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# Incidence of Non-Gonococcal Infection in Childbearing and Pregnant Women in Ardabil

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ABSTRACT

Non-gonococcal infections causes are made up of *Chlamydia trachomatis* (one of the most common infection Non-Gonococcal), *Ureaplasma urealyticum* (a possible causal in acute pyelonephritis, pelvic inflammatory disease, curiuamniutis, bacterial vaginosis, preterm birth, Non-Gonococcal urethritis, miscarriage, infertility and spontaneous abortion), *Gardnerell*a *vaginalis* and *Streptococcus agalactiae* (could be lead to colonization and disease in newborns). This study was achieved on 100 pregnant women that were conferred to the Alavi hospital of Ardabil city and done at the winter 1389 to late spring 1390. Three swab of vaginal discharge and 2 ml of blood were obtained from each woman. The samples were cultured on specific environments and analyzed by enzyme linked immunosorbent assay (ELISA). Finally, *Ureaplasma urealyticum*, *Chlamydia trachomatis*, *Gardnerella vaginalis* and *Streptococcus agalactia*e were isolated. In 26 cases *Chlamydia trachomatis* (%28.6), 18 cases *Ureaplasma urealyticum* (%19.8), 28 cases *Gardnerella vaginalis* (%30.8) and 18 cases *Streptococcus agalactiae* (%19.8), were isolated. Women who were spending their third trimester, had high outbreak chance rather than other women to these infections.

## 1. Introduction

Recently, non-Gonococcal infections have been changed to one of the most common and lesions sexually transmitted diseases in different communities (Weatherall et al., 1992). Possibility causes of this infections are made up of *Chlamydia trachomatis*, *Ureaplasma urealyticum*, a number of other organisms such as *Gardnerell*a *vaginalis* and *Streptococcus agalactiae*, which they are rarely isolated (Abdul-Karim et al., 1986). *Chlamydia trachomatis* is a gram-negative, small coccoidal and none-moved bacterium (Malek zade et al., 1383). This bacterium is one of the most common infection of non-Gonococcal in the worldwide and it is estimated that, the frequency of the disease increasing 20 percent annually (Workows-ki et al., 2006). Symptoms of *chlamydia* infection in women consist of vaginal discharge; dysuria and lower abdominal pain (Paavonen et al., 1999). It is believed that the effects of these bacteria in pregnancy are pre-term birth and premature rupture of the membrane (Low et al., 2009). Among the methods that are exploited for detection of

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*Chlamydia trachomatis*, are the identification of bacterial genome with PCR on urine specimens and antibodies of *Chlamydia trachomatis* in the serum (Jeddi Tehrani et al., 1999). *Ureaplasma urealyticum* is found commonly in the preparation of women who are healthy and sextually active. The prevalence of infection is related to menstrual periods and pregnancy. *Ureaplasma urealyticum* is a possible cause of acute pyelonephritis, pelvic inflammatory disease, uriuamniutis, bacterial vaginosis, preterm birth, non-Gonococcal urethritis, miscarriage, infertility and spontaneous abortion. Therefore, this probably sounds that the risks of these complications for mother and fetus during pregnancy could be equivalent, important and so serious (Domingues et al., 2003; Arnold et al., 1995; Nancy et al., 1991; Robertson et al., 1986; Bejar et al., 1981). *Gardnerell*a *vaginalis* is a genus of gram-negative and oxidase-negative organisms, which can be discovered in the female genital and urethra tracks (Reyes-Carcamo et al., 2000). In the most of cases, vaginal discharges of these women have the rotten fish smell and may contain of several anaerobic bacteria associated with *Gardnerell*a *vaginalis*. In this case, the vaginal pH is more than 4.5 (Bruox et al., 2007). Another things that we could mention about the possible role of *Gardnerell*a *vaginalis* in relation of women infections is that *Gardnerella vaginalis* could be efficacious agent in the childbirth pain. In addition the majority of women have to receive adequate treatment. For this reason, *Gardnerell*a *vaginalis* is one of the essential agents of preterm delivery (Mosby et al., 1998).

