Abstract

Introduction

Trichomonas vaginalis (TV) rates in women are increasing and many are asymptomatic. Nucleic acid amplification tests (NAATs) are becoming the 'gold standard' for diagnosis. We aimed to establish our asymptomatic TV rates by testing all women attending Oxfordshire's Sexual Health service, regardless of symptoms, using the BD ProbeTec^{TM} TV Q^{x} NAATs (BDQ^{x}).

Methods

During BDQ^{x}'s verification process, the sensitivity and specificity were calculated using results of 220 endocervical samples from symptomatic women, compared with culture. BDQ^{x} was subsequently implemented and prospectively evaluated over 6 months in female attendees. Wet mount microscopy was also performed in symptomatics. Demographic and clinical characteristics of those diagnosed were analysed.

Results

From 220 samples tested by BDQ^{x} and culture: 5 were positive on both and one solely using BDQ^{x}, giving a sensitivity and specificity of 100% and 99.53% respectively. In the prospective cohort, of 5775 BDQ^{x} tests, 33 (0.57%) were positive. 11/33 (33%) patients were asymptomatic. All patients diagnosed had risk factors: age >25 years (85%), residence in a deprived area (79%) and black ethnicity (21%).

Conclusion

Despite BDQ[x] being highly sensitive and specific, with our low TV prevalence universal screening may not be justified. Targeted screening using local demographic data merits further investigation.

Key words: Diagnosis; trichomoniasis (Trichomonas vaginalis), screening, epidemiology

Detecting asymptomatic Trichomonas vaginalis in females using the BD ProbetecTM Trichomonas vaginalis Qx nucleic acid amplification test

Introduction

In the UK, data from the Genitourinary Medicine Clinic Activity Dataset v2 (GUMCADv2) has shown rates of *Trichomonas vaginalis* (TV) in women rose by 14% between 2010-14[1]. Women are up to fifteen times more likely to be infected than men and up to 50% are asymptomatic[2, 3]. Untreated infection can be associated with sequelae such as pelvic inflammatory disease (PID), increased susceptibility to HIV, and obstetric complications[2, 4, 5].

Previous British Association for Sexual Health and HIV (BASHH) guidelines recommended TV testing for symptomatic women using wet-mount microscopy, acridine orange staining with fluorescence microscopy (AOS) or culture[6]. In the last 2 years two commercially available nucleic acid amplification tests (NAATs) have become available (BD ProbeTec™ TV Qx NAATs (BDQx) and Gen-Probe APTIMA TV assay (ATV)); these may have contributed to the rise in reported TV cases. They are the most sensitive test available, BDQx has reported sensitivities and specificities of >98% and >99% respectively[4, 7]. NAATs are now replacing culture as the current 'gold standard' and current BASHH guidelines recommend NAATs in symptomatic women where resources allow[4]. The burden of asymptomatic TV may, however, be underestimated, and its role in sustaining transmission is therefore uncertain[2].

In Oxfordshire's Sexual Health Service, TV tests were previously only performed in symptomatic women using wet-mount microscopy and/or AOS (ProLab diagnostics,

England). In January 2015, the BDQ^{x} assay became available by inclusion in the existing *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoea* (GC) platform. It is processed on the BD Viper™ System and uses Strand Displacement Amplification technology to detect TV DNA in endocervical or self-taken lower vaginal swabs. It can be used in asymptomatic and symptomatic females[8].

Following verification of BDQ^{x} by the laboratory, AOS was discontinued and BDQ^{x} implemented as the standard of care for TV diagnosis in females, regardless of symptoms. The primary aim of this study was to investigate local rates of asymptomatic TV using BDQ^{x}. In addition we wanted to determine whether demographic and clinical data could help inform future screening policies.

Methods

As part of the BDQ^{x} verification process, the microbiology laboratory evaluated its use. Specimens from 220 consecutive symptomatic females who had already had endocervical samples taken for CT/GC NAATs and AOS microscopy as part of their clinical care were collected. TV was additionally tested for on the CT/GC NAATS (BDQ^{x}) sample using the BD Viper^{TM} System and the AOS sample inoculated into culture (CM0161, Oxoid Ltd, England). To calculate the sensitivity and specificity, BDQ^{x} results were compared to culture, rather than AOS as this was the 'gold standard' in previous UK guidelines[(6)].

Following verification, BDQ^{x} was implemented and over 6 months all women presenting for STI tests were offered BDQ^{x} (CT/GC/TV), regardless of symptoms.

