Evaluation of Serum Arabinitol as a Diagnostic Test for Candidiasis

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Gas-liquid chromatography was used to quantitate the arabinitol concentration in the sera of patients with candidiasis and in that of control patients. Serum arabinitol was elevated in 59% (n = 34) of patients with Candida sepsis, in 39% (n = 38) with Candida colonization, in 14% (n = 62) with bacterial sepsis, and in 0% (n = 11) of normal persons. The above patients were subsequently divided on the basis of renal function. Of those with decreased renal function, serum arabinitol was elevated in 89, 92, and 50% of patients with Candida sepsis, Candida colonization, and bacterial sepsis, respectively. Of those with normal renal function, serum arabinitol was elevated in only 23 and 14% of patients with Candida sepsis and Candida colonization, respectively. When serum arabinitol/creatinine ratios were calculated for patients with both increased arabinitol and increased creatinine, elevated ratios were obtained in 69, 36, and 0% of patients with Candida sepsis, Candida colonization, and bacterial sepsis, respectively.

Candida infections are becoming more prevalent owing to the increasing use of broad-spectrum antibiotics, immunosuppressive agents, cytotoxic therapies, and intravenous infusions (12). Many Candida infections elude diagnosis because clinical symptoms are often obscure. Also, depending on the site of specimen collection, the mere isolation of Candida sp. from clinical specimens can be confusing as this may reflect contamination from the host's microbial flora. Currently, the diagnosis of Candida disease can be made only after repeated positive blood cultures, persistent yeast isolation from sterile sites (e.g., urine or spinal fluid), or detection of yeasts in tissue. However, the isolation of Candida sp. is not a sensitive diagnostic test, as evidenced by the observation that positive blood cultures are obtained from <50% of patients with invasive disease (5). A more rapid and accurate assay is needed for early diagnosis and treatment, which is vital to the successful management of fungal infections. Physicians are reluctant to prescribe antifungal agents in the absence of a specific diagnosis because these agents are toxic to mammalian cells. Consequently, many researchers have investigated serological methods for early, rapid diagnosis of candidiasis (1, 4, 6, 9, 10), but as yet no serological method has had widespread acceptance.

In 1979, Kiehn et al. (7) reported that many Candida sp. excrete D-arabinitol, a relatively unusual metabolite, and that the serum arabinitol concentration might be used as an assay for the detection of candidiasis. We have recently completed a study in which we monitored the concentration of serum arabinitol in patients suspected of having candidiasis. Since few institutions have a population of high-risk patients large enough to carry out such a study, we felt that our results should be reported.

MATERIALS AND METHODS

Sera were obtained (January 1979 through April 1981) from patients at the University of Minnesota Hospitals, Minneapolis. The sera were categorized into four groups: (i) Candida septicemia as determined by positive blood cultures; (ii) Candida colonization with isolation of the organism from other sites, such as urine, sputum, vagina, peritoneal fluid, bronchial washing, and oral cavity; (iii) bacterial septicemia; and (iv) normal controls (hospital personnel). All microbial isolates were identified by the Clinical Microbiology Laboratory of the University of Minnesota Hospitals. During the time of this study, pertinent fungal isolates were identified as follows: Candida albicans (62%), Torulopsis glabrata (12%), C. tropicalis (12%), C. parapsilosis (5%). Candida species (8%). These Candida species were isolated in proportionate numbers from the patients in the Candida septicemia and the Candida colonization groups. C. albicans, C. tropicalis, C. parapsilosis, and C. pseudotropicalis have been shown to excrete arabinitol (2). Patients' renal function was determined by the serum creatinine concentration, and ≥1.5 mg/dl was considered indicative of impaired renal function.

Serum arabinitol was quantitated by the method of
TABLE 1. Serum arabinitol in control patients and in patients with candidiasis

<table>
<thead>
<tr>
<th>Patient status</th>
<th>No. of patients</th>
<th>% with elevated serum arabinitol*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida sepsis</td>
<td>34</td>
<td>59</td>
</tr>
<tr>
<td>Candida colonized</td>
<td>38</td>
<td>39</td>
</tr>
<tr>
<td>Bacterial sepsis</td>
<td>62</td>
<td>14b</td>
</tr>
<tr>
<td>Normal</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>

* Arabinitol > 1.0 μg/ml.

b Of these nine patients, five had Candida sp. isolated from other sites and two had systemic candidiasis diagnosed at autopsy.

