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Diagnosis of Dengue Virus Infection by Detection of Specific Immunoglobulin M (IgM) and IgA Antibodies in Serum and Saliva

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To evaluate alternative approaches to the serological diagnosis of dengue virus (DEN) infection, the detection of DEN-specific immunoglobulin M (IgM) and IgA antibodies in serum and saliva specimens was assessed in 147 patients with symptoms of DEN infection seen at the Ministry of Health in Nicaragua. Seventy-two serum samples were determined to be positive for anti-DEN antibodies by IgM capture enzyme-linked immunosorbent assay, the routine diagnostic procedure. Serum and saliva specimens were obtained from 50 healthy adults as additional controls. IgM was detected in the saliva of 65 of the 72 serum IgM-positive cases, 6 of the 75 serum IgM-negative cases, and none of the control group, resulting in a sensitivity of 90.3% and a specificity of 92.0% and demonstrating that salivary IgM is a useful diagnostic marker for DEN infection. Detection of IgA in serum may be another feasible alternative for the diagnosis of DEN infection, with serum IgA found in 68 (94.4%) of the IgM-positive cases. In contrast, detection of IgA in saliva was not found to be a useful tool for DEN diagnosis in the present study. Further studies of the kinetics of antibody detection in another set of 151 paired acute- and convalescent-phase serum samples showed that DEN-specific IgA antibodies were detected in more acute-phase samples than were IgM antibodies. Thus, we conclude that DEN-specific IgA in serum is a potential diagnostic target. Furthermore, given that saliva is a readily obtainable, noninvasive specimen, detection of DEN-specific salivary IgM should be considered a useful, cheaper diagnostic modality with similar sensitivity and specificity to IgM detection in serum.

The four serotypes of dengue virus (DEN) cause the most prevalent arthropod-borne illness in humans, ranging from the self-limited but debilitating dengue fever (DF) to the life-threatening dengue hemorrhagic fever-dengue shock syndrome (DHF/DSS). One hundred million cases of DF and 250,000 to 500,000 cases of DHF/DSS are estimated annually, with 2.5 billion people at risk for DEN infection (12, 18). In many tropical regions, dengue is endemic, with intermittent explosive epidemics. DF is characterized by fever, headache, myalgias, arthralgias, rash, and occasionally hemorrhagic manifestations (3). These nonspecific symptoms necessitate specific diagnostic tests to differentiate dengue from other diseases, such as leptospirosis, rubella, influenza, or rickettsial infections, that can have similar clinical presentations (10, 12). Dengue epidemics, which often occur in urban settings, result in tens of thousands of cases, requiring high-throughput diagnosis.

The immune response to dengue varies in primary versus secondary infections and from person to person; therefore, several techniques are often used in combination to confirm a case of dengue. Not all infections result in detectable immunoglobulin M (IgM), even several days after the infection has cleared. Therefore, in addition to IgM seroconversion, other methods are used, such as viral detection—viral isolation, reverse transcription-PCR, and antigen detection—in acute-phase specimens and measurement of IgG titers in paired acute- and convalescent-phase samples (11, 26). Virus isolation can be compromised by the difficulty of proper transportation and storage of the specimen required to protect the labile RNA virus. Antibody titration via enzyme-linked immunosorbent assay (ELISA) or the "gold standard," hemagglutination inhibition (HI) (4), is useful; however, diagnosis by antibody titration requires paired samples, which are often difficult to obtain. Due to this problem, IgM detection, using IgM capture ELISA (MAC-ELISA) (24) in a single sample, is the most commonly used diagnostic assay. However, this results in a "probable" case rather than a "confirmed" case of dengue, which requires paired sera or detection of virus in an acute-phase specimen (26). Recently, rapid diagnostics such as immunochromatographic cards have been developed (23) but have yet to be thoroughly evaluated in terms of cost, efficiency, and accuracy for widespread use in dengue-endemic countries, such as Nicaragua.

Since dengue is a major public health problem in Nicaragua, we performed a series of investigations with the objective of improving the diagnosis of DEN infection (2, 13). The need for a venous blood sample is a major drawback to all of the serological assays, particularly where children are concerned, in

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whom venipuncture can be problematic. Therefore, we investigated the use of saliva (oral fluid) as a clinical specimen in a large number of patients and found this approach to be very promising. Furthermore, because the IgA response in DEN infection has not been well investigated, we examined the kinetics of DEN-specific IgA in serum and saliva samples and show that detection of IgA in serum may be a useful alternative to IgM detection.