Women were informed of the routine tests available and consented for investigation, as is usual practice in any screen. As BDQ[x] was the standard test available, specific consent was not obtained; patients not wanting TV testing could opt out. Asymptomatic women were offered self-taken lower vaginal swabs, unless they were having a speculum examination e.g. for cervical cytology, when an endocervical specimen was taken. For symptomatic women, a clinician-taken posterior fornix swab for wet-mount microcopy and endocervical swab for BDQ[x] was recommended; in those who did not consent or could not tolerate speculum examination a lower vaginal swab was done, by the patient or clinician examining.

TV diagnoses were prospectively evaluated over the 6-month period following BDQ[x] implementation. The laboratory provided a database for all tests performed and their results. However, results did not distinguish between vaginal/endocervical swabs. The notes for those with a positive result were reviewed and the following data recorded: age, ethnicity (black/mixed black ethnicity), country of birth, relative level of deprivation by postcode (index of multiple deprivation scores (IMD) scores), symptoms, signs, type of test (endocervical/vaginal), concurrent infection, previous TV and the number and ethnicity of sexual partners in the last 6 months.

Data analysis

The relationship between symptoms of TV and infection risk factors was evaluated using Fisher's exact test. The data collected were tabulated into Microsoft Excel (Redmond, WA, US) and statistical analyses conducted using GraphPad QuickCalcs (http://www.graphpad.com/quickcalcs/ConfInterval1.cfm).

Study Results

Results from laboratory verification of BDQ^{x}

220 endocervical samples from symptomatic women were included in the preliminary laboratory work, 5 (2.27%) were positive for TV on BDQ^{x}, culture and AOS microscopy. One sample only tested positive on BDQ^{x}. This patient subsequently re-attended with symptoms still in keeping with TV; the BDQ^{x} repeated and was still positive. All patients diagnosed were treated as per clinic guidelines. Calculated sensitivity and specificity of the BDQ^{x} compared to culture were 100% and 99.53% respectively. If it is assumed the patient only positive on BDQ^{x} was a false positive, the positive predictive value (PPV) of BDQ^{x} was 83.3% and the negative predictive value (NPV) 100%, using culture as the gold standard.

Detection of TV by BDQ^{x} in female clinic attenders

During the 6 month study 6114 women had 6951 new or rebooked attendances (837 had more than one episode). Of the 6951 STI screens undertaken, 5814 were BDQ^{x} CT/GC/TV tests. In 1137 episodes the clinician did not request TV on the CT/GC sample. Thirty-nine BDQ^{x} samples could not be processed, therefore 5775 results were reviewed. (Figure 1). Of those tested with BDQ^{x}, 33/5775 (0.57%) were positive for TV. A further 3 patients did not have a BDQ^{x} sample taken and were diagnosed using wet-mount microscopy alone, giving a total of 36 TV infections during the study period (Table 1).

Of the patients diagnosed with TV, 19/36 (53%) were tested with both wet-mount microscopy and BDQ^{x}. 11/19 (58%) had concordant positive results. 8/19 (42%) had discordant results (all microscopy negative, BDQ^{x} positive). The remaining 17/36 (47%) were only screened by one test, see Table 1.

Of those diagnosed using BDQ^{x}, 11/33 (33%) were asymptomatic, of whom 8 (73%) had self-taken lower vaginal swabs. Three patients had endocervical swabs as they had speculum examinations for other reasons. Twenty-two patients (67%) were symptomatic, 16/22 (73%) had endocervical swabs and 6 (27%) lower vaginal swabs, either because the patient refused or could not tolerate a speculum.

Identified risk factors for TV infection in those diagnosed with BDQ^{x}

Demographic and clinical characteristics of the patients diagnosed are shown in Table 2. Of the eleven without signs or symptoms documented, 9/11 (81%) were screened using BDQ^{x} only. The remaining two patients tested positive by BDQ^{x} but were negative on microscopy.

All patients diagnosed had at least one risk factor for infection: age >25 years and residence in an area classified as deprived were the most frequent. 7/33 (21%) of patients were of black/mixed black ethnicity. In approximately one third of cases ethnicity of sexual partners was not documented. If those without documented ethnicity of partner(s) are excluded (n=13), 10/20 (50%) had sexual partners of black/mixed black ethnicity. Country of birth was not analysed as it was rarely documented. Concurrent diagnoses, both sexually and non-sexually transmitted, were detected in 11

(33%) patients, the most frequent being bacterial vaginosis and PID (both 8%). One (3%) patient was HIV positive. A minority were also CT/GC positive (6% and 3% respectively).

