LB-21

A Novel Method to Determine Immunity to *Vibrio Cholerae* In A Previously Immunologically-Naïve Population

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ABSTRACT

Background: Gingival crevicular fluid (GCF) sampling for the detection of cholera infection in previously naïve population has not yet been investigated. This would represent a highly innovative method of making the retrospective diagnosis of *v. cholerae* when conducting serologic surveys, in settings where cholera has been newly introduced.

Aim: To examine the correlation between serum and mucosal immune response in patients recently infected with *v. cholerae* in Haiti.

Method: We compared the serum and mucosal immune responses of 13 patients living in Haiti, with culture confirmed cholera (CCC) and 13 healthy controls (HC) living in a cholera non-endemic region. Serum and gingival crevicular fluid (GCF) was collected from HCs and patients with CCC. We then measured GCF and serum levels of IgG and IgA specific cholera toxin B (CTxB - IgG/IgA) and vibriocidal antibodies for evaluation of comparative endpoints.
Results: Serum CTxB-IgG response was detected in all 13 CCCs and none of the HCs at dilutions as high as 1:400. Serum CTxB-IgA was detected in only 1 CCC case (0.08%) and none of the HCs at a dilution of 1:100. GCF CTxB- IgG response was detected in 10 CCC (76%) and none of HCs at 1:5 dilution. GCF CTxB-IgA was detected in only 1 (0.085%) CCC patient at 1:5 dilution. Serum vibriocidal antibody response was detected in 12 (92%)CCCs and 1(0.085%) HCs. GCF vibriocidal antibody was detected in none of the CCCs or HCs.
Conclusion: Higher levels of CTxB-IgG were detected in serum than in GCF in patients with CCC. Although CTxB-IgG was detected in GCF, it is not a sensitive method for use in serologic surveys.

INTRODUCTION

Cholera seroepidemiologic surveys play an important role in understanding transmission patterns, identifying vulnerable groups and monitoring immune response to vaccines. The best studied correlate of protection against *v. cholerae* is the vibriocidal antibody, a complement fixing bacteriocidal antibody. The antibody to the B-subunit of toxin produced by *v.cholerae* (CTxB) is also a sensitive indicator of recent infection. Until the October 21st 2010 outbreak in Haiti, *v. cholerae* has never previously figured as a cause of infectious diarrhea. At the onset of the epidemic, no cases of acute diarrhea would be expected to have high background titers of antibody to cholera. Therefore, detection of significantly elevated cholera antibody titers a few weeks after the acute illness, would represent evidence of recent infection. Both vibriocidal and CTxB antibodies have been used extensively in serum serological surveys. The relationship between gingival crevicular fluid (GCF) and serum antibody titers has been well established and is adapted for diagnosis of HIV and other infectious diseases. CTxB has a mucosal immunopotentiating effect which has been shown to stimulate mucosal IgA immune response after intestinal infection. Cholera serological surveys are usually conducted by measuring serum cholera antibodies, but the use of GCF for cholera antibody detection in a previously naïve population has yet to be investigated. Use of this type of sampling could potentially afford clear advantages such as an easy, noninvasive method of sample collection not requiring specialized personnel. This study has examined the detection of GCF sampling for the retrospective diagnosis of cholera which would represent a highly innovative surveillance method during cholera epidemics in previously immunologically naïve populations.

AIM

To examine the correlation between gingival and serum cholera antibody response for retrospective diagnosis in an immunologically naïve population .

STUDY POPULATION

Over 4,000 patients were admitted to GHESKIO's cholera treatment center (CTC), located in Port-au-Prince Haiti, for acute diarrhea between October 21st 2010 to August 7th 2011. During the first 3 months of the outbreak, 37 patients were found to have bacteriologic evidence of cholera infection, of which 13 patients were seen in clinic for follow up at 2-15 weeks post admission. Patient were subsequently interviewed before obtaining the blood and GCF samples used in this analysis.

