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RESEARCH ARTICLE

SIGNIFICANT OF MACROSCOPIC and MICROSCOPIC DETECTION FOR DIAGNOSIS OF BLOODY DIARRHEA AMONG CHILDREN UNDER FIVE YEARS IN KHARTOUM STATE

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ABSTRACT

Objective: To assess the significance of the Integrated Management of Childhood Illness (IMCI) guidelines in diagnosis of bloody diarrhea.
Methods: Four hundred and thirty two children patients with diarrhea attending Khartoum Pediatric Hospital and Omdurman Pediatric Hospital Khartoum Sudan were enrolled into the study during the period of 2005 to 2007. Stool specimens collected were processed by wet preparation and cultured into XLD medium, sorbitol MacConkey medium and Skirrow's medium. The suspected pathogenic colonies were tested for oxidase and urease production then identified by analytical profile index (API 20 E), and agglutination with specific antisera.
The results: The sensitivity and specificity of gross blood in faeces as diagnostic tools were 61.4% and 60.1% respectively ($P<0.05$). RBCs were detected in 112 (25.9%) of specimens with positive culture compared with 305 (70.6%) that revealed negative culture. The sensitivity of presence of RBCs in faeces as diagnostic tools were was high 98.2% while its specificity was very low 3%. The sensitivity and specificity of pus cells in faeces as diagnostic tools were high that represent 99.1% and low 4% respectively.
Conclusions: Microscopical examination of stool specimen as simple diagnostic tool was high sensitive and its specificity was moderate.

INTRODUCTION

Diarrhea remains a major public health problem in developing countries, an estimated 12 or more diarrheal episodes per child occur within the first five years of life. Annually, approximately 4.6 million pediatric deaths approximately 25-30% of all deaths among children less than 5 years of age can be attributes to acute diarrhea (Al-Gallas *et al*., 2007). Invasive bacteria is the terminology used to refer to diarrhea caused by bacterial pathogens that invade the bowel mucosa, causing inflammation and tissue damage. This causes numerous polymerphonuclear leucocytosis (PMNs), and sometimes red blood cells, to appear in the stools, which can be detected by microscopic examination. The stools may also contain visible blood, but this is not necessary to diagnose invasive bacteria. When visible blood is present, the episode could also be termed bloody diarrhea or dysentery (sWHO, 2005; Cheryl *et al*., 1999; Murray *et al*., 2004). Compared with watery diarrhea, bloody diarrhea generally lasts longer, associated with more complications, more likely to adversely affect a child's growth, and has a higher case fatality rate (Murray *et al*., 2004; WHO, 2004). The IMCI guidelines rely on detection of cases based on simple clinical signs with simple or without laboratory tests, and offer empirical treatment. A careful balance has been struck between sensitivity and specificity in the classification of illness using as few clinical signs as possible (Ingle and Malhotra, 2007).

METHODS

The study patients were children age 2 months up to 5 years presenting to Khartoum Pediatric Hospital and Omdurman Pediatric Hospital during the period of May 2005 to July 2007. Declaration was approved by the ethical committee board of Al Neelain University. Each mother of patient has signed informed consent.

Specimen collection and processing

Stool specimens were collected in a wide mouth plastic disposable containers and placed into Carry and Blair transport medium. Samples were processed directly for microscopic presence of ova, parasites, white blood cells and red blood cells (Collee *et al*., 1996). From Cary-B lair media all specimens were inoculated into XLD medium, sorbitol MacConkey medium and Skirrow's media. The cultured plates of XLD and MacConkey were incubated aerobically at 37 ^{O}C for 18-24 hours where as Skirow *Camylobacter* selective medium was incubated microaerobically at 42 ^{O}C for up to 48 hours. The significant growth colonies were examined morphologically for size, consistency, shape, and ability to ferment lactose. The suspected pathogenic colonies were tested for oxidase and urease production then identified by analytical profile index (BioMérieux's API 20E).The serotypes of all *Shigella* spp. and diarrhoeagenic *E. coli* isolates were determine with commercially variable polyclonal antisera (Mast Co. Merseyside, UK) against *Shigella* serotypes. The data based on 432 cases was analyzed using the Statistical Package for Social Sciences software version 17 for Windows ® (SPSS Inc, Chicago, IL, USA). Frequencies of age group, gender, diarrheal duration, fever, presence of blood, mucus, pus, red cells, and bacterial isolates were obtained. Cross tabulation and chi-square tests for macroscopic examination*bacterial isolates, microscopic examination were used. Sensitivity and specificity of macro and microscopic examination were calculated.

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RESULTS

Out of 432 children patients 34% were represent with diarrheal duration of two days followed by duration of 3 days (21.3%). Frequency of gross blood was 45.4% (196/432) and mucus was 46.9% (203/432) as shown in Table (1)

Table (1). The frequency of some laboratory and clinical findings among the study group of children under 5 years with acute diarrhea

(Khartoum and Omdurman Pediatric Hospitals n= 432)

Clinical and other characterization	n (%)
Duration of diarrhea:	
1 day	81 (18.7)
2 days	148 (34.2)
3 days	92 (21.3)
> 3 < 7 days	75 (17.4)
1 week less than 2 weeks	36 (08.3)
Gross blood in stool:	
Yes	196 (45.4)
No	236 (54.6)
Mucus in stool	
Yes	203 (46.9)
No	229 (53.1)
RBCs	
Yes	417 (96.5)
No	15 (3.5)
Pus cells	
Yes	418 (96.8)
No	14 (03.2)

