

# **PREVALENCE OF *TRICHOMONAS VAGINALIS* INFECTION AMONG WOMEN IN ERBIL GOVERNORATE, NORTHERN IRAQ: AN EPIDEMIOLOGICAL APPROACH**

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## **Abstract**

Trichomoniasis is one of the sexually transmitted diseases with worldwide prevalence. It is a public health problem and associated with adverse outcomes of pregnancy and increased risk of HIV transmission. This analytic cross sectional study aimed to assess the epidemiological and clinical parameters concerning trichomoniasis in women attended Maternity Teaching Hospital in Erbil over the period from September 2012 till July 2013. Vaginal discharge swabs were collected from 440 women their ages ranged between 16-60 years using randomized sampling method. Out of 440 women, 14 (3.18%) and 12(2.73%) revealed positive results for trichomoniasis using culture technique and direct wet mount, respectively. The infection rate was non-significantly higher among women from rural settings (4.9%) than urban setting people (3.2%) and in housewives (3.8%) more than employed women (1%). Furthermore, Non- pregnant (3.4%) women were more susceptible to infection than pregnant group (0%), and there were no significant differences among married (3.1%), widow (7.7%) and divorced women (0%). Trichomoniasis was comparably higher in illiterate women (4.1%) than those with school and University qualifications (2.44%). A significant proportion of infected women experienced gray colored (50%) vaginal discharge and followed by green colored (16.7%) discharge. The infection rate was 4%, 3.3% and 3.1% among women with age groups of 16-26 years, 27-37 years and 38-48 years, respectively. Furthermore, trichomoniasis was non-significantly associated with number of parity and neonate abnormality. However, it's significantly ( $P = 0.016$ ) associated with history of abortion. Moreover, Women who were immediately post menstruation (9.3%) had significantly ( $P < 0.05$ ) higher

infection rate than those were at pre- (3.2%), immediately pre- (5.7%) and menses women (0%).

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**Keywords:** *Trichomonas vaginalis*, trichomoniasis, epidemiology, abortion, vaginal discharge, sexually transmitted diseases.

## Introduction

Human trichomoniasis is a widely prevalent sexually transmitted infection worldwide, causing roughly the same number of sexually transmitted diseases as *Chlamydia trachomatis*, the most prevalent sexually transmitted bacterial pathogen. Worldwide prevalence of *T. vaginalis* varies from 2% to greater than 50% depending on region, country, gender and demographic parameters of the study populations as well as the diagnostic procedures that used in various studies (Ginocchio *et al.* 2012). An estimated 180 million new infections acquired annually worldwide (Coleman *et al.* 2013).

Nearly half of all women with *T. vaginalis* are asymptomatic. Signs of infection in symptomatic women include malodorous vaginal discharge, edema or erythema and Strawberry cervix (punctate hemorrhagic lesions), vulval irritation and inflammation. Other complaints include dysuria, pruritis, dyspareunia and lower abdominal pain (Johnston and mabey 2008; Donbraye *et al.* 2010). 2007). Therefore, serious complications may be associated with trichomoniasis such as premature rupture of the placental membranes, premature labor and low birth weight in pregnant women infection (Johnson 2009), infertility, pelvic inflammatory disease, and enhances predisposition to immunodeficiency virus (HIV) transmission and acquisition (Donbraye *et al.* 2010; Masese *et al.* 2011; Giordani *et al.* 2012) and treatment of trichomoniasis resulted in a 4.2 fold reduction in the quantity of HIV-1 in vaginal secretions (Wang *et al.* 2001). Some recent studies provided evidence that trichomoniasis predispose to certain malignant disease such as prostate and cervical cancer (Schwebke and Burgess 2004; Stark *et al.* 2009).

In Iraq several authors provided data concerning *T. vaginalis* infection epidemiology, risk factors and diagnostic procedures (Al-Habib *et al.* 2005; Kadir and Fattah 2010; Al-Saeed 2011; Kadir *et al.* 2014). However, accurate data about its incidence, risk factors and complications in both pregnant and non-pregnant women as well as gestational age and menstrual cycle influence are not precisely reported in Erbil governorate, Northern Iraq since this infection is not reportable condition. This study aimed to provide accurate data regardind the epidemiology and risk factors associated with *Trichomonas vaginalis* infection in pregnant and non pregnant women in Erbil.

