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A New Direct Pancreatic Function Test in Pediatrics

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Summary: Traditional methods for collecting duodenal fluid are time consuming and technically difficult. A simple endoscopic method is proposed in this report as a means of collecting duodenal fluid to perform exocrine pancreatic function tests. Thirty-five patients between 24 and 36 months of age were studied for pancreatic exocrine function. Twenty-seven presented with chronic diarrhea and 8 with failure to thrive. In 20 patients (group 1), duodenal fluid was collected by means of a double-lumen tube and sequential administration of pancreozymin (PZN) and secretin (SEC). The rest (group 2) had duodenal aspiration from the level of the papilla of Vater through a fiberoptic endoscope following administration of SEC only. The procedure took approximately 3 h in group 1 and 45 min in group 2. Secretin administration produced comparable levels of enzymes in both groups. Pancreozymin produced the highest enzyme levels, but this was only significantly higher than SEC-induced levels in the case of lipase. Endoscopic collection of duodenal fluid following SEC administration is a safe, quick, and reliable method of collecting pancreatic secretion.
Key Words: Exocrine pancreatic function test—Duodenal fluid—Pancreozymin—Secretin.

A variety of investigations are currently used to study exocrine pancreatic function (1–5) but direct collection of pancreatic secretions following administration of exogenous hormones remains the method of choice (6). The pancreatic secretions are usually collected by duodenal intubation with a double-lumen tube (7,8). Correct positioning of the tube requires fluoroscopy, can be technically difficult, and may be time consuming, resulting in prolonged periods of patient discomfort in some cases. Recently, endoscopic cannulation of the pancreatic duct has been used to collect pancreatic secretions. This method permits aspiration of pure pancreatic juice (9,10), but requires technical expertise, is time consuming and expensive, and carries a greater risk of iatrogenic trauma.

An alternative method of collecting pancreatic secretions would be by aspiration of duodenal fluid at the level of the papilla of Vater under direct visualization using a flexible fiberoptic endoscope. The procedure is technically less difficult and should take less time than other methods. This report describes a method of endoscopic collection of pancreatic secretions in young children and compares the technique to that using duodenal intubation with a double-lumen tube. Because the use of pancreozymin (PZN) for clinical testing is now prohibited in the United States, stimulation of pancreatic secretion in those children undergoing endoscopy was by means of intravenous administration of exogenous secretin (SEC) only. To assess the reliability of using SEC alone by this method of collection, the results of the enzyme analysis in these children were compared to those of a group of children with similar symptoms who had previously undergone pancreatic function testing using sequential administration of PZN and SEC.

PATIENTS

Thirty-five patients (24 males) between the ages of 24 and 36 months underwent pancreatic function testing to exclude pancreatic exocrine insufficiency as a cause for either chronic diarrhea (n = 27) or failure to thrive (n = 8). The first 20 patients (group

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1) were studied by means of aspirating duodenal fluid using a double-lumen tube and administration of both PZN and SEC. The remaining 15 patients (group 2) underwent endoscopic aspiration of duodenal fluid following administration of SEC only. The procedure was approved by the hospital Institutional Review Board and informed consent was obtained from the parents in all cases.

METHODS

Following a 4-h fast, the patient was sedated with a mixture of Demerol–Phenergan–Thorazine containing 12.5, 25, and 12.5 mg/ml, respectively. The administered dose was 0.1 ml/kg of body weight to a maximum dose of 2.0 ml. In group 1 infants, a double-lumen tube was passed nasogastrically. Under fluoroscopic control, the tube was manipulated into the second part of the duodenum with the proximal opening situated in the stomach to aspirate gastric fluid. A sample of duodenal fluid (±1.0 ml) was aspirated to determine basal levels of pancreatic enzymes. PZN (Kabi Diagnostica) was then administered intravenously in a dose of 2 units/kg body weight and ±1.0 ml aliquots of duodenal fluid were aspirated at 10-min intervals for the next 30 min. Thereafter, the patient was given an intravenous injection of secretin (Kabi Diagnostica) in a dose of 2 units/kg of body weight and a further three aliquots of duodenal fluid were collected at 10-min intervals. To ensure that the distal end of the tube remained in the duodenum throughout the study, each sample of fluid aspirated was inspected for bile discoloration and checked for alkaline pH. After completing the study, the tube was withdrawn from the patient. In each case, the duration of the entire procedure was recorded.

