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Original Research Article

Hand-Washing, Subclinical Infections, and Growth: A Longitudinal Evaluation of an Intervention in Nepali Slums

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Objective: We conducted a longitudinal study to assess the impact of a hand-washing intervention on growth and biomarkers of child health in Nepali slums. This is the first study to evaluate the impact of hand-washing on markers of subclinical, asymptomatic infections associated with childhood growth faltering.

Methods: We recruited a total sample of infants in the target age-range (3–12 months) living in the eight largest Kathmandu slums, allocating them to intervention ($n = 45$) and control ($n = 43$) groups. In intervention areas, a small-scale community-based hand-washing program was implemented for six months; in control areas, mothers continued their normal practices. Time series linear regression was used to assess the impact of the intervention on levels of morbidity, mucosal damage, immune stimulation and growth.

Results: As expected, children with higher levels of mucosal damage exhibited worse growth over the period of the intervention ($P = 0.01$, <0.001 and 0.03 for height-for-age, weight-for-age, and weight-for-height z-scores, respectively). We observed a 41% reduction in diarrheal morbidity ($P = 0.023$) for the intervention group relative to control. However, the hand-washing intervention did not lower levels of mucosal damage or immune stimulation, nor slow growth faltering.

Conclusions: Reducing exposure to pathogens is an important global health priority. This study confirms the importance of hand-washing campaigns for reducing childhood morbidity. Yet our data suggest that promoting hand-washing is necessary *but not sufficient* to address chronic, subclinical infections. From a human biology standpoint, tackling the root causes of childhood infections is needed to address growth faltering in the context of highly contaminated slum environments. Am. J. Hum. Biol. 23:621–629, 2011. © 2011 Wiley-Liss, Inc.

The relationship between growth and health in childhood is very simple: healthy children grow well; sick children do not. Because of its remarkable sensitivity to environmental insults, growth is recognized as the best global indicator of child well-being (de Onis et al., 2000). Since the 1980s, significant progress has been made across the globe in reducing rates of growth faltering: the prevalence of stunting in developing countries, for example, has fallen from 47% in 1980% to 33% in 2000 (*ibid*). However, despite this progress, it is estimated that about a third of children remain stunted, one in four is underweight, and one in 10 is wasted (UNICEF, 2009). The negative consequences of growth faltering for health in the short-, medium- and long-term are well documented (Calder and Jackson, 2000; Norgan, 2000); finding ways to alleviate this problem is therefore a pressing public health priority.

The two major causes of poor growth in childhood are malnutrition and infection, which interact synergistically to create a vicious cycle of growth faltering. Much attention has focused on the importance of diarrheal disease in causing growth faltering. Diarrhea, of course, is not a disease per se, but rather a symptom of an underlying pathology, often indicating damage and inflammation of the mucosal lining of the gastrointestinal tract. Pathogenic damage to the lining of the intestine can lead to maldigestion and malabsorption of food, resulting in less energy being absorbed into the body. Such damage also results in the stimulation of inflammatory and immune responses to repair damaged tissue and fight off infection, thus leaving less energy available for processes such as growth (Campbell et al., 2003; Lunn, 2000).

However, it is important to recognize that such intestinal damage occurs along a spectrum and not all will result in diarrheal symptoms: much of this damage occurs at a subclinical level. In other words, it occurs at a level not severe enough to produce clinically visible symptoms such as diarrhea, but nonetheless results in some amount of functional impairment to the digestive and absorptive capacity of the intestinal mucosa (Lunn, 2000). In much of the developing world, poor environmental conditions result in children being frequently exposed to pathogenic organisms and thus the prevalence of sub clinical infections is likely to be high (Adelekan et al., 2003). While, by definition, such infections are less severe than those resulting in diarrhea, the potentially chronic nature of subclinical infections suggests they may be an important factor in childhood growth faltering.

Indeed, some studies have indicated that subclinical infections may be more important than diarrhea per se. A study conducted in a peri-urban shanty town in Lima, Peru by Checkley et al. (1997) investigated the importance of both symptomatic and asymptomatic (i.e., subclinical)

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cryptosporidiosis on child growth. Although asymptomatic infections retarded weight gain to a lesser extent than those resulting in diarrheal symptoms, the former were almost twice as common. Thus the authors concluded that it was the asymptomatic infections that had the most detrimental impact on child growth, due to their much greater prevalence within the population. Similarly, Lunn (2000) reported no association between diarrhea and growth faltering in young Gambian children, but levels of mucosal damage and its associated stimulation of the immune system accounted for almost half of the observed deficits in height and weight. Finding ways to prevent subclinical infections that result in mucosal damage and immune stimulation may therefore be an important means of preventing growth faltering in early childhood.

An obvious means of preventing such infections is to limit children's exposure to the pathogenic organisms that cause damage. The link between improved hygiene and reduced spread of infection is well established (Aiello and Larson, 2002). In particular, hand-washing with soap at specific junctures (after contact with fecal material or before contact with food) has been shown to be highly effective in reducing diarrheal and respiratory infections. Meta-analyses conducted by Curtis and Cairncross (2003) and Rabie and Curtis (2006) have demonstrated that hand-washing with soap reduces the risk of diarrheal disease in children and adults by 42–47% and acute respiratory infections by 16%. However, the impact of hand-washing on the biological pathways that link subclinical infections and childhood growth faltering remains untested (Humphrey, 2009). Could this simple intervention have an additional benefit in also reducing levels of mucosal damage and immune stimulation? We hypothesized that an increase in hand hygiene would not only reduce clinical morbidity, but would also lead to a reduction in mucosal damage, markers of immune stimulation and ultimately growth faltering in young children. Here we report results from a small-scale community-based hand-washing intervention implemented in Kathmandu, Nepal designed to test this hypothesis for the first time.

