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Evaluation of the Pyruvate Kinase isoenzyme tumor (Tu M2-PK) as a tumor marker for cervical carcinoma

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Abstract

Various isoforms of the glycolytic enzyme pyruvate kinase are expressed in different cell types. One of these isoforms, Tu M2-PK, is over-expressed in tumor cells and released into body fluids. Plasma determination of Tu M2-PK has been shown to discriminate between benign and malignant lesions. Tu M2-PK was quantitated in the plasma of 50 patients with cervical carcinoma, 10 patients with chronic cervicitis and 10 healthy controls. The concentration of Tu M2-PK was determined by commercial kits using a sandwich enzyme linked immunosorbent assay based on two monoclonal antibodies (clone I and II) specific for Tu M2-PK. The sensitivity of the test for discrimination of malignant from non-malignant condition was 82% with a specificity of 60%. Highly significant statistical difference was found in the means of three groups ($P = 0.0002$). The present results indicate that Tu M2-PK can be used as a tumor marker in follow-up of patients with cervical carcinoma.

Key words: cervical carcinoma, Pyruvate Kinase, tumor M2-PK.

Introduction

Pyruvate kinase is a key enzyme in glycolysis, which determines the relative amount of glucose that is channeled into synthetic process or used for glycolytic energy production. Several isoforms of pyruvate kinase are tissue specifically expressed in tetrameric form. The isoenzyme M2-PK is shifted to a dimeric form in a variety of tumors. Since the dimeric form is over-expressed in tumor cells, it is called pyruvate kinase tumor M2 (Tu M2-PK).[1] Tu M2-PK is present in body fluids also, most likely released from tumor cells by tumor necrosis and cell turnover.[2] It has been previously shown for various tumors that Tu M2-PK determination in the circulation provides excellent discrimination between benign and malignant disease or may provide additional information regarding sensitivity to chemotherapy.[3–5] Cervical cancer is the most common cancer in females in India comprising 23.5% of all female cancers, and is mainly associated with HPV-16.[6,7] The dissociation of tetrameric to dimeric form of M2-PK has been shown to be caused by HPV-16 E7 oncoprotein.[8] This prompted us to evaluate Tu M2-PK as a tumor marker in patients with cervical carcinoma.

Materials and Methods

Fifty patients with histologically proven squamous cell carcinoma of the cervix were the subjects of this study. Five milliliters of blood from each patient was collected before instituting any therapy. There were three patients in Fig. 1 stage I, 23 in stage II, 22 in stage III and two in stage IV. Ten patients with chronic cervicitis and 10 healthy females with normal Pap smear were

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Table 1 Values of Tu-M2 PK (U/mL) in patients with cervical carcinoma, chronic cervicitis and healthy controls

Patients	No. patients	Mean	SD	Median	Range
Cervical carcinoma	50	49.94	31.27	39.50	3–100
Chronic cervicitis	10	24.00	15.92	19.00	1–50
Healthy controls	10	21.80	16.77	15.50	0–56

SD, standard deviation.

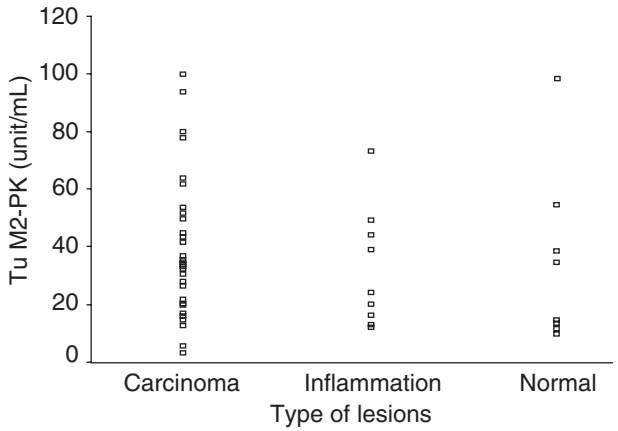

Figure 1 Tu M2 PK levels in patients with cervical carcinoma, chronic cervicitis and healthy controls.

included as controls. Samples were collected as ethylenediamine tetraacetic acid (EDTA) blood, followed by centrifugation (2000 g, 10 min) and removal of the supernatant plasma. The concentration of Tu M2-PK was determined immunologically using a sandwich enzyme linked immunosorbent assay based on two monoclonal antibodies (clone I and II) specific for the Tu M2-PK. Antibodies do not cross react with other isoforms of the pyruvate kinase. The test kit (ScheBo Tech, Germany) requires 50 µL of 1 : 100 diluted plasma per sample and all the tests were performed in duplicate according to the manufacturer's instructions.

Results

Tu M2-PK levels in the three groups are shown in Table 1 and Fig. 1. The preliminary cut-off value suggested by the manufacturer was 15 U/mL with 15–20 U/mL as gray zone. Using the receiver-operating curve, we selected a cut-off of 17 U/mL to give the best sensitivity and specificity. Forty one of 50 patients with cervical carcinoma had elevated levels of Tu M2-PK. However five of 10 patients with chronic cervicitis and three of 10 healthy controls had high levels giving a sensitivity of 82% for cervical carcinoma (as related to the non-malignant group comprising chronic cervicitis and healthy controls) and a specificity of 60%. The significance of the difference of the means was calculated using ANOVA and was found to be statistically significant between the three groups ($P = 0.002$). On further analysis for difference in the means between individual groups, the difference was found to be significant for both cervical carcinoma *versus* inflammatory ($P = 0.01$) and cervical carcinoma *versus* healthy controls ($P = 0.007$). Patients were not stratified according to the stage of the disease because the number of patients in stage I and IV were too small. No definitive correlation between Tu M2-PK plasma levels and the stage of the disease was seen; however, there was a tendency in the stage III patients to have higher levels than II patients and all the patients who had levels above 75 U/mL and above were in stage III.

