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CLINICAL EVALUATION OF RADIOIMMUNOASSAY OF DIGOXIN

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Serum digoxin concentration was measured by radioimmunoassay in 129 adult in-patients on maintenance doses of digoxin and compared with the clinical status of the patient.

The mean digoxin concentration for 108 nontoxic patients was 1.1 ± 0.1 (1 s.e.) ng/ml whereas that of 21 toxic patients was 3.8 ± 0.5 ng/ml ($p < 0.005$). The serum digoxin concentration of 2 ng/ml best discriminated toxicity with minimum overlap and with the overall accuracy 89%. Ninety-seven (90%) of 108 nontoxic patients had digoxin levels of 2 ng/ml or less whereas 19 (86%) of 21 toxic patients had levels above 2 ng. In 11% of the patients studied, the method failed to differentiate toxic and nontoxic patients.

The data indicate that the determination of serum digoxin concentration by radioimmunoassay can be of help in the diagnosis of digoxin toxicity.

An undesirably high incidence of digitalis toxicity has long been recognized and recent studies reported it to be above 20% of hospitalized patients receiving his drug (*1,2*). Clinicians are in need of better methods to help them judge proper therapeutic doses and diagnose digitalis toxicity. The variable biologic availability of digoxin preparations reported by Lindenbaum, et al (*3*) adds emphasis to this problem.

Since radioimmunoassay of digoxin has been developed by Smith, et al (*4*), a few controversial observations have been reported regarding its clinical application. Several studies indicated the determination of serum or plasma digoxin level by this method is useful in differentiating toxic from nontoxic patients (*1,3,5–7*), but Fogelman, et al (*8*) found no significant difference in the mean plasma digoxin level in the two groups and emphasized the fallibility of the plasma-digoxin level. These differing observations led us to reevaluate the clinical usefulness of digoxin radioimmunoassay in our hospital patients.

METHODS AND SUBJECTS

Radioimmunoassay of serum digoxin concentration was performed with a commercially available Schwartz/Mann (Orangeburg, N.Y.) Kit using ^{125}I-labeled 3-0-succinyl digoxigenin tyrosine as the labeled antigen, a modified method after Oliver, et al (*9*). Subjects were 129 adult patients admitted to a medical ward of the University of Cincinnati Medical Center during the period of January through May 1972, for whom a serum-digoxin level was requested and other randomly obtained medical patients who were on a maintenance dose of digoxin.

Each patient was examined by one of us and an EKG was obtained at the time or within 24 hr of sampling. Serum digoxin radioimmunoassay was performed on blood samples that were drawn between 5 and 30 hr after the previous dose of digoxin. On most of the patients a simultaneous assay of serum potassium and creatinine was performed. Patients were classified according to the criteria of Beller, et al, based on EKG data (*1*), as "nontoxic" (108 patients) and "definitely toxic" (21 patients).

RESULTS

The minimum detectable amount of digoxin by radioimmunoassay using the Schwartz/Mann Kit and a 0.05-ml serum sample was 0.4 ng/ml serum. Digoxin solution in 30% ethanol (2.0 ng per 0.025 μl per assay tube) was used to determine the reproducibility of this assay technique. Ten replicate analyses gave a 2 standard deviation of ±7.1% (within-day reproducibility) whereas the 2 standard devia-

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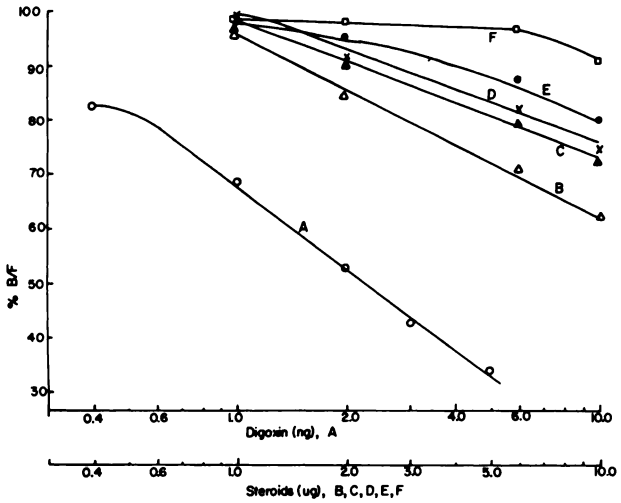

FIG. 1. Inhibition of binding of ^{125}I-labeled 3-0-succinyl digoxigenin tyrosine to digoxin antibody by various steroids. (A) digoxin; (B) progesterone; (C) cortisone; (D) testosterone; (E) cortisol; (F) cholesterol. Ratio of antibody-bound radioactivity to free radioactivity (B/F), in absence of various compounds mentioned above but in presence of 0.05 ml of digoxin free serum, was assumed to be 100%.

tion for 14 assays performed over a month's period was ±30% (day-to-day reproducibility). As shown in Fig. 1, the digoxin antiserum (Lot No. YN 6202 of Schwartz/Mann Kit) also cross reacts with some steroids. Ten micrograms of progesterone, cortisone, testosterone, cortisol, and cholesterol inhibited the binding of ^{125}I-labeled 3-0-succinyl digoxigenin tyrosine to the antibody by 37, 28, 25, 20, and 9%, respectively, whereas 5 ng of digoxin inhibited the binding by 67%. One microgram of each steroid showed no appreciable effect on the binding.

