Cytological features of pap smear of multiparous women with mycoplasma hominis and ureaplasma urealyticum infection at outpatient clinic in medan

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Abstract. Mycoplasma hominis and Ureaplasma urealyticum were commensal pathogenic-potential bacteria in the female urogenital tract and may cause of newborns death. Several studies have suggested a correlation between cervical cytology and Mycoplasma genitalium and Ureaplasma urealyticum infections. It was a cross sectional design carried out on 50 multiparous women in an outpatient clinic in Medan in 2018. The cervical cytology features observed were reactive cellular change (RCC), cannonballs, predominance of coccoid bacteria, clue cells, and large number of neutrophils. All cytology features were negative from 3 (6.0%) subjects that had Mh infection. 9 (18.0%) subjects with Uu infection showed negative result of RCC, 9 (100.0%) were positive cannonballs, 3 (33.3%) were predominance of coccoid bacteria, 1 (11.1%) were positive clue cells and 7 (77.8%) had large number of neutrophils. Cytology finding of 38 (76.6%) subjects with no infection of Mh and Uu revealed 10 (26.3%) had RCC, 28 (73.7%) were positive cannonballs, 19 (50.0%) were predominance of coccoid bacteria, 2 (5.3%) were positive clue cells and 16 (42.1%) had large number of neutrophils. Non-specific cytological features were found in Mycoplasma infection. Specific features found in Ureaplasma infection but need to be traced to the infection of other organisms in group non Mycoplasma and Ureaplasma infections.

1. Introduction
Since the implementation of Papanicolaou (Pap) test, rates of cervical cancer in the United States have decreased from 14.2 per 100 000 in 1973 to 7.8 per 100 000 in 1994 [1]. Some criteria that lead women should do Pap smear screening are several risk factors which classically associated with cervical cancer as follows: early age of first intercourse, multiple sexual partners, and increasing parity [2] [3]. Unfortunately, the pap smear screening programs were nonexistent in many parts of the world. Fewer than 5% of women in developing countries have ever had a Pap test [4]. Cytological screening programmes have not reached the goal to reducing the incidence and mortality resulting from cervical cancer [5]. Previous studies revealed that knowledge about cervical cancer, emotional support and
informational support from the husbands about pap smear gave positive contribution for women to get pap smear examination [6].

Although Pap smear have been known as one of the most efficient and successful methods of cervical cancer screening, there was limitation of accuracy of Pap smear in diagnosing cervical precancerous lesions, as showed by several studies which revealed that the sensitivity of Pap smear remains low [7] [8]. Even if cytology was not the best method of detecting cervicovaginal infections but will sometimes incidentally recognize them and can alert the clinician to an unsuspected infection [9].

Association between HPV infection with cervical cancer have been already known, even though several studies found Mycoplasma hominis and Ureaplasma urealyticum involvement to contribute the risk of HPV infection to occure abnormal cervical cytology [10] [11]. Mycoplasma hominis and Ureaplasma urealyticum were found 30 ± 80% of women urogenital tract as a commensal organism which have pathogenic potential and can trigger premature labor and several infections in fetus in pregnant woman [12]. A previous study found that Mycoplasma genitalium was detected in a sample previously negative for bacterial vaginosis[13]. Another study in Malaysia showed that Ureaplasma is the most commonly discovered pathogen (positive in 90.5% of women and 47.5% of newborns), followed by Mycoplasma (32.5% and 7.5%), and the rest are chlamydia, trichomonas and gonococcus [14]. Several studies revealed that Mycoplasma hominis and Ureaplasma urealyticum played a role in abnormal cervical cytology associated with the development of precancerous cervical lesions [15].

The purpose of this study was to look at cervical cytology features in women with Mycoplasma hominis and Ureaplasma urealyticum infections.

2. Material and Methods

2.1. Clinical samples

The study was a descriptive study with a cross sectional design. A total of 50 nonpregnant multiparity women of reproductive age who were examined at a outpatient services of primary health care clinic in Medan North Sumatera in 2018. The Ethics Committee of University of Sumatera Utara approved the study, and informed consent forms were obtained from all subjects. Cervical swabs were collected from patients who underwent Pap smear screening. Mycoplasma hominis (Mh) and Ureaplasma urealyticum (Uu) were tested by Mh-Uu duplex PCR assessment using Verity 96-well Thermal Cyclcer, AppliedBiosystem. According to the Duplex PCR result, samples were divided into 3 groups as follows: Mycoplasma hominis infection, Ureaplasma urealyticum infection, and not both infections.

