



Occurrence of *Trichomonas vaginalis* and *Candida* spp. infections among married women attending General Brack Hospital and private gynaecology clinics in Brack Al-Shati region, a cross sectional study

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ABSTRACT

The focus of the current study was on exploring the epidemiological profiles of *Trichomonas vaginalis* and *Candida* spp. infections among married women admitted gynaecology clinics in Brack Al-Shati region. 234 of vaginal swab samples were collected and subjected to direct microscopic examination for microorganisms' characterization. 45 (19.23%) of overall samples examined were found to be *T. vaginalis* and *Candida* spp. positives. Occurrence of each infection agent was individually compared. Infection with *Candida* spp were statistically ($p=0.003$) raised 32 (13.7%) over that of *T. vaginalis* infection 13 (5.6%). Co-infections were observed in only 2 (4.4%) of overall positives. No significant associations were observed between these infections and women age categories. Women had history of miscarriages and those had university levels were statistically linked ($p<0.05$) to the two infection agents although the former was only linked ($p<0.05$) to *T. vaginalis* infection. *T. vaginalis* infection was the only agent driving significance ($p<0.05$) when associated with vaginal secretion presence. Overall occurrence of *T. vaginalis* and *Candida* spp infections revealed in the present work was relatively high. Interestingly, a number of significant association between both infection profiles and women's risk factors was also observed.

وقوع الاصابة بعدوى طفيل المشعرة المهبلية و الأنواع الفطرية لجنس الكانديدا بين النساء المتزوجات المترددات على عيادات النساء بمستشفى براك العام وبعض العيادات الخاصة بمنطقة براك، دراسة مقطعية

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المخلص

ركزت الدراسة الحالية على اكتشاف الصور الباثية لكل من طفيل المشعرة المهبلية و الانواع الفطرية الواقعة تحت جنس الكانديدا في النساء المتزوجات المترددات على عيادات النساء الواقعة في منطقة براك الشاطي. 234 عينة جمعة من المسحات المهبلية وخضعت للفحص الميكروسكوبي المباشر للتعرف على خصائص تلك الكائنات. 45 (19.23%) من اجمالي العينات المفحوصة كانت مصابة بأي من هذه الميكروبات المعدية. عند مقارنة وقوع الإصابة الفردية لكل ميكروب، فطريات الكانديدا سجلت إحصائياً أعلى معدل إصابة 32 (13.7%) من مما هو في المشعرة المهبلية 13 (5.6%). الإصابة الثنائية لوحظت في فقط 2 (4.4%) من العينات الموجبة. المقارنة الإحصائية لوقوع كلا الكائنين المعديين والفئات العمرية لم يثبت وجود أية فروق معنوية. علاوة على ذلك، التاريخ الاجهاضي كان اكثر ارتباطاً احصائياً ($p<0.05$) بطفيل المشعرة المهبلية من فطريات الكانديدا ($P>0.05$). أيضاً تم تحليل وقوع الإصابة بهذه الكائنات مع المستويات التعليمية المختلفة للنساء والفرق الجوهري الوحيد لوحظ عند مقارنة الكائنين معاً و المستوى الجامعي. هذا الاختلاف تسبب بفعل الإنخفاض في الإصابة بطفيل

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المشعرة المهبلية (4.44%) من تلك التي في الكانديدا (17.77%). تم أيضا ربط الإصابة بكلا الكائنين وظهور الإفرازات المهبلية حيث أن الإصابة بالمشعرة المهبلية كانت مرتبطة احصائياً ($p < 0.05$) بتلك العرض. النسبة العامة للإصابة بطفيل المشعرة المهبلية وفطريات الكانديدا المسجلة في هذه الدراسة كانت مرتفعة نسبياً. عند تحليل العلاقة بين الإصابة وعوامل الخطر المختلفة لوحظ وجود عدد من الفروق الجوهرية.