A comparison of risk factors for infection between asymptomatic and symptomatic women with TV was undertaken; however, there were no statistically significant associations (Table 2).

Discussion

Although the ATV assay has been evaluated in different UK healthcare settings[9-11]; our study is, to our knowledge, the first prospective analysis of the BDQ^{x} assay in clinical practice that aimed to ascertain the burden of asymptomatic infection.

Results from the verification exercise found a prevalence of 2.27%, significantly higher than in the prospective BDQ^{x} analysis (0.57%). This may be because all patients in the preliminary work were symptomatic. Both the sensitivity and specificity of BDQ^{x} were very high. Measured against culture, the previous gold standard, the calculated PPV was 83.3%. However, the patient just positive on BDQ^{x}, but negative on culture (and AOS) had symptoms and risk factors for TV, as well as a second positive BDQ^{x} test. As culture has shown to be less sensitive than NAATS, we suggest this represents failure of culture to pick up the infection, rather than a false positive BDQ^{x} result[7]. However, when designing screening protocols false-positive results need to be considered, especially in asymptomatic patients in low prevalence populations. Reassuringly, of the

twenty-two patients diagnosed only by BDQ[x] (no concurrent microscopy result), the 50% who were asymptomatic all had risk factors.

In 1137 patients, the clinician did not test for TV. Unfortunately, it was not possible to review the notes to ascertain why. Possible reasons include clinician choice (because the woman was asymptomatic and perceived low risk) omission in error, or the woman may have declined the test. While infections may have been missed as a result, our overall local prevalence of 0.57% is comparable to Mahto et al. (0.8%) in their ATV study in Macclesfield[9].

Few UK clinics routinely screen for TV in women not reporting symptoms; therefore the national rates of asymptomatic infection are largely unknown[2]. Hathorn et al. found 46.3% of females diagnosed with TV in their Birmingham study were asymptomatic[10]. Comparison of our TV data obtained in 2015 by BDQ[x] with data from the previous year, when only symptomatic patients were tested using AOS, showed similar prevalence rates (0.57%, compared to 0.7%, p = 0.39, Fisher's exact test). However, limited conclusions can be drawn as no reference screening method was used during both periods. Nevertheless, a third of patients diagnosed using BDQ[x] were asymptomatic, suggesting the prevalence in our clinic may have been underestimated prior to 2015.

No studies to date have assessed the optimal screening frequency in asymptomatic women and the risk of clinical sequelae if they remain undiagnosed[12]. One-third of asymptomatic women with TV will develop symptoms within 6 months and those who remain asymptomatic may be more likely to remain sexually active[13, 14]. TV is

associated with both HIV acquisition and transmission and its treatment decreases vaginal HIV viral shedding. HIV patients are also more likely to develop complications such as PID[12].

It was not possible to perform a detailed cost analysis for this study, however, our laboratory estimates an opportunity for savings on the current BDQ[x] price if it fully implemented throughout the county and AOS discontinued. The results of an economic evaluation of the ATV assay are awaited[15]. Meanwhile a preliminary assessment of the cost implications of TV NAATs suggests that while initial outlay costs may be high if the relevant processing system is not already in place, there may be savings in overall labour costs[16]. Without national prevalence data on the burden of asymptomatic infection, determining a prevalence threshold at which to offer universal screening is difficult. However, it is probably not justified to continue in our very low prevalence setting. As Hathorn and Turner et al. describe, targeted screening may be more appropriate[10, 11].

No statistical difference was found in terms of the identified risk factors for TV between those with and without symptoms. Our results are consistent with risk factors already established to be associated with infection[2]; given the background demographics of those attending our service (Table 2), patients of black/mixed black ethnicity, aged >25 years and living in areas of deprivation were overrepresented among TV diagnoses. However, if ethnicity alone had been used to target asymptomatic screening, ten infections (30%) would have been missed. In terms of informing our asymptomatic screening policy, age >25 years, HIV positivity and black ethnicity are factors to consider, with a large transient student population, it is impractical to use postcode as

criteria for targeted screening. Other factors such as partner ethnicity are poorly documented and concurrent diagnoses such as CT/GC would not be known at the time of screening.