Discussion

The loss of tissue specific isoenzyme of pyruvate kinase and subsequent expression of the M2 isoenzyme is one of the first steps in multistep carcinogenesis. M2-PK occurs in a tetrameric form with a high affinity to its substrate phosphoenolpyruvate, and in a dimeric form with a low affinity to phosphoenolpyruvate. The transition between both conformations regulates the glycolytic flux in tumor cells. At later stages of carcinogenesis, the expression of M2-PK is upregulated and subsequently, the enzyme is shifted from a tetrameric to dimeric state.[1]

Plasma determination of Tu M2-PK has been shown in various tumors to provide discrimination between benign and malignant disease. In pancreatic cancer, Tu M2-PK has been shown to discriminate pancreatic cancer from benign lesions and there was good correlation between Tu M2-PK levels and tumor metastasis. Also, it showed better correlation to metastasis than CA-19–1 and carcinoma embryonic antigen.[3] However, Pezzilli *et al*. studied Tu M2-PK in neuroendocrine tumors of the pancreas and found it to be as sensitive as chromogranin but much less specific limiting its diagnostic value.[9] In advanced renal cell and breast carcinoma,

a positive correlation between Tu M2-PK levels and response to chemotherapy was found.[5,10] Tu M2-PK levels proved useful as a diagnostic aid for therapy control in carcinoma lung patients and for detecting tumor relapse after treatment.[11] In a study of Tu M2-PK in gastrointestinal cancers, there was a highly significant difference between GI cancer Vs healthy controls as well as with Crohn/colitis patients. The most prominent difference was observed in cases of colorectal cancer and cholangiocarcinoma.[4] Hardt *et al*. demonstrated utility of measurement of fecal Tu M2-PK levels to determine cases of colorectal carcinoma and suggested that it can be an interesting screening tool for colorectal cancer.[12] In hematological malignancies, plasma determination of Tu M2-PK was not useful but may have a role in serial monitoring of patients and their response to therapy.[13]

The dissociation of tetrameric form to dimeric form is induced by oncoproteins. The first oncoprotein that was found to interact with M2-PK was the activated $pp60^{v-src}$ kinase.[14] Another oncoprotein that leads to dimerization of M2-PK is HPV-16 E7.[8,15] The E7 N-terminus mediates binding to proteins of the retinoblastoma gene family and, thereby, contributes to deregulation of the cell cycle, and the C-terminal domain binds to M2-PK. Investigations of HPV-16 E7 mutants and the non-oncogenic HPV-11 subtype suggest that the interaction of HPV-16 E7 with M2-PK may be linked to the transforming potential of the viral oncoprotein. E7 protein physically interacts with and stabilizes the dimeric form of M2-PK. Expression of E7 ensures the channeling of glucose carbons to synthetic processes and reduces the cell's requirement for oxygen; two important properties of tumor cells.

In humans, cancer of the cervix is linked to infection by human papillomaviruses (HPV) of the high-risk group, whereas viruses of the low-risk group are not associated with cancers *in vivo* and fail to transform human cells *in vitro*.[16] Pap smear is the main stay of cervical screening for the early detection of cervical carcinoma and its precursor lesion, and now, HPV DNA testing for high risk viruses has been approved for screening in conjunction with Pap smear in women over 30 years of age.[17] The combined use of Pap and HPV DNA testing improves the ability of clinicians to quantify a woman's risk of cervical carcinoma or its precursor lesion. Tu M2-PK may serve as a surrogate marker for HPV infection and malignant change.

Our results indicate that in squamous cell carcinoma of the cervix, plasma concentration of Tu M2-PK is a good marker to differentiate malignant lesions from non-malignant condition with a sensitivity of 82%; however, its specificity was only 60%. There was highly significant difference in the means of the three groups of patients of cervical carcinoma, chronic cervicitis and healthy controls. Tu M2-PK levels have been shown to have different concentrations in healthy persons according to the type of specimen used, presence of lipaemia, hemolysis, elevated C-reactive protein and proteinuria.[18] None of the factors were present in our controls. However, infection by HPV-16 leading to elevated Tu-M2-PK but unassociated with cytologic abnormalities cannot be ruled out as yet because HPV status in this study was not studied. It will be interesting to see the Tu M2-PK levels in patients with intraepithelial neoplasia of the cervix who are positive for HPV-16.

The other tumor markers which have been shown of some value in cervical carcinoma are squamous cell carcinoma antigen (SCC Ag), CA-125, tissue specific polypeptide antigen.[19,20] Serum concentration of SCC Ag correlates well with the stage of the disease, presence or absence of risk factors, course of the disease and the effect of treatment. Patients in complete remission have normal SS levels in 96% of the cases, whereas during follow-up, 85% of the cervical cancer patients with recurrent or residual disease show elevated levels at some time. However, Esajas *et al*. found routine SCC Ag analysis in follow-up to be too insensitive to detect recurrent disease at a stage in which effective therapy is still possible.[21] The good sensitivity of the assay suggests that determination of Tu M2-PK might be useful in the follow-up of treated patients for early detection of relapse. Future work will be done to confirm the present data and the role of Tu M2-PK in follow-up of patients on treatment.

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