ANALYSIS OF FECAL LEUKOCYTES AND ERYTHROCYTES IN *SHIGELLA* INFECTIONS IN URBAN BANGLADESH

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Abstract. We evaluated the usefulness of enumeration of fecal leukocytes and erythrocytes in making an early diagnosis of *Shigella* infection, where *Shigella* is a leading cause of invasive diarrhea. Stool specimens from 561 invasive diarrhea patients were submitted for microscopic examination. A presumptive diagnosis of shigellosis based on microscopic examination was made in 389 of them; 227 had stool cultures positive for *Shigella* spp (*Shigella* patients). One hundred sixty-two patients with no detectable *Shigella* infection (non-*Shigella* invasive diarrhea cases) served as a comparison group. Two hundred twenty-seven randomly selected *Shigella* patients and 227 non-*Shigella* infectious diarrhea cases from the surveillance system database of the hospital constituted another group for comparative evaluation. The stool specimens of the patients were examined under the microscope, and isolation, biochemical characterization and serotyping of *Shigella* were performed. In comparison with non-*Shigella* invasive diarrhea cases, the presence of >50 WBC/hpf in association with any number of RBC in the fecal sample had a modest sensitivity of 67%, specificity of 59%, positive predictive value of 70%, negative predictive value of 56%, accuracy of 64%, and positive likelihood ratio of 1.6 in predicting shigellosis. Comparison between *Shigella* and non-*Shigella* infectious diarrhea patients revealed the presence of >20 WBC/hpf was a less accurate predictor of shigellosis (sensitivity 51%, specificity 88%, positive predictive value 81%, negative predictive value 64%, accuracy 69%, and positive likelihood ratio 4.1). Direct microscopical examination of stool specimens for the presence of WBC and RBC may facilitate the early diagnosis of shigellosis, and may be a cheap alternative to stool culture in this setting.

INTRODUCTION

The impact of shigellosis on health, nutrition, and mortality is a serious global health concern (Bennish *et al*, 1990; Bennish and Wojtyniak, 1991; Shears, 1996; Anonymous, 1997; Kotloff *et al*, 1999). It has been estimated that 164.7 million episodes of *Shigella* infections occur globally each year, among which 163.2 million episodes occur in developing countries and are associated with 1.1 million deaths (Kotloff *et al*, 1999). Effective antimicrobial therapy for shigellosis shortens the duration of illness, hastens clinical recovery, lessens severity of the disease, and may prevent the development of life-threatening complications, particularly those which occur later in the course of illness (Salam and Bennish, 1991). Delay in recognizing *Shigella* infection may also result in delay in initiating effective antimicrobial therapy. *Shigella* is known to penetrate the large gut mucosa, where it initiates an intense acute inflammatory response resulting in fecal excretion of leukocytes and erythrocytes (Mathan and Mathan, 1991). The possible value of fecal quantitative and qualitative cell counts in di-

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agnosing specific bacteria has been reported. Several authors (Harris *et al*, 1972; Pierce *et al*, 1974; Pickering *et al*, 1977; Korzeniowski *et al*, 1979; Stoll *et al*, 1983; Hossain and Albert, 1991) have emphasized the presence of leukocytes on microscopic examination of the stool as a rapid, reliable, and inexpensive method for distinguishing diarrhea due to invasive microbial infection (*Shigella*, *Salmonella* or *Campylobacter*) from other causes of diarrhea, such as those due to viruses, parasites or bacterial toxins (Harris *et al*, 1972; Pierce *et al*, 1974; Pickering *et al*, 1977; Korzeniowski *et al*, 1979; Stoll *et al*, 1983; Hossain and Albert, 1991). Investigators in Bangladesh (Stoll *et al*, 1983; Hossain and Albert, 1991; Bardhan *et al*, 2000) demonstrated the importance of the presence of both WBC and RBC in stool as a simple and inexpensive method for diagnosing shigellosis or invasive diarrhea in Bangladesh. Changes in the proportions of various *Shigella* serogroups over time has been observed in Bangladesh (Stoll *et al*, 1982a,b; Hossain *et al*, 1998). In recent years, several new subserotypes of *Shigella* have been identified in Bangladesh and in other developing countries (Simmons and Romanowska, 1987; Carlin *et al*, 1989; Talukder *et al*, 2001). We reassessed the comparative diagnostic values of fecal WBC and RBC in differentiating *Shigella* from non-*Shigella* invasive diarrhea cases and *Shigella* symptomatic infections from other non-*Shigella* infectious diarrhea cases, to serve as a rapid screening test to identify patients whose stool should be cultured for isolation of *Shigella* and to identify patients who would benefit from antimicrobial therapy.