Introduction

The protozoan parasite *Trichomonas vaginalis* was firstly described as a sexual transmitted disease in the mid of 21th century and it has been reported as the most common none-viral sexual transmitted organism [1]. Occurrence of this parasite varied between countries with global occurrence ranged between 170 and 190 million cases annually [2]. Sexual intercourse regarded as the main rout of transmission [3]. *Candida* spp. are also infectious microorganisms inhabit the same as *T. vaginalis* infection site and in some studies, it constituted the most prevalent sexually transmitted diseases [4]. Vaginitis and vaginal discharges found to be the most common symptoms linked to both infectious organisms, although 15-20% of the annual vaginitis cases were mainly associated with the parasitic infection *Trichomonas vaginalis* [5]. Microscopic examination of wet mount prepared vaginal swab recorded to be more utilised technique over that others (culture, staining and molecular techniques) in developing countries where time consuming and costs are taken into account [6]. Several global studies have been concerned with the occurrence of these infectious agents either among reproductive women [7]–[9], vaginal discharged (symptomatic) women [10]–[12], or pregnant women [1], [4], [13], [14]. However, reports considered the occurrence of these infections among married (non-pregnant) women still in paucity in Libya generally and in Wadi Al-Shati region in particular. Therefore, the current study was broadly aimed to explore the occurrence of *T. vaginalis* and *Candida* spp. infections among married women attending gynaecology clinics in Brack Al-Shati, Southern Libya.

Materials and methods

During a period of 10 months (March to November 2019), occurrence of *T. vaginalis* and *Candida* spp. infections was assessed targeting a total of 234 married (non-pregnant) women. Two private gynaecology clinics (Tasneem; Alafia) and one general gynaecology departments (Brack General Hospital) were targeted as a source of sample collection. Sampling was aseptically performed from vaginal walls using a sterile cotton tipped swabs which then replaced in its

specified tube specified for this purpose as previously described [15], [16]. All women involved in this study were asked to provide an informed consent before sampling and demographic information (age, education, miscarriage history and presence of symptoms such as vaginal secretions) were also gathered. Other criteria which may influence microorganisms' presence such as treatment, using pregnant barriers, subjection to special control methods... et al. were also ruled out. Upon collection, sample-contained swabs were immediately prepared (wet mount preparation) and examined utilizing light microscope (low power; x100 and high power; x400). Characteristics *T. vaginalis* and *Candida* spp were microscopically observed as previously reported [15].

Data obtained in this work were statistically analysed applying WinPepi statistical software (WINPEPI, UK) version 11.65 [17] statistical software. Chi-squared (χ^2) test at probability (p) of (<0.05) were utilized to determine statistical significance.

Results

In present study, all vaginal smears collected (n=234) were subjected to microscopic examination for *Trichomonas vaginalis* and/or *Candida* spp identification. Of overall samples examined, 19.23% (n=45); 95% C. I. (14.3-24.8) found to be infected with any of these microorganisms. Infection profile of each microorganism was 5.6% (13/234) and 13.7% (32/234) for *T. vaginalis* and *Candida* spp, respectively. Differences in the occurrence between these infections were statistically compared and *Candida* spp were recorded significantly greater infection rate than that of *T. vaginalis* ($p = 0.003$). Presence of co-infection with *T. vaginalis* and *Candida* spp was also assessed, and 2 (0.9%) of overall examined and 2 (4.4%) of overall positives, were co-infected. The lowest occurrence of co-infection reported in this study was the main driver of statistical significance ($p < 0.05$) when it compared with single infections due to either *T. vaginalis* or *Candida* spp. (Table 1).