The mean serum-digoxin concentration in 108 nontoxic patients was 1.1 ± 0.1 (s.e.) ng/ml and was significantly lower than the mean value of 3.8 ± 0.5 ng/ml in 21 toxic patients ($p < 0.005$, standard t tests), as shown in Fig. 2. At an arbitrary level of 2 ng/ml (Fig. 2), the sum of the number of false-positive (nontoxic patient with higher digoxin level) and false-negative cases (toxic patients with lower digoxin level) was minimal. Among 21 toxic patients, 18 or 86% had digoxin levels of 2 ng/ml or above, whereas 97 patients or 90% of the 108 nontoxic patients had digoxin levels of less than 2 ng/ml.

No correlation was found between the serum digoxin level and serum creatinine level ($p > 0.1$) in either toxic (21 patients) or nontoxic group (102 patients). As shown in Table 1, the mean serum concentrations of creatinine and potassium of toxic patients were not significantly different from those of nontoxic patients.

DISCUSSION

Our finding that serum-digoxin level is significantly higher in the toxic group than in the nontoxic group is in agreement with the results of many other investigators (*1,3,5–7*) but is in contrast to the observation by Fogelman, et al (*8*).

Some of the known conditions associated with increased prevalence of digitalis toxicity are diminished renal function, loss of body potassium, hypoxia due to pulmonary disease, myocardial infarction, and hypothyroidism. We found no significant difference in serum creatinine or potassium concentration between toxic and nontoxic patients. The latter finding was also observed by Beller, et al (*1*). This is not surprising because serum concentration of potassium may not necessarily reflect the intracellular potassium level (*10*). It is of interest to note that Wotman, et al (*11*) found no significant difference in the plasma potassium level but there was a significantly high salivary potassium concentration in digitalis toxic patients. Some studies have noted digoxin intoxicated patients have higher blood urea nitrogen or serum creatinine level compared to that of nontoxic patients (*1,2,4,5*) whereas others have not (*8,11*).

We observed considerable overlap between the drug level of toxic patients and that of nontoxic patients as in other studies. Similar overlap was also observed in the digoxin assay using Na-K-ATPase system (*12*). This probably inevitable overlap may be due—other than to the limited specificity of the antibody—partly to the individual difference in the metabolism of and sensitivity to the digoxin and partly to the difficulty of classifying patients objectively and thus using ECG findings as a sole criteria. Other factors may be different sampling time (5–30 hr) after the last dose of digoxin, the presence of radioactivity in the plasma from other nuclear medicine procedures, and the use of steroid medica-

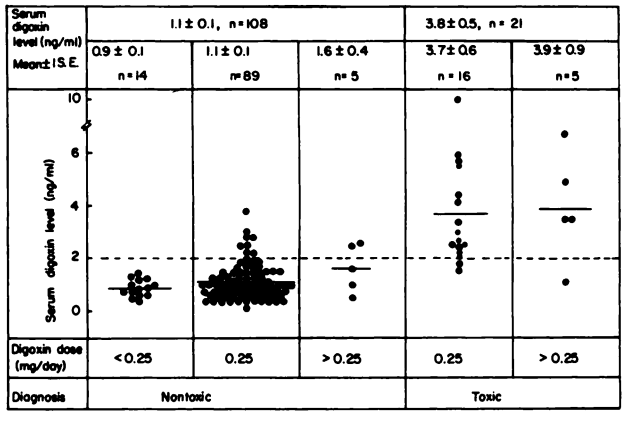

FIG. 2. Serum digoxin levels in patients receiving various doses of digoxin. Horizontal bars represent mean values and dotted line a arbitrary level that best discriminates digoxin toxicity with minimum false-positive and false-negative (n: number of patients studied. 1 s.e.: one standard error of mean).

TABLE 1. SERUM CREATININE AND POTASSIUM CONCENTRATION IN PATIENTS RECEIVING DIGOXIN THERAPY*

	Nontoxic	Toxic
No. of patients	102	21
Creatinine (mg/100 ml) Mean ± s.e.	1.6 ± 0.2	2.1 ± 0.5
Potassium (mEq/L) Mean ± 1 s.e.	4.5 ± 0.1	4.1 ± 0.2

* 1 s.e.: one standard error of the mean.

tions that may compete with the digoxin in the radioimmunoassay system. The results shown in Fig. 1 indicate that the presence of cortisol, testosterone, cortisone, and progesterone with serum concentration greater than 2 μg per 0.05 ml (per assay tube) or 40 μg/ml could give falsely high digoxin levels. The degree of specificity shown in Fig. 1 was determined by the use of the antiserum (Lot No. YN 6202) provided in the ^{125}I digoxin radioimmunoassay kit by Schwartz/Mann. Each different lot of antiserum should be evaluated for its specicificity in each laboratory. The serum levels of these steroids under a normal physiological condition are not expected to interfere with the assay. However, in patients receiving massive doses of steroid medications, a falsely high digoxin concentration may result. In our series, the medication chart of each patient was carefully scrutinized. We confirmed that the nontoxic patients with relatively high serum-digoxin level (greater than 2 ng/ml) had not received previous steroid medications. Also, none of the toxic patients with relatively low serum-digoxin level (less than 2 ng/ml) had received any radionuclide contamination from other nuclear medicine procedures.

We conclude that determination of serum-digoxin level by radioimmunoassay can be a valuable adjunct to a clinician in the diagnosis and followup of digoxin toxicity when the result is interpreted with a knowledge of the limitations of the test.

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