STUDY BACKGROUND

The importance of mucosal damage, immune stimulation, and early growth faltering has been recently documented in Nepal. One cross-sectional study documented very high levels of intestinal permeability among 0- to 60-month-old children in poor areas of Kathmandu (Goto et al., 2002). Subsequently, a longitudinal study in younger cohorts of 3- to 18-month-olds was able to demonstrate prospective associations between mucosal damage and growth faltering, and evaluate the relative importance of nutrition and infection pathways responsible for growth faltering (Panter-Brick et al., 2009). This study builds on such previous work, with the specific aim of evaluating the impact of a hand-washing intervention. The hand-washing intervention was informed by a systematic body of work assessing previous community-based hand-washing interventions in developing countries (Curtis et al., 1997, 2001), and was underpinned the Theory of Planned Behavior (Fishbein and Ajzen, 1975) which focuses on the social and psychological determinants of behavioral change. This theory suggests that an intention to perform a certain behavior is a product of the interaction between a person's attitudes, subjective norms, and perceptions of self-efficacy. The intervention thus aimed to promote a positive attitude towards hand-washing, establish hand-washing as a social norm and remove barriers that might hinder hand-washing practices. Informed by qualitative findings from interviews and focus groups, the primary motivational message of the intervention was that washing hands prevents sickness and protects one's family. However, this was also backed up by subsidiary messages aimed at changing attitudes and social norms and creating a "demand" for hygiene; thus hand-washing was promoted to the mothers as something that "good," "clean," and "respected" mothers do, while also emphasizing the personal benefits of making them feel fresh and clean and giving them soft, nice-smelling hands.

METHODS

Study design

We focused this study on a target group of 3- to 12-month-olds, to capture the critical period of growth faltering (6–18 months) and allow recruitment of children moving into this age range over the six-month period of study. We also focused on poor children living in slum settlements, having identified these children as experiencing very high levels of mucosal damage, immune stimulation and growth faltering in a previous study (Panter-Brick et al., 2009).

Data from this previous study (Panter-Brick et al., 2009) were used to determine sample size, based on a test with 80% power, a 95% level of significance and a 30% reduction in levels of mucosal damage, immune stimulation and growth faltering. Sample size was calculated to be 88 children (44 intervention, 44 control). To mitigate potential attrition, target sample size was increased to 100 children.

We used the most recently available geographical and demographic data to identify all existing slum settlements within Kathmandu (Shrestha and Shrestha, 2005): a total of 118 settlements were listed, the majority of which were small settlements of less than 50 households. Because of Kathmandu's geographical spread, the logistics of traveling within this overcrowded city, and frequent and unpredictable strikes associated with Nepal's recent political upheaval, we focused our study on the largest slum settlements in order to maximize the number of children in the target age range over the smallest number of field sites. We thus selected the largest slum settlements (n = 8) to achieve required sample size, after excluding five communities that were inaccessible (n = 3), declined to participate (n = 1), or recently benefited from improvements in water provision and sanitation (n = 1). House-to-house surveys were conducted in each of the selected settlements to determine the number of children in the target age-range. The mothers of these children were invited to a recruitment meeting and agreed to participate. The sampling strategy for this study therefore consisted of a purposive sampling of the eight largest slum settlements in Kathmandu, followed by a total sample of children aged 3–12 months living in these communities.

The eight slum settlements under consideration were divided into two separate groups based on broad geographical areas ("north" and "east") and then randomly allocated (by flipping a coin) to intervention or control groups. It was not possible to randomly allocated each separate settlement to control/intervention conditions as many sites were situated very close to one another (e.g., separated by just a road or stream) such that the intervention message could easily have crossed over into the control settlements.

The first author was present throughout the period of study, having already codirected 10-months of growth and biomarker data collection in Kathmandu (Panter-Brick et al., 2009). The local field team consisted of three Nepali research assistants, helping with the project and acting as translators where necessary; five Community Motivators, well-known and respected women from each study slum area, responsible for the implementation of the hand-washing intervention; and 10 fieldworkers, who after completing a three-week intensive training program, undertook all structured observations of hand-washing behavior and collected weekly morbidity data.

Baseline data collection

Structured questionnaires were used to collect demographic and socio-economic data on all participants, including data on parental levels of education, the family's housing situation and access to certain facilities (such as sanitation), household income and ownership of valuable material possessions (such as television, radio, etc). These data were used to create a composite socio-economic score for each household.

We assessed base-line hand-washing practices in both intervention and control sites through structured observations and questionnaires. Fieldwork assistants undertook structured observations of hygiene behavior for a random sample of households ($n = 75$), recording the mother's hand-washing behavior at five specific junctures: (1) after visiting the toilet, (2) after cleaning the baby's bottom, (3) before preparing food, (4) before feeding the baby, and (5) before eating food. The 3-h observation periods began as soon as the family woke up, usually at 6 am. To limit reactivity, mothers were told the purpose of the observations was to learn about the life and work of Nepali women: hand-washing was never mentioned. Further structured observations conducted at the end of the study to assess changes in hand-washing practices were not possible as mothers in intervention communities would have associated these observations with the hand-washing intervention, thus biasing any data collected.