MATERIALS AND METHODS

Study population

This study was conducted at the Dhaka Hospital of the ICDDR, B between January 2000 and September 2001. Invasive diarrhea was defined as having 3 or more loose stools within 24 hours with or without abdominal cramps, and with visible blood and/or mucus in the stool. Consecutive patients of all age groups and both sexes, attending the hospital (between 6:00 AM and 7:30 PM hours each day of the week) with a history of diarrhea of less than 96 hours duration and the presence of blood and/or mucus in the stool (noted by the study physician) were identified and their stool specimens were sent to the laboratory for microscopic examination. Those with a history of antibiotic use for the current illness at home were excluded from initial screening. A presumptive diagnosis of shigellosis was made as a patient with invasive diarrhea with the presence of >10 WBC and any number of RBC/high power field (hpf) on microscopic examination of her/his freshly passed stool specimen. This was based on findings of a previous study that demonstrated the presence of both WBC and RBC in stool could improve the reliability of the diagnosis of *Shigella* infections (Hossain and Albert, 1991). Stool specimens from a total of 561 diarrhea patients with blood or mucus were submitted for microscopic examination, and a presumptive diagnosis of shigellosis based on microscopic examination was made in 389, of which 227 had stool cultures positive for *Shigella* spp. Another 162 with no detectable *Shigella* infection (non-*Shigella* invasive diarrhea cases; Fig 1) served as a comparison group. Each patient was interviewed by a trained research assistant and examined by a study physician. Data were recorded from a structured questionnaire.

The Dhaka Hospital of ICDDR, B has maintained a Diarrheal Disease Surveillance System since 1980. The system enrolls a systematic sample (2%, or every 50th patient) of all patients presenting to the hospital, and records clinical, etiological and epidemiological information. The detailed process and methodology of enrollment has been described elsewhere (Stoll *et al*, 1982b). From the hospital surveillance system database, 227

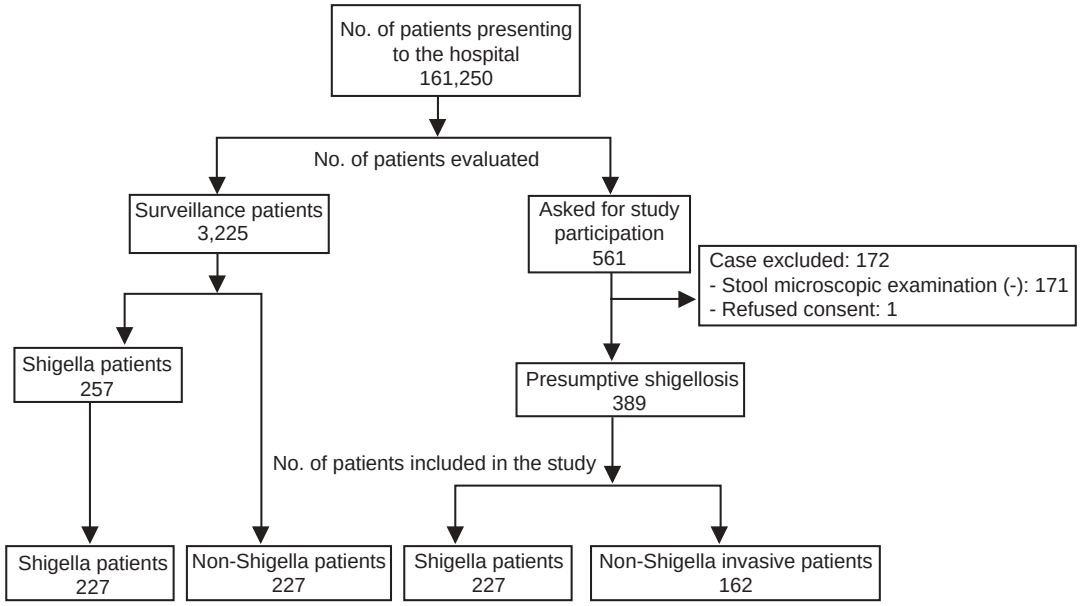

Fig 1–Trial profile.