## **Mterials ans methods**

### **Study design and population**

Four hundred and forty women were enrolled in this analytic cross sectional study, with ages ranged between 16-60 years old (average of 34.2 years) and who attended Maternity Teaching Hospital in Erbil Governorate, Northern Iraq. From those 290 experienced vaginal discharge and 150 were asymptomatic. The participants were selected randomly among women referred to the above mentioned hospital over a period of 10 months starting from September 2012 to July 2013. All women interviewed personally before collecting vaginal swabs with assistance of gynecologists. For each subject a well structured questionnaire was filled carefully and included: patient's name, phone number, age, address, occupation, marital status, educational level, gestation, symptoms, menstrual cycle, history of abortion, number of parity, history of antibiotics and antiprotozoal drugs and history of other diseases. This study approved by Medical Ethics Committee in College of Medicine, Hawler Medical University, Erbil and an informed consent was taken from the participants subsequently they presented for clinical examination and vaginal discharge sample collection.

### **Collection of vaginal discharge and Parasite identification**

For each subject, two high vaginal swabs were collected using a sterile cotton swab. First swab placed in 2 ml of sterile saline solution (0.85 % NaCl in sterile distilled water) for direct wet mount preparation as described elsewhere (Nourian *et al.* 2013). Briefly, a single drop of well homogenized vaginal swab content in normal saline placed on a clean microscopic glass slide. The slide initially scanned at 100X, looking for motile flagellates, subsequently at 400X to confirm the parasite motility, flagella movement and morphologic features of the organism. Furthermore, other microscopic findings such as red blood cells, pus cells, epithelial cells, Bacteria and Fungi were also reported.

The second vaginal swab content was cultivated on Trichomonas Media (Oxoid, UK) supplemented with 8 % heat inactivated horse serum (Oxoid, UK) following the manufacturer's instructions. To suppress bacterial and fungal growth, 500 µg/ml of streptomycin (Cox pharmaceutical LTD, UK) and 100 µg /ml of chloramphenicol (OXOID, UK) were added to the complete medium. The PH of complete working medium was adjusted using 1 N HCl and the working medium was sterilized using 0.2 µm milipore filter unit. Inoculated cultures were incubated at 37° C and followed up, microscopically for the presence of motile trophozoites at 24, 48 and 72 hours of incubation.

The collected data was entered into computer and analyzed using SPSS version 18. Descriptive statistics, percentages and Chi-square test,

were used to make comparisons among categorical variables. P values less than or equal to 0.05 was considered statistically significant.

## Results

Out of 440 vaginal discharge samples that were collected from women who attended Maternity Teaching Hospital in Erbil city, 14 (3.18%) revealed positive culture for *T. vaginalis*, however, results of direct microscopic examination being non-consistent with culture technique since the number of positive cases was reduced to 12 (2.73%) by direct wet mount preparation (Table 1).

The prevalence of *T. vaginalis* infection was 3.2%, 1.5% and 4.9% among women who were from urban, sub-urban and rural areas, respectively. Comparably, the infection rate was higher among women with rural residents. However statistical analysis revealed no significant ( $p=0.537$ ) association of trichomoniasis and residency of the patients (Table 2).

Table 3 describes the prevalence of trichomoniasis among studied women according to their occupation. No significant ( $P=0.208$ ) differences was observed between housewives and women with official jobs. Furthermore, all infected women (no. 14) who had trichomoniasis, were non-pregnant (Table 3). The results indicated that the majority of the cases were symptomatic (85.71%) and profuse malodorous vaginal discharge associating with intense itching being the most common symptoms (data not shown). Furthermore, highly significant ( $p=0.00048$ ) association was detected between vaginal discharge color and *Trichomonas vaginalis* infection and most cases (50 %) experienced gray colored vaginal discharge followed by green (16.7%) and yellowish (1.5%) discharge (Table 5). The association of *T. vaginalis* infection and marital status was as shown in Table 4. The infection rate was 7.7%, 3.1% and 0% among married, widow and divorced women, respectively. However, statistical analysis revealed no significant differences ( $P > 0.05$ ) among those three groups in term of trichomoniasis.

The association of *T. vaginalis* infection and educational level was as shown in Table 5. Comparably, the educated women had less infection rate 6 (3.3%) than illiterate women 8 (4.1%), however, there was no significant ( $P=0.0259$ ) differences between them.