Cultures of 432 of specimens yielded 114 (26.4%) bacterial pathogens, out of 114 bacterial isolates, *Shigella spp*. were the most common isolated bacteria 61/114 (53.5%) followed by diarrhoeagenic *E. coli* 37/114 (31.6%), non-typhoidial salmonella 12/114 (10.3%) and *Campylobacter spp*. 4/114 (3.5%). The most frequently isolated pathogens were *S. flexneri* 31 (7.1%) followed by EIEC 24 (EIEC 5.6%). *S. dysenteriae* 13 (3.0%) *S. sonnei* 12 (2.8%), non-typhoid *Salmonella* 12 (2.8%), ETEC 9 (2.1%), S. *boydii* 5 (1.2%), *Campylobacter* spp. 4(0.9%), EPEC 3 (0.6%) and VTEC 1(0.2%). Gross blood was detected in 70 (16.2%) of specimens with positive culture compared with 127 (29.4%) that revealed negative culture (Table 3). The sensitivity and specificity of blood in faeces as diagnostic tools were 61.4% and 60.1% respectively (P<0.05). The sensitivity and specificity of mucus in faeces 68.2% and 61.3% respectively (P<0.05). The sensitivity and negative predictive value of macroscopical examination as a diagnostic tool was high 92.9% and 93.7% respectively (Table 2). The sensitivity and of microscopical examination (both pus cells and RBCs) was high 98.2% and specificity was 73.1% (Table 2).

Table (2). The sensitivity and specificity of macroscopical and microscopical examination as a simple diagnostic tool in diagnosing bloody diarrhoea among the study group of children under 5 years

(Khartoum and Omdurman Pediatric Hospitals n= 432)

	Sensitivity %	Specificity %	PPV %	NPV %
Macroscopical examination				
Gross blood	61.4	60.1	35.5	81.2
Mucus	68.4	61.3	84.4	68.4
Al least one positive	92.3	38.1	34.4	93.7
Microscopical examination				
RBCs	98.2	4.1	26.8	86.6
Pus cells	99.1	4.1	27.1	92.8
Both	98.2	73.1	26.1	99.3

DISCUSSION

The IMCI guidelines rely on detection of cases based on simple clinical signs without laboratory tests, and offer empirical treatment. A careful balance has been struck between sensitivity and specificity in the classification of illness using as few clinical signs as possible. In this study a potential enteropathogen was detected in 26.3% of all screened patients. This result is in agreement with study in Ethiopia in 2001 (Mitike, 2001) whom reported that the enteropathogen was detected in 22.3% in study group, and quite similar to Talan and his colleagues (David *et al*., 2001) who in 2001 studied 877 episodes of bloody diarrhea and reported that enteropathogens were identified in 168 episodes 30.6% (David *et al*., 2001). The prevalence point of enteropathogens in the current study was low compared with that reported in Tehran 2008 (Jafari *et al*., 2008), whom reported in study of 808 patients with diarrhoea, the prevalence of enteropathogens was 45%, and in Tunisia 2007 (Al-Gallas *et al*., 2007) that represents about 33%m whereas the rate is lower than the other part in Korea (Steele TW, McDermott, ?). *Shigella* is an important pathogen that causes acute bloody diarrhoea in Sudan and in many developing countries. Accordingly, in the current study, the frequency of *Shigella* spp. was 14.1% among patients in the study group and 52% among enteropathogens isolated. This may have been due to tendency of these organisms to cause severe diarrhea (Jafari *et al*., 2008). In the current study *Shigella flexneri* was predominate isolate among all *Shigella* spp. isolates (50.8%) and this findings was equivalent to that of previous study conducted in Iran 2007 (Alizadeh *et al*., 2007) and Tanzania 2007 (Temu *et al*., 2007) whom reported that *Shigella flexneri* was the most common among *Shigella* spp. isolates, whereas discordant results were reported in other previous studies as in Iran 2008 (Jafari *et al*., 2008) and in Turkish 2007(Karacan *et al*., 2007), whom reported that *Shigella sonnei* was most predominate among *Shigella* spp. Isolates The sensitivity and specificity of pus cells in faeces as diagnostic tools were high that represent 99.1% and low 4% respectively, whereas RBCs were detected in 112 (25.9%) of specimens with positive culture compared with 305 (70.6%) that revealed negative culture.

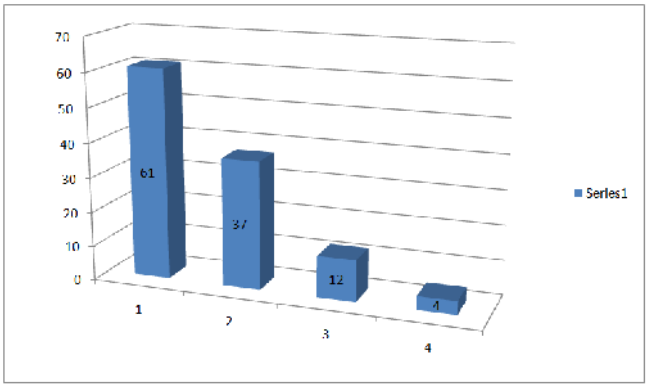

Fig. (2). The frequency of pathogenic bacteria isolates from the study group of children under 5 years 1-Shigella spp, 2: Diarrheagenic E. coli, 3: Non-typhoid salmonella, 4: Campylobacter

The sensitivity of presence of RBCs in faeces as diagnostic tools were was high 98.2% while its specificity was very low 3%. Chi-square analysis statistically showed significant relation (P<0.05). Microscopic examination of fresh stool specimens for the presence of faecal leukocytes to support the initial clinical diagnosis of shigellosis is an age-old practice. The test is also helpful in determining the presence and nature of gut pathology (Khan *et al*., 2006). 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 faecal leukocytes are also present in idiopathic ulcerative colitis that is associated with loss of mucosal integrity and

inflammatory. The findings of the current study 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. The results of the present 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.

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