Shigella cases were randomly selected from 257 *Shigella* patients detected during the same study period, and their information were extracted. Information from a randomly selected study population comprised of 227 non-*Shigella* infectious diarrhea patients was also extracted. These were selected from 3,225 culture proven non-*Shigella* diarrhea patients (comprised of both invasive and non-invasive diarrhea cases) between January 2000 and September 2001 and constituted another comparison group (non-*Shigella* infectious diarrhea patients, Table 1).

This study was approved by both the Research Review Committee and Ethical Review Committee of the ICDDR, B.

Laboratory methods

A single, fresh stool specimen from each study patient was examined within an hour of collection for the presence of visible blood and/or mucus, and tested for pH (acidic or alkaline). A small portion of stool, preferably from the part containing visible blood and mucus or only mucus, was collected using an applicator stick, placed on a microscope glass slide, and thoroughly mixed with two drops of Loeffler methylene blue stain. A cover slip was placed over the stained mixture and the preparation was allowed to settle for 2-3 minutes. An experienced technician examined the preparation to count the numbers of red and white blood cells per field. The technician first examined the slide under low power to determine areas of increased cell count, and examined at least ten high power fields to enumerate cells. The numbers of RBC and WBC present were counted and expressed as the average numbers under the following categories: 0, 1-10, 11-20, 21-50, and >50 per hpf. A portion of each stool sample from each patient was plated onto MacConkey's and *Salmonella-Shigella* agars (Difco, Becton Dickinson and Company, Sparks, MD) in the microbiology laboratory. The plates were incubated overnight at 37ºC and examined for colonies of*Shigella* by standard methods (WHO, 1987). *Shigella* isolates were biochemically identified and serotyped by slide agglutination test using commercially available anti-

Table 1
Comparison of stool microscopy results between *Shigella* and non-*Shigella* patients in Dhaka, Bangladesh, 2000-2001.

Variables	*Shigella* patients n=227 (%)	Non-*Shigella* invasive patients n=162 (%)	*Shigella* surveillance patients n=227 (%)	Non-*Shigella* surveillance patients n=227 (%)
Naked eye examination				
Blood in stool	85 (37.4)	48 (29.6)	29 (12.8)	3 (1.3)
Mucus in stool	225 (99.1)	157 (96.9)	208 (91.6)	177 (77.9)
pH				
Alkaline	195 (85.9)	134 (82.7)	142 (62.6)	110 (48.5)
Acidic	32 (14.1)	28 (17.3)	85 (37.4)	117 (51.5)
Microscopical examination				
>5 macrophages/hpf	47 (20.7)	6 (3.7)	19 (8.4)	1 (0.4)
No. of RBC/hpf				
> 20	134 (59.0)	55 (34.0)	45 (19.8)	2 (0.9)
> 50	88 (38.8)	40 (24.7)	30 (13.2)	2 (0.9)
No. of WBC/hpf				
> 20	206 (90.7)	120 (74.1)	117 (51.5)	29 (12.8)
> 50	152 (67.0)	66 (40.7)	68 (30.0)	8 (3.5)
>20 WBC/hpf + presence of RBC	206 (90.7)	120 (74.1)	116 (51.1)	28 (12.3)
>50 WBC/hpf + presence of RBC	152 (67.0)	66 (41.0)	68 (30.0)	8 (3.5)
>20 WBC/hpf +>20RBC/hpf	128 (56.4)	50 (31.1)	45 (19.8)	2 (0.9)
>50 WBC/hpf +>20RBC/hpf	114 (50.2)	38 (23.6)	42 (18.5)	1 (0.4)
With visible blood in stool	n=85 (%)	n=48 (%)	n=29 (%)	n=3 (%)
No. of RBC/hpf				
> 20	75 (88.2)	38 (79.2)	26 (89.7)	2 (66.7)
> 50	64 (75.3)	34 (70.8)	19 (65.5)	2 (66.7)
No. of WBC/hpf				
> 20	80 (94.1)	35 (72.9)	29 (100)	2 (66.7)
> 50	68 (80.0)	25 (52.1)	25 (86.2)	1 (33.3)
With mucus in stool	n=225 (%)	n=157 (%)	n=208 (%)	n=177 (%)
No. of RBC/hpf				
> 20	133 (59.1)	55 (35.0)	45 (21.6)	2 (1.1)
> 50	87 (38.7)	40 (25.5)	30 (14.4)	2 (1.1)
No. of WBC/hpf				
> 20	205 (91.1)	119 (75.8)	116 (55.8)	29 (16.4)
> 50	152 (67.6)	66 (42.0)	68 (32.7)	8 (4.5)
With alkaline pH	n=195 (%)	n=134 (%)	n=142 (%)	n=110 (%)
No. of RBC/hpf				
> 20	131 (67.2)	54 (40.3)	45 (31.7)	2 (1.8)
> 50	88 (45.1)	40 (29.9)	30 (21.1)	2 (1.8)
No. of WBC/hpf				
> 20	179 (91.8)	100 (74.6)	95 (66.9)	25 (22.7)
> 50	145 (74.4)	61 (45.5)	61 (43.0)	8 (7.3)

