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Diagnostic and prognostic value of tumor M2-pyruvate kinase levels in patients with colorectal cancer

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Background/aims: *Screening for precancerous lesions is important for the diagnosis and treatment of colorectal tumors. We investigated M2-pyruvate kinase levels in patients with colorectal polyps and carcinoma and assessed factors affecting M2-pyruvate kinase levels.* ***Materials and Methods:*** *Eighty-five patients who had undergone colonoscopic examination and who were diagnosed with a neoplastic lesion were included. Patients were divided into two groups according to the macroscopic diagnosis of polyp or carcinoma. According to histopathological evaluation, specimens were grouped as nonneoplastic lesions, tubular adenoma, tubulovillous adenoma and adenocarcinoma. M2-pyruvate kinase levels were measured with the Tumor M2-pyruvate kinase ELISA kit.* ***Results:*** *Mean M2-pyruvate kinase levels were 76.1±57.73 (13.1-288.22) IU/ml. We did not find a correlation between M2-pyruvate kinase levels and age, gender, smoking, alcohol and aspirin consumption and colorectal cancer family history. There was a relationship between body mass index and M2-pyruvate kinase level (p=0.022). The carcinoma group had the highest levels of M2-pyruvate kinase both endoscopically and histopathologically (p=0.009, p=0.019 respectively). M2-pyruvate kinase levels of patients who died were significantly higher than patients who survived (p=0.001). Enzyme values were significantly lower in diabetic patients than nondiabetics (p=0.04); and chronic renal failure patients had higher levels (p=0.045).* ***Conclusion:*** *Serum M2-pyruvate kinase levels may be useful in distinguishing malignant and benign lesions of the colon and may provide insight in terms of survival.*

Key words: Colorectal cancer, tumor M2- pyruvate kinase, diabetes mellitus, coronary heart disease, chronic renal failure

Kolorektal kanserli hastalarda tümör M2-pirüvat kinaz düzeyinin tanısal ve prognostik değeri

Amaç: *Amaç: Kolorektal tümörlerin tanı ve tedavisinde prekanseröz lezyonların taranması önemlidir. Biz kolorektal polip ve karsinomlu hastalarda M2-pirüvat kinaz düzeyini ve bu düzeyi etkileyen faktörleri incelemeyi amaçladık.* ***Gereç ve Yöntem:*** *Kolonoskopik inceleme yapılarak neoplastik lezyon tanısı konan 85 hasta çalışmaya alındı. Hastalar makroskopik tanılarına göre polip ve karsinom olarak iki gruba ayrıldı. Histopatolojik incelemeye göre biyopsi örnekleri nonneoplastik lezyon, tübüler adenom, tübülovillöz adenom ve adenokarsinom olarak gruplandı. M2-pirüvat kinaz düzeyleri tümör M2-pirüvat kinaz ELIZA kiti ile ölçüldü.* ***Bulgular:*** *Ortalama M2-pirüvat kinaz düzeyi 76.1±57.73 (13.1-288.22) IU/mL idi. Yaş, cinsiyet, sigara ve içki kullanımı, aspirin tüketimi, ailede kolorektal kanser öyküsü ile M2-pirüvat kinaz düzeyleri arasında korelasyon bulunmadı. Vücut kitle indeksi ile M2-pirüvat kinaz düzeyi arasında ilişki gözlendi (p=0.022). Karsinom grubu gerek endoskopik gerekse histopatolojik olarak en yüksek enzim değerlerine sahipti (sırasıyla: p=0.009, p=0.019). M2-pirüvat kinaz düzeyleri bir yıl içinde ölen hastalarda yaşayanlara göre daha yüksekti (p=0.001). Diyabetik hastalarda nondiyabetiklere göre anlamlı olarak daha düşük (p=0.04); kronik böbrek hastalarında ise daha yüksek değerler gözlendi (p=0.045).* ***Sonuç:*** *Serum M2-pirüvat kinaz düzeylerinin kolonun malign ve benign lezyonların ayırt edilmesinde yararlı olduğunu ve sürvi açısından öngörü sağlayabileceğini düşünüyoruz.*

Anahtar kelimeler: Kolorektal kanser, tümör M2-pirüvat kinaz, diabetes mellitus, koroner kalp hastalığı, kronik börek yetmezliği

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INTRODUCTION

Colorectal cancer (CRC) is one of the most prominent causes of cancer related morbidity and mortality (1). It is generally accepted that most CRC originate within previously benign adenomas (2); therefore early detection and removal of lesions is of crucial importance. Approximately 92% of colon cancer patients undergo surgical resection, which is the primary treatment (1). As CRC may be prevented by detection and removal of precancerous lesions, screening is paramount in reducing disease burden (3).