MATERIALS AND METHODS

Study population and specimen collection. Patients or guardians granted permission for saliva specimens to be obtained and used, along with the routinely collected blood samples, for the present study; all personal identifiers were removed. Serum samples were collected conventionally via venipuncture by using Vacutainer tubes (Becton Dickinson, Franklin Lakes, N.J.), and saliva specimens were collected in a plastic receptacle normally used for sputum collection. Both types of specimens were stored and transported at 4°C to the National Center of Diagnosis and Reference (CNDR) in Managua, Nicaragua, where they were frozen at −70°C until processing.

Group 1: single samples classified by serum IgM status. A total of 147 serum and saliva specimens were collected from patients manifesting signs and symptoms suggestive of DEN infection at different health centers pertaining to the Ministry of Health in Nicaragua from July 1999 to November 1999. Both serum and saliva samples from 117 persons were collected within the first 7 days since the onset of symptoms; samples from 27 persons were collected between days 8 and 30 since the onset of symptoms; and samples from the remaining 3 persons were taken between days 31 and 78 since onset of symptoms. For some of the IgM-negative samples, additional methods (e.g., reverse transcription-PCR and inhibition ELISA) were used to determine whether the case was ultimately classified as a confirmed-positive, confirmed-negative, or indeterminate result.

Group 2: confirmed paired samples. A second collection of 151 paired acute (0 to 7 days since the onset of symptoms)- and convalescent (8 or more days since symptom onset)-phase serum samples from another series of patients with confirmed DEN infection was analyzed. The acute- and/or convalescent-phase sera from these cases were confirmed as DEN infections by using an in-house IgM capture ELISA and/or an inhibition ELISA that measures total anti-DEN antibody levels.

Group 3: healthy controls. Fifty specimens of serum and saliva were collected as described above from healthy adults, constituting a separate control group for the study; consent was granted as described above, and personal identifiers were removed for analysis.

Antigen production. *Aedes albopictus* C6/36 cells (15) were grown in four 150-cm³ flasks, each containing 100 ml of minimum essential medium (Gibco-BRL, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (Sigma Chemical Co., St. Louis, Mo.) and 1% glutamine, vitamins, and nonessential amino acids. After 5 days of incubation at 28°C, the medium was decanted, and each flask was inoculated with one of the four DEN serotypes (DEN1, Hawaii strain, obtained from the Centers for Disease Control, Atlanta, Ga., and DEN2 [NIC9622/98], DEN3 [NIC5392/98], and DEN4 [NIC1554/99], isolated in the CNDR laboratory in Nicaragua). After 1 h, 100 ml of medium (as described above but with 2% fetal bovine serum) was added to each flask, which was incubated for 7 days at 28°C. At this point, the medium was decanted and the cells were washed three times with phosphate-buffered saline (PBS). Then, 1 ml of PBS was added to each flask, and seven freeze-thaw cycles were performed. The contents of the four flasks, each containing one of the four serotypes, were mixed together and treated with 1% Tween 20. The antigen was inactivated with a 1:2,000 dilution of formaldehyde (0.0185% final concentration) and stored at −70°C until use.

Capture ELISA for IgM and IgA. The method described by Kuno et al. (17) was used with minor modifications. Briefly, strips of polystyrene microwells were coated with rabbit anti-human IgM (100 μl; Sigma Chemical Co.) or anti-human IgA immunoglobulins (100 μl; Sigma Chemical Co.) at a concentration of 10 μg/ml in sodium carbonate-bicarbonate buffer (pH 9.5) and incubated for 30 min at 50°C. Each serum specimen was diluted 1:20 in PBS plus 0.05% Tween 20 (PBST), whereas the saliva specimens were not diluted. All samples were processed in duplicate. The samples (50 μl) were added to the wells and incubated for 30 min at 37°C. A mixture of antigens from the four DEN serotypes was diluted 1:40 in PBST with 2.5% negative human serum (NHS), added to the wells (50 μl), and incubated for 1 h at 37°C. A human anti-DEN immunoglobulin was conjugated in-house to horseradish peroxidase (HRP; Sigma Chemical Co.) as