Table 7 shows distribution of *Trichomonas vaginalis* infection in different age groups. Comparably, higher infection rate was detected among women with age groups of 16-26 (4%) and 27-37 (3.3%) years old. While oldest women showed lower incidence of trichomoniasis. It means that the incidence of trichomoniasis was higher among childbearing ages. Nevertheless, statistically there were no significant ( $p>0.05$ ) differences among the studied age groups.

The highest incidence of trichomoniasis was seen among women with 1-2 parity (4.5%). However, there was no significant association of number of parity ( $p = 0.739$ ) and trichomoniasis on one hand and number of gestation ( $P = 0.453$ ) and trichomoniasis on the other hand (Tables 8, 9). In contrast there was significant association ( $P = 0.016$ ) between history of abortion and incidence of trichomoniasis (Table 10).

As it can be shown in Table 11, only one (0.23%) of all women with positive result for *T. vaginalis* infection had history of neonate abnormality. Thus, there was no significant ( $p > 0.05$ ) association of *T. vaginalis* infection and neonate abnormality.

Table 12 shows the association of *Trichomonas vaginalis* infection and stages of menstrual cycle. The infection rate was significantly ( $P = 0.013$ ) higher a day immediately post- menstruation (9.3%).

## Discussion

The incidence of trichomoniasis that detected in this study being lower than that obtained and recorded by Kadir and Fattah (2010), but it still higher than that obtained by Kadir *et al.* (2014) in Kerkuk. Interestingly, the frequency of *T. vaginalis* infection has diminished markedly than most other previous studies and this might be due to a wide prescription of metronidazole as anti-diarrheal and anti-bacterial medication. On the other hand improvement of personal hygiene and sanitary habits in addition to relatively higher sexual education at least at educated women, all the factors that may contribute to such lower incidence of trichomoniasis. However, such lower rate of infection may also be attributed to some technical limitations. Such as Participant selection and sample size in addition to the procedures that have been used to identify the parasite. (Swygard *et al.* 2004; Mairiga *et al.* 2011; Dahab *et al.* 2012).

It is already established that a minimal concentration of  $10^4$  organisms per milliliter of vaginal fluid appears to be necessary for identification of the protozoa by wet mount (Sood *et al.*, 2007). The greater positive rate of infection in culture media (3.2%) than direct wet mount technique (2.5%) reflects the efficacy of culture media than direct method for diagnosis of the parasite and this can be supported by Ohlemeyer *et al.* (1998); Mazlouni *et al.* (2008); Kadir and Fattah (2010).

As evidenced by this study, the prevalence of trichomoniasis was higher among women from rural areas comparing with urban residents which may be attributed to lack of knowledge about the disease and its transmission routes among rural residents as well as limited health services in rural communities. In this study the housewives were more susceptible to infection than employer women. This finding may be explained by the fact that the employer women may use vaginal washing and antiseptics after

coitus and/ or to the existence of health education programmers about sexually transmitted infections arranged by the maternal care office (Khalil *et al.* 2012; Nourian *et al.* 2013; Kadir *et al.* 2014).

In the present study, the infection rate in non-pregnant women (3.4%) was higher than that found in pregnant women (0%), This might be attributed to lack of menstrual cycle during gestation which has negative impact on the growth and maintenance of *Trichomonas vaginalis* trophozoites. Excessed level of Iron during menstruation increases growth of trichomonads and synthesis and surface placement of adhesins that bind to specific receptors on vaginal epithelial cells (Garcia *et al.* 2005; Ardalan *et al.* 2009). Furthermore, a unique feature of *T.vaginalis* genome is the duplicity for the majority of genes, of which 117 are up regulated in iron-rich environments, and 78 in iron-restricted environments. This feature may facilitate survival of the parasite in iron-rich environments and adherence of *T.vaginalis* to the genital tract epithelium, like the vagina in menstruating women, where haeme from the breakdown of menstrual blood provides an abundant supply of iron (Poole and McClelland, 2013).

All women who enrolled in this study were married women and among them the infection rate was non-significantly higher among widows (7.7%) than women who live with their husbands (3.1%). The high infection rate that obtained among widows in this study might be explained by some technical limitations such smaller sample size. However, the infection may also be acquired non-venereal through handling contaminated toilet articles and medical equipment such as gynecologist's gloves and colposcopy or through underclothing exchange.

