Chancroid- The most frequent cause of genital ulcer disease: A prospective study by using PCR assay

Hoque MM, Uuda MN, Mamun SA, Ali CM, Hassan T, Uddin MJ

Abstract

Aim: Since the etiology of genital ulcer disease (GUD) in Bangladesh is based on clinical ground and syphilis serology only, the present study was designed to provide information on the etiology of GUD by using recommended laboratory methods. Methods: To demonstrate ulcer etiology 98 male patients with clinically diagnosed GUD reporting at DMCH Bangladesh were prospectively studied for serologic evidence of syphilis (RPR & TPHA, T pallidum IgG and IgM antibodies), culture and PCR proven chancroid and PCR proven genital herpes. The outcome of laboratory diagnosis were compared with the clinical diagnosis. Results: A definite microbial etiology of 88 infection in 72 of the 98 patients evaluated was found. H. ducreyi (65%), herpes simplex (13%) and T pallidum (11%), alone or in combination, were the most frequent diagnosis, whereas 27% had no laboratory diagnosis. Seventeen patients (65%) of the latter group had clinically genital scabies with high eosinophil count, seems to be responsible for ulcer infection. Excluding the mixed infection, the sensitivity of the clinical diagnosis of chancroid was 30.6% and syphilis was 57%. The number of single herpes infection was too small for evaluation. Laboratory proven chancroid was frequently observed among the remaining clinical diagnosis. Conclusion: All GUD patients should be treated for chancroid including all those having reactive syphilis serology.

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Introduction

Genital ulcer disease (GUD) constitute a major risk factor for the sexual transmission of HIV. In many developing countries syphilis, chancroid and genital herpes was the most frequent diagnosis among the patients with GUD by using recommended laboratory methods. Genital Scabies "Pyogenic infection", syphilis and chancroid have been claimed to be major causes of GUD in Bangladesh. Diagnosis is almost exclusively based on clinical grounds and syphilis serology only. It was reported that the clinical diagnosis can be made with reasonable certainty only for a minority of cases even if performed by experienced clinician.

Reactive syphilis serology (RPR & TPHA) showed 83-90% specificity and 66-72% sensitivity when taking PCR as the standard for diagnosis, so there exist a chance of overestimation or missing of active syphilis. Use of culture for laboratory confirmation of chancroid has high specificity and low sensitivity in terms of gold standard methods. Since nutrition requirements of H. ducreyi are very complex, lack of essential substance in the culture media may have been responsible for the poor yield of H. ducreyi. Therefore when a culture of H. ducreyi is negative, there exist a chance of false negative results.

PCR technique for the diagnosis of syphilis, chancroid and herpes has high specificity and sensitivity and used as a gold standard for assessing the validity of a diagnostic procedure. In this study the PCR technique were used for the detection of H. ducreyi and herpes simplex: virus. The aim of the present study was to provide information on the etiology of GUD in Bangladesh by using recommended laboratory methods.

Patients and methods

Patients
The study was carried out at the Skin and VD outpatient Department of Dhaka Medical College Hospital and at the Laboratory Sciences Division of ICDDR,B in Dhaka, Bangladesh throughout the months of March and April, 1999. A total of 98 patients with GUD were selected for the study. All subjects were male and at least 15 years of age. Each patient was ensured confidentiality and anonymity and asked to participate in an interview regarding socio-demographic variables, sexual behavior and information was collected about the use of the medicines, the presence of symptoms such as pain, itching, recurrent character of the lesion, the presumed source of infection and a history of STI in the past. All patients under went a physical examination of the external genitals, the inguinal regions and the rest of the body.

Clinical diagnosis
The etiological diagnosis base on clinical sign complex attributed to syphilis (painless, indurated, clean-based ulcer) chancreoid ulcer (a deep, undermined purulent ulcer), genital herpes ulcer (multiple, grouped, shallow, tender ulcer) and genital scabies ulcer (multiple itchy purulent, tender ulcers, and/or presence of burrow especially on the genitalia and adjacent areas and characteristic distribution of scabies lesions else where in the body) .Donovanosis was considered when the lesion had a beefy aspect.