described by Wilson and Nakane (25). This conjugate was diluted 1:4,000 in PBST with 2.5% NHS, added to the wells (50 μl), and incubated for 30 min at 37°C. The substrate, tetra-methyl-bencidine (TMB; Sigma Chemical Co.), was added (50 μl), and the strips were held at room temperature for 10 min until the reaction was terminated by the addition of 50 μl of 12.5% sulfuric acid. The optical density was read at 450 nm in an ELISA reader (Humanreader, HUMAN Gesellschaft fur Biochemica and Diagnostica, Taunusstein, Germany). After each incubation period, the wells were washed 4 times with PBST. The cutoff was determined as 2 times the average of the negative controls for IgM and 1.8 times the average of the negative controls for IgA. All of the samples with absorbance values above the cutoff were considered positive.

Inhibition ELISA. The inhibition ELISA was performed as described by Fernandez and Vazquez (8) with minor modifications. Briefly, 96-well polystyrene plates were coated with 100 μl per well of human anti-DEN immunoglobulins at a protein concentration of 10 μg/ml and incubated overnight at 4°C. After three washes with PBST, 150 μl of 1% bovine serum albumin (BSA) diluted in PBST was added to each well, followed by incubation for 30 min at 37°C. The mixture of the four DEN antigens was diluted 1:80 in PBST, and 100 μl was added to each well, after which the plates were incubated for 1 h at 37°C. After four washes with PBST, 100 μl of the specimen (serially diluted in PBST with 0.4% BSA from 1:20 to 1:20,480) was added. The negative controls were added at a dilution of 1:20, and the positive controls were added at a dilution of 1:5,120. The plates were then incubated for 1 h at 37°C. After four washes with PBST, 100 μl of HRP-conjugated human anti-DEN antibody diluted 1/6,000 in PBS with 2.5% NHS was added to each well. The plates were incubated for 1 h at 37°C and washed four times with PBST, and then 100 μl of TMB was added to each well. The plates were held at room temperature for 10 min, the reaction was stopped with 50 μl of 12.5% sulfuric acid/well, and the optical density at 450 nm was determined in an ELISA reader. Again, all samples were processed in duplicate. The titer of each sample was calculated as the last dilution for which the percent inhibition (%I) was ≥50. The %I was calculated by using the following formula: $\%I = [1 − (\text{absorbance of the sample/average absorbance of the negative controls})] \times 100$.

Definitions and statistical analysis. Serological confirmation and classification as primary or secondary infection were determined as described previously (14). Briefly, primary infection was defined by inhibition ELISA by an antibody titer of <20 in acute-phase samples (equivalent to a HI titer of <10) or of <5,120 in convalescent-phase samples (equivalent to an HI titer of <2,560). Secondary infection was defined by inhibition ELISA by an antibody titer of ≥20 in acute-phase samples (equivalent to an HI titer of ≥10) or ≥5,120 in convalescent-phase samples (equivalent to an HI titer of ≥2,560). The data were analyzed by using Epi-Info, version 6 (Centers for Disease Control and Prevention, Atlanta, Ga.).

RESULTS

Determination of IgM antibodies in saliva specimens from group 1 (single samples classified by serum IgM status) and group 3 (healthy controls). A total of 147 samples of serum and saliva were collected from patients who presented with symptoms indicative of DEN infection. Since the most common diagnostic assay for DEN infection in single samples is the IgM capture ELISA (24), detection of IgM in saliva was compared to that of serum IgM in the study population for evaluation of the utility of salivary IgM detection as a diagnostic tool. Serum and saliva samples were also collected from 50 healthy donors who were confirmed to be serologically negative for DEN infection, constituting an additional control group (group 3). Of the 147 cases, 72 were positive for serum anti-DEN IgM. IgM was present in the saliva of 65 (90.3%) of the 72 serum IgM-positive cases, in 6 (8%) of the 75 serum IgM-negative cases, and in none of the healthy controls. The six serum IgM-negative, saliva IgM-positive cases all yielded results for saliva IgM close to the cutoff value and were therefore borderline positive for saliva IgM. All six were negative for IgA in sera, and five of the six were negative for IgA in saliva. As shown in Table 1, the IgM saliva assay, compared to the IgM serum assay, resulted in a sensitivity of 90.3%, a