The majority of the cases experienced gray colored (50%) vaginal discharge followed by green colored discharge (16.7%) then Yellowish discharge (15%). The variable discharges that noticed in this study might be resulted from co-infection of trichomoniasis with other microbial pathogens (Ginocchio *et al.* 2012).

Women at ages 16-26 years old had a higher infection rate than other age groups, followed by ages ranged between 27-37 and 38-48 years old. In contrast, no infection was detected in women who were above 49 years. Trichomoniasis as one of the sexually transmitted infection commonly associated with patients at child-bearing ages since this ages are more sexually active (Miranda *et al.* 2014). The highest rate of infection in young ages (16-25 years) could be correlated with the marriage status at females. In our society this age rang is a preferable age of marriage. So the high incidence of infection occurs at these sexually active ages (Chalechale and Karimi, 2010). In addition to that some physiological factors may also be contributed to various rate of infection among women with different age groups, such as pH of vagina, secretion of estrogen and progesterone

hormones for maintaining the pH of the vagina through the birthing age as well as abortion and frequency of pregnancy and immunodeficiency of the body defense after menstruation period. At this reproductive age the estrogen hormone level is higher than other ages so that vaginal environment become more suitable for the parasite growth (Westhoff *et al.* 1996). In contrast the lowest infection rate occurred at older ages (46-55) might be explained by the fact that after menopause, there will be fluctuations in the level of estrogen production. Other factor that may contribute to low incidence of infection at the older ages is that, the pH begins to fluctuate back and forth causing an imbalance (Vliet 2002). Glycogen and lactic acid production also begins to dwindle all that changes in vaginal environment may create suitable conditions for *T. vaginalis* growth.

The highest percentage of *T. vaginalis* infection was observed in multipara women (4.5%) and the lowest percentage of infection in nulliparous women (1.7%). This, relatively, high rate of infection that observed in multipara women may be related to that multi- delivery meaning higher chance to get infection through contaminated equipment or may be through contaminated medical articles and physician's gloves during gynecological examination (Nourian *et al.* 2013).

In the present study there was a significant association of trichomoniasis and history of abortion. The higher percentage of infection was noticed among women with one or more history of abortion, however, some studies showed adverse findings (Mazloumi *et al.* 2008; Khalil *et al.* 2012). Concerning menstrual cycle the higher infection rate was detected in vaginal discharge that collected from women immediately after menstruation (9.3%), followed by in the samples that collected immediately before menstruation (5.7%). This is might be due to increased level of estrogen immediately after menstruation which in turn makes the pH of vagina stable for *T. vaginalis* growth (Poole and McClelland, 2013). Furthermore, high level of estrogens may create an immunosuppressed environment through depressing cell-mediated immunity, impairing the activity of natural killer cells, and suppressing some aspects of neutrophil function (Styrt and Sugarman, 1991). Other possible reason that may contribute to high incidence of trichomoniasis immediately after menstruation is that iron that released from destructed red blood cells during menstruation increases growth of trichomonads since iron is an essential nutrient for *T. vaginalis*, on the other hand iron is also a crucial co-factor that enhances expression of surface adhesion molecules on the parasite (Garcia *et al.* 2005; Kucknoor *et al.* 2005). Moreover, vaginal mucosa may be a poor nutritional medium for microbes. Since the ability to synthesize lipids is lacking in *T. vaginalis*, erythrocytes may be a prime source of fatty acids that are demanded by the parasite (Ozcelik *et al.* 2012).

## Conclusion

Incidence of *Trichomonas vaginalis* infection is significant among women at child-bearing age in Erbil, Northern Iraq and trichomoniasis is significantly associated with history of abortion. On the other hand neonate abnormality is non-significantly associated with *Trichomonas vaginalis* infection.

## Acknowledgements

We would like to acknowledge Maternity Teaching Hospital staff in particular, the gynecologists for their kind assistance during taking vaginal swabs and also we would like to thank the participated women for their cooperation and willingness to be involved in this research. Finally, we would like to acknowledge staff of department of Microbiology, College of Medicine, Hawler Medical University and Central Public Health Laboratory in Erbil for their assistance in laboratory analysis.