Laboratory diagnosis
Isolation of H. ducreyi:
For the isolation of H. ducreyi a cotton tipped swab rolled in the ulcer, after cleaning with the back of a hemostylet, and plated on-

a) Mueller-Hinton medium (Becton Dickinson, Cockeysville, Maryland, USA), supplemented with 1% hemoglobin (Becton Dickinson), 1% Iso VitaleX (Becton Dickinson), 5% sterile fetal calf serum (Life Technologies Inc, N. V. USA) and 3 μg of vancomycin/ml;

b) GC-agar base (Becton Dickinson) enriched with the same ingredients. All plates were incubated for seven days at 33°C in a candle jar. Presumptive identification of H. ducreyi was based on the typical colonial morphology, Gram staining, a negative catalase test and a positive oxidase test with tetramethyl-p-aminophenylene diamine. All strains were tested for the presence of a β-lactamase with the chromogenic cephalosporin test (Nitrocefin R Becton Dickinson).

Detection of H. ducreyi by PCR technique:
A dacron tipped swab was subsequently rubbed against the ulcer base and stored in a sterile I molar phosphate buffered saline solution (PBS; 50 mM sodiumphosphate, 0.15M sodiumchloride; pH 7.5), but without sodium chenodeoxycholate18 and stored at -70°C until shipped to the Laboratory. Three different primers were used for the detection of the H.ducreyi genome19,20. Only samples giving a positive result with three primers were considered as being positive.

Detection of herpes simplex virus by PCR technique:
Detection and typing of herpes simplex virus was performed with two different primers22. Only samples giving a positive result with two primers were considered as being positive and further typing of herpes simplex virus was done according to Kimura et al23

Serology for syphilis:
For the diagnosis of syphilis, serum specimens were tested with the rapid plasma reagin test (RPR nosticon, Organon Teknika, Turnhoul, Belgium) which was titrated till the end point, as well as with the 7: pallidum haemagglutination assay (TPHA nosticon, Organon Teknika). T.pallidum IgG and IgM antibodies were detected with an ELISA technique (Treponema pallidum, IgM EIA, Treponema pallidum IgG Comfort EIA Meddens Diagnostics, Brummen, The Netherlands).Patients were considered as having primary syphilis if the RPR was reactive in the presence of a positive TPHA test and in all cases where IgM antibodies to T.pallidum were diagnosed. All patients with a positive IgM test were screened for the presence of the
rheumatoid factor (Serodia-RA, Fujirebio, Tokyo, Japan).

Detection of C. donovani: With the back of the haemostylet smear from the ulcer was prepared for specific staining and microscopy. Total and differential WBC count were performed in all cases. Microscopic detection of the scabies mite was not performed since it is not expected to be fruitful in scrapings of heavily infected lesions.

Results  
Socio-demographic data: 
Summarized in table-1.

Table 1: Socio-demographic profile and sexual risk behaviour of the GUD patients DMCH, Bangladesh

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N=98</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years ± SD)</td>
<td>24±8.5</td>
<td></td>
</tr>
<tr>
<td>Civil Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>25</td>
<td>(25.5)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>71</td>
<td>(72.4)</td>
</tr>
<tr>
<td>Divorced</td>
<td>2</td>
<td>(2.0)</td>
</tr>
<tr>
<td>Socio-Economic Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td>54</td>
<td>(55.1)</td>
</tr>
<tr>
<td>Middle</td>
<td>38</td>
<td>(38.8)</td>
</tr>
<tr>
<td>Higher</td>
<td>6</td>
<td>(6.1)</td>
</tr>
<tr>
<td>Sexual Partner</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>1</td>
<td>(11.2)</td>
</tr>
<tr>
<td>Multiple</td>
<td>72</td>
<td>(73.5)</td>
</tr>
<tr>
<td>No</td>
<td>15</td>
<td>(15.3)</td>
</tr>
<tr>
<td>Sex worker as source of infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>14</td>
<td>(56.0)</td>
</tr>
<tr>
<td>Unmarried / separated man</td>
<td>31</td>
<td>(42.5)</td>
</tr>
<tr>
<td>History of sexually transmitted infection</td>
<td>14</td>
<td>(14.3)</td>
</tr>
</tbody>
</table>

The mean age of the patients was 24 years±8.5 years. A majority of the GUD patients were unmarried (72.4%). Most patients belonged to the lower socioeconomic strata of Dhaka and were small traders, rickshaw pullers, drivers, unskilled daily wagers or factory workers. However 20% of patients were students. A large proportion of patient reported of having multiple sexual partners. Forty-six percent of patients indicated sex worker as source of infection and 56% (14/25) of the married men reported recent sexual contact with commercial sex worker. Fourteen percent had history of STD.

