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Diagnosis of *Helicobacter pylori* Infection in Children: Comparison of a Salivary Immunoglobulin G Antibody Test with the [^{13}C]Urea Breath Test

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The prevalence of *Helicobacter pylori* infection in a population-based sample of 477 children (mean age ± standard deviation, 5.8 ± 0.5 years) determined by the [^{13}C]urea breath test ([^{13}C]UBT) was 10.7% (95% confidence interval [CI], 8.1 to 13.8%), and that determined by salivary enzyme-linked immunosorbent assay (ELISA) was 11.9% (95% CI, 9.2 to 15.2%). Compared to the [^{13}C]UBT, the sensitivity and specificity of the salivary ELISA were 80.9% (95% CI, 66.3 to 90.4%) and 95.3% (95% CI, 92.7 to 97.1%), respectively.

For the diagnosis of *Helicobacter pylori* infection in children, endoscopy is not clinically indicated and is not feasible in most studies. Noninvasive diagnosis of *H. pylori* infection can be done by measuring specific anti-*H. pylori* immunoglobulin G (IgG) antibodies in serum or saliva with an enzyme-linked immunosorbent assay (IgG-ELISA), with the [^{13}C]urea breath test ([^{13}C]UBT), and with an enzyme immunoassay (HpSA) for antigens in stools (3, 12–14, 16–18, 21–25). Whole saliva as a test sample is easily accessible, and despite some less encouraging results with *H. pylori* testing (9, 19, 23), some studies seem to be promising (1, 6, 10, 11, 15).

The [^{13}C]UBT has been shown to be an extremely accurate method of detecting *H. pylori* infection because it has the advantage of evaluating the gastric mucosa as a whole, thereby avoiding the sampling errors inherent in biopsy (4, 5, 7). Furthermore, as previously shown, the [^{13}C]UBT is an excellent diagnostic test in children 5 years of age and older and can be considered another "gold standard," especially if endoscopy is not indicated (5, 7, 8).

In this study, we investigated the salivary anti-*H. pylori* IgG immune response in a population-based sample of school-age children. The performance of the salivary assay was assessed against the [^{13}C]UBT as the gold standard for establishing *H. pylori* infection.

Study subjects were 477 randomly selected children 5 to 7 years old (mean age ± standard deviation, 5.8 ± 0.5 years) (Table 1) living in Ulm, Germany, who were examined for school fitness by the Public Health Service in 1998. A total of 71.7% of the children were of German nationality, 13.0% were of Turkish nationality, and 15.3% were of other than German or Turkish nationality. Participation in the study was voluntary, and informed consent of parents was obtained for each child. The study was approved by the Ethics Board of the University of Ulm.

The [^{13}C]UBT was performed as described previously (2, 20). Sixty milligrams of [^{13}C]urea (99.5% C; Mass Trace, Woburn, Mass.) was dissolved in 200 ml of apple juice (pH 2.2 to 2.4). Breath samples were collected into plastic bags before and 30 min after intake of the apple juice and were analyzed with an isotope-selective nondispersive infrared spectrometer (Wagner Analytical Systems, Bremen, Germany). A test was regarded to be *H. pylori* positive if the difference between the baseline $^{13}CO_2/^{12}CO_2$ ratio and the 30-min $^{13}CO_2/^{12}CO_2$ ratio exceeded 4‰.

To minimize the possibility of false-negative [^{13}C]UBT results, children who had received antibiotic treatment within the previous 4 weeks, which could influence the [^{13}C]UBT, were excluded from the analysis. None of the children had received proton pump inhibitors, H_2 blockers, bismuth salts, or antacids within the prior 4 weeks.

Before the [^{13}C]UBT was conducted, whole saliva was collected with a special saliva sampling device (Salivette; Sarstedt, Nürnberg, Germany) according to the manufacturer's instructions. Children were asked to chew thoroughly a cotton wool swab for 1 min. The cotton wool swab was then placed into the suspended insert of the sampling device, and the saliva was

TABLE 1. Various demographic characteristics of the study population

Variable	*n*	%
Age (yr)		
5	124	26.0
6	330	69.2
7	23	4.8
Sex		
Male	225	47.2
Female	252	52.8
Nationality		
German	342	71.7
Turkish	62	13.0
Other	73	15.3
Total	477	100

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TABLE 2. Performance of salivary ELISA versus [^{13}C]UBT in 477 children

Salivary ELISA result	No. with the following [^{13}C]UBT result:		
	Positive	Negative	Total
Positive	38	19	57
Indeterminate	4	20	24
Negative	9	387	396
Total	51	426	477

centrifuged (3,500 rpm for 5 min) and frozen at −80°C until analysis.