Kiern et al. (7), with some modifications. In a 2-ml glass vial, 0.1 ml of serum was mixed with 0.2 ml of 50 μg of α-methyl-d-mannoside (Sigma Chemical Co., St. Louis, Mo.) per ml in acetone as the internal standard. The mixture was vortexed and centrifuged at 8000 x g for 10 min. A 50-μl portion of the supernatant was transferred to a clean vial, dried under nitrogen, and redissolved in 0.1 ml of freshly prepared silylating reagent, which consisted of pyridine-hexamethyldisilazane-trimethylchlorosilane, 6:4:2 (all components of the silylating reagent were purchased from Sulpelco, Inc., Bellefonte, Pa.). This mixture was capped, vortexed for 20 s, and then reacted at room temperature for 20 min. Samples were either refrigerated at 4°C if assayed the same day or frozen at −70°C for a maximum of 3 days. A 0.5-μl portion of the silylated sample was analyzed on a Perkin-Elmer 3920B gas chromatograph (Perkin-Elmer Corp., Norwalk, Conn.) with a Hewlett-Packard 3380A integrator (Hewlett-Packard Corp., Avondale, Pa.). Samples were injected onto a 25-m open tubular capillary column, wall coated with OV101 as the stationary phase (Perkin-Elmer). The nitrogen carrier gas flow rate was 30 ml/min, and the column temperature was programmed from 140 to 200°C at 4°C/min. All peak areas were reported with a retention time between 4 and 15 min. The arabinitol peak was identified and quantified in relation to the internal standard. The relative response factor of the detector to arabinitol versus the internal standard was not considered in the calculation. Arabinitol concentrations of >1.0 μg/ml were considered elevated. For calculations of arabinitol/creatinine (a/c) ratios, a nondetectable arabinitol concentration was assigned a value of 0.1 μg/ml. A control solution, consisting of 2.5 μg of arabinitol per ml mixed with the internal standard in acetone, was assayed at the beginning and end of each run. The range of 36 consecutive measurements of this control solution (selected at random) was 1.8 to 2.9. Assuming a confidence level of 0.05 (α = 0.05), the 95% confidence interval for the mean was 2.2 to 2.5 μg/ml (t-distribution).

RESULTS

Serum arabinitol. Serum arabinitol was elevated in 59% of patients who had Candida sp. isolated from the blood, whereas 39% of patients who had Candida sp. isolated from other sites also had elevated serum arabinitol (Table 1). Of 62 patients with bacterial sepsis, only 9 had elevated arabinitol, and the majority of these patients were subsequently discovered to be colonized with Candida sp. (see footnote b, Table 1). None of the control patients had elevated serum arabinitol.

TABLE 2. Serum arabinitol in control patients and in patients with candidiasis analyzed according to renal function

<table>
<thead>
<tr>
<th>Patient status</th>
<th>No. of patients</th>
<th>Renal function</th>
<th>% with elevated serum arabinitol*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida sepsis</td>
<td>18</td>
<td>Decreasedb</td>
<td>89</td>
</tr>
<tr>
<td>Candida sepsis</td>
<td>13</td>
<td>Normalc</td>
<td>23</td>
</tr>
<tr>
<td>Candida colonized</td>
<td>12</td>
<td>Decreased</td>
<td>92</td>
</tr>
<tr>
<td>Candida colonized</td>
<td>22</td>
<td>Normal</td>
<td>14</td>
</tr>
<tr>
<td>Bacterial sepsis</td>
<td>8</td>
<td>Decreased</td>
<td>50</td>
</tr>
<tr>
<td>Bacterial sepsis</td>
<td>29</td>
<td>Normal</td>
<td>11</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
<td>Decreased</td>
<td>NAd</td>
</tr>
<tr>
<td>Normal</td>
<td>11</td>
<td>Normal</td>
<td>0</td>
</tr>
</tbody>
</table>

* Arabinitol > 1.0 μg/ml.

b Serum creatinine ≥ 1.5 mg/dl.

c Serum creatinine < 1.5 mg/dl.

d NA, Not applicable.

Renal function and serum arabinitol. Many of the patients listed in Table 1 had serum creatinine determinations as an indicator of renal function. Of those with Candida sepsis, only 23% of patients with normal renal function had elevated serum arabinitol, whereas 89% of those with decreased renal function had elevated serum arabinitol (Table 2). The data on Candida-colonized patients were similar. Of those with decreased and normal renal function, elevated serum arabinitol was noted in 92 and 14%, respectively (Table 2). When all patients were divided on the basis of renal function, it was evident that those with elevated arabinitol tended to have decreased renal function (Table 2). The individual arabinitol concentrations from Table 2 are presented as a scattergram in Fig. 1. a/c ratios. Recent studies by Wong et al. (16, 17) have indicated that the a/c ratio might be a more useful measurement of increased arabinitol production. According to Wong et al. (16, 17) an increased a/c value was defined as one that is >2 standard deviations greater than the mean value of the control group. Table 3 presents a summation of the a/c ratios taken from the data depicted in Table 2 and Fig. 1. Only the patients with elevated serum arabinitol were considered here since those with normal arabinitol values would not be considered infected with Candida sp. and also these patients occasionally have a misleadingly elevated a/c ratio. Of those with an elevated arabinitol and an elevated creatinine, an increased a/c ratio was noted in 69% of those with Candida sepsis, in 36% of those with Candida colonization, and in 0% of those with...
bacterial sepsis (Table 3). All patients with an elevated arabinitol and a normal creatinine had an increased a/c ratio; this ratio was most elevated in those with Candida sepsis and least elevated in those with bacterial sepsis (Table 3).