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**Table 1: Prevalence of *T. vaginalis* infection among women according to the identification method**

| Diagnostic method            | No. examined | No. positive samples (%) | No. negative samples (%) | P value                                |
|------------------------------|--------------|--------------------------|--------------------------|----------------------------------------|
| Direct wet mount preparation | 440          | 12 (2.73)                | 429 (97.5)               | P = 0.686<br>df =1<br>$\chi^2 = 0.371$ |
| Culture                      | 440          | 14 (3.2)                 | 426 (96.8)               |                                        |

**Table 2: Distribution of *T. vaginalis* infection according to the residency of the patients**

| Residency | No. examined | No. positive samples (%) | No. negative samples | P value                                       |
|-----------|--------------|--------------------------|----------------------|-----------------------------------------------|
| Urban     | 311          | 10 (3.2)                 | 301                  | P = <b>0.537</b><br>df = 2<br>$\chi^2 = 1.24$ |
| Sub-Urban | 68           | 1 (1.5)                  | 67                   |                                               |
| Rural     | 61           | 3 (4.9)                  | 58                   |                                               |
| Total     | 440          | 14 (3.18)                | 426                  |                                               |

**Table 3: Prevalence of *T. vaginalis* infection according to occupation and gestational status**

|                    | No. examined | No. positive samples (%) | No. negative samples (%) | P value                                        |
|--------------------|--------------|--------------------------|--------------------------|------------------------------------------------|
| Occupation         | 341          | 13 (3.8)                 | 328 (96.2)               | P = 0.208, df = 1,<br>$\chi^2 = 1.95$          |
| Housewife          | 99           | 1 (1)                    | 98 (99)                  |                                                |
| Employee           | 440          | 14 (3.18)                | 426 (96.82)              |                                                |
| <b>Total</b>       | 341          | 13 (3.8)                 | 328 (96.2)               |                                                |
| Gestational status |              |                          |                          | P = <b>0.611</b><br>df = 1<br>$\chi^2 = 1.112$ |
| Pregnant           | 27           | 0                        | 27 (100)                 |                                                |
| Non-pregnant       | 413          | 14 (3.4)                 | 339 (96.6)               |                                                |
| Total              | 440          | 14 (3.18)                | 426 (96.82)              |                                                |

**Table 4: Prevalence of *T. vaginalis* infection according to marital status**

| Marital status | No. examined | No. positive samples (%) | No. negative samples (%) | P value                               |
|----------------|--------------|--------------------------|--------------------------|---------------------------------------|
| Married        | 420          | 13 (3.1)                 | 407 (96.9)               | P > 0.05<br>df = 2<br>$\chi^2 = 1.09$ |
| Widow          | 13           | 1 (7.7)                  | 12 (92.3)                |                                       |
| Divorced       | 7            | 0                        | 7 (100)                  |                                       |
| Total          | 440          | 14 (3.18)                | 426 (96.82)              |                                       |

**Table 5: Vaginal discharge color and *T. vaginalis* infection**

| Educational level            | No. examined | No. positive sample (%) | No. negative sample (%) | P value                                      |
|------------------------------|--------------|-------------------------|-------------------------|----------------------------------------------|
| Illiterate                   | 194          | 8 (4.1)                 | 186 (95.5)              | P = <b>0.259</b><br>df = 2<br>$\chi^2 = 2.7$ |
| Primary and secondary School | 181          | 6 (3.3)                 | 175 (96.7)              |                                              |
| University                   | 65           | 0                       | 65 (100)                |                                              |
| Total                        | 440          | 14 (3.18)               | 426 (96.82)             |                                              |

**Table 6: Association of *T. vaginalis* infection and educational level**

| Discharge color | No. examined | No. positive samples (%) | No. negative samples (%) | P value                                                 |
|-----------------|--------------|--------------------------|--------------------------|---------------------------------------------------------|
| Yellow          | 358          | 6 (1.5)                  | 379 (98.5)               | <b>P = 0.00048</b><br><b>df = 4</b><br>$\chi^2 = 96.78$ |
| Green           | 12           | 2 (16.7)                 | 10 (83.3)                |                                                         |
| Gray            | 12           | 6 (50)                   | 6 (50)                   |                                                         |
| Frothy          | 1            | 0                        | 1 (100)                  |                                                         |
| Blood tinged    | 30           | 0                        | 30 (100)                 |                                                         |
| Total           | 440          | 14 (3.18)                | 426 (96.82)              |                                                         |