Table 1: Overall occurrence of *T. vaginalis* and *Candida* spp infections

| Overall samples examined | Single infections | | | | | |
|--------------------------|------------------------------|-------------------------------|--------------------|---------------------------------|---|-------------------------------|
| | <i>Trichomonas vaginalis</i> | | <i>Candida</i> spp | | Coinfections (<i>T. v</i> + <i>Candida</i> spp) | |
| | No. infected | Infected % (C. I.) | No. infected | Infected % (C. I.) | No. infected | Infected % (C. I.) |
| 234 | 13 | 5.6 (3.3-9.3) ^L | 32 | 13.7 (9.5-18.8) ^M | 2 | 0.9 (0.1-3.1) ^N |

Spp= species, No.= number, T. v= *Trichomonas vaginalis*; statistical comparison: L and M: $\chi^2 = 9.1$, df= 1, $p = 0.003$; L and N: $\chi^2 = 9.28$, df= 1, $p = 0.002$; M and N: $\chi^2 = 33.99$, df= 1, $p = 0.000$

Presence of *T. vaginalis* and *C. spp.* was also analysed with respect to individual's age groups. Women aged 32-39 years found to be more vulnerable to the infection with either *T. vaginalis* 9.23% (6/65) or *C. spp.*

18.46% (12/65) compared to the other age categories. However, statistical comparison of each infection agent among different age categories revealed no significance ($p > 0.05$) (Table 2).

Table 2: *T. vaginalis* and *C. spp* infections with relation to women age

| Age group | Examined | Infected (%), (C. I.) | |
|-------------|----------|------------------------------------|-------------------------------------|
| | | <i>T. v</i> | <i>C. spp</i> |
| 18-24 | 39 | 2 (5.12%), (0.6-17.3) ^A | 7 (17.94%), (7.5-33.5) ^E |
| 25- 31 | 85 | 4 (4.70), (1.3-11.6) ^B | 8 (9.41%), (4.2-17.7) ^F |
| 32-39 | 65 | 6 (9.23), (3.5-19) ^C | 12 (18.46), (9.9-30.0) ^G |
| 40 and over | 45 | 2 (4.44), (0.5-15.1) ^D | 6 (13.33), (5.1-26.8) ^H |

T. v= *Trichomonas vaginalis*, *C. spp*= *Candida* species; statistical comparison: A, B, C and D= $\chi^2 = 1.7$, df= 3, $p = 0.63$; E, F, G and H= $\chi^2 = 3.06$, df= 3, $p = 0.38$; statistical comparison of both microorganisms in each individual age group revealed no significant differences $p < 0.05$.

As shown in Table 3, data of present study were allowed to be classified into three categories based on women's history of miscarriage. 80% of overall samples examined (n=234) were from

women with no history of miscarriage, while the remaining (20%) were from those subjected to either one or more than one-time abortion. Indeed, occurrence *T. vaginalis* infection found to be more

linked (21.1%; 4/19) to women had more than one-time miscarriage than those with no miscarriage history (3.74%; 7/187). Comparison of these differences was statistically significant ($p < 0.05$). There were

no significant differences ($P > 0.05$) in the occurrence of *C. spp* between these categories.

Table 3: *T. vaginalis* and *C. spp* infections with relation to women abortion /miscarriage

| Overall samples examined | History of miscarriages | No. samples examined | <i>T. v</i> infection (%), (C. I.) | <i>C. spp</i> infection (%), (C. I.) |
|--------------------------|-------------------------|----------------------|------------------------------------|--------------------------------------|
| 234 | No. H. M | 187 | 7 (3.74) ^A , (1.5-7.6) | 25 (13.4) ^L , (8.8-19.1) |
| | yes (1 time) | 28 | 3 (10.7) ^B , (2.3-28.2) | 7 (25) ^M , (10.7-44.9) |
| | yes (more than 1 time) | 19 | 4 (21.1) ^C , (6.1-45.6) | 1 (5.26) ^N , (0.1-26) |
| Total | | 234 | 14 (6), (3.3-9.8) | 33 (14.1), (9.9-19.2) |

No. H. M= no history of miscarriages; statistical comparison: A, B and C: $\chi^2 = 7.65$, $df = 2$, $p = 0.022$; multiple statistical comparison: A and B= $\chi^2 = 2.09$, $p = 0.14$, A and C= $\chi^2 = 6.58$, $p = 0.010$, B and C= $\chi^2 = 0.94$, $p = 0.33$; L, M and N: $\chi^2 = 3.25$, $df = 2$, $p = 0.13$