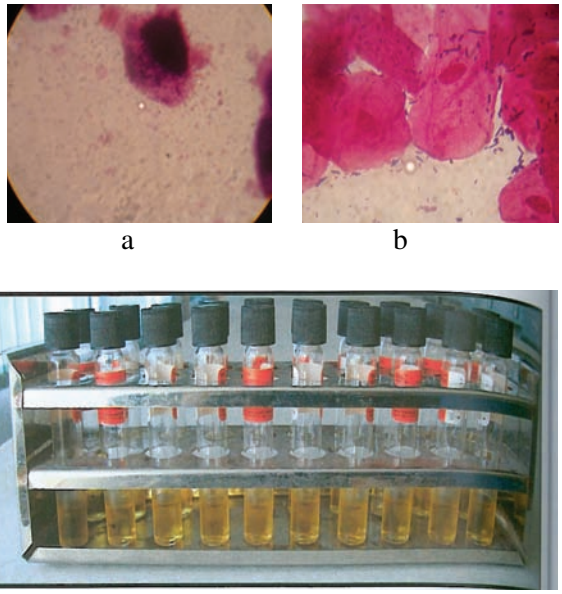
Another bacterium is *Streptococcus agalactiae* that it was examined in this study. This bacterium is a Gram-positive coccus that is arranged in the form of pair or chain shapes. Considering that group B *Streptococcus* in women constitute 5-25 percent of vaginal flora, experts assume that this type of *Streptococcus* sp. in pregnant women, with cross-channel delivery, can lead to colonization and disease in newborns (Artz et al., 2003). It has the mortality rate of 10 to 20 percent in the neonates (Artz et al., 2003). In spite of the fact that the prevalence of non-Gonococcal infections are rising in the human population, the cost of diagnostic disease constitute small fraction of the cost of the treatment. Therefore, diagnosis of the disease reduces the prevalence of reproductive tract infections and so, the cost of complications (Gerbase et al., 1998). The aim of this study was to investigate the effects of non-Gonococcal infections in pregnant women.

## 2. Materials and Methods

Present research was a descriptive study and done at the winter 1389 to late spring 1390. This study was achieved on 100 pregnant women that they conferred to the Alavi hospital of Ardabil city during 8-40 weeks of their pregnancy. The questionnaire includes demographic information such as age; gestational age; previous obstetric history of abortion; genital infections before pregnancy; education and employment for each of the participants in the project. Pregnant women with symptoms of spotting and bleeding and intrauterine fetal death were excluded in this study. Sampling was done in the front part of the vagina with sterile swab. The sampling was carried out by specialist obstetrics gynecology. Three swab of vaginal discharge and 2 ml of blood were taken from each woman, (Due to the possibility of shock damage to the fetus and increased risk of miscarriage in pregnant women, the sampling was barred with Speculum) and the samples were immediately transferred to the laboratory. One swab was transported to the laboratory in transport medium for *Mycoplasma* (*Mycoplasma* base broth with 10% yeast extract= PPLO). In addition, another swab was carried to laboratory via selective medium of Gram-positive bacteria LIM (Liquid Granada Medium Group B Strep Broth; GIBCO Laboratories Madison, Wis) (Figure. 1c). 2 ml of blood sample of pregnant women was collected and then was centrifuged for serological testing method ELISA. The concentration of serum antibodies against *Chlamydia* were measured with Euroimmun kits (EUROIMMUN AG. D-23560 Lubeck, Deutschland). In the laboratory, the PPLO medium (*Mycoplasma* base broth + 10% Yeast, Merk Co.) containing vaginal discharges was transfered from the filters (0.45 micron, Sartruos) that the *Mycoplasma sp.* were rejected and then the PPLO medium containing vaginal discharges were cultured in a substrate of U. broth (*urealyticum* broth, BESiMiK LTD. S.T.L, Turkey). The medium (Memmert Co.) containing the sample was incubated at 37°C for a week. The pH was

controlled and the change of color from yellow to purple was checked daily. Pink and purple in the haze-free environment should be created in this environment (Maitrayee et al., 1994). We used two diagnostic test in order to recognize *Gardnerella vaginalis*. For the first one, we prepared a solution of sterile KOH 10% and added a sample of vaginal swab to the tube and subsequently about 4-5 drops of the KOH solution was added. In the positive result, the tubs containing vaginal swabs and KOH solution had sickening smell of amine (rotten fish smell). This smell may be due to changes to vaginal pH. In another test that we mentioned above, samples of vaginal discharges were explored by a microscope (NICON, YS-100, Japan). Thus we polluted a slide by the contamination swab via rolling