Questionnaires were also used to determine mothers' reported hand-washing behavior. Though reported data cannot give reliable data on *actual* practices (Cousens et al., 1996), they are useful in assessing mothers' knowledge of *when* hand-washing should occur. Once base-line structured observations were completed, the first author implemented a structured questionnaire with all mothers in intervention and control areas to assess reported hand-washing on the previous day at the five key junctures mentioned above. This questionnaire was repeated at the end of the intervention period.

Design of hand-washing intervention

To inform the design of the hand-washing intervention, we conducted both in-depth interviews and focus groups. In-depth interviews ($n = 26$), conducted with a random selection of the mothers from the intervention group, were focused on local understandings of child health and hygiene. These were conducted in Nepali by the first author (RL) with assistance from a research assistant where necessary. Focus groups ($n = 3$), each comprising of six to eight women, were conducted to discuss these topics in a group setting. These were moderated by a Nepali research assistant and lasted between 1.5 and 2 h.

The research team (RL, three Nepali research assistants, and five community motivators) discussed all available data generated from the observations, interviews and focus groups in order to determine the intervention message and plan intervention activities. The hand-washing program was launched in intervention areas at a community meeting organized in each local area (June 2007). This meeting included an interactive educational session, a discussion led by the Community Motivator, and a short play, commissioned specifically for this intervention and performed by actors from the slum communities.

The intervention was then intensively promoted for six months. The launch meeting was followed up by daily home visits by Community Motivators to each mother to encourage the establishment of a new hand-washing regime. These visits continued on a daily basis for two weeks, and then decreased in frequency until the mothers were visited just once or twice a week throughout the six-month intervention period. Mothers' group meetings were held in each area, with their local Community Motivator, every two weeks throughout the study period. This provided the women an opportunity to socialize with one another, while promoting the hand-washing message. The Community Motivators distributed a new bar of soap to each mother at these meetings to encourage hand-washing practices in the family. In addition, locally designed posters were distributed to all families in the intervention areas, as well as being displayed prominently throughout the settlements.

Health and growth measures

Data collection schedule. Baseline health and growth data were collected during May, 2007. Following the launch of the intervention, monthly health checks (June to November) were conducted in both intervention and control areas to assess mucosal damage, immune stimulation, and growth performance. Morbidity reports for each child were collected on a weekly basis.

Morbidity. Morbidity data for each child focused on the most commonly reported symptoms—diarrhea, coughs/colds, and fevers. Following the WHO (2005), diarrhea was defined as at least three loose stools in a 24-h period. Details of any sickness were collected from the children's mothers by trained local fieldworkers using a symptom checklist. To prevent bias in data collection, these fieldworkers were never involved in any aspect of the program to promote hand-washing.

Subclinical infection

Mucosal damage. Mucosal damage was assessed using the lactose:creatinine urinary test (L:C)—a method which had been successfully employed in a previous study in Nepal (Panter-Brick et al., 2009). Breastfed infants ingest lactose from their mother's breast milk; in a normal gut the majority of this sugar is hydrolyzed by the mucosal enzyme lactase. The resulting monomers are passively absorbed into the bloodstream; only very small amounts of undigested lactose are absorbed into the body and excreted in the urine. However, pathogenic damage to the mucosa leads to the loss of lactase resulting in less lactose being hydrolyzed; thus a greater amount of undigested lactose will be recovered in the urine. The proportion of lactose to creatinine in the urine therefore acts as an index of mucosal damage.

Urine was collected on a sterile urine pad (Newcastle Urine Collection Pack, Ontex UK Ltd, Corby, UK) placed inside a locally purchased nappy. Once the child had urinated, the pad was removed and two 2 ml samples of urine were extracted using a syringe. These samples were preserved with one or two drops of bacteriostat (chlorhexidine digluconate, 2 g/L solution) and then frozen at -20°C until shipment to the UK. An enzymatic assay (Northrop et al., 1990) was used to analyze levels of urinary lactose and a Jaffe technique for creatinine levels (Randox Creatinine Assay Kit; Randox, Crumlin, Antrim, UK).

Immune stimulation. Stimulation of the immune system was assessed through analysis of protein markers in dried blood spots—α-1-acid glycoprotein (AGP) and immunoglobulin G (IgG)—which both increase as a result of infection or inflammation. In addition, hemoglobin (Hb) and albumin were measured, providing information on the nutritional status of the child.

Single-use lancets (Hemocue, Dronfield, Derbyshire, UK) were used to prick the child's finger and obtain the blood-drop samples. One drop of blood was used to obtain an on-the-spot hemoglobin result using a hemoglobinometer (Hemocue, Dronfield, Derbyshire, UK). Subsequent blood drops were collected on 903-protein saver collection cards (Whatman Plc, Maidstone, Kent, UK). When completely dry, the cards were sealed in individual zip-lock bags with desiccant and frozen at -20°C until shipment to the UK. Samples were analyzed using techniques described in Panter-Brick et al. (2009).