**Table 7: Association of *T. vaginalis* infection and age groups**

| Age groups | No. examined | No. positive sample (%) | No. negative sample (%) | P value                                              |
|------------|--------------|-------------------------|-------------------------|------------------------------------------------------|
| 16-26      | 100          | 4 (4)                   | 96 (96)                 | <b>P = 0.749</b><br><b>df = 3</b><br>$\chi^2 = 1.21$ |
| 27-37      | 181          | 6 (3.3)                 | 175 (96.7)              |                                                      |
| 38-48      | 129          | 4 (3.1)                 | 125 (96.9)              |                                                      |
| 49-60      | 30           | 0                       | 30 (100)                |                                                      |
| Total      | 440          | 14 (3.18)               | 426 (96.82)             |                                                      |

**Table 8: Association of *T. vaginalis* infection and number of parity**

| Parity | No. examined | No. positive samples (%) | No. negative samples (%) | P value                                              |
|--------|--------------|--------------------------|--------------------------|------------------------------------------------------|
| 0      | 58           | 1 (1.7)                  | 57 (98.3)                | <b>P = 0.739</b><br><b>df = 3</b><br>$\chi^2 = 1.25$ |
| 1-2    | 134          | 6 (4.5)                  | 128 (95.5)               |                                                      |
| 3-4    | 134          | 4 (3)                    | 130 (97)                 |                                                      |
| ≥ 5    | 114          | 3 (2.6)                  | 111 (97.4)               |                                                      |
| Total  | 440          | 14 (3.18)                | 426 (96.82)              |                                                      |

**Table 9: Association of *T. vaginalis* infection and number of gestation**

| Gestation | No. examined | No. positive samples (%) | No. negative samples (%) | P value                                              |
|-----------|--------------|--------------------------|--------------------------|------------------------------------------------------|
| 0         | 37           | 2 (5.4)                  | 35 (94.6)                | <b>P = 0.453</b><br><b>df = 3</b><br>$\chi^2 = 2.62$ |
| 1-2       | 126          | 6 (4.8)                  | 120 (95.2)               |                                                      |
| 3-4       | 119          | 3 (2.5)                  | 116 (97.5)               |                                                      |
| ≥ 5       | 158          | 3 (1.9)                  | 155 (98.1)               |                                                      |
| Total     | 440          | 14 (3.18)                | 426 (96.82)              |                                                      |

**Table 10: Association of *T. vaginalis* infection and number of abortion**

| Abortion | No. examined | No. positive samples (%) | No. negative samples (%) | P value                                               |
|----------|--------------|--------------------------|--------------------------|-------------------------------------------------------|
| 0        | 266          | 7 (2.6)                  | 259 (97.4)               | <b>P = 0.016</b><br><b>df = 3</b><br>$\chi^2 = 10.29$ |
| 1-2      | 147          | 6 (4.1)                  | 141 (95.9)               |                                                       |
| 3-4      | 24           | 0 (0)                    | 24 (100)                 |                                                       |
| ≥ 5      | 3            | 1 (33.3)                 | 2 (66.7)                 |                                                       |
| Total    | 440          | 14 (3.18)                | 426 (96.82)              |                                                       |

**Table 11: Association of *T. vaginalis* infection and neonate abnormality**

|                  | No. examined | No. positive samples (%) | No. Neonate abnormality |
|------------------|--------------|--------------------------|-------------------------|
|                  | 385          | 13 (3.37)                | 0                       |
|                  | 49           | 1 (2.04)                 | 1                       |
|                  | 6            | 0 (0)                    | >1                      |
| Total            | 440          | 14 (3.18)                |                         |
| $\chi^2 = 0.432$ | df=2         | p>0.05                   |                         |

**Table 12: Prevalence of *T. vaginalis* according to menstrual cycle of the patients**

| Menstrual cycle              | No. examined | No. positive sample (%) | No. negative samples (%) |                                                       |
|------------------------------|--------------|-------------------------|--------------------------|-------------------------------------------------------|
| Pre-menstruation             | 199          | 2 (3.2)                 | 197                      | <b>P = 0.013</b><br><b>df = 5</b><br>$\chi^2 = 19.21$ |
| Immediately pre-menstruation | 70           | 4 (5.7)                 | 66                       |                                                       |
| Menstruation                 | 25           | 0                       | 25                       |                                                       |
| Immediately Pos-menstruation | 86           | 8 (9.3)                 | 78                       |                                                       |
| None                         | 60           | 0                       | 60                       |                                                       |
| Total                        | 440          | 14 (3.18)               | 426                      |                                                       |