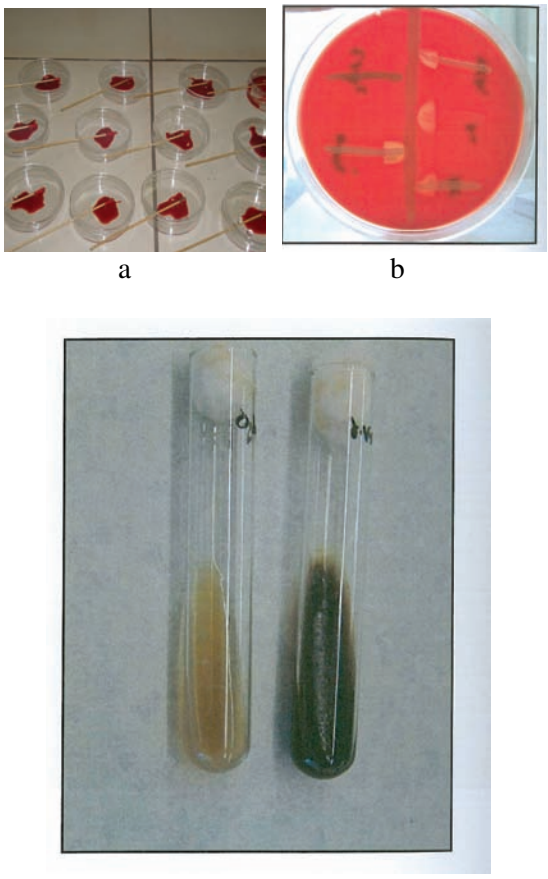
Method to determine the absence of lactobacilli; decreased acidity in order to find if the vaginal cells have the classic appearance of clue cells (Connie et al., 2000) (Figure 1a)



a b c

**Figure 1.** a) Clue cells b) Epithelial cells without bacterial vaginitis c) LIM transport medium

The third swab that had been prepared from the vaginal discharges of pregnant women was placed in a LIM transport medium and was transferred to the laboratory by transfer media LIM broth (Figure. 1c). The samples were inoculated on sheep blood agar by pure plate method (Figure 2a). Identification of the *Streptococcus* has been done by careful examination of colony morphology and hemolytic patterns (Figure 2c; Figure 2b). Statistical analysis of the data was carried out with SPSS (version 16).



a b c

**Figure 2.** a) Pureplate b) CAMP-Test c) Bile Esculin Hydrolysis

## 3. Results

After culturing the samples on specific environments and ELISA procedure, *Ureaplasma urealyticum*, *Chlamydia trachomatis*, *Gardnerella vaginalis* and *Streptococcus agalactia* were isolated. According to table 1, from 3 swab samples that had been prepared from 100 patients, in 26 cases *Chlamydia trachomatis* (28.6 percent), 18 cases *Ureaplasma urealyticum* (19.8 percent), 28 cases *Gardnerella vaginalis* (30.8 percent), and 18 cases *Streptococcus agalactiae* (19.8 percent), were isolated. The age of patients was in the 15 to 40 years. Results showed that except the age of

pregnancy, other values such as age, job and education has not significant relation with these infections. In current study we have shown that the risk factors that cause to sexual transferable infections are preceding childbirth, miscarriage record and the pre-pregnancy infections. In this study we have found that there is no relation between these factors and infections.

**Table 1**. Incidence of Non-Gonococcal Infections in Pregnant Women

| | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|
| Valid *C.trachomatis* | 26 | 28.6 | 28.9 | 28.9 |
| *U. urealyticum* | 18 | 19.8 | 20.0 | 48.9 |
| *S. agalactiae* | 18 | 19.8 | 20.0 | 68.9 |
| *G. vaginalis* | 28 | 30.8 | 31.1 | 100.0 |
| Total | 90 | 98.9 | 100.0 | |
| Missing System | 1 | 1.1 | | |
| Total | 91 | 100.0 | | |

Our results revealed that the only variable that has relation with infections in statistical analysis was the age of the pregnancy. There is no doubt that, women who were spending their third trimester, had high outbreak chance rather than other women to these infections (Figure 3). According to the T-test statistical examination, 71 members in their third trimester, 9 members in their second trimester and 4 members in their first trimester, were showed the infection. We conclude that 6 specimen have been polluted to these infections at the time of delivery.