Evaluation of saliva IgG against *H. pylori* antigens was performed with a modification of a commercially available ELISA kit (HM-CAP; Enteric Products, Inc., Stony Brook, N.Y.) in blinded fashion. Serological tests based on this kit have been shown to have high agreement with the [^{13}C]UBT in adults (13, 14) and in children (4, 24). One hundred microliters of whole saliva was added to the wells of a modified HM-CAP antigen preparation without dilution and incubated for 2 h at room temperature. The wells were washed three times, and 100 μl of peroxidase-conjugated anti-human IgG was added and incubated for 20 min at room temperature. The wells were further washed three times, and 100 μl of TMB substrate was added and incubated for 10 min at room temperature. The reaction was stopped with 100 μl of 1 N sulfuric acid, and results were read on a spectrophotometer at 450 nm. The results were calculated from a three-point linear regression curve and extrapolated into ELISA values (values of >2.2 were positive, values of <1.8 were negative, and values of >1.8 and <2.2 were indeterminate).

Table 2 shows that *H. pylori* infection could be detected in 51 children (10.7%; 95% confidence interval [CI], 8.1 to 13.8%) as determined by the [^{13}C]UBT and in 57 children (11.9%; 95% CI, 9.2 to 15.2%) as determined by the salivary ELISA. Twenty-four of the 477 saliva test results (5%) were indeterminate and were excluded from further analysis. Compared to the results of the [^{13}C]UBT, the sensitivity and specificity of the salivary antibody ELISA in children were 80.9% (95% CI, 66.3 to 90.4%) and 95.3% (95% CI, 92.7 to 97.1%), respectively (Table 3). Overall, the resulting positive predictive value (PPV) was 66.7% (95% CI, 52.8 to 78.3%), and the negative predictive value (NPV) was 97.7% (95% CI, 95.6 to 98.9%).

As the prevalence varied between children of German nationality (3.2% [95% CI, 1.6 to 5.7%] as determined by [^{13}C]UBT) and children of other than German nationality (29.6% [95% CI, 22.1 to 38.1%] as determined by [^{13}C]UBT), we also calculated the test performance separately in the two groups. The sensitivity and specificity in children with German nationality and in children with other than German nationality were very similar, but the PPV was much higher in non-German children (81.1% [95% CI, 64.3 to 91.4%]), whereas the NPV was higher in German children (99.0% [95% CI, 96.9 to 99.7%]), due to the different prevalences in the two groups.

In this population-based study of children, we could show that compared to [^{13}C]UBT, the accuracy of salivary ELISA was very high, indicating that it seems to be a reliable, convenient, and easy-to-use tool, which may especially be valuable in the context of epidemiological studies.

Studies performed with saliva of adult patients show an overall sensitivity of 81 to 94% and a specificity of 70 to 90% compared with endoscopy-based methods (6, 10, 12, 17, 20, 24). The PPV and the NPV range from 76 to 84% and from 60 to 80%, respectively. Data obtained from children referred for endoscopy show comparable sensitivity (93%) and specificity (82%) when tested against histology and the urease test (10). Two studies investigated asymptomatic children by using either a serology-based test (sensitivity, 64%; specificity, 87%) or the [^{13}C]UBT (sensitivity, 65%; specificity, 95%) (1, 12). The results obtained with the HM-CAP ELISA kit in this study for asymptomatic children (sensitivity, 81%; specificity, 95%) compared to the [^{13}C]UBT are promising. In particular, the high NPV (98%) confirms the suitability of the test to exclude *H. pylori* infection with a high probability.

The choice of a test for noninvasive diagnosis of *H. pylori* infection depends mainly on the accuracy, the availability, and the costs of the test. The salivary ELISA is a very reasonable device in this respect, but there is a need to evaluate the test further with young children in whom the immune response against *H. pylori* is not fully developed (18).

In conclusion, whole-saliva-based IgG-ELISA may be of particular interest as a tool to study *H. pylori* infection in children in epidemiological studies, as it allows screening of a large number of children with a noninvasive method with high acceptance and very good practicability.

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TABLE 3. Salivary IgG-ELISA compared to [^{13}C]UBT

Parameter	Result[a] for children of the following nationality:		
	German	Other	Overall
H. pylori prevalence according to [^{13}C]UBT	3.2 (1.6–5.7)	29.6 (22.1–38.1)	10.7 (8.1–13.8)
Sensitivity	72.7 (39.3–92.7)	83.3 (66.5–93.0)	80.9 (66.3–90.4)
Specificity	96.2 (93.2–97.9)	92.4 (84.4–96.6)	95.3 (92.7–97.1)
PPV	40.0 (20.0–63.6)	81.1 (64.3–91.4)	66.7 (52.8–78.3)
NPV	99.0 (96.9–99.7)	93.4 (85.7–97.3)	97.7 (95.6–98.9)

[a] Values are percentages. Data in parentheses are 95% CIs.

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