DISCUSSION

Since the publication of Kiehn's work on arabinitol as a metabolite of Candida sp. (7), there have been two reports of studies in which the concentration of serum arabinitol in patients suspected of having candidiasis was monitored. In a relatively small study, Roboz et al. (14) obtained promising results with mass spectroscopy quantification of serum arabinitol. Elevated serum arabinitol was found in 82% (n = 11) of patients with diagnosed (autopsy or positive blood culture) invasive disease, and normal serum arabinitol was found in all patients (n = 6) colonized (oropharyngeal or vaginal) with Candida sp. This latter observation was not consistent with our finding of elevated serum arabinitol in 39% of patients colonized with Candida sp. (Table 1). However, nearly all (92%) of our Candida-colonized patients with elevated serum arabinitol also had decreased renal function (Table 2). Roboz et al. (14) stated that severe kidney dysfunction resulted in elevated serum arabinitol, but no data were presented. In another study by Eng et al. (3), gas-liquid chromatography was used to prospectively monitor the serum arabinitol in 31 patients with suspected candidiasis. (Nine of these patients had Candida sp. isolated from the blood and 20 patients had Candida sp. isolated from other sites.) Eng et al. (3) concluded that since the serum arabinitol concentration was elevated in patients with renal disease, the arabinitol concentration was a useful diagnostic tool in patients with normal renal function, but a normal arabinitol level did not rule out invasive disease. Before this report, the findings of Roboz et al. (14) and of Eng et al. (3) had not been evaluated in conjunction with data collected from a large patient population.

One serious difficulty exists with most studies that assess the reliability of a diagnostic test for candidiasis. In this report and in the reports of others (3, 14) some of the patients with Candida sepsis were identified solely on the basis of positive Candida blood cultures. This group most likely contains two types of patients, since those with invasive candidiasis cannot be separated from those with Candida sp. contaminating a parenteral line. Autopsy confirmation or tissue biopsy is needed to reliably identify patients with invasive systemic disease. This is often difficult to obtain. Thus, some of our patients identified as having Candida sepsis might not have had invasive disease. We were able to obtain autopsy or biopsy confirmation of invasive candidiasis in five patients. (C. albicans was isolated from lung tissue from two patients, from kidney tissue from one patient, from an abdominal aneurysm from one patient, and from bile fluid and a subhepatic abscess from one patient.) Interestingly, all five patients were
grouped as Candida colonized and all had a serum arabinitol level of <1.0 μg/ml. We find it difficult to speculate on the reason(s) for this, but low serum arabinitol could be due to antifungal agents interfering with Candida sp. metabolism. Also, others (7, 17) have noted low serum arabinitol in patients with candidiasis.

Serum arabinitol was elevated in 59 and 39% of patients with Candida sepsis and Candida colonization, respectively (Table 1). These results must be interpreted with caution. It has been shown that normal individuals can excrete low levels of arabinitol (15) and that the serum arabinitol can increase with decreased renal function (as measured by elevated serum creatinine) (3). Since the kidney is often the primary focus of infection in invasive candidiasis (13), many patients with invasive disease will also have an elevated serum creatinine. When the patients with Candida sepsis or Candida colonization were divided on the basis of renal function, those with elevated serum arabinitol tended to have decreased renal function and those with normal serum arabinitol tended to have normal renal function (Table 2, Fig. 1). In the absence of renal impairment, elevated arabinitol was associated with Candida sepsis in only a small fraction (23%) of patients with blood cultures positive for Candida sp. (Table 2). Thus, it appeared that, since serum arabinitol was affected by kidney function, the arabinitol assay would often be difficult to interpret and crucial specificity would be lost.

In an attempt to compensate for the effect of decreased renal function on the concentration of serum arabinitol, the a/c ratios were calculated as defined by Wong et al. (16, 17). As expected, in patients with increased serum arabinitol and normal renal function, elevated a/c ratios were obtained in all patient groups (Table 3). Assuming that increased arabinitol concentrations were indicative of candidiasis in those with normal renal function, calculation of a/c ratios would be unnecessary in this patient population. However, in patients with increased serum arabinitol and decreased renal function, calculation of a/c ratios might aid in the interpretation of the serum arabinitol concentration. In this latter group, all patients by definition had elevated serum arabinitol, but elevated a/c ratios were observed in only 69, 36, and 0% of patients with Candida sepsis, Candida colonization, and bacterial sepsis, respectively. Thus, calculation of a/c ratios might have a normalizing effect on the arabinitol concentration in patients with decreased renal function.

The data from this study indicated that the serum arabinitol concentration by itself was not a reliable indicator of candidiasis. However, after calculation of a/c ratios the percentage of positive patients was decreased in all groups of patients with compromised renal function. This decrease was greatest in the group with bacterial sepsis, and none of these patients had an elevated a/c ratio. It was intriguing to note that more patients in the Candida sepsis group (69%) had elevated a/c ratios than did the patients in the Candida-colonized group (36%). It is therefore tempting to speculate that a/c ratios might be helpful in the diagnosis of candidiasis. Although prospective studies of autopsy or biopsy proven cases of candidiasis are difficult to accomplish, it seems that such a study involving a/c ratios is warranted. This study, possibly coupled with analysis of other fungal products such as mannose (8, 11) and mannan (9), has the potential of providing definitive diagnosis of systemic Candida infection.

LITERATURE CITED