Although the gold standard for the early detection of colorectal cancer is colonoscopy; the acceptance of this costly and invasive method is low even in well-developed countries such as Germany. For an effective cancer screening programme, an easy, fast and economic screening technique is necessary. The guaiac- based fecal occult blood test (FOBT) is the most widely used test for colorectal cancer screening. Despite its low sensitivity, it has been shown to be cost-effective for screening. Although newer immunological FOBT tests have shown higher sensitivities, nonbleeding tumors cannot be detected by this method (4,5).

Biomarkers of gastrointestinal cancer are used for the purpose of cancer screening, diagnosis and post-treatment surveillance. There are some genetic and protein based markers available in diagnostic medicine. One of these markers is tumor M2- pyruvate kinase. The glycolytic isoenzyme pyruvate kinase type M2 (M2-PK) plays a major role in tumor cells and is expressed in human tumor tissues. The tissue-specific izoenymes are replaced by M2-PK during the neoplastic process. In tumor cells there is a shift from the tetrameric form to a nearly inactive dimeric form. This predominantly raised M2-PK level also correlates with the presence of metastases (6).

Tumor M2-PK has been investigated by many groups as a marker of cancer since the 2000s. We aimed to evaluate the diagnostic importance of plasma M2-PK in clinical practice. For this purpose, we assessed plasma tumor M2-PK levels along the adenoma-carcinoma sequence and evaluated its levels in comobidities such as diabetes mellitus (DM), coronary heart disease (CHD), chronic renal failure (CRF).

MATERIALS and METHODS

In this study, 85 patients with an endoscopically pathological lesion who had undergone colonoscopic examination were included. Patients who had a second malignancy, rheumatic disease, inflammatory bowel disease, sepsis, a history of trauma, and patients who were unable to cooperate or who were in poor general health were excluded. Age, gender, body mass index (BMI), alcohol and smoking history, aspirin consumption, comorbidities such as DM, CHD, CRF, survival after one year of colonoscopy, family history of CRC, endoscopic diagnosis and histopathological diagnosis were recorded. The study was approved by the Ethical Committee on Human Research of Istanbul Education and Research Hospital. Written informed consent was obtained from all subjects.

All colonoscopic procedures were performed in our endoscopy unit with Fujinon EC-450WL5. Biopsy specimens were fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Tissue sections were prepared at 4 µm thickness and stained with H&E. All microscopic slides were reviewed by an experienced pathologist. The adenomas were classified as tubular, tubulovillous, villous type and categorized according to the presence of low-grade and high-grade dysplasia.

Blood was drawn after 12 -14 hours of fasting in the morning from each patient into uncoated serum tubes (3 ml) and ethylenediaminetetraacetic acid (EDTA)-containing plasma tubes (3 ml), respectively. After a resting period of at least 30 min and a maximum of 60 min, the tubes were centrifugated at 2500G for 10 min, after which the plasma was extracted. Serum and plasma samples were stored at -80 °C until the assay was performed. All icteric or hemolytic blood samples were discarded.

Measurement of plasma M2-PK activity

For determination of this metabolic marker, an ELISA with monoclonal antibodies which specifically recognize the dimeric form of tumor M2-PK has been developed (Tumor M2-PK ELISA kit, Catalog No: 08 ScheBo® • Biotech AG, Giessen, Germany).The test kit requires 50 µL of 1:100 diluted plasma per sample and all tests were performed in duplicate according to the manufacturer's instructions. The cut-off value suggested by the manufacturer was 15 U/ml with values of 15-20 U/ml classified as being in the gray zone. The standard curve revealed linearity for M2-PK concentrations between 5 and 100 U/ml. The coefficients of intra- and inter-assay variations for M2-PK activity were 4.3% (n = 10) and 5.1 % (n = 10), respectively.

Statistical analysis

Statistical analysis was performed with the NCSS 2007 package programme. Apart from descriptive statistic methods (mean, standard deviation) one-way variance analyses was used for between group comparisons and Tukey test for sub-group comparisons. We used t test and Chi-squared test for quantitative comparisons. P<0.05 was considered statistically significant.