TABLE 1. Evaluation of IgM in saliva and IgA in serum and saliva as diagnostic tools compared to IgM in serum from group 1 (single samples categorized for serum IgM status)

Immunoglobulin group	IgM serum			Sensitivity	Specificity	Concordance	PPV[a]	NPV[b]
	No. positive	No. negative	Total					
Saliva IgM								
Positive	65	6	147	90.3	92.0	91.2	91.5	90.8
Negative	7	69						
Serum IgA								
Positive	68	19	147	94.4	74.7	84.4	78.2	93.3
Negative	4	56						
Saliva IgA								
Positive	51	24	147	70.8	68.0	69.4	68.0	70.8
Negative	21	51						

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.

specificity of 92.0%, a positive predictive value of 91.5%, a negative predictive value of 90.8%, and a concordance of 91.2%.

Determination of IgA antibodies in serum and saliva specimens from group 1 and group 3. IgA was detected in 68 (94.4%) serum samples from the serum IgM-positive cases and in 19 (25.3%) of the serum samples from the serum IgM-negative cases. This resulted in a sensitivity of 94.4%, a specificity of 74.7%, and a concordance of 84.4% (Table 1). IgA was detected in 51 (70.8%) saliva samples from the serum IgM-positive cases and in 24 (32.0%) of the saliva samples from the serologically negative cases. All serological markers yielded negative results in the healthy control group. Thus, the sensitivity, specificity, and concordance of IgA in serum was significantly better than that of salivary IgA (Table 1).

Kinetics of IgM and IgA in serum and saliva specimens from serum IgM-positive samples of group 1. The kinetics of IgM in saliva and IgA in serum and saliva samples were evaluated according to the number of days since reported onset of symptoms in the 72 cases that were positive for serum IgM DEN antibodies (Table 2). The rate of positivity of DEN-specific IgM in saliva was not significantly different in samples collected in the first 7 days (45 of 50, 90%) than in samples collected after 7 days since symptom onset (20 of 22, 90.9%). In contrast, the rate of positivity of DEN-specific IgA in serum was higher in samples collected in the first 7 days (48 of 50, 96%) than after 7 days (20 of 22, 90.9%) since the onset of symptoms.

Of the serum IgM-positive samples in group 1, 46 (63.8%) were classified as secondary infections. Table 2 shows the distribution by day since symptom onset according to immune status. The rates of positivity for IgA in serum were significantly higher in secondary infections than in primary infections (100 versus 84.6%) compared to those of salivary IgM (93.5 versus 84.6%) or those of salivary IgA (69.6 versus 73.1%).

Kinetics of IgM and IgA in serum samples according to days of illness in group 2 (confirmed paired samples). The kinetics of IgM and IgA detection were evaluated in a second sample set consisting of 151 paired acute- and convalescent-phase serum samples from a different group of patients, who were all serologically confirmed for DEN infection. DEN-specific IgA and IgM were measured in the acute-phase sample (collected

TABLE 2. Determination of IgM in saliva and IgA in sera and saliva in samples of serum IgM-positive cases of group 1 (single samples categorized for serum IgM according to the number of days since symptom onset and the status of infection)

Immunoglobulin group	No. of samples at day[a]:																											
	1			2			3			4			5			6			7			>7			Total		
	All	1°	2°	All	1°	2°	All	1°	2°	All	1°	2°	All	1°	2°	All	1°	2°	All	1°	2°	All	1°	2°	All	1°	2°
Total	2	0	2	7	6	1	12	1	11	9	1	8	12	2	10	2	0	2	6	2	4	22	14	8	72	26	46
Saliva IgM																											
Positive	2	0	2	6	5	1	11	1	10	7	1	6	12	2	10	2	0	2	5	1	4	20	12	8	65	22	43
Negative	0	0	0	1	1	0	1	0	1	2	0	2	0	0	0	0	0	0	1	1	0	2	2	0	7	4	3
Serum IgA																											
Positive	2	0	2	5	4	1	12	1	11	9	1	8	12	2	10	2	0	2	6	2	4	20	12	8	68	22	46
Negative	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	4	4	0
Saliva IgA																											
Positive	2	0	2	5	4	1	8	1	7	6	0	6	8	1	7	2	0	2	3	2	1	17	11	6	51	19	32
Negative	0	0	0	2	2	0	4	0	4	3	1	2	4	1	3	0	0	0	3	0	3	5	3	2	21	7	14

[a] "Day" refers to the day since symptom onset. Immune status is indicated as all types (All), primary (1°), or secondary (2°).

in the first 7 days since onset of symptoms), and the percentage of specimens positive by each method was graphed according to the day since onset of symptoms. IgA was consistently detected in more samples than was IgM, since IgA was found in 80 (53.0%) and IgM was detected in 71 (47.0%) of the 151 samples (Fig. 1A).

