

Patterns of Presentation in Gonococcal Urethritis and Assessment of Antibiotic Sensitivity in Gonorrhoea

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Abstract

Objective: To see various features of Gonococcal Urethritis and assess the results of Gram stain and culture tests in cases of Gonorrhoea and to see the antibiotic resistance pattern in them, in our local population (PNS Shifa hospital Karachi, Pakistan).

Design: Prospective case series.

Place and duration of study: Skin department of PNS Shifa Hospital Karachi, Pakistan. June 2004 to December 2007.

Materials and methods: The study was carried out in the outpatient dermatology department of PNS Shifa hospital Karachi during the period from June 2004 to Dec 2007. It was a prospective study. One hundred clinically suspected patients of Gonococcal urethritis were included in the study. Detailed history including history of sexual contact was taken. General and systemic physical examination was carried out in all the patients. Dermatological examination including examination of external genitalia was also done. In all these patients following investigations were carried out. Urine complete examination, complete blood counts, pus sample was collected from external urethral meatus by two sterile inoculating loops on to a clean glass slide for Gram staining which was seen under the microscope for the presence of gram-negative diplococci. The other loop was inoculated onto chocolate agar plate and was incubated in an atmosphere of 5% CO₂ at 37°C for 24-48 h. The growths obtained were identified with colony morphology, Gram staining, oxidase reaction and rapid sugar fermentation. The Gonococci isolated were tested for antibiotic sensitivity using modified Kerby buer technique. The antibiotics included Penicillin G, Tetracycline, Cefotaxime, Cefuroxime, Ceftriaxone and Ciprofloxacin. HIV test was done in all patients. The results of two types of tests and their merits and demerits were assessed and compared.

Results: A total of 100 patients having urethral discharge and dysuria were studied. All the patients were males. The ages varied from 20 to 40 years, the average being 29.2 years+5.8 years. History of illicit sexual exposure was elicited in 43 (43%) patients. The duration of period of symptoms ranged between 4 and 30 days with a mean of 12.8 days and a median of 12 days. The patients with gonorrhoea presented with purulent discharge in 66 (66%) cases, and dysuria in 49 (49%) cases. No case had anal or oral symptoms. Examination showed a reddened external urethral meatus in 53(53%) cases. Eighty seven (87%) cases showed intracellular diplococci on direct microscopy of Gram stained smear. Sixty-two (62%) cases showed growth of gonococci on culture of urethral pus. No case was seen to be HIV positive. Tetracycline resistance was seen in 34(55%) strains. Penicillin resistance was seen in 25 (41%) strains of Neisseria Gonorrhoea. Five strains (11.5%) showed resistance to Ciprofloxacin. No resistance was seen with Cephalosporins.

Conclusion: Gram staining and culture for Neisseria Gonorrhoea are both useful diagnostic tests for Gonococcal urethritis. Drug resistance of Neisseria Gonorrhoea is high with various antibiotics except for Cephalosporins stressing the need for culture based therapy and enthusiastic prevention of Gonorrhoea in our society.

Keywords: Gonorrhoea; Gram stain; Culture; Urethral discharge

Introduction

Gonorrhoea is a common sexually transmitted disease, which is caused by *Neisseria gonorrhoea*. This is transmitted between individuals by direct, usually sexual contact. It is the second most commonly reported communicable disease, with more than 350,000 cases reported annually [1]. In males the most common manifestation of infection is acute urethritis characterized by abrupt onset of dysuria and a purulent urethral discharge. The symptoms usually appear within 2 to 10 days of infection, but the incubation period is sometimes much longer, and some men never develop symptoms. The incidence of gonorrhoea declined significantly in most developed countries during the 1980s, but remains common in some urban centers in the world. It is prevalent in many developing countries. The male: female ratio is 2:1 [2].

Neisseria gonorrhoea is highly susceptible to adverse environmental influences such as temperature extremes and drying and it does not survive long outside its natural host, which is man. Infection with Gonorrhoea increases the risk of becoming infected with HIV. This is likely

due to weakening of the mucosal surface secondary to the Gonorrhoea infection [3]. The Gram stain and the culture test are the two standard tests for Gonorrhoea [4]. The Gram stain involves placing a smear of the discharge on a slide where it is stained with Gram stain and examined for Gonococcal diplococci under a microscope. It has high sensitivity and specificity (over 90% in males) for the diagnosis [5]. The culture test is more reliable but takes longer. It involves taking a swab of the discharge, rolling it on to a culture plate, and incubating it under special laboratory

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Received August 03, 2016; Accepted August 18, 2016; Published August 25, 2016