RESULTS

Patients' characteristics are described in Table 1. Our study group consisted of 25 female and 60 male subjects. Mean age was 62.7 (33-87) years. No patient in our group was diagnosed as pure villous adenoma histopathologically. Mean M2-PK level was 76.1±57.73 (13.1-288.22) IU/ml. There was no correlation between M2-PK levels and age, gender, smoking, alcohol and aspirin consumption and colorectal cancer family history. There was a relationship between BMI and M2-PK level (p=0.022).

M2-PK levels were significantly lower in diabetic patients than non-diabetics (p=0.04). Patients with CRF had higher levels (p=0.045). No difference was found between patients with ischemic heart disease and patients without ischemic heart disease (p=0.643) (Table 2).

M2-PK levels of the patients who died were significantly higher than patients who survived (p=0.001) (Table 3).

As noted in Table 4 and Table 5, both endoscopically and histopathologically, the carcinoma group had the highest levels of M2-PK (p=0.009, p=0.019 respectively).

The carcinoma group had significantly higher levels of M2-PK than the groups with tubular adenoma and tubulovillous adenoma (p=0.003, p=0.044); but there was no significant difference between other groups (p> 0.05).

Colonoscopic examination revealed the presence of polyps in 45 patients and the presence of carcinoma in 40 patients. There was a significant difference between these groups with regard to M2-PK levels (p=0.009). The area under the curve was 0.664 and the cut-off value was 108 IU/ml. The sensitivity was 35% and specifity was 99.33%.

DISCUSSION

For the early detection of adenoma and colorectal carcinoma, M2-PK levels can be measured by ELISA in fecal specimens (4) and in serum or tumor homogenates (7).

Table 1. Characteristics of patients.

		n	%
Gender	Female	25	29.4
	Male	60	70.6
Endoscopy	Polyp	45	52.9
	Carcinoma	40	47.1
	Nonneoplastic lesion	7	8.2
	Tubular adenoma	33	38.8
Pathology	Tubulovillous adenoma	9	10.6
	Carcinoma	36	42.4
Smoking	(-)	46	54.1
	(+)	39	45.9
Alcohol	(-)	73	85.9
	(+)	12	14.1
Family history	(-)	65	76.5
	(+)	20	23.5
DM	(-)	69	81.2
	(+)	16	18.8
CHD	(-)	66	77.6
	(+)	19	22.4
CRF	(-)	78	91.8
	(+)	7	8.2
Mortality	Survivor	77	90.6
	Exitus	8	9.4
Aspirin	(-)	58	68.2
	(+)	27	31.8

DM: Diabetes mellitus. CHD: Coronary heart disease. CRF: Chronic renal failure.

Table 2. M2-PK and comorbidity.

		M2-PK U/ml Mean±SS	M2-PK U/ml Median (IQR)	p
DM	(-)	79.63±58.53	57.49 (42.37-100.42)	0.04
	(+)	60.86±53.22	38.71 (32.61-80.6)	
CHD	(-)	69.67±43.91	54.2 (37.49-89.07)	0.643
	(+)	98.43±88.97	56.02 (35.29-138.22)	
CRF	(-)	71.5±52.17	52.13 (36.58-89.01)	0.045

DM: Diabetes mellitus. CHD: Coronary heart disease. CRF: Chronic renal failure.

Table 3. M2-PK and mortality.

Mortality	M2-PK U/ml Mean±SS	M2-PK U/ml Median (IQR)	P
Survivor	68,01±50,54	50,66 (35,9-80,66)	
Exitus	153,95±67,96	146,15 (114,8-219,5)	**0,001**

Table 4. Endoscopic diagnosis and M2-PK levels.

Endoscopy	M2-PK U/ml Mean±SS	M2-PK U/ml Median (IQR)	P
Polyp	58,33±35,74	49,44 (35,17-69,56)	
Carcinoma	96,09±70,46	60,78 (41,08-141,51)	**0,009**

Table 5. Histopathology and M2-PK levels.

Pathology	M2-PK U/ml Mean±SS	Median (IQR)	p
Nonneoplastic lesion	72,82±39,31	59,93 (49,44-104,07)	
Tubular adenoma	52,76±27,26	46,27 (34,93-62,86)	
Tubulovillous adenoma	55,07±23,83	49,44 (39,31-67,24)	
Carcinoma	103,39±74,36	77,37 (39,68-149,07)	**0,019**

Oremek at al. showed that serum levels of M2-PK were higher than levels in plasma. Hemolytic, icteric and lypemic materials showed similar higher results. Rheumatic disease and nephropathy also influence M2-PK concentrations (8). Additionally, lymphocytes contain small amount of tumor M2-PK. As they might release this enzyme in heparin-plasma and serum, EDTA- plasma is the most appropriate sample for obtaining the diagnosis of this tumor marker (9). We measured enzyme levels in EDTA-plasma samples.