## 4. Discussion

In spite of development in diagnosis methods expansion, the number of NGU cases with unknown diagnosis is increasing. In order to control and prevention of these diseases, it is undeniable to pay attention to contagious diseases science and change of the meaning of remedial methods in sexual transmitted disease (Weatherall et al., 1992). In this current study, using serological investigation (ELISA) incidence of *Chlamydia* infection was 28.9%. Up to now in the world, different results were presented depending to the pregnant women using the various diagnosis methods. In the studies on the frequency of *chlamydia* infection by Chamani in Tehran (Chamani et al., 2008), 11.2%, Chen and colleagues in china (Chen et al., 2006), 10.1%, Kirke in England (Kirk et al., 2008) 2.2% and sulivan in Australia by PCR method (Sullivan et al., 2003), 21.5% cases were positive.

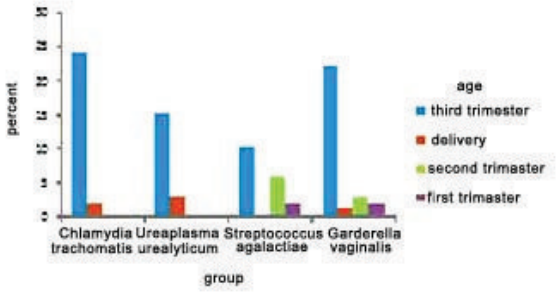



**Figure 3.** Relationship between age of pregnancy and incidence of infections

The result shows that the incidence of *Ureaplasma urealyticum* in this study is 20 percent. In an investigation that has conducted by Najjar-pirayeh in medical school of Tarbiyat Modares university, *Ureaplasma urealyticum* was isolated from 15 percent (Najjar-pirayeh et al., 1383). Samimi and his colleagues found these bacteria in 41% of non-generating women (Samimi et al., 1372). To another investigation that had been conducted by Karamastchi in Tehran, 48 specimen (19.2%) polluted to *Ureaplasma urealyticum* (Karamastchi et al., 1381).

In the other part of this study, we have investigated the incidence of *Streptococcus agalactiae* (GBS) in pregnant women population in Ardebil. Of 100 pregnant women, 18 women were carrier of GBS. In the other studies this level has been recorded from 5 to 40%. Mc Donald, collected vaginal swabs of 962 populations who were on 24$^{th}$ week of their pregnancy and cultured them and reported 91 positive cases (McDonald et al., 1989). In eastern area of Turkey, Kadanali and his colleague studied 150 pregnant women to determine the incidence of GBS. The samples were prepared from vaginal and rectum. 48 people (32%) of pregnant women had been colonized with GBS (Kadanali et al., 2005). According to our results, the incidence of *Gardnerella vaginalis* in this investigation in all cases was 31.1% and in the majority of the studies, the incidence of *Gardnerella* was similar to our result. This level was reported 18.8% in villager women with 16-22 years old in Tamil Nadu city of India. In another study that was conducted in Molana Azad college of Dehli in India by Jasmin Helen and colleagues in 1996 and 1997,

the incidence of this infection was reported 18% in 15 to 49 years old women (Sadri et al., 1387). In Kucinskeine's study that was conducted to gain the statistics of this infection in relation with demographic variables, age was the main risk factor (Kucinskiene et al., 2006). In the other study, the high incidence of *Chlamydia* infection in employed non- pregnant women of Tehran with elementary education was reported (Chamani et al., 2008). In kirke's study in England, the *Chlamydia* incidence was shown to have relation with demographic variables. In Najjar pirayeh's study, there was a relation between the separation level of *Ureaplasma urealyticum* and the age of the patients on the basis of statistical analysis (Kirk et al., 2008). In the investigated patients there was a meaningful differences between the isolated *Ureaplasma urealyticum* compared to women who have recorded of 1 to 10 time miscarriage and women who did not have recorded miscarriage (Najar-apiraye et al., 1385). In a study in Brazil no relation between the variables and GBS colonization has been reported (Costa et al., 2008). Kadanali, in eastern part of Turkey stated that maternal colonization in younger ages was considerably increased (Kadanali et al., 2005). In a study that conducted by Sadri in Ardebil university, the highest level of infection of *Gardnerella vaginalis* was among the ages between 20 to 35 and it was reported about 5.59% (Sadri et al., 1387). In the other study, the number of deliveries were 3 to 4 (41%) as compared to control group (16%) (Kazem zade et al., 1389). It is concluded that women who were spending their third trimester, had high outbreak chance rather than other women to these infections.

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