Citation: Saleem K, Azim W (2016) Patterns of Presentation in Gonococcal Urethritis and Assessment of Antibiotic Sensitivity in Gonorrhoea. J AIDS Clin Res 7: 613. doi: 10.4172/2155-6113.1000613

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conditions for 16 to 48 hours to let the Gonococci multiply [6]. For the diagnosis of gonorrhoea, appropriate sites for specimen collection depend to some extent on the age, sex and sexual practices of the individual and the clinical features of the infection. The commonest sample is pus from genitourinary passage. If standard culture media are available at the collection site, the specimen is directly inoculated and is placed in an atmosphere containing 5% CO₂ at 37°C. It is then transported to laboratory. Gonococci are fastidious in their growth requirements. Most of the times these are present along with normal flora [7]. The culture media used are, therefore, both enriched and selective. For routine culture chocolate agar base medium of good quality with 9% Saponin lysed horse/sheep blood and inhibitors (Vancomycin to suppress the growth of Gram positive, Colistin to suppress Gram negative organisms, Nystatin to suppress yeast multiplication and Trimethoprim is added to inhibit swarming by *Proteus* spp.) [8]. Antibiotic treatment is an essential control measure for Gonorrhoea. Antibiotic treatment is usually administered as a standard treatment of a single dose of antibiotic. The standard treatment regimen should cure more than 95% of cases. Antibiotic resistance in *Neisseria gonorrhoea*, to agents used for treatment, has been a continuing problem. So a surveillance of antibiotic resistance with a change in treatment regimens when resistance occurs in >5% of isolates is recommended. Antimicrobial resistance in Gonorrhoea including both chromosomal resistance and plasmid mediated resistance has increased worldwide recently [9].

The aim of the study was to see the pattern of presentation, to assess the results of Gram stain and culture tests in cases of Gonorrhoea in our population (reporting to PNS Shifa hospital Karachi, Pakistan) and to see the antibiotic resistance pattern of Tetracyclines, Penicillins, Quinolones and Cephalosporins in them.

Materials and Methods

The study was carried out in the outpatient dermatology department of PNS Shifa hospital Karachi, Pakistan, during the period from June 2004 to Dec 2007. It was a prospective case study. One hundred clinically suspected patients of Gonococcal urethritis were included in the study. Only male patients of all ages were included in the study. Patients who had received systemic treatment for their complaints and those having other inter-current illnesses were excluded from the study. Detailed history of symptoms and history of sexual contact was taken. General and systemic physical examination was carried out in all the patients. Local examination including examination of external genitalia, testis, oral cavity, eyes and anus was also done. In all these patients investigations carried out were urine complete examination and complete blood counts along with ESR were done. Genitalia were cleaned up with normal saline and a slight pressure on penis was exerted to exude a drop of pus at external urethral meatus. The pus was collected by two sterile inoculating loops. One loop was rolled up on a clean glass slide to prepare a thin and homogenous film which was allowed to air dry before it was Gram stained. This was seen under microscope for the presence of Gram-negative diplococci. The other loop was inoculated onto a 1 cm circular area of the chocolate agar plate. The chocolate agar contained Vancomycin and was incubated in an atmosphere of 5% CO₂ at 37°C for 24-48 h. The growths obtained were identified with colony morphology, Gram staining, oxidase reaction and rapid sugar fermentation. HIV test was performed in all the patients using Elisa method. The Gonococci isolated were tested for antibiotic sensitivity using modified Kerby buer technique [10]. The antibiotics included Penicillin G, Tetracycline, Cefotaxime, Cefuroxime, Ceftriaxone and Ciprofloxacin. Culture testing was done by growing bacteria on a nutrient plate and then exposing them to known amounts of an

antibiotic to determine the bacterial susceptibility to the antibiotic. The results of two types of tests and their merits and demerits were assessed.

Results

A total of 100 patients having urethral discharge were studied. All the patients were males. The age varied from 20 to 40 years, the average being 29.2 years+5.8 years. Majority of patients seen were in the age group of 21-30 years (60%). History of illicit sexual exposure could be elicited in 36 (36%) patients whereas rest of the patients denied it. There was history of solitary sexual exposure in 26 (26.3%) patients and multiple exposures in 10 (10%) patients. The duration of symptoms ranged between 4 and 30 days with a mean of 12.8 days and a median of 12 days. The patients with Gonococcal Urethritis presented with purulent discharge (both frank and scanty) in 66 (66%) cases, and dysuria in 49 (49%) cases, 2 (2%) cases had unilateral swelling of testis, 1 (1%) patient had fever and lower abdominal pain, 23 (23%) patients had erythema and swelling at external urethral meatus. No case had anal, eye or oral symptoms. Thirty four (34%) cases had an elevated white blood cell (WBC) count, in the range of 10,000-15,000/ μ L. Forty one (41%) cases had elevated erythrocyte sedimentation rate (ESR) in the range of 20-50 mm fall at the end of 1st hour. Eighty seven (87%) cases showed intracellular diplococci on direct microscopy of Gram stained smear. Sixty-two (62%) cases showed growth of gonococci on culture of urethral pus. No case was seen to be HIV positive. Tetracycline resistance was seen in 34(55%) strains. Penicillin resistance was seen in 25 (41%) strains of *Neisseria Gonorrhoea*. Five strains (11.5%) showed resistance to Ciprofloxacin. No resistance was seen with Cephalosporins.