Group 2 infants were fasted for the same period and received the same sedation as those in group 1. Following intravenous administration of SEC (2 U/kg of body weight), a flexible pediatric fiberoptic endoscope (Olympus GIF P3) was passed into the patient's duodenum. Prior to starting the procedure, the suctioning apparatus of the endoscope was modified to include a Leuken's collection trap as shown in Fig. 1. All gastric juice was aspirated before passing the endoscope into the duodenum. To further minimize contamination of the first sample with gastric juice, an aliquot of approximately 2 ml of duodenal juice was initially aspirated and this together with the gastric juice already in the Leukens trap was discarded. Once the papilla of Vater

FIG. 1. Leuken's collection trap connected to the suction system of a fiberoptic pediatric endoscope. Group 1 has a double-lumen tube that was passed nasogastrically. Group 2 has a flexible pediatric fiberoptic endoscope passed into the duodenum.

was visualized, a clean Leuken's trap was installed and samples of duodenal fluid (±1 ml/aliquot) were aspirated at 10-min intervals. Thereafter, the endoscope was withdrawn and the duration of the procedure was recorded.

ENZYME DETERMINATION

Following the procedure, all specimens were stored at −20°C until analysis. Enzyme assay of the duodenal fluid was performed within 24 h of collection in all cases. The pH of each specimen was measured by means of an Accumet pH meter 915 (Fisher Scientific, Pittsburgh, PA, U.S.A.).

Trypsin activity was measured by the liberation of *p*-nitroaniline from the substrate benzoyl–DL-arginine–*p*-nitroaniline at pH 8.2 and a temperature of 25°C, according to the method of Erlanger et al. (11). Units were expressed as nanomoles of *p*-nitroaniline produced per minute.

Chymotrypsin activity was determined from the rate of hydrolysis of *N*-benzoyl–DL-tyrosine ethyl acetate ester measured by the change in absorbance at 256 nm with time, as described by Hummel (12). Units were expressed as micromoles of substrate hydrolyzed per minute.

Lipase activity was determined by the potentiometric titration (at a constant pH = 8.0) of ionized fatty acids liberated from a triglyceride (olive oil) emulsion with 0.05 N NaOH following the method of Smeriva et al. (13). Units were expressed as micromoles of acid equivalent liberated per minute.

TABLE 1. *Mean (±SEM) of peak values for pH pancreatic enzymes (U/ml/min) of amylase, lipase, trypsin, and chymotrypsin*

	Group 1			Group 2, secretin
	Basal	PZN	Secretin	
pH	7.13 ± 0.49	7.71 ± 0.48[a]	8.08 ± 0.42[a]	9.15 ± 0.28
Amylase	105.23 ± 15.51	187.62 ± 34.51[a]	161.51 ± 18.05[a]	168 ± 30.47
Lipase	916.73 ± 392.84	2,093.05 ± 744[a]	1,089.77 ± 217.9	557.08 ± 166.2
Trypsin	124.06 ± 25.23	491.69 ± 37.89[a]	379.84 ± 62.17[a]	431.72 ± 97.75
Chymotrypsin	7.93 ± 1.65	20.75 ± 3.51[a]	11.89 ± 1.31[a]	19.90 ± 2.85[b]

[a] Significance when compared with basal levels ($p < 0.05$).
[b] Significance when compared with secretin stimulation in group 1 ($p < 0.05$).

This measured the lipase activity, independent of the colipase activity.

α-Amylase was determined from the colored product obtained by reduction of 3,5-dinitrosalicylic acid by maltose liberated in the hydrolysis of starch (14). Units were expressed as micromoles of maltose liberated per minute.

Each enzyme assay was performed in duplicate and the assay was repeated if the two results differed by more than 5%. The highest pH and enzyme levels recorded from the series of aspirates in each patient were used to assess pancreatic exocrine function in that individual.

The mean ± SEM of the peak pH and enzyme measurements was calculated for the basal aspirate in the group 1 patients and compared to those following administration of PZN and SEC. The corresponding values were also calculated for the group 2 patients and the results were compared to those of group 1. Where applicable, comparisons were by means of the Student's *t* test.