One possible explanation for the difference between IgA and IgM is that IgA, like IgG, may rise rapidly in a secondary DEN infection, occasionally overshadowing the IgM response (14); therefore, the relation of antibody detection to immune status was examined in this group of specimens as well. Consistent with our previous results with group 1, a trend was observed of higher rates of positivity with IgA than with IgM in serum in secondary infections (Fig. 1B). The discordant results, where IgA did not correspond with IgM, were evaluated with respect to immune status. The IgM and IgA results differed in acute samples in 17 of the 151 cases, of which 13 were IgA positive and IgM negative. Of these, 12 were secondary cases. The remaining four discordant cases, which were IgM positive and IgA negative, consisted of two primary cases and two secondary cases. The majority of cases (128 of 151, 88.6%) were classified as secondary dengue infections.

DISCUSSION

This study establishes the utility of saliva for the diagnosis of DEN infection and describes the characteristics of DEN-specific IgM and IgA antibodies in both serum and saliva. The collection of saliva specimens is not invasive and therefore presents an attractive alternative for the diagnosis of DEN infection. The utility of this type of specimen has been reported for the diagnosis of a number of infectious diseases (6, 7, 9, 20, 21), including dengue (1, 5). However, earlier studies of the detection of anti-DEN antibodies in saliva were performed on limited numbers of specimens; our study examines over three times more samples than in previous reports.

To evaluate its potential in dengue diagnosis, detection of DEN-specific IgM in saliva was compared to that of IgM antibodies in serum by using the MAC-ELISA (group 1), yielding a sensitivity and a specificity of 90.3 and 92.0%, respectively. These results are consistent with those reported by Cuzzubbo et al. (5), who found an overall sensitivity of 92.0%, and are higher than those reported by Artimos de Oliveira et al. (1), who detected anti-DEN IgM in 65.8% of saliva samples. Of the 72 serum IgM-positive cases, there were 7 false-negative results as determined by saliva IgM. Of these seven, there was no discernible trend in number of days since onset of symptoms or in immune status. It should be noted that neither protease inhibitors nor any other type of preservative was added to the saliva specimens due to cost considerations; this could have contributed to the discordance.

Of the 75 cases that were serologically IgM negative, there were 6 that were borderline positive for IgM in saliva. Of these six, four had convalescent-phase samples that were tested in our laboratory for national surveillance, and no additional data were available on the remaining two. Analysis of the four convalescent-phase samples demonstrated that these were in fact cases of dengue (data not shown). One potential explanation for positive saliva IgM-negative serum IgM results could be that salivary IgM may not be bound to antigen and therefore may be detected better in the assay, whereas serum IgM may be bound in complexes with the dengue antigen. That the six original samples also tested negative for serum IgA may further support the theory that all available anti-DEN serum antibodies in the specimen were saturated in immune complexes.

Thus, detection of anti-DEN IgM in saliva specimens provides an alternative diagnostic marker that has the benefit of easy sample collection, requiring neither special materials nor technical expertise in phlebotomy. This is particularly important in dengue-endemic, resource-poor countries such as Nicaragua, where many children become infected annually with DEN, since collection of venous blood can be difficult and even dangerous in small children. In addition, saliva is a more cost-effective test sample than serum since saliva specimens are cheaper to collect and require no additional processing.