Growth faltering

Lengths and weights of children were measured using standard anthropometric techniques, as described by Lohman et al. (1988). Children were measured by a single investigator (RL), to the nearest 0.5 cm for length and 0.01 kg for weight, using a SECA stadiometer or weighing scale (Milton Keynes Scales, Leighton Buzzard, Bedfordshire, UK). Duplicate measures of length and weight for 20 children were taken to calculate technical errors of measurement (Ulijaszek and Kerr, 1999) which yielded coefficients of reliability of 0.99 and 0.98 for weight and length, respectively.

Ethical approval

Ethical approval was gained from the Nepal Health Research Council and from Durham University's Research Ethics Committee. All procedures were demonstrated to mothers at the start of the study to secure informed consent. Mothers were offered a small gift (200 rupees, approximately £1.50) at each monthly measurement to compensate them for their time. At the end of the study all children were given a gift of clothing and provided with medical treatment by a local hospital where appropriate. In addition, mothers in the control group were invited to attend a session on hygiene and handwashing and offered free bars of soap.

Statistical analyses

Height-for-age (HAZ), weight-for-age (WAZ) and weight-for-height (WHZ) z-scores were calculated using Epi Info™ (version 3.3.2) based on the National Centre for Disease Statistics, 2000 growth curves. Mucosal damage (L:C) values were normalized by $\log_{10}$ transformation after adding '2' to each value to prevent negative scores. Relationships between categorical demographic or socioeconomic variables were assessed using χ^2 tests, and between continuous variables by two-tailed independent t-tests and linear regression. Weekly morbidity scores were summed to provide morbidity profiles for each child over the whole period of study. These scores were nonnormally distributed and thus were assessed using nonparametric Mann-Whitney U tests. Data were analyzed using SPSS for Windows statistical package (versions 14-15; SPSS Inc., Chicago, IL) and Stata (Intercooled) (Version 8.2; Statacorp, College Station, TX). Statistical significance was set at the 5% level.

Time-series linear regression analysis was used to assess the impact of the intervention longitudinally, to account for the interdependence of repeated measures for each participant and to examine between- and within-subject variation. We built multivariable models based on initial univariable analyses to evaluate predictor variables. Age was included as a covariate in all analyses. Differences in baseline biochemical variables between intervention and control groups were controlled for in regression models. Because we were interested in changes in biochemical and growth variables over the course of the intervention, we also tested for interactions between time and group (intervention/control).

RESULTS

A total of 99 children were recruited into the study, with 88 children providing complete datasets. No differences between the intervention and control group were observed for any demographic variable nor for the composite socioeconomic score (Table 1). Over half (53%) of the mothers in the study were illiterate, while 18% had received primary level education and 28% had attended at least some secondary level schooling. Education levels were, as expected, higher among the fathers: about a quarter (27%) were illiterate, 21% had received primary school education and half (52%) had continued to secondary level education.

Just over half (55%) of the families owned their own house (but not the deeds to the land), while the rest lived in rented accommodation. Most houses were simple brick constructions roofed with corrugated iron sheets, though some houses were only walled with woven bamboo or plastic sheeting. The houses were generally small, dark and over-crowded. Over half (57%) of families lived in just one room, which served as kitchen, bedroom and general living area for the entire family. Access to drinking water was limited in all areas: three settlements had public taps that provided water for just a few hours on alternate days; the remaining settlements relied primarily on water from

TABLE 1. Household demographic and socio-economic characteristics of control and intervention groups

Households	All (n = 88)	Control (n = 43)	Intervention (n = 45)	P
Age of child (months)				
Mean	7.6	7.5	7.7	0.72
SD	2.4	2.5	2.3	
Sex of child %				
Male	48.0	46.5	48.9	0.50
Female	52.0	53.5	51.1	
Maternal education %				
None	53.4	51.1	55.6	0.91
Primary	18.2	18.6	17.8	
Secondary+	28.4	30.2	26.6	
Paternal education %				
None	27.3	25.6	28.9	0.08
Primary	20.5	11.6	28.9	
Secondary+	52.2	62.8	42.2	
Tenure %	54.5	53.5	55.6	
Own house	54.5	53.5	55.6	0.51
Rent house	45.5	46.5	44.4	
Rooms in house %				
One room	56.8	44.2	68.9	0.02
Two+ rooms	43.2	55.8	31.3	
Toilet %				
Own	18.2	16.3	20.0	0.43
Shared/Public	81.8	83.7	80.0	
Fuel type %				
Firewood	35.6	23.3	67.7	0.02
Kerosene	34.5	34.9	34.1	
Gas	29.9	41.9	18.2	
Income per month (Rs)				
Median	4500	4500	4000	0.65
IQ range	3,000–6,300	3,000–7,200	3,000–5,300	
Possessions				
Median	2	2	1	0.14
IQ range	1–3	1–3	1–3	
SES Score				
Median	5	6	5	0.08
IQ range	3–9	4–10	3–7.5	

P from χ^2 test, two-tailed t-tests, or Mann-Whitney U tests.

public or private tube wells and deep wells. Water for hygienic purposes, however, was always available from these tube and deep wells. The majority of families (82%) did not have access to a private toilet, but instead shared sanitary facilities with several families or used public toilets. Families owned few valuable possessions; a median of just two items, with almost a quarter (24%) of families owning none at all. The most common possessions were televisions and radios, with 64% and 50% of families owning these items, respectively.