Clinical findings: Depicted in table -2. Ninety-six patients were circumcised. Ulcers were localized on the glans or skin of the penis or on the scrotum, only one patient had anal and peri-anal Twenty nine (29.6%) patients had a single ulcer, 1. (14.3%) had 2 ulcers and 22 (56.1%) had more than 2 ulcers. Five (5.1 %) men had balanitis, 28(28.6%) inguinal lymphadenopathies, 5 of them with buboes. One patient had generalized chicken pox and 95 (45.9%) patients showed scabies lesions elsewhere or the body. More then half of the patients (54%) had waited more then two weeks before attending the out patient department. The clinical diagnosis was genital scabies (n=49), chancroid (n=30}, syphilis (n=12) genital herpes (n=3}, traumatic ulcer (n=2), donovanosu (n= 1) and chickenpox (n= 1).

Table2: Clinical data of 98 genital ulcer patients DMCH, Bangladesh

Laboratory diagnosis: Represented in table -3.  
A definite microbial etiology of 88 infection was found ir 72 of 98 patients evaluated. H. ducreyi was isolated from 22 patients, 42 additional H. ducreyi infection being discovered by the PCR technique. Four of the five (80%) patients with inguinal buboes had a positive H. ducreyi PCR test and all were negative for any other laboratory diagnosis. All 22 culture positive samples were PCR positive. Therefore laboratory diagnosis of

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chancroid was confirmed in 64 (65%) cases with GUD, among them 49 had . Chancroid infection, *H. Ducreyi* were isolated or detected in 6 of 12 (50%) cases which were clinically diagnosed as syphilis, in 30 of 49 (61%) cases which were recognized clinically as genital scabies and in 2 of 3 (67%) cases of clinically diagnosed genital herpess. One of the patients with a traumatic ulcer was culture and PCR positive for *H. ducreyi*. The patient with generalized chicken pox had chancroid and the patient with clinically diagnosed donovanosis harbored chancroid as mixed infection with genital herpess. After exclusion of mixed infection, not expected to be recognized .clinically, the sensitivity of the clinical diagnosis of chancroid was 30.6% (15/49). Culture of *H.ducreyi* had 34.4% sensitivity when taking PCR as the standard for diagnosis.

Table 3 : Association between clinical and laboratory diagnosis

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Laboratory diagnosis</th>
<th>N=50</th>
<th>Chancroid</th>
<th>Syphilis</th>
<th>Herpes</th>
<th>Mixed infection</th>
<th>No diagnosis</th>
<th>N=26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scabies</td>
<td></td>
<td>49</td>
<td>26</td>
<td>2</td>
<td>4</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chancroid</td>
<td></td>
<td>30</td>
<td>15</td>
<td>4</td>
<td>1</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syphilis</td>
<td></td>
<td>12</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herpes</td>
<td></td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donovanosis</td>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean % eosinophil</td>
<td>s(5D)</td>
<td></td>
<td>0.33</td>
<td>0.45</td>
<td>0.32</td>
<td>0.32</td>
<td>0.47</td>
<td>0.32</td>
</tr>
</tbody>
</table>

There were 11 (11.2%) serology proven syphilis, among them 9 patients showed a reactive RPR test in the presence of a positive TPHA test. Two patients negative for RPR (up to titer 1:32) and 7: *pallidum* IgG antibodies, were positive for TPHA and IgM. Both patients were negative for the RA factor and considered as having active syphilis. Single syphilis infection were detected in 7 (7.1%) cases. Excluding the mixed infection, the sensitivity of clinical diagnosis of syphilis was 57% (4/7).