Parameters		Patients with culture confirmed cholera (CCC) N=13	Healthy Controls (HC) N=13
Age (yr) median		27 (1.5-53)	NA
Gender	Male	8	6
	Female	5	7
Level of Dehydration on admission at CTC	Mild	4	NA
	Moderate	1	NA
	Severe	7	NA
Duration of Illness prior to Presentation (mean, range)		3.5 days (1-8)	NA

METHOD

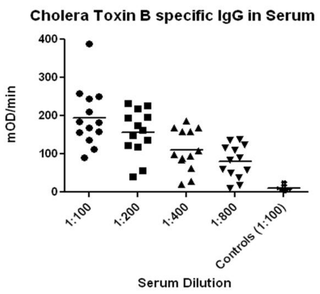
Humoral immune responses in gingival crevicular fluid and serum of 13 patients with culture confirmed cholera (CCC) and 13 volunteers living in a cholera non-endemic region were measured as follows:
Cholera Toxin B specific IgG/IgA (CTxB-IgG/IgA): Gingival fluid was collected using the Orasure® system. Gingival crevicular fluid and serum IgG responses to the recombinant cholera toxin B subunit (CTxB) were measured by kinetic enzyme-linked immunosorbent assay (kELISA).
Vibriocidal Assay: Serial dilution of samples and comparative endpoints of serum and mucosal samples were determined. Cultured strains of the Haitian isolate serotype of *V. cholerae O1* El Tor Ogawa (BAA-2163) and three additional international strains (MQ1795, O395, and N16961) were used.
Sample Interpretation: Since cholera has never previously been identified as a cause of diarrhea in Haiti, the detection of significant cholera antibodies would represent evidence of recent cholera infection.
CTxB detection: Optic density values less than 30mOD/min in serum and less than 10 mOD/min in gingival crevicular fluid were read as negative
Vibriocidal IgG detection: Vibriocidal titers <1:16 were read as negative as this was the highest serum level detected in negativecontrols (reported as the reciprocal of the highest serum dilution) .

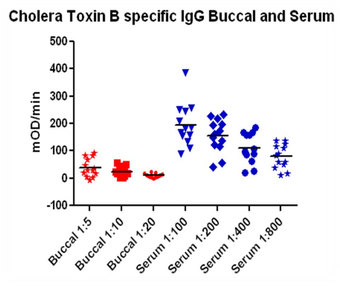
RESULTS

Serological Results	Participants with Culture Confirmed Cholera (%)	Healthy Controls
Serum CTxB-IgG > 30mOD/min	13 (100%)	0
Serum CTx-IgA >30mOD/min	1 (0.08%)	NA
Gingival CTxB-IgG >30mOD/min	10 (76%)	0
Gingival CTxB-IgA >10mOD/min	1 (0.08%)	NA
Serum Vibriocidal titer to BAA2163* >1:16	12 (92%)	1(0.08%)
Gingival Vibriocidal titers to BAA2163* >1:16	0	0

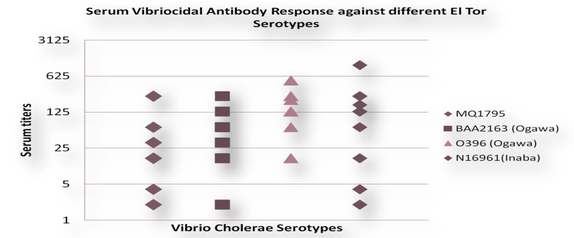
Antibody Response and parameter	Value for Response in patients with CCC	
	CTxB IgG/IgA Response	
	Serum	Gingival
CtxB-IgG Response (Mean, mOD/min)	195 (1:100 dilution)	39.1 (1:5 dilution)
95% CI	148.2-241	19.2-59
p value Compared to HC	0.000229	0.0011
CTxB-IgA Response (Mean mOD/min)	5.2 (1:100 dilution)	0.08 (1:5 dilution)
95% CI	-1.2-11	-1.7-1.9
Antibody Response and parameter	**Vibriocidal Response**	
Vibriocidal Titer To BAA2163* (Mean)	156	None
95% CI	88-223	NA
p value compared to HC	0.000608	NA
Vibriocidal Titer To BAA2163* (Mean)	156	None
95% CI	88-223	NA

*Haitian Strain of V.Cholera El Tor Ogawa





RESULTS



SUMMARY

- Serum and oral mucosal CTxB-IgG response exceeded CTxB-IgA response in patients with CCC
- Patients with CCC who had detectable GCF CTxB specific IgG had a 20 fold lower concentrations compared to serum
- GCF CTxB-IgA was undetectable in 12 of 13 patients
- 92% of patients with CCC demonstrated significant serum vibriocidal antibody titers with cross reactivity amongst 4 different Vibrio Cholera El Tor strains .
- Gingival crevicular fluid vibriocidal antibody was not detected using described methods

CONCLUSION

CTxB-IgG was detected in gingival crevicular fluid of patients recently diagnosed with cholera. Even in an immunologically naïve population, this method may not be sensitive enough to retrospectively diagnose recent cholera infection.

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