Impact on hand-washing practices

Data from the preintervention structured observations suggested that hand-washing at the five key junctures was uncommon. Indeed, so few hand-washing events were actually observed that we did not conduct statistical comparisons between the intervention and control groups. Only a fifth of mothers were observed to wash hands with soap after going to the toilet, and just 14% were seen to wash their hands with soap after cleaning the baby's bottom. Hand-washing before cooking or feeding the baby was virtually non-existent (data not shown).

As expected, self-reported rates of hand-washing were much higher than those suggested by observations (Table 2). At baseline almost all mothers (95%) reported hand-washing with soap after defecation and over three-quarters reported using soap after cleaning the baby's bottom. Hand-washing was reported much less frequently in both groups before cooking food or feeding the baby and very infrequently before eating a meal.

By the end of the study, however, reported hand-washing after cleaning the baby's bottom or before cooking, eating or feeding the baby had increased in mothers from the intervention areas (McNemar's test, $P < 0.01$ for all four junctures), while hand-washing in control areas remained unchanged (Table 2). Thus, by the end of the study, mothers in the intervention areas were significantly more likely than control mothers to report hand-washing at these four junctures ($P < .01$ for all four junctures). Only for one juncture ('after visiting the toilet') was there no significant increase in reported behavior among intervention mothers ($P = 0.50$) and no difference in hand-washing rates between the two groups ($P = 0.053$).

Impact on morbidity

Children from intervention areas reported fewer episodes of diarrhea over the study period (3.0 vs. 4.33 episodes for intervention and control, respectively; $P = 0.049$), experiencing, on average, 31% fewer episodes of diarrhea than control counterparts. Intervention children also experienced 41% fewer days of diarrhea than children in control areas, (9.67 vs. 16.33 days for intervention and control groups, respectively; $P = 0.023$). There were no differences between the two groups in episodes or days of coughs/colds or fevers (Fig. 1a,b).

Associations between biochemical and growth variables

Associations between biochemical and growth variables are presented in Table 3. Markers of immune stimulation—AGP and IgG—were positively associated with one another over the study period ($P < 0.001$). Similarly, hemoglobin and albumin, showed a weak, but positive correlation ($P < 0.001$). L:C (mucosal damage) was not associated with any of the immune or nutritional markers. However, children with higher levels of mucosal damage over the period of study had significantly lower HAZ ($P = 0.01$), WAZ ($P < 0.001$) and WHZ z-scores ($P = 0.034$). Albumin was associated with better WAZ and WHZ scores over the period of study ($P = 0.001$ and $P = 0.002$, respectively), while children with higher levels of AGP had poorer scores for these two variables ($P < 0.001$ for both). Elevated levels of IgG, indicative of long-term stimulation of the immune system, were associated with poorer HAZ scores ($P = 0.029$). Hb showed no association with any growth variable.

Impact of intervention on biochemical markers

Time series regression analysis revealed no differences between intervention and control groups with respect to L:C, AGP, albumin or hemoglobin, after controlling for age and baseline differences. A significant interaction between group and time was observed for IgG ($P = 0.002$, Table 4), indicating that children in the intervention and control groups were changing in different ways. IgG levels increased in both groups, but the slope of the regression line for the intervention group is steeper, indicating that, even after correcting for baseline differences, IgG levels were increasing at a faster rate in the intervention group (coef. = 0.621, $P < 0.001$) than in the control group (coef. = 0.237, $P < 0.001$) (Fig. 2a). However, despite

TABLE 2. Changes in mothers' reported hand-washing practices over the six month intervention period

Hand-washing junctures	Baseline Control (n = 43)	Baseline Intervention (n = 45)	Baseline Group differences (P value)[a]	Endline Control (n = 43)	Endline Intervention (n = 45)	Endline Group differences (P value)[a]	Change in HW from baseline to endline (P value)[b] Control	Change in HW from baseline to endline (P value)[b] Intervention
1. After visiting toilet	95.2	95.5	0.674	90.7	100	0.053	0.625	0.500
2. After cleaning baby's bottom	76.2	86.4	0.175	83.7	100	0.005	0.549	0.031
3. Before cooking	10.3	13.6	0.449	2.3	71.1	<.001	0.125	<0.001
4. Before feeding	17.6	33.3	0.104	18.6	62.2	<.001	0.500	0.004
5. Before eating	4.8	22.7	0.016	0	60	<.001	0.100	0.003

[a]χ^2 tests.
[b]McNemar's test.

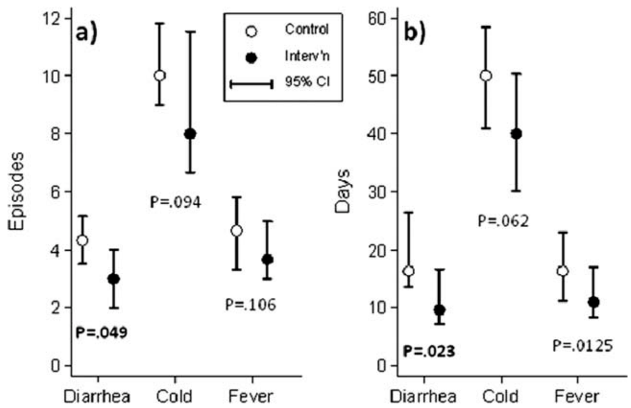

Fig. 1. (a,b) Impact of hand-washing on child morbidity (episodes and days of sickness) over six month intervention period. Circles indicate median values.

this difference in trajectory, the two groups were not significantly different from one another (group coef. = −0.235, P = 0.56).