The behavior of IgA as a serological marker for DEN infection was also evaluated and found to be a promising alternative diagnostic tool to detection of IgM in serum. IgA was detected in the serum of 94.4% of the IgM-positive samples (group 1). The four discordant cases (IgM positive and IgA negative) were all cases of primary infection; therefore, it is probable that class switching had not yet occurred, since IgM must be produced prior to IgA in a primary infection. Of the 75 cases that were serum IgM negative, 56 were also IgA negative, whereas 19 were IgA positive. However, when the 19 IgA-positive, IgM-negative serum samples were further investigated (either through an available convalescent-phase sample or viral detection in an acute specimen), 11 were found to be positive and 6 were negative for DEN infection. No additional information was available for the two remaining samples. Thus, the specificity calculation for the serum IgA diagnostic assay is complicated by the fact that a number of the IgM-negative, IgA-positive samples were shown by other assays to be cases of DEN infection. This brings up the possibility that, at least in secondary infections, the detection of IgA may be more sensitive than IgM detection in serum.

The high percentage (96.0%) of DEN-specific IgA in acute-phase sera of group 1 led us to investigate IgA kinetics in a second set (group 2), which were all confirmed as positive for DEN infection with a convalescent-phase sample. It was found that IgA antibodies were detected in more acute-phase sera than were IgM antibodies. One explanation for our results is that IgA levels are higher in secondary infections, which constituted the vast majority of our samples. In a primary infection, IgM must be generated before IgA class switching can occur; however, in a secondary infection, cross-reactive boosting of an IgA response to a previous infection may occur, analogous to the situation with IgG, and IgA levels may rise prior to IgM. In fact, we found that IgA was detected in a higher percentage of secondary than primary cases. In contrast, Talarmin et al. (22) found that IgA levels became elevated after the levels of IgM increased; however, the majority of their samples were from primary infections. The high rates of secondary DEN infections observed in these sample sets are consistent with results from our previous hospital-based studies (14) and seroprevalence data from an ongoing prospective study in schoolchildren in Managua (A. Balmaseda, S. Hammond, and E. Harris, unpublished data). The overall sensitivity we obtained for IgA in serum was higher than that reported
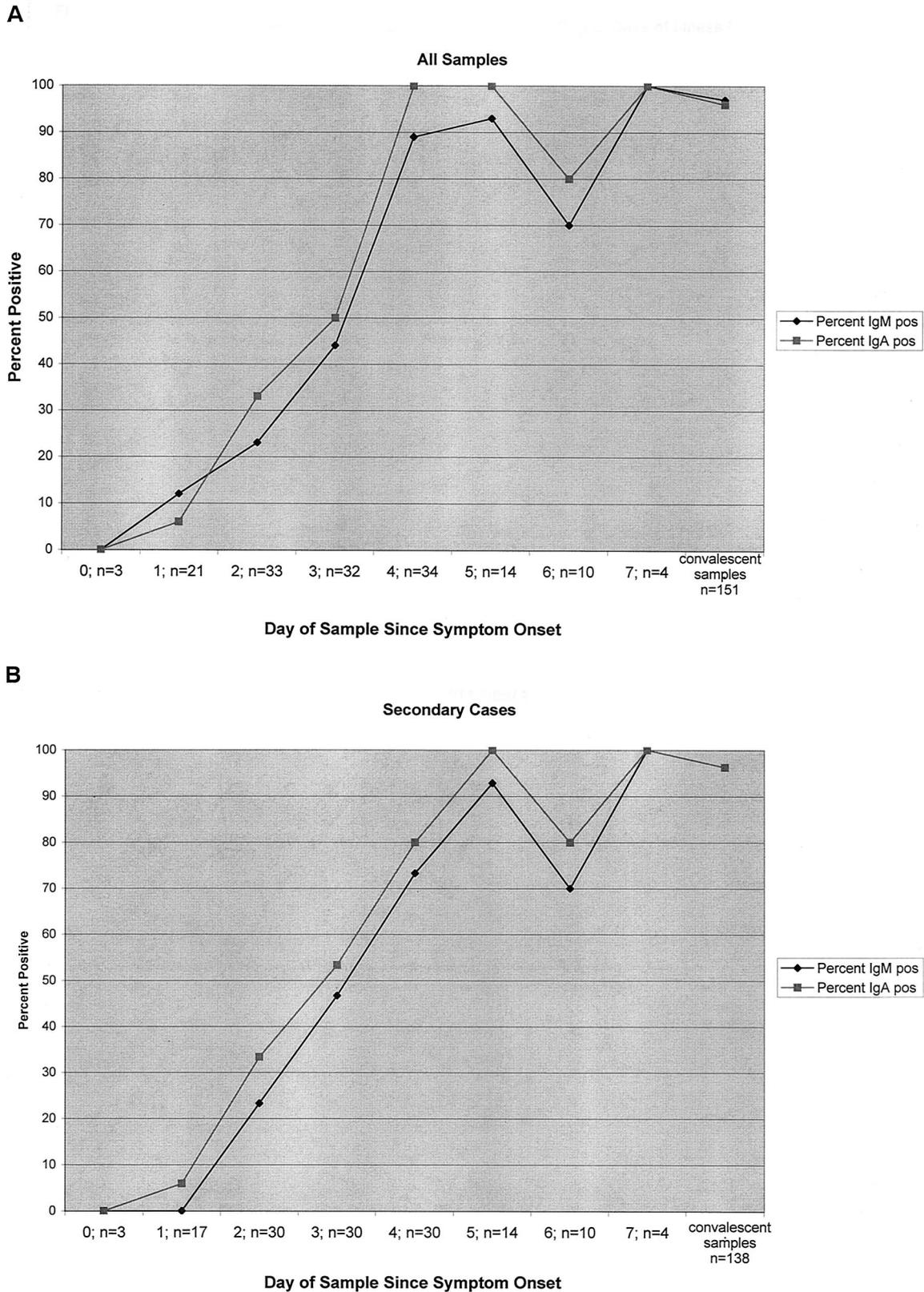
A

