U/ml it could significantly discriminate cases > N1 with 75 % sensitivity and 89.2 % specificity, with AUROC = 0.83. This is in agreement with Tonus et al., (2006), Koss et al., (2008).

Faecal tumour M2-PK™ level showed a statistically significant positive correlation with the pathological grades. The mean value of grade I was (6.25 U/ml), grade II (33.79 U/ml) and grade III (47.9 U/ml) (P < 0.05). This was in agreement with Hardt et al., (2004) and Tonus et al., (2006).

Also, in the present study, the mean value of faecal tumour M2-PK™ level showed a statistically significant difference between different TNM stages (P < 0.05) with a statistically significant positive correlation. The mean values in T1, T2, T3 and T4 were 11.80, 31.87, 40.23 and 53 U/ml, respectively. This comes in agreement with Hardt et al., (2004) who found the mean value of T1, T2, T3 and T4 were 11.1, 23.8, 55.5 and 132.7 U/ml, respectively.

The mean value of faecal tumour M2-PK™ level showed a statistically significant difference between different Duke's stages (P < 0.05) in the present study with a statistically significant positive correlation. The mean values in Duke's A, B and C were 12.7, 33.33 and 42.23 U/ml, respectively. This was similar to Hardt et al., (2004) who found the mean value of Duke's A, B and C were 19.4, 31.5 and 57.1 U/ml, respectively.

In the current study, there was a statistically significant correlation between Tumour M2-PK™ level with BMI and diabetes mellitus. M2-PK™ levels were significantly lower in diabetic patients than non-diabetics but no significant correlation with smoking. This was in agreement with Schneider et al. (2000).

In addition the current study showed that no statistically significant correlation was found between Tumour M2-PK™ level and either age or sex. This comes in agreement with Koss et al., (2008) who assessed 32 CRC patients with a median age 66 Yrs (male to female ratio was 3:1) with no association between faecal tumour M2-PK™ level and patients’ age or sex.

In the present study, there was a statistically highly significant positive correlation between tumour M2-PK™ level and serum albumin in the studied cases (r = -0.33 and P < 0.05). This is in agreement with Hamilton et al., (2010).

In the present study, there was a statistically highly significant positive correlation between tumour M2-PK™ level and both serum CEA and CA19-9 levels. However, faecal M2-PK™ was more sensitive than CEA and CA19.9 in detection of CRC. This comes in agree with Hardt et al., 2000 who suggested that when other biomarkers such as CA19-9, CEA and M2-PK™ were compared, the sensitivity of M2-PK™ was 70% showing the best results in CRC with a higher sensitivity when compared with CEA and CA19-9. They also
concluded that M2-PK™ should be used in combination with CEA to increase the sensitivity. Also this is in agreement with Liu et al., (2014) who told that the pooled sensitivity of CEA for diagnosis of CRC was only 46 % and the specificity was 89 %. In conclusion the present work showed that is important in early detection of CRC.

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