RESULTS

The mean age of the patients in group 1 was 27.1 months; of the 20 children in this group, 12 had chronic diarrhea and 8 had failure to thrive. The findings were similar to those of the group 2 patients, who had a mean age of 28.4 months, with 10 having chronic diarrhea and 5 failure to thrive. The procedure was completed in all cases without any complications. On average, the study took 3 h to complete using the double-lumen tube in group 1 patients, and 45 min for those undergoing endoscopy in group 2. On the basis of the results, none of the children had evidence of pancreatic exocrine insufficiency. The means (±SEM) of the pH, trypsin, chymotrypsin, lipase, and α-amylase measurements for group 1 and group 2 patients are shown in Table 1 and illustrated graphically in Figs. 2–6. In group 1, use of both PZN and SEC resulted in a significant increase in pH over basal levels. Secretin administration in the group 2 patients produced the highest pH levels recorded.

Pancreozymin resulted in the greatest increase over basal levels for all four enzymes assayed. Secretin also increased the levels of all enzymes over basal measurements in group 1 patients; with the exception of lipase, the increase was similar to that following PZN administration. In group 2 patients, SEC administration resulted in levels of trypsin, chymotrypsin, and amylase that were higher than those following SEC in the group 1 patients but significantly so only for chymotrypsin. As was found in the group 1 infants, SEC had a minimal effect on the lipase levels.

DISCUSSION

Direct enzyme assay of duodenal aspirates remains the best means of assessing pancreatic exocrine function, but the methods currently used to obtain the specimens are either time consuming and extremely uncomfortable for the patient or require a

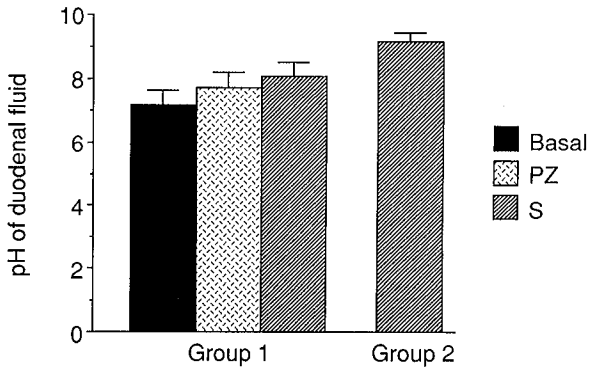

FIG. 2. pH values in groups 1 and 2 (mean ± SEM). Group 1 had a double-lumen tube that was passed nasogastrically. Group 2 had a flexible pediatric fiberoptic endoscope passed into the duodenum.

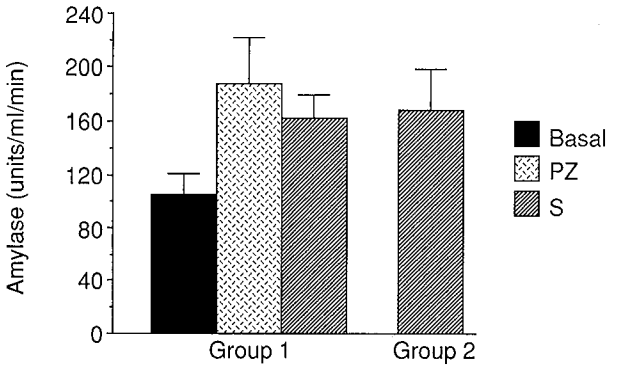

FIG. 3. Peak values of amylase activity in groups 1 and 2 (U/ml/min, mean ± SEM). Group 1 had a double-lumen tube that was passed nasogastrically. Group 2 had a flexible pediatric fiberoptic endoscope passed into the duodenum.