Occurrence of *T. vaginalis* and *C. spp* was also compared based on women’s educational level. Approximately, one half of the samples were collected from women had secondary school qualification. Statistical comparison of each infection agent between different levels revealed no significance ($p > 0.05$). However, upon comparing

both infection agents in each individual education level, women had university graduation found to be significantly ($p < 0.05$) lower infected with *T. vaginalis* (4.44%) than *C. spp* (17.77%) (Table 4).

Table 4: *T. vaginalis* and *C. spp* infections with relation to women’s educational levels

| Educational levels | Examined | Infected (%), (C. I.) | |
|--------------------|----------|-------------------------------------|-------------------------------------|
| | | <i>T. v</i> | <i>C. spp.</i> |
| Primary | 32 | 1 (3.12) ^p , (0.1-16.2) | 5 (15.62) ^q , (5.3-32.8) |
| Secondary | 157 | 11 (7.00) ^r , (3.5-12.2) | 20 (12.73) ^s , (8-19) |
| University | 45 | 2 (4.44) ^t , (0.5-15.1) | 8 (17.77) ^u , (8-32.1) |

Statistical comparison: p, q and r: $\chi^2 = 0.95$, $df = 2$, $p = 0.62$; s, t and u: $\chi^2 = 0.8$, $df = 2$, $p = 0.67$; p and s: $\chi^2 = 2.94$, $df = 1$, $p = 0.86$; q and t: $\chi^2 = 2.90$, $df = 1$, $p = 0.089$; r and u: $\chi^2 = 4.05$, $df = 1$, $p = 0.044$.

The association between both microorganisms and presence of the most prominent vaginal symptoms (vaginal discharges) was also analysed. Women suffering from vaginal secretion recorded significantly ($p < 0.05$) higher (9.15%) *T. vaginalis* infection than those had no vaginal discharges (1.08%). Conversely, infection with

C. spp was significantly ($p < 0.05$) raised (20.65%) in women with no vaginal

secretions compared to those suffering from that symptoms (9.85%) (Table 5).

Table 5: *T. vaginalis* and *C. spp* infections with respect to women symptoms

| Vaginal discharges | Samples examined | <i>T. vaginalis</i> | | <i>Candida</i> spp | |
|--------------------|------------------|---------------------|----------------------------|--------------------|--------------------------------|
| | | Infected | Infected % (CI) | Infected | Infected % (CI) |
| Present | 142 | 13 | 9.15 ^A (5-15.1) | 14 | 9.85 ^C (5.5-16) |
| Absent | 92 | 1 | 1.08 ^B (0-5.9) | 19 | 20.65 ^D (12.9-30.4) |

Statistical comparison: A and B: $\chi^2 = 6.46$, $df = 1$, $p = 0.011$; C and D: $\chi^2 = 5.37$, $df = 1$, $p = 0.021$; A and C: $\chi^2 = 0.04$, $df = 1$, $p = 0.84$; B and D: $\chi^2 = 18.17$, $df = 1$, $p = 0.000$