Discussion

The duration of symptoms in our study ranged between 4 and 30 days with a mean of 12.8 days and a median of 12 days. Some other studies in this regard have shown duration of symptoms to be 8 days to 2 weeks [11]. Men with Gonococcal urethritis may present with any combination of symptoms [12]. In our study the patients with gonorrhoea presented with purulent discharge (both frank and scanty) in 66 (66%) cases, and dysuria in 49 (49%) cases, 2 (2%) cases had unilateral swelling of testis, 1 (1%) patient had fever and lower abdominal pain, 23 (23%) patients had erythema and swelling at external urethral meatus (Table 1). The discharge was mostly present spontaneously at

Age	20-40 years.Average-29.2 years+5.8 years. 60% - Maximum: 21-30 years
Gender	Males
H/O Illicit Sexual Contact	36%
Duration of Symptoms	4-30 days Mean-12.8 days Median-12 days
Purulent Discharge	66%
Dysuria	49%
Epididymitis	2%
Fever & Lower abdominal pain	1%
Erythema at External urethral meatus	23%
Elevated WBC count, in the range of 10,000-15,000/ μ L.	34%
Elevated ESR in the range of 20-50 mm fall at the end of 1st h	41%
Gonococci seen on Gram stain of Smear	87%
Positive Gonococcal Culture	62%

Table 1: Presentation pattern in gonorrhoea patients, n=100.

the urethral meatus and in a few cases was elicited by exerting a slight pressure on penis. The discharge was scanty and copious, purulent or muco-purulent in consistency. In another such study discharge was present in 82 percent of cases and dysuria in 53 percent [13]. Unilateral testicular pain and swelling may be the sole presenting complaints of men with epididymitis, with concomitant urethritis often discovered during the history and physical examination as it was seen in 2(2%) cases in our study. No extra genital symptoms were seen in our study. History of sexual exposure was scarce as it could be elicited only in 36% cases which may be due to considering of illicit sexual contact as a taboo in this part of world. In our study the highest incidence (60%) was found to be in the age group of 21-30 years which is almost similar to certain other studies in this regard [14]. All the cases in our study had urethritis. No case had oral or anal symptoms.

Specific culture of a swab from the site of infection is a criterion standard for diagnosis at all potential sites of Gonococcal infection. In male patients with urethritis, the diagnosis can be made by direct microscopy of stained smears of urethral discharge [15]. Cultures are particularly useful when the clinical diagnosis is unclear as in asymptomatic males and in females, when a failure of treatment has occurred, when contact tracing is problematic, and when legal questions arise. Following culture of specimens obtained from the genital tract using a highly selective medium, a presumptive diagnosis can be made based on colony morphology; Gram stain and the detection of cytochrome C oxidase [16]. A number of swab types are suitable for collecting specimens of *N. gonococci*. These include serum/albumin-coated swabs, calcium-alginate swabs and some modern rayon fiber-tipped swabs. Sterile inoculating loops can also be used. It is always preferable to collect two swabs, one for microscopic examination and the other for culture. If standard culture media are available at the collection site, the specimen can be inoculated at the site and placed in an atmosphere containing 5% CO₂ at 37°C. The inoculated plates can then be transported at a convenient time [17]. In our study the positive Gram stained smears were seen in 87% cases of suspected Gonococcal urethritis. It was easy to perform the test in OPD or on the bedside of the admitted patient. Results were received quickly. The cost of this test was quite low (Table 2). This test was found to be quite accurate for men but is considered not good in women. It is generally known that only one in two women with gonorrhoea have a positive stain [18]. In one study, a Gram stain diagnosed 94 percent of cases in symptomatic men however the sensitivity dropped to 81 percent in asymptomatic subjects. Some other reports have shown that performance of Gram stain in a man is similar to that of culture, with sensitivities of 89 to 94 percent and specificities of 94 to 97% [19]. Thus, in symptomatic men, urethral Gram stain is sensitive, specific, and cost effective.