Impact of intervention on growth

No differences between intervention and control groups were detected for HAZ. However, for both WAZ and WHZ, the two groups changed in different ways over time (P = 0.012 and 0.019, respectively for interaction terms, Table 4). Both groups experienced a decrease in WAZ over the period of study (Fig. 2b) but children from intervention areas declined at a faster rate than the control group (coef. = −0.149 and −0.027, for intervention and control, respectively). Despite these different trajectories, overall WAZ scores for the two groups were not significantly different from one another (group coef. = −0.068, P = 0.76). A similar pattern was observed for WHZ (Fig. 2c). WHZ scores declined at a faster rate in the intervention group (coef. = −0.079 and −0.034 for intervention and control, respectively), although the overall difference between the two groups remained nonsignificant (group coef. = 0.24, P = 0.16).

DISCUSSION

This study confirms the role of hand-washing as an important means of preventing diarrheal disease. Children

TABLE 3. Associations between biochemical and growth variables (n = 88)

	Predictor	Coef.	Std. Err.	P	95% CI	Rho
IgG	Age	0.448	0.033	<0.001	(0.384, 0.512)	0.459
	AGP	1.598	0.215	<0.001	(1.177, 2.018)	
	Alb	0.130	0.011	<0.001	(0.109, 0.151)	
	Hb	0.015	0.009	0.101	(−0.003, 0.034)	
	Constant	−4.839	0.979	<0.001	(−6.757, −2.921)	
AGP	Age	−0.012	0.006	0.041	(−0.023, 0.000)	0.092
	IgG	0.043	0.006	<0.001	(0.030, 0.050)	
	L:C	1.036	0.540	0.055	(−0.023, 2.095)	
	Alb	0.000	0.002	0.964	(−0.004, 0.004)	
	Constant	0.287	0.201	0.153	(−0.107, 0.681)	
Albumin	Age	−0.022	0.109	0.839	(−0.235, 0.191)	0.121
	Hb	0.213	0.031	<0.001	(0.153, 0.272)	
	Constant	11.696	3.395	0.001	(5.042, 18.350)	
HAZ	Age	−0.093	0.006	<0.001	(−0.104, −0.082)	0.945
	L:C	−1.162	0.452	0.010	(−2.049, −0.276)	
	IgG	−0.011	0.005	0.029	(−0.021, −0.001)	
	Constant	−0.047	0.191	0.805	(−0.422, 0.328)	
WAZ	Age	−0.159	0.006	<0.001	(−0.170, −0.147)	0.939
	L:C	−1.932	0.525	<0.001	(−2.960, −0.904)	
	AGP	−0.219	0.036	<0.001	(−0.289, −0.149)	
	Alb	0.006	0.002	0.001	(0.003, 0.009)	
	Constant	0.739	0.224	0.001	(0.300, 1.178)	
WHZ	Age	−0.081	0.008	<0.001	(−0.096. −0.066)	0.844
	L:C	−1.499	0.705	0.034	(−2.881, −0.117)	
	AGP	−0.251	0.048	<0.001	(−0.346, −0.157)	
	Alb	0.007	0.002	0.002	(0.002, 0.012)	
	Constant	1.163	0.277	<0.001	(0.619, 1.707)	

Time series linear regression analysis. Rho = between-subject variability explained by the model. Only significant models (P < 0.05) are presented.

in the hand-washing areas experienced a significant reduction in diarrheal episodes, resulting in 41% fewer days of diarrheal symptoms over the period of study than their control counterparts. The magnitude of this impact is in line with results reported from other studies (Curtis and Cairncross, 2003).

This is the first study that sought to look beyond the impact of hand-washing on reported morbidity such as diarrhea, to examine its impact on subclinical rates of infection. We found that although the hand-washing intervention significantly reduced diarrheal disease in children from the intervention group, it failed to have an impact on levels of mucosal damage and immune stimulation that are associated with poorer growth performance in young children. No differences were observed between intervention and control children for any biochemical variable, nor for any growth indicator over the period of study.

TABLE 4. Impact of hand-washing on biochemical and growth variables (n = 88)

	Predictor	Coef.	Std. Err.	*P*	95% CI	Rho
IgG	IgG (baseline)	0.463	0.060	<0.001	(0.345, 0.582)	0.178
	Age (baseline)	0.108	0.056	0.054	(−0.002, 0.218)	
	Group	−0.235	0.397	0.555	(−1.013. 0.544)	
	Time	0.384	0.055	<0.001	(0.277, 0.491)	
	Time*group	0.237	0.077	0.002	(0.087, 0.387)	
	Constant	1.349	0.514	0.009	(0.341, 2.357)	
WAZ	Age (baseline)	−0.263	0.045	<0.001	(−0.352, −0.175)	0.932
	Group	−0.068	0.219	0.755	(−0.497, 0.361)	
	Time	−0.122	0.008	<0.001	(−0.137, −0.107)	
	Time*group	−0.027	0.011	0.012	(−0.049, −0.006)	
	Constant	1.065	0.374	0.004	(0.333, 1.798)	
WHZ	Age (baseline)	−0.225	0.034	<0.001	(−0.292, −0.158)	0.814
	Group	0.241	0.172	0.162	(−0.097, 0.579)	
	Time	−0.045	0.010	<0.001	(−0.065, −0.024)	
	Time*group	−0.034	0.014	0.019	(−0.062, −0.006)	
	Constant	1.699	0.285	<0.001	(1.140, 2.258)	

Time series linear regression analysis, controlling for baseline differences between groups where appropriate. Rho = between-subject variability explained by the model. Only significant models ($P < 0.05$) are presented.