Our study is limited by the small number of patients diagnosed with TV therefore information regarding risk factors could be related to chance. Also, asymptomatic women were not examined, and signs of infection could have been missed. The absence of a negative control group meant that we could not establish whether there were differences between reported symptoms and risk factors in those with and without infection. In addition, we could not review the notes of all 5775 women tested using BDQ[x]. As laboratory results did not distinguish between self-taken (suggesting absence of symptoms) or clinician taken samples, we could not establish how many tested overall were asymptomatic. Also, as patients did not have concurrent endocervical and lower vaginal swabs, no conclusions can be made regarding whether type of swab might have affected the number of infections detected.

We would suggest that in such a low prevalence population, universal asymptomatic screening is probably not justified. However, a targeted approach of testing all symptomatic women and screening those asymptomatics who are HIV positive, of black ethnicity or aged >25 years, could be an appropriate strategy.

Figure 1: number of female attendances, type of STI screen performed and results

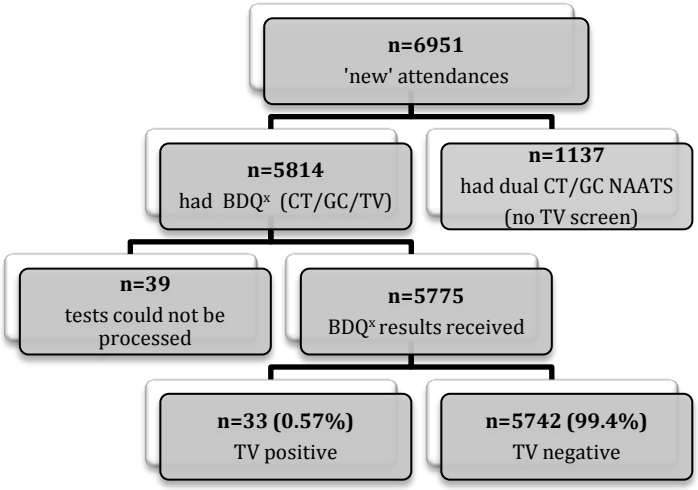

Table 1: Direct microscopy and BDQ^{x} results

	Number (%)
Total no. of patients coded 'C6a'	36
Total no. of BDQ^{x} performed	5775
Total no. of BDQ^{x} positive	33 (0.57)
Microscopy +ve ; BDQ^{x} +ve	11
Microscopy +ve; BDQ^{x} –ve	0
Microscopy -ve; BDQ^{x} +ve	8
Microscopy +ve; BDQ^{x} not done	3
Microscopy not done; BDQ^{x} +ve	14

Table 2: Patient characteristics of those diagnosed with NAATS, comparing those both with and without symptoms

	All patients attending in 6 months n=6114 (%)	All patients diagnosed using BDQ[x] n=33/5775 (%)	Symptomatic patients diagnosed n=22 (%)	Asymptomatic patients diagnosed n=11 (%)	P-value (Fisher's exact test)[*]
Overall Prevalence	-	**0.57**	[**]	[**]	-
Signs/symptoms: vulval irritation, discharge, dysuria, pelvic pain	-	22 (67)	22 (100)	0	-
Swab: I. Lower vaginal swab II. Endocervical swab	-	 14 (42) 19 (58)	 6 (27) 16 (73)	 8 (73) 3 (27)	-
Patients with at least one risk factor for infection:	-	33 (100)	22 (100)	11 (100)	-
I. Age: over 25 years	2928 (48)	28 (85)	17 (77)	11 (100)	0.14
II. Postcode: classified 'deprived'	596 (10)	26 (79)	17 (77)	9 (82)	1.0
III. Ethnicity: black/mixed black	285 (5)	7 (21)	6 (27)	1 (9)	0.38
IV. Multiple Sexual Partners: >3 in 6/12	-	6 (18)	2 (9)	4 (36)	0.15
V. Partner of black ethnicity[♯]	-	10 (30)	7 (32)	3 (27)	1.0
VI. Other sexually transmitted infection, PID, bacterial vaginosis or candida	-	11 (33)	8 (36)	3 (27)	0.71
VII. Previous TV infection	-	2 (6)	2(9)	0	0.54

[*] Comparison between risk factors for infection in those with/without symptoms. Significance = p-value <0.05

[**]Unable to calculate specific prevalence as no data regarding total number of symptomatic/asymptomatic presentations

[♯] Ethnicity of sexual partner only documented in 20/33 (61%) patients

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