sera (National Committee for Clinical Laboratory Standards, 1990; Talukder *et al*, 2002). A culture-proven case was defined as a patient with the presence of a *Shigella* organism in the stool culture either as a single or mixed infection.

Statistical methods

Analyses of data were performed using SPSS for Windows (version 10.2; SPSS Inc, Chicago). Differences in proportions were compared by the chi-square test and the Fisher's exact test was performed when the expected number of cells was less than 5. A probability of <0.05 was considered significant. Sensitivity and specificity, positive and negative predictive values, the accuracy and the likelihood ratio were calculated using standard formulae (Anonymous, 2005).

RESULTS

Among *Shigella* patients, *S. flexneri* was the most commonly isolated species (54%) followed by *S. dysenteriae* (20%), *S. boydii* (16%), and *S. sonnei* (10%) (Khan *et al*, 2004). The presence of >20WBC/hpf and >20RBC/hpf was more common than ≤20WBC/hpf and ≤20RBC/hpf (56%, n=128 *vs* 7%, n=16). Sixty-seven percent and 39% of study patients had >50WBC/hpf and >50RBC/hpf, respectively (data not presented). Non-*Shigella* invasive diarrhea cases were not evaluated for etiolologic agents.

There were no significant differences in the presence of blood or mucus in stool between *Shigella* and non-*Shigella* invasive diarrhea patients. There were significant ($p<0.001$) differences in the presence of blood or mucus in stool between the *Shigella* and non-*Shigella* patients selected from the surveillance system. Irrespective of excretion of RBC, 52% of the *Shigella* infected patients in comparison to 13% of non-*Shigella* patients had >20 WBC/hpf ($p<0.001$). Patients infected with *Shigella* were more likely ($p<0.001$) to have macrophages (>5/hpf) than non-*Shigella* cases, and were more likely to have >20RBC/hpf and >50 RBC/hpf ($p<0.001$) (Table 1).

The sensitivity and specificity, positive and negative predictive values, and the accuracy and likelihood ratio of various cutoff values for these fecal microscopical findings in predicting shigellosis are shown in Table 2. In comparison with non-*Shigella* invasive diarrhea patients, the presence of >50 WBC/hpf in association with any number of RBC in the fecal sample had a sensitivity of 67%, specificity 59%, and a positive predictive value of 70% in predicting shigellosis. When comparison was made between shigellosis cases and non-*Shigella* infectious diarrhea patients from the surveillance system, the presence of >20 WBC/hpf with the presence of RBC in any numbers was a modest predictor for identifying patients with *Shigella* infection (sensitivity 51%, specificity 88%, positive predictive value 81%, negative predictive value 64%, accuracy 69%, and positive likelihood ratio 4.1).

DISCUSSION

Microscopic examination of fresh stool specimens for the presence of fecal leukocytes (fecal leukocyte test) to support the initial clinical diagnosis of shigellosis (bacillary dysentery) is an age-old practice, which reached its peak during World War I when field diagnosis was critical. The test is also helpful in determining the presence and nature of gut pathology (Korzeniowski *et al*, 1979). In Western countries, to make the laboratory investigation more productive and cost-effective, only stools positive for RBC or WBC are cultured for enteropathogens.

The presence of WBC and RBC in most cases signifies an inflammatory process due to invasive pathogens. Numerous fecal leukocytes are also present in idiopathic ulcerative colitis that is associated with loss of mucosal integrity and inflammatory response in the gut

Table 2
Sensitivity, specificity, predictive values, overall accuracy, and likelihood ratio of fecal white and red blood cells in detecting *Shigella*.