Genital herpess were detected in 13 (13.3%) patients. Type-2 infection observed in 7 (7.1%) patients, type - 1 in 4 (4.1%) patients and 2 patients harbored a mixed (type -1 and type-2) infection. The single genital herpess infection was detected in one patient which was clinically diagnosed as a traumatic ulcer. Remaining all 12 infection represented as mixed infection with chancroid and only one with syphilis.

Laboratory proven mixed infection was observed in 15 cases.Chancroid detected genital herpess in all cases,genital herpess in 12 cases and syphilis in 4 cases. The 26 cases of GUD with no demonstrable ulcer etiology included the 17 cases of clinically diagnosed genital scabies. The mean leucocyte count was 10357/cmm. The mean percentages of eosinophil was 4.0±3.0 among the patients with proven infections and 7.1±5.0 in patients with no laboratory diagnosis. Among the latter group 17 patients with clinically diagnosed scabies had the highest eosinophil level (8.6±1.2) observed in 15 cases.Chancroid detected genital herpess in all cases,genital herpess in 12 cases and syphilis in 4 cases. The 26 cases of GUD with no demonstrable ulcer etiology included the 17 cases of clinically diagnosed genital scabies. The mean leucocyte count was 10357/cmm. The mean percentages of eosinophil was 4.0±3.0 among the patients with proven infections and 7.1±5.0 in patients with no laboratory diagnosis. Among the latter group 17 patients with clinically diagnosed scabies had the highest eosinophil level (8.6±1.2)

Discussion
This study confirmed chancroid as the leading cause of GUD in Bangladesh. Similar observation were drawn in many developing countries where chancroid was the most frequent diagnosis. The relative frequency of chancroid and syphilis is in contrast to our previous observation and confirmed the lack of accuracy of the clinical etiologic diagnosis of genital ulcers. The socio-demographic profile and sexual risk behavior described earlier remained unchanged in our present study.
A low frequency of syphilis raises a dilemma in the etiological ranking of GOD in Bangladesh. The rate of syphilis observed in our study may not very precisely represent the actual prevalence of syphilis in the community as some patients with GOD reporting to the primary health care providers erroneously consider all cases of genital ulcers as syphilis and treat them. The wide spread use of antibiotics before attending the clinic may have prevented the development of T.pallidum antibodies, on the other hand, the sensitivity of syphilis serology (RPR & TPHA) indicating that some infections were missed and leading to underestimation of syphilis.

The number of single herpes infection was too small for evaluation. Herpes Simplex virus may secondarily invades the pre-existing genital ulcers without altering the clinical sign complex of primary infections. On the other hand the spontaneous resolution of genital herpes ulcer prevents many patients from seeking treatment. Perhaps these patients come to the clinic with concomitant infection that does not heal inherently and difficult to recognize clinically. These might be the interpretations for most of the genital herpes patients with mixed infection.

Sixty five percent (17/26) of the patients with no laboratory diagnosis had clinically genital scabies and significantly high eosinophil count. Therefore genital scabies as single infection seems to be responsible for the ulcer infection among this group. However in the remaining 65% (32/49) clinically diagnosed genital scabies cases it cannot be excluded that pre-existing genital scabies lesions became secondarily infected with H. ducreyi, T. pallidum or herpes simplex virus resulting in a clinical picture of secondarily infected scabies. A high eosinophil count would be expected which was not observed among this group. Therefore such mixed infection remains unproven.

The high prevalence of chancroid and their presence in the most of the clinically diagnosed genital scabies, syphilis and genital herpes cases is the most striking finding of the present study. The study showed point prevalence of different etiologic causes of GOD and stressed on future study on large sample size representative of GOD population of Bangladesh. Since the sensitivity of the clinical diagnosis of chancroid is very low, many infection are missed, on the other hand as the culture of H. ducreyi had low sensitivity there exist a chance of many false negative results. Therefore it will be justifiable to treat all GUD patients for chancroid including all those having reactive syphilis serology.

References
7. Sadeque JBMJ, Rahmatullah H. A study of sexually Transmitted diseases in relation with the socio-economic condition of the potenl. Bangladesh Journal of Dennalology,
23 Kimura H, Shibala M, Kuzzushima K, Nishikawa K, Nishiyama 1; Morishima 7: Delection and direct typing of herpes simplex virus by polymease chain reaction. Med Microbial Immunoll990; 179; 177-84.