B

FIG. 1. (A) Kinetics of detection of DEN-specific IgM and IgA antibodies with respect to symptom onset. The percentage of serum specimens positive for DEN-specific IgM or IgA antibodies is graphed according to when the sample was collected in relation to the number of days since onset of symptoms (*x* axis). Acute-phase specimens from the set of 151 paired serum samples were analyzed in this graph. (B) Kinetics of detection of DEN-specific IgM and IgA antibodies with respect to symptom onset in cases of secondary infection only.

previously (22), while the specificity was lower. This difference could be attributed to the fact that we diluted samples only 1:20 as opposed to 1:100 (22) or to the assignation of cutoff values, since Talarmin et al. (22) chose to maximize the specificity of their assay whereas we placed more emphasis on sensitivity. Certainly, the behavior and duration of IgA for both secondary and primary infection warrants further investigation.

The decision to compare detection of IgA in both serum and saliva to detection of IgM in serum was based on the reasoning that IgM detection is the routine diagnostic method and, as such, should be the benchmark for diagnostic techniques. However, our results strongly indicate that to better assess the specificity and sensitivity of a DEN-specific IgA assay, studies assessing the sensitivity and specificity of IgA detection in confirmed (e.g., paired) samples would be worthwhile. The kinetics of IgA response to DEN infection clearly needs additional study, since previous reports suggest that DEN-specific IgA is found in serum for only about a month (22), whereas our preliminary data suggest that DEN-specific IgA may actually persist longer than IgM (Balmaseda et al., unpublished). Interestingly, Koraka et al. (16) reported recently that titers of DEN-specific IgA were significantly higher in acute-phase serum from DSS patients as opposed to DF patients and thus may correlate with clinical outcome. In our study, IgA titers were not determined, so this analysis could not be performed. Nonetheless, these data suggest that detection of IgA in serum may be a new and effective tool for the diagnosis of dengue.

Using the currrent configuration of a capture ELISA, IgA in saliva apparently does not constitute a very useful diagnostic marker of DEN infection, resulting in a sensitivity and specificity of ~70% compared to IgM in serum. The low sensitivity of this marker could be due to the high concentration of nonspecific IgA present in saliva that can compete with DEN-specific IgA; it has been reported that the concentration of IgA in the saliva is 20 mg/ml compared to that of IgM and IgG, which are found at 1.4 and 0.2 mg/ml, respectively (19). Therefore, prior to dismissing salivary IgA as a diagnostic marker, an ELISA where the antigen captures DEN-specific IgA (indirect method) is worthy of investigation; these studies are currently ongoing.

In conclusion, the present study demonstrates the utility of a DEN-specific IgM assay using saliva as a cheap, noninvasive means of dengue diagnosis. In addition, the data offer some insight into the behavior of the DEN-specific IgA response and suggest specific IgA detection as an alternative diagnostic tool for dengue infections.

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