Presence of cells[a]	Comparison group	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Overall accuracy	Likelihood ratio of a positive test	Likelihood ratio of a negative test
>20 WBC/hpf and presence of RBC	Non-*Shigella* patients	51 (47-55)	88 (84-91)	81 (74-86)	64 (61-67)	69	4.1	0.6
	Non-*Shigella* invasive patients	91 (87-94)	26 (21-30)	63 (61-65)	67 (55-77)	64	1.3	0.4
> 50 WBC/hpf and presence of RBC	Non-*Shigella* patients	30 (27-32)	97 (93-98)	90 (80-95)	58 (56-59)	63	8.5	0.7
	Non-*Shigella* invasive patients	67 (63-71)	59 (53-65)	70 (65-74)	56 (50-62)	64	1.6	0.6
>20 WBC/hpf and >20 RBC/hpf	Non-*Shigella* patients	20 (18-21)	99 (97-100)	96 (85-99)	55 (54-56)	59	22.5	0.8
	Non-*Shigella* invasive patients	56 (52-60)	69 (63-75)	72 (66-77)	53 (48-57)	62	1.8	0.6
>50 WBC/hpf and >20 RBC/hpf	Non-*Shigella* patients	19 (16-19)	99 (97-100)	98 (87-100)	55 (62-72)	59	42.0	0.8
	Non-*Shigella* invasive patients	50 (46-54)	77 (71-82)	75 (69-81)	52 (54-55)	61	2.1	0.7

Results are expressed as percentages and 95% confidence intervals in the parentheses
[a]Number of cells per high power field of microscope

(Harris *et al*, 1972). Apparently, healthy individuals in the tropics may have some degree of subclinical gut inflammation leading to excretion of cells (Mathan and Mathan, 1985). The absence of leukocytes among healthy controls or in asymptomatic *Salmonella* cases in different settings may be explained by the lack of gut inflammation (Harris *et al*, 1972). In our study, we correlated the numbers of WBC and RBC excreted in the stool with *Shigella* infection and found a significant association between them. Our findings (>20WBC/hpf, present in 91% of Bangladeshi shigellosis patients) are different from those observed among Western populations (more than 25 WBC/hpf in 68% of shigellosis and invasive *E. coli* stools) where enteric illnesses are more often due to non-invasive bacteria and viral agents, or with non-infectious causes, less associated with fecal leukocytes (Harris *et al*, 1972; Pickering *et al*, 1977; Hossain and Albert, 1991). Host response differentials including impairment of cell mediated immunity and under-nutrition, variations in the disease severity, endemicity and repeated enteric infections, failure to detect the causative invasive pathogens, and mixed infections are among factors that may influence the numbers of WBC in the stools of patients in Bangladesh (Stoll *et al*, 1983a,b). The number of fecal leukocytes depends on the anatomic site of involvement and the extent of the inflammatory process rather than the etiology (Pickering *et al*, 1977). Shigellosis is endemic in Bangladesh, and the presence of relatively low numbers of fecal WBC and RBC among Bangladeshis may indicate partial host defense, or early small intestinal phase of the disease, while the presence of numerous fecal leukocytes and erythrocytes may indicate inflammation of the colon to a large extent. Our findings were different from those of Hossain *et al* (1991) who observed higher sensitivities and specificities (particularly of WBC and the presence of RBC/hpf) in predicting

shigellosis in Bangladesh. This could be due to changes in the proportions of isolation of species, serotypes and subserotypes of *Shigella* over period, as observed in our study.

The results of our study indicate that microscopical examination of a freshly collected stool specimen to determine the presence and number of WBC and RBC may facilitate early diagnosis of shigellosis and initiation of effective antimicrobial therapy. This may be a reasonable alternative to stool culture where fecal culture is not possible in a resource limited setting, particularly in *Shigella* endemic locations and during epidemics of dysentery. Stool cultures are often negative if the patient has already received antimicrobial therapy, but stool microscopy may still be useful in the diagnosis of shigellosis. The test has only modest sensitivity but was still able to differentiate two thirds (sensitivity 67%) of the shigellosis cases from invasive diarrheas. If we consider the positive predictive value, then 70% of positive tests have shigellosis. About two thirds of invasive diarrhea patients would benefit by the treatment of shigellosis in developing countries where shigellosis in malnourished children is a major health problem. Early diagnosis and initiation of effective antimicrobial therapy can hasten recovery, prevent or reduce the rates of complications, and prevent or lessen transmission of infection to others. However, adequate training of laboratory personnel is necessary for reliable results. PCR technology can detect *Shigella* cases from cases negative by conventional culture, however, is expensive and only a few facilities can afford it. We conducted this study in a *Shigella* endemic country. There is a need to conduct similar studies in other geographical locations where the population is likely to benefit from early diagnosis and antimicrobial therapy.

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