Discussion

T. vaginalis infection has widely been reported to cause various vaginal disease and symptoms including impairment of the protective content of the vagina and it increases the risk of other opportunistic infections such as papilloma virus and human immune deficiency virus (HIV) [1]. *Candida* spp. infections have also been associated to cause several vaginal problems, starting from changing the color of vaginal secretion, and presence of deep erythema in the valve and vagina (Matini & Mohebali, 2012). Data of the current study highlighted the overall occurrence and risk factors associated with two medically important microorganisms, *T. vaginalis* and *C. spp.*, among married women attending two private and on general gynecology clinics in Brack Al-Shati region. A relatively high (5.6; 13.7%) rate of infection was recorded for *T. vaginalis* and *C. spp.*, respectively. In this study *C. spp.* were reported a significantly ($p < 0.05$) increased infection than *T. vaginalis* infection. Previous work carried out in Libya revealed concurred findings. Indeed, studies exploring the infection profiles of these infections in Libyan women showed different outcomes. In south Libya, for instance, only 1.84% out of 489 pregnant women found to be infected with *T. vaginalis* [19], whereas Younis & Elamami, (2016) reported that 24.5% of 110 vaginal swab were positive in women suffering from vaginal discharges and type2 diabetes. Bernawi et al., (2016) found a similar results (4.1-5.3%) of *T. vaginalis* in married women in Sebha, compared with present study findings. The increased occurrence of *T. vaginalis* reported by others may potentially owe to the differences in the population targeted since these studies (previous studies) were mainly focused either on type2 diabetes and/or vaginal discharged or women during pregnancy. The higher occurrence

(13.7%) of *Candida* spp. infection over that of *T. vaginalis* was also

reported by others. Bernawi et al., (2016) has found similar findings in married women in Sebha. In immune-competent (type2 diabetic) women, *Candida* spp. infections occurred in lower percentage compared to those infected with *T. vaginalis* infection [20]. Data of current study have allowed assessing the presence of coinfection, and only 0.9% of the total samples examined and 4.25% of overall positives were coinfecting with *Candida* spp. and *T. vaginalis*. Few studies considered coinfection profiles of these microorganisms in married un-pregnant women. In one study [1], although utilizing molecular based techniques, presence of co-infection reported in only 0.17% of overall examined (n=2203) and 7% of overall positives (n=127). Other previous studies separately addressed each infection agent individually [10], [18], [20], [21]. The association between women’s age and *T. vaginalis* and *Candida* spp. infections was also considered in this study. 81% of vaginal swabs were sampled from women categorised under a reproductive age (18-39), while the remaining (19%) were grouped either equal or more than 40 years. Although increased percent of both microorganisms were recorded in reproductive age women, these differences were not statistically significance. Increased level of *T. vaginalis* in reproductive age (25-34) women over those older than 35 has also been reported previously [22], [23], and this was statistically significant. Indeed, in study involved very large sample size (n= 20356) in Brazil, infection with *T. vaginalis* and *Candida* spp. was associated with women’s age group, and the infection lowered as their ages raised [24]. Non-

significant association between infections and women ages reported in the current study may potentially linked to that the majority (81%) of samples targeted were from those aged 18-39. In attempt to inspect differences in *T. vaginalis* and *Candida* spp. occurrence based on women's history of miscarriage, interestingly, women aborted more than one-time found to be significantly more susceptible to *T. vaginalis* infection compared with those had no miscarriage. However, these differences were not significant in terms of *Candida* spp. occurrence. Indeed, a significant relationship between *T. vaginalis* infection and miscarriage has also been observed previously [25]. On inspecting *T. vaginalis* and *Candida* spp. presence with relation to women's education, data showed that these infections have not been significantly influenced by women's educational level. The only significance observed when comparing these agents in women had university educations, and this was due to the increased occurrence of *Candida* spp. Samples examined in this study were collected from women regardless of their symptoms. This sampling strategy allowed associating the infection profile of both organisms with the main vaginal symptom observed, vaginal discharges. Interestingly, *T. vaginalis* infection was more associated ($p < 0.05$) with women suffering from vaginal secretion compared to those absent from this symptom. Conversely, *Candida* spp. infections were significantly associated with women had no vaginal discharges.

Conclusion

Data obtained in this study highlighted a relatively higher percentage of *T. vaginalis* and *Candida* spp. infections. Various significant associations were observed between the infections and presence of vaginal discharges, abortion and women's education level. Data revealed in this work highlighted that the presence of *T. vaginalis* and *Candida* spp. infections in the tested samples raised the necessity of establishing a proper control strategies and hygiene practices. Future researches are also required targeting larger sample size and associating more risk factors with both microorganisms.

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