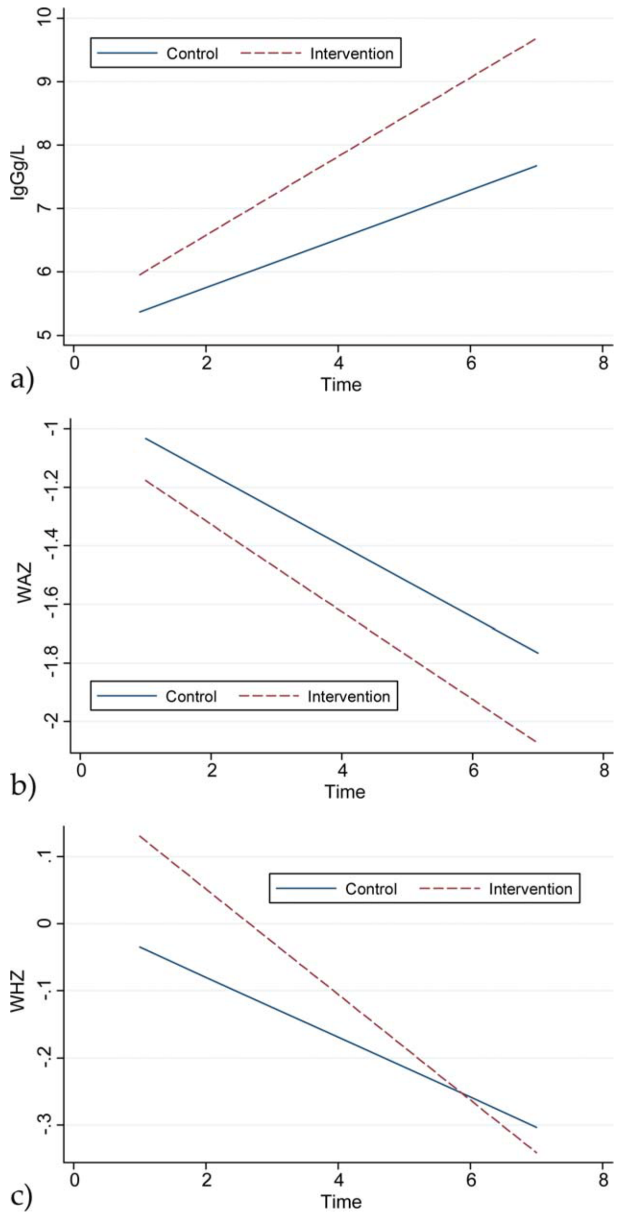

Fig. 2. Changes in IgG, WAZ and WHZ over the six month intervention. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Our data therefore appear to suggest that while hand-washing can reduce more severe forms of infection that result in diarrheal symptoms, it may have little or no effect on sub clinical, yet often chronic, forms of infection. This lack of impact has to be interpreted with caution given the two main limitations of this study. First, this is a small-scale prospective study. While we recruited a total sample of children in the target age-range living in the eight largest slum settlements of Kathmandu, our sample size was constrained by the number of target-aged children available in the communities at the time. We obtained repeated and multiple measures on a cohort of 88 participants, suffering an attrition rate of only 11 children from 99 originally recruited. Second, the intervention was monitored for a six-month period. This was perhaps not long enough to detect the full impact of changes in hand-washing practice. However, any reduction in a child's exposure to pathogenic organisms should lead to a fairly swift reduction in immune stimulation and, in the absence of further pathogenic damage, mucosal crypt cells might be expected to regenerate within a few weeks (Noone et al., 1986). Biochemical indicators were therefore likely to have been sensitive enough to detect some impact within the intervention period, yet no such change was observed. Thus, while a full-scale cluster randomized controlled trial is required to provide more conclusive evidence, our results suggest that hand-washing may have a limited impact on subclinical infections in young children living in such conditions.

Given the context of the slums, this lack of impact on sub-clinical infections is perhaps not surprising. Fecal contamination of the hands is undoubtedly a significant threat to health, and the 41% reduction in days of diarrhea in the intervention group confirms its importance as a highly effective intervention. However, for children living in poor slum conditions contamination of the hands is just one of many pathways by which they are exposed to pathogenic organisms. In this context children are put at risk of infection through consumption of bacterially contaminated food and water, as well as living in poor quality and over-crowded environments that promote the rapid spread of disease. Hand-washing may interrupt one particular route of transmission, but if children continued to be exposed to pathogens via a multitude of other pathways, it is possible that no significant reduction in mucosal damage and immune stimulation would occur. Thus, while hand-washing has the potential to reduce incidence of acute cases of diarrheal disease, it is perhaps insufficient to affect the daily exposure to infectious organisms that cause subclinical infection which are a regular part of life in urban slums.

