Serum and Urine Polyamines in Cancer

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ABSTRACT

Polyamines in serum and urine have been found to be elevated in patients with cancer. A variety of methods employed for these measurements are discussed. Normal values obtained by the most recent methods are presented, and a survey has been made of polyamine levels in cancer patients.

Introduction

It has been the goal of laboratory scientists for many years to develop specific tests for the detection of cancer. Such tests would hopefully provide a diagnosis early in the course of the disease. In addition, it would be extremely desirable to have such a test for monitoring the cancer patient undergoing therapy.

In 1971, Russell and coworkers reported a relationship between urinary polyamines and cancer. Elevated concentrations of urinary polyamines were observed in patients with various types of solid tumors and leukemias. For example, increased urine polyamines levels were seen in rectal carcinoma, lymphosarcoma, Hodgkin's disease, osteogenic sarcoma and acute myelocytic leukemia. The latter patient, after chemotherapy, was found to excrete normal levels of polyamines in the urine.

It has been well established that in both normal and neoplastic rapidly growing tissues, the accumulation of polyamines or their derivatives parallels the cellular proliferation rate. Polyamines have been implicated in the regulation of ribonucleic acid (RNA) synthesis and, therefore, of course with protein synthesis. This regulation may occur in a number of ways, — possibly by increasing RNA polymerase activity or by stabilizing messenger RNA or by activating transfer RNA and increasing its susceptibility to methylation.

The polyamines which have been linked with the regulation of the growth process are putrescine, spermidine and spermine whose chemical structures are shown in table I.

Methods for the Measurement of Polyamines

Until very recently, there were no convenient sensitive and specific analytical procedures for the assay of all the polyamines present in biological fluids. In 1950, Tokuoka described a colormetric method for the quantitation of spermine in serum which involved heating diluted serum in the presence of CuCO₃ and observing the reddish-purple-blue color. This test like
other colorometric tests suffered from a lack of specificity. A greater degree of specificity was obtained using the technique of ion-exchange chromatography. This method, although widely used, was relatively insensitive and time consuming.

More recent techniques have involved paper electrophoresis, paper chromatography, thin-layer chromatography and serological methods. An enzymatic method which measured the products of enzymatic degradation by spermidine dehydrogenase or serum amine oxidase has also been used. Although these methods offer a considerable improvement over earlier assays, in most cases not all of the polyamines are measured by each procedure, nor is adequate sensitivity and specificity obtained. Two recent developments in methodology involve gas-liquid chromatography and automated ion-exchange techniques. These methods offer greatly improved accuracy and sensitivity for the measurement of polyamines in biological materials.

The gas chromatographic procedure described by Gehrke and coworkers for urinary polyamines involves an initial acid hydrolysis of the sample followed by clean up on cation exchange resin. The trifluoroacetyl derivatives of the amines are then formed and injected onto the column of the gas chromatograph.

To improve the speed and sensitivity of the cation-exchange method reported by Bremer and Kohne, several workers have utilized an amino acid analyzer. The urine specimens are again hydrolyzed to obtain the free amines and applied to the amino acid analyzer column for analysis. The eluate from the column is passed through a colorimeter where the polyamines are detected by their reaction with ninhydrin.

A new approach to the measurement of polyamines involves the powerful new technique of gas chromatography-mass spectrometry (GC-MS). The first application of this technique was reported by Walle who demonstrated the mass spectrometer to be a very sensitive and specific detector for the gas chromatograph.

The use of multiple ion detection or mass fragmentography combined with stable isotopes of the amines as internal standards would allow quite sensitive and rapid quantitation of the polyamines on very crude biological extracts.

Normal Values

Normal values for the measurement of serum and urine polyamines reported by the three analytical techniques of high voltage electrophoresis, classical ion exchange chromatography (CIE) and gas chromatography (GLC) are given in table II. It is apparent that the level of spermine in urine is overestimated by the high voltage electrophoresis method. Marton et al discuss this overestimation in urine as being caused by interferences from an unknown compound that migrates with spermine on the electrophoretogram. With the exception of spermine levels measured by CIE, there is generally good agreement between the various analytical approaches to estimating polyamine levels. Many more normal sera and urines need to be analyzed and a rigorous statistical analysis of the data made before a true normal range can be established.

Levels of Polyamines in Patients with Cancer

The detection, in association with cancer, of elevated levels of polyamines in biological samples has been made by several investigators. Tokuoka, using the
CuCO₃ colorimetric test for the detection of spermine in sera, obtained a positive reaction in 85.6 percent of patients with cancer. Bachrach and Robinson⁴ observed a 65 percent positive with cancer using this colorimetric test, with 11 percent of normal sera also producing a positive color.

Kosaki et al¹⁸ have isolated a spermine-containing phospholipid, malignolipin, from blood and have indicated that levels of this compound are elevated in cancer patients. It has also been reported, using a serological method, that spermine can be detected in the sera of patients with cancer and chronic infections. Considerable interest in polyamines was aroused by reports by Russell and coworkers²⁴,²⁵ who analyzed urine using high voltage electrophoresis. These workers noted considerable elevations in the urinary excretion of putrescine, spermidine and spermine in several patients with diagnosed leukemia, lymphoma, brain tumor and metastatic solid tumors. Elevations in spermidine and spermine were noted most frequently with levels being several-fold the normal range. Of special interest was the observation of a patient with a large ovarian tumor mass and another with acute myelocytic leukemia. Both patients had profound elevations of polyamines before surgery or chemotherapy and exhibited normal levels after treatment.

Recent investigations have provided results which are not quite as impressive as the original work of Russell and coworkers.²⁴ It is now evident that the high voltage electrophoresis system used by Russell lacked sensitivity and was subject to serious interferences when applied to urine analysis. The more specific and sensitive techniques of gas chromatography and automated ion exchange chromatography, which have been applied recently to polyamine measurements, give close agreement in normal values for urine polyamines. The application of one of these methods, automated ion exchange to chromatography to the analysis of urine samples from cancer patients, demonstrated elevations of urinary polyamines in many of these cancer patients although no trends were observed. A summary of these results is given in table III.
TABLE IV

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
<th>(nmol per ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>0.18-0.46</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mediastinal</td>
<td>0.25-0.75</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td>0.15-0.25</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>0.24-0.44</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Malignant teratoma</td>
<td>0.15-0.25</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td>0.25-0.44</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td>0.15-0.25</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Hodgkin's disease</td>
<td>0.10-0.15</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

In table IV are shown findings of polyamines in serum reported in one study using this same technique of automated ion exchange chromatography. Consistent elevations of serum spermidine levels were certainly observed with occasional elevations of putrescine and spermine. Spermidine was only normal in two of the cases studied.

Conclusions

The relationship between polyamines and cancer appears to have been well established, and there is sufficient data to indicate that measurements of polyamines in biological materials could provide a valuable test in the diagnosis of cancer and for monitoring therapy. At the present time, there is a need for the development of precise and accurate methods with ultimate emphasis being placed on simplicity in order that large scale studies can be carried out. Normal values of polyamines in serum and urine need to be established and further extensive clinical correlations are required.

References