We excluded patients who had a second malignancy, rheumatic disease, inflammatory bowel disease, sepsis or history of trauma which could affect our results. Eigenbrodt et al. measured M2-PK content in the serum of 666 healthy subjects. The values for the 50% percentiles (median) of blood donors were 10.8 U/ml and 55.0 U/ml for colon carcinoma (7). Although there are inter-study variations in cut-off points, elevated levels correspond to the extent of disease; plasma and stool M2-PK appears to be a promising test for colon cancer (6). Kim et al. accepted the cut- off level as 20 U/mL using an ELISA assay (10). Kumar et al. used cut of levels as 15 IU/mL for M2-PK by electrochemiluminesence immunoassay in patients with colorectal metastases undergoing liver resection (11). In our study, the mean M2-PK level was 76.1+57.73 (13.1-288.22) IU/ml. Although patients with carcinoma had higher enzyme levels, there were overlapping values between groups. Kumar et al. also showed that age and gender did not influence the levels as in our study in contrast to previous research showing higher fecal tumor M2-PK levels in older patients (12). We accepted 20 U/ml as the upper limit as shown in the manufacturers kit. But we do not have a control group of healthy subjects. This is one of the limitations of our study.

There was no relation between smoking, alcohol and tumor M2-PK levels in our research. There are some studies which suggest a positive correlation between adenoma and colorectal cancer risk and smoking and alcohol use (13-17). We did not observe a difference with regard to adenoma or carcinoma in current smokers or nonsmokers. We did not either observe a relation with alcohol consumption. As our groups were small, we did not divide them to subgroups to evaluate the dose-response relationship according to pack-years of smoking; and according to the history of quantity and frequency of alcohol consumed.

The risk of CRC in first degree relatives is increased two to three-fold (5). We did not find such a relationship in accordance with Haug's study (3).

Higher tumor M2-PK levels were found in patients with higher BMI. The relationship between obesity and carcinogenesis is well known (16,18-20). Number and size of adenomas were found to correlate with visceral fat accumulation (21). In our study we observed a relationship between BMI and M2-PK levels; but there was no relation between obesity and histopathological evaluation. Haug et al. did not observe a relationship between fecal M2-PK levels and BMI (3).

Hathurusinghe et al. reported cut-off plasma levels for tumor M2-PK ranging from 15 to 19.8 U/ml; when ROC curves were provided, the AUC value was 0.807 in patients with colorectal cancer without distant metastases. They also observed that levels of M2-PK were significantly elevated in colorectal cancer patients correlating to Duke's stage (6). We did not evaluate the relationship of M2-PK level and Duke's staging in our study. When control subjects, patients with adenoma and CRC were compared, Hardt et al. (22) showed that fecal tumor M2-PK levels did not rise in patients with adenoma as much as in CRC patients. In our study colonoscopic examination revealed that 45 patients had polyps and 40 patients had carcinoma. There was a significant difference between these groups with regard to M2-PK levels. (p=0.009). The area under curve was 0.664 and cut-off value was 108 IU/ml. The sensitivity was 35% and specifity was 99.33%. This means that the probability of having CRC is 5.25 times higher

in a patient with M2 –PK levels above 108 IU/ml. When we analyzed each patient individually, we observed 6 patients in whom malignancy was confirmed histopathologically despite a lack of diagnosis of carcinoma by colonoscopic evaluation. Similarly, 2 patients were histopathologically diagnosed with carcinoma among 45 patients with colonoscopically diagnosed polyps. These 6 patients with a non-malignancy diagnosis had lower M2-PK values than 108 IU/ml. One patient with biopsy-proven carcinoma had an M2-PK level of 194 IU/ml, the other patient had DM, and the enzyme level was 37 IU/ml. We also observed lower M2-PK values in other patients with DM. We think that M2-PK levels in diabetic patients should be interpreted more cautiously. Many studies indicate that type -2 diabetic patients have an increased risk for developing cancer (15,16,18,23-25). We could not find a relation between diabetes mellitus and histopathological diagnosis. Eight of our diabetic patients were diagnosed as having CRC and six of them had values under our cut-off levels (<108 IU/ml of malignancy of our results; but > 20 IU/ml of normal subjects); whereas 2 patients had higher enzyme levels consistent with other studies showing association between malignancy and altered M2-PK levels. One of the major differences observed between cancer cells and normal cells is in how they metabolize glucose; most cancer cells primarily metabolize glucose by glycolysis whereas most normal cells completely catabolize glucose by oxidative phosphorylation (26). This shift to aerobic glycolysis with lactate production (also known as the Warburg effect), coupled with increased glucose uptake is likely used by proliferating cells to promote the efficient conversion of glucose into macromolecules required for the construction of a new cell (27). The glycolytic enzyme pyruvate kinase is alternatively spliced to produce either the M1 (PKM1) or M2 (PKM2) isoform (28). The splice-isoform of pyruvate kinase expressed in cells influences the extent to which glucose is metabolized either by aerobic glycolysis or oxidative phosphorylation (26-28). We suggest that unexpectedly lower levels may be explained by this metabolic difference.