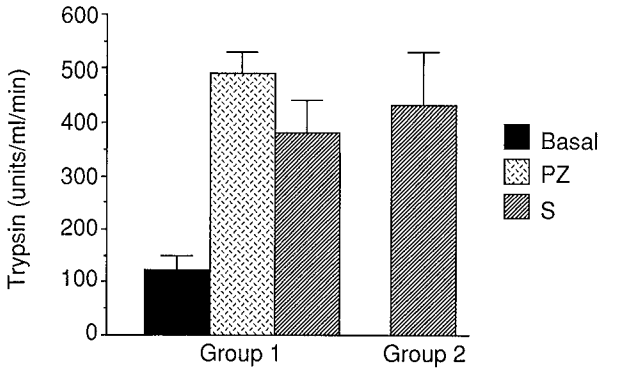

FIG. 5. Peak values of trypsin activity in groups 1 and 2 (U/ml/min, mean ± SEM). Group 1 had a double-lumen tube that was passed nasogastrically. Group 2 had a flexible pediatric fiberoptic endoscope passed into the duodenum.

high level of technical expertise. The procedure described in this report offers a suitable alternative to other methods. Aspiration of duodenal fluid under direct vision through a flexible fiberoptic endoscope is relatively simple and safe and can be completed in much less time than it takes using a double-lumen tube. Furthermore, the investigator can be certain that the fluid is collected from the correct position in the duodenum by visualizing the papilla of Vater at all times. An additional advantage of this method is that intestinal biopsies can be taken during the same procedure. These are often necessary as part of the investigation of children suspected to have malabsorption, and completing both studies at the same time is not only less traumatic for the patients but represents a major potential saving in costs.

Comparison of the peak pH and enzyme levels between group 1 and group 2 illustrates that the endoscopic method is also a reliable means of obtaining pancreatic secretions. The peak levels recorded for group 2 patients were similar to those following SEC administration in group 1 and also to established norms for children of the same age (15). In both groups, SEC resulted in lower peak enzyme levels than those obtained with PZN but the difference was only significant for lipase. Only PZN produced a significant increase in the levels of lipase over basal measurements. This suggests that administration of SEC without PZN may not provide a sensitive means of studying pancreatic lipase production. As the use of PZN for clinical testing is now prohibited in the United States, alternative methods of studying pancreatic lipase production may have to be found.

In conclusion, endoscopic aspiration of duodenal fluids is a relatively simple and quick method of obtaining pancreatic secretions. Stimulation of pancreatic secretion with administration of intravenous SEC is a reliable means of assessing production of trypsin, chymotrypsin, and amylase but may be less useful for assessing lipase production.

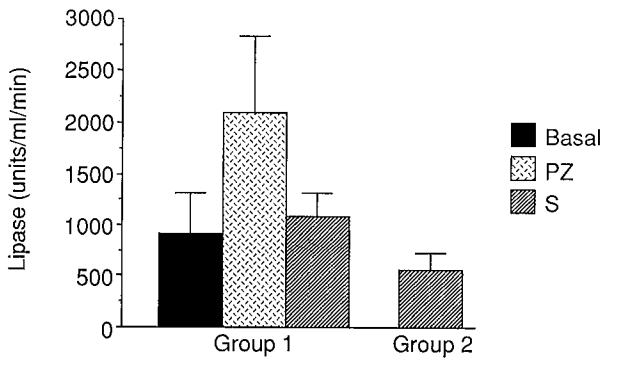

FIG. 4. Peak values of lipase activity in groups 1 and 2 (U/ml/min, mean ± SEM). Group 1 had a double-lumen tube that was passed nasogastrically. Group 2 had a flexible pediatric fiberoptic endoscope passed into the duodenum.

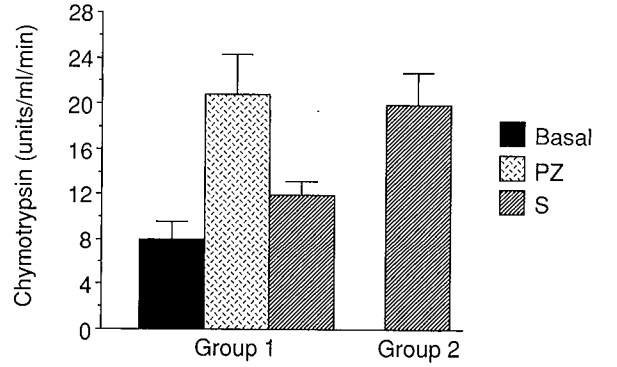

FIG. 6. Peak values of chymotrypsin activity in groups 1 and 2 (U/ml/min, mean ± SEM). Group 1 had a double-lumen tube that was passed nasogastrically. Group 2 had a flexible pediatric fiberoptic endoscope passed into the duodenum.

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