	Microscopy	Culture
Time Required	Rapid	24-48 h
Cost	Inexpensive	Moderately Expensive
Utility	Ineffective for females, rectal and pharyngeal samples	Effective for all types of samples
Level of use	In OPD and on bed side	Laboratory facilities are required
Ease of performance	Easy	Moderate
Equipment	Light microscope	Transport Medium, Culture medium, incubator, light microscope, candle jar
Antibiotic sensitivity	Can not be determined	Can be determined

Table 2: Comparison of gram stain and culture for detection of *Neisseria gonorrhoea*.

However, if the Gram strain is negative in an asymptomatic man, additional testing should be performed if Gonorrhoea is suspected due to risk factors. Recently developed tests using cycling probe technology, such as examination of urine by PCR, allow diagnosis to be made without the need for culture. However, these tests are costly and culture is essential for surveillance of antimicrobial susceptibility. The positivity of the culture in our study was found to be 62%. Culture test was more difficult to perform than the Gram stain smear and required the patient to be referred to the laboratory as the availability of transport media was sparse and difficult. The culture test was more reliable but required 24-48 hrs for the results. The cost of the test was higher than the smear test. Costs for culture are moderate compared with more expensive DNA amplification techniques (Table 3).

Over the last decade, strains of *Neisseria gonorrhoea* have been reported to develop high levels of resistance against several antimicrobial agents which have been used previously for the treatment of gonorrhoea [20]. Gonorrhoea has progressively developed resistance to various antibiotics in our population also. Fortunately the sensitivity to Cephalosporins is still preserved. No patient had positive HIV test in our study. However high level of HIV positivity was found in cases of Gonorrhoea in certain other studies. Khopakar et al reported 14% positivity rate in all STD cases and Bhushanam et al 2.3% [21]. The resistance to Cephalosporins is also being reported from certain other areas of world [22]. Furthermore the frequent travel is also spreading the multi-drug resistant strains across the world. The reason for resistance seems to be the ability of gonococcus to mutate and develop efflux pump, a structure that evolved in several types of bacteria to pump antimicrobial compounds out of the organism before they can do any harm [23]. The emergence of cephalosporin resistance would substantially limit treatment options and would be a significant public health concern, in particular because untreated gonorrhoea can prompt to costly and severe public health consequences. The untreated Gonorrhoea can lead to urethral strictures, pelvic inflammatory disease and infertility both in males and females. In USA there are approximately close to 700,000 cases of Gonorrhoea each year. The drug resistant strains of gonorrhoea were 1 in 10 cases of sexually transmitted diseases in 2010 in Europe [24]. In this perspective it becomes all the more imperative to develop newer effective antibiotics against the organism and stress upon the prevention of disease in our society. A major challenge to monitoring emerging antimicrobial resistance of *N. gonorrhoea* is the substantial decline in the use of gonorrhoea culture by many clinicians, as well as the reduced capability of many laboratories to perform gonorrhoea culture techniques required for antibiotic susceptibility testing. Culture testing is when the bacteria is first grown on a nutrient plate and is then exposed to known amounts of an antibiotic to determine the bacteria's susceptibility to the antibiotic. The decline in culture testing results from an increased use of newer non-culture-based laboratory technology, such as a diagnostic test called the Nucleic Acid Amplification Test (NAAT). Currently, there is no well-studied reliable technology that allows for antibiotic susceptibility testing from non-culture specimens. Increased laboratory culture capacity is needed. CDC recommends that all state and local health department labs maintain or develop the capacity to perform gonorrhoea

Drug	Sensitivity
Tetracyclines	34 (55%)
Penicillins	25 (41%)
Quinolones	5 (11.5%)
Cephalosporins	Nil

Table 3: Sensitivity pattern of *Neisseria gonorrhoea*.

culture [25]. CDC's 2015 STD Treatment Guidelines now recommend only one regimen of dual therapy for the treatment of gonorrhoea—the injectable cephalosporin ceftriaxone, plus oral azithromycin. Dual therapy is recommended to address the potential emergence of gonococcal cephalosporin resistance [26]. A safe and effective vaccine for gonorrhoea would greatly decrease the urgency of developing newer antibiotics for N.Gonorrhoea. Dr. Cynthia Cornelissen, an NIAID-funded investigator at Virginia Commonwealth University, is working toward that goal [27].

Conclusion

Gram staining and culture for *Neisseria gonorrhoea* are both useful diagnostic tests for Gonococcal urethritis. Drug resistance of *Neisseria Gonorrhoea* is high with various antibiotics except for Cephalosporins stressing the need for culture based therapy and development of newer antibiotics along with enthusiastic prevention of Gonorrhoea in our society.

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