Oremek et al. (29) measured high false-positive levels in patients with diabetic nephropathy over the cut-off 25 IU/ml. Seven of our patients had CRF. These patients had significantly higher levels. We think this may be due to impaired clearance of the enzyme. Diabetes may also increase these levels. We could not find any other research that has previously addressed this issue.

Quantitative detection of tumor M2-PK may serve as a valuable tumor marker for lung cancer (30), hematological malignancies and solid tumors; but it should be kept in mind that a number of false positive results may be detected in healty individuals (31). Increased levels of M2-PK may be measured in many different tumors. When other biomarkers such as CA19-9, carcinoembryonic antigen (CEA) and M2-PK were compared, the sensitivity of M2-PK was 70% for GIS tumors. But M2-PK showed the best results in colorectal carcinoma (32). Schulze et al. reported a diagnostic specifity of 89% with a higher sensitivity when compared with CEA, CA19-9 and CA72-4. They also concluded that M2-PK should be used in combination with CEA to increase the sensitivity (33). The combined use of M2-PK and CEA increases the sensitivity but adding a third marker does not improve it and cannot be recommended with respect to costs (34). We could measure CEA levels in only 13 patients. In this subpopulation mean M2-PK and CEA levels were 90.53±73.69 IU/mL, and 15.23±44.46 ng/mL respectively. We did not evaluate other biomarkers. We did not find a correlation between M2-PK and CEA levels r=0.37, p=0.26). This may be due to the limited number of patients and also due to a high standard deviation. Shastri et al. suggested that at a cut-off value of 4 U/mL, fecal M2-PK test was found a poor screening marker for CRC (35); Ewald et al. observed that M2-PK is better for detecting colorectal cancer and large adenomas, compared to FOBT or genetic testing; they also measured high levels in acute and chronic inflammatory bowel diseases (36).

A number of studies have shown that the level of the enzyme increases with tumor size, stage and metastatic potential (4,6,37). A study conducted by Koss at al. also showed that M2-PK levels fell significantly after surgery (37). Kumar et al. could not find an association with enzyme levels and tumor stage (11). We did not evaluate the data with regard to tumor size, stage and presence of metastases in patients. We observed that M2-PK levels were significantly associated with one-year mortality (p=0.001). The cut-off level for this relationship was 111.15 with a sensitivity 87.5% and a specificity of 88.31%. The probabilty of dying in the first year was 7.49 times higher for a patient with M2-PK levels above 111.15 IU/ml. Eight of 85 patients in our group died, all of whom had carcino-

ma histopathologically, at the end of one year. M2-PK levels were significantly higher in these subjects than in survivors. We could not find any previous research that addressed this issue.

The data on the relationship between aspirin use and colorectal neoplasias is conflicting. Aspirin may play a role in decreasing the development of adenomatous polyps whereas there is no evidence of reduction in the incidence of colorectal cancer (38). There are some other studies showing that prolonged use of aspirin may reduce the risk of gastrointestinal cancer and recurrence of adenoma (39-41). Benamouzig et al. reported a decrease in adenoma recurrence at one year despite no differences being found at four years (42). In our study, 27 patients used aspirin 100 mg daily for cardiac protection. 17 of them were diagnosed carcinoma histopathologically. We did not find a relationship with M2-PK levels and aspirin consumption. We could not obtain a detailed history about the duration of aspirin use. This may also be a limitation of our study.

In conclusion, screening tests are important for the early diagnosis of colorectal cancer. Plasma M2-PK levels may be used as a screening bio-marker and also for surveillance. We observed that tumor M2-PK values are useful in differentiating malignant and benign lesions, and that M2-PK levels correlate with mortality. Easy use and high specifity of the test may potentiate its routine clinical use in the future.

Conflict of Interests

None of the authors have conflict of interests, disclosures, or funding sources related to this paper.

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