The influence of wider environmental conditions on levels of mucosal damage and immune stimulation is also highlighted in a recent study from Bangladesh. Goto et al. (2008, 2009) implemented a double-blind randomized controlled trial to test the effects of anti-*Giardia* and anthelminthic treatment on biochemical and growth indicators in a sample of 222 children aged between 3 and 15 months. Children were assigned to one of four groups (anti-*Giardia*, anthelminthic, anti-*Giardia* + anthelminthic, or placebo) and were followed for 36 weeks. No significant differences for intestinal permeability, biochemical or anthropometric variables were found between the intervention groups—a surprising finding given the number of studies reporting associations between *Giardia* and intestinal helminths and poor growth (Crompton and Nesheim, 2002; Farthing et al., 1986; Gupta and Urrutia, 1982; Oberhelman et al., 1998; Simsek et al., 2004). The authors, however, point to the high endemicity of *Giardia* within this population, suggesting that children were rapidly reinfected after treatment due to the very poor standards of hygiene within these communities. The results from both this and our own study suggest that behavioral and pharmacological interventions may be unlikely to have a significant impact on the biochemical and growth status of children in the absence of more comprehensive improvements to general living conditions.

To put this into a global context, it is worth reminding ourselves of the scale of this problem: currently, almost a billion people in developing countries lack access to safe water and 2.6 billion lack basic sanitation facilities (WHO, UNICEF, 2010). In addition, the poorest people often pay a disproportionately greater amount to use these resources where they are available. Slum dwellers often pay between five to ten times more per liter of water than richer people living in the same city (UNDP, 2006:10). Without addressing these structural issues, not only will it remain difficult for the poorest people to improve their hand-washing practices, but in the context of significant and persistent environmental contamination that this lack of access creates, the potential impact of hand-washing on health may be constrained.

One further aspect of the results from this study requires comment. While there was no overall difference between the intervention and control groups for any variable, they did show differences in trajectory for IgG, WAZ, and WHZ. These divergences were not large enough to produce significant absolute differences between the two groups, but this may have emerged with a longer observational period. Unexpectedly, these differences in trajectory were in the opposite direction to that predicted: IgG levels rose with age, WAZ and WHZ worsened with age, at a faster rate in the intervention group relative to controls. These trends demand careful evaluation.

Could the hand-washing program have had an unexpected negative impact on the biochemical and growth status of children in the intervention group? It is difficult to envisage a plausible mechanism by which a poorer trajectory could be attributed to increases in maternal hand-washing practices. Although it has been suggested that an exaggerated concern for hygiene can impede the development of a healthy and functioning immune system (Strachan, 1989), this has primarily focused on the rise in atopic diseases such as eczema and asthma in the developed world (Okada et al., 2010; Sherriff and Golding, 2002) and not the types of infectious disease to which urban slum children would be exposed.

A more likely scenario is that there was something significantly different about the two groups at the start of the study and it was this difference that continued to exert pressure on the biochemical and growth status of children in the intervention areas, despite improvements in hand-washing practices. This interpretation is supported by the fact that there were significant differences between the groups at baseline for IgG, AGP, and albumin. Although these differences were accounted for in longitudinal analyses, one might expect these differences between the two groups to persist if whatever factor caused these differences in the first place was unaffected by the hand-washing intervention.

Some evidence points to subtle differences between intervention and control areas that may signal greater socio-economic deprivation in households recruited to the hand-washing campaign. The groups were comparable with respect to the composite scale constructed to assess socio-economic status, yet differed with respect to two particular items (Table 1). Thus over-crowding was greater in intervention areas with 69% of intervention families living in just one room that served as kitchen, bedroom and living area, compared with 44% of families in control areas ($P = 0.02$). Intervention families were also more likely to use the cheapest form of cooking fuel (fire wood) than controls (68% vs. 23% respectively, $P = 0.02$). The poorer health trajectory of children in the intervention group may perhaps reflect conditions of over-crowding and poverty, leading to greater pathogen exposure through pathways unaffected by hand-washing behaviors.

What these results signal is that even within slum communities that are generically thought of as "poor," there is significant heterogeneity between households and that slum children are differentially exposed to a wide variety of stressors (both nutritional and pathological) that can impact on their health and growth. This emphasizes the point that growth faltering is a highly complex and multifaceted problem which will likely require comprehensive interventions (tackling environmental sanitation as well as hygiene behavior) rather than programs that target behavioral change alone. Our study underscores previous work that argues that many carefully conducted health interventions have limited health impact, even where wholesale behavioral change to minimize exposure to pathogenic infections is achieved (Panter-Brick et al., 2006; Tomlinson et al., 2010).

CONCLUSION

Hand-washing is a highly effective means of reducing diarrhea in young children; indeed, so effective that it has been promoted as a "do-it-yourself" vaccine against childhood infections (Curtis et al., 2005). However, the results from our preliminary study suggest that its impact on the more subtle, yet often chronic, forms of infection may be limited. For children living in highly contaminated, overcrowded environments, with poor access to clean water and sanitation, hand-washing may be necessary, *but not sufficient* to reduce levels of subclinical mucosal damage and immune stimulation that are strongly associated with growth faltering.

From the standpoint of human biology and health, what are needed are comprehensive, structural interventions that address the root causes of these infections—poverty and poor living conditions. Focusing attention solely on hygiene interventions that target individual behaviors, in the context of recurrent infections in slum environments and in the absence of improvements to wider living conditions, may have limited global and local health impacts.

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