2.2. Cytological features

All of conventional cervical smears were prepared by Papanicolaou staining, followed by microscopic examination to observed cervical cytology features, which were reactive cellular change (RCC), the presence of cannonballs, predominance of coccoid bacteria, the present of clue cells and large number of neutrophils.

Reactive cellular change involving superficial, intermediate and parabasal cells that formed with clear cell boundaries, smooth core spreads, slight increase in the core and cytoplasmic ratio, with the nucleus slightly enlarged, rounded, smooth chromatin, nucleoli found, mitosis often seen, many cells with pale to dense cytoplasm and sometimes covered with neutrophils[16]. Cannonballs were formed as compact clusters of neutrophils that surrounding squamous cells. Positive criteria for cannonballs when 30 or more compact clusters of neutrophils appeared on the squamous cells per specimen [17] [18]. Predominance of coccoid bacteria characterized by presence of epithelial cells covered with blue-stained coccoid bacteria [18]. Characterized of clue cells occured after glycogen cytolysis contained in squamous cells and parabasal cells, presented moth-eaten images in surface of the squamous cell that was obscured by a large accumulation of bacilli [18]. Positive criteria for clue cells when the number of squamous cells with moth-eaten images accounted for 20% or more of overall
squamous cells [17]. Large number of neutrophils was considered if the total number of neutrophils observed in the five high-power field (40× objective) was 1,000 or more [17].

3. Result and Discussion

The data were summarized in Table 1. According to PCR result there were 3 groups with each cytological findings as follows: 3 women (6.0%) had positive Mh, 9 women (18.0%) had positive Uu infection and the rest 38 (76.6%) had no infection of both bacterials. All cytology findings were negative from subjects that had Mh infection otherwise cytology findings from subjects that had Uu infection were as follows: 0 (0.0%) had negative result of RCC, all of 9 subjects (100.0%) were positive cannonballs, 3 (33.3%) were predominance of coccoid bacteria, 1 (11.1%) were positive clue cells and 7 (77.8%) had large number of neutrophils. Cytology finding of 38 subjects with no infection of Mh and Uu revealed 10 (26.3%) had RCC, 28 (73.7%) were positive cannonballs, 19 (50.0%) were predominance of coccoid bacteria, 2 (5.3%) were positive clue cells and 16 (42.1%) had large number of neutrophils.

| Cytological findings                  | Mh infection | Uu infection | Negative Mh & Uu infection |
|--------------------------------------|--------------|--------------|----------------------------|
| No. of cases (%)                     | 3 (6.0)      | 9 (18.0)     | 38 (76.0)                  |
| RCC +                                | 0 (0.0)      | 0 (0.0)      | 10 (26.3)                  |
| Cannonballs +                        | 0 (0.0)      | 9 (100.0)    | 28 (73.7)                  |
| Predominance of coccoid bacteria     | 0 (0.0)      | 3 (33.3)     | 19 (50.0)                  |
| Clue cells +                         | 0 (0.0)      | 1 (11.1)     | 2 (5.3)                    |
| Large number of neutrophils          | 0 (0.0)      | 7 (77.8)     | 16 (42.1)                  |

Reactive cellular change is a part of benign abnormal change which is common finding in cytological smear, according to various condition and epidemiological factors. The previous study reported various frequencies, ranging 20% to 70% [8] [19] [20]. This is consistent with this study figure which is 26.3% RCC found in subjects with negative Mh & Uu infection. Unexpected result found from the subjects with Mh infections and Uu infections that were all negative result of RCC. This is unsimilar with previous studies finding which found positive result of RCC in subjects with Mh or Uu infections although with different frequencies. Several studies even traced further about the association of RCC in Ureaplasma infection with squamous abnormality that leading to malignancy although the clinical significance of RCC on Pap smears since a higher prevalence of squamous abnormalities is still controversial [17] [21] [22] [23] [24].

Cannonballs are common related to cervical inflammation. It has been reported that during Ureaplasma infection as well as Trichomonas infection role, interleukin-8 (IL-8) level are elevated, that induces neutrophil migration. The previous study by Okodo et al even suggest that it might be one cytological feature of Uu infection while cannonballs appear in Pap smears without Trichomonas infection [17]. Unspecifically finding in this study, cannonballs were found higher in subjects with Uu infections as well as subjects with negative Uu or Mh infections (100% and 73.7%). Relevan cytological feature of cannonballs is large number of neutrophils which almost similar with cannonballs finding, showed 77.8% in subjects with Uu infections and 42.1% in subjects with negative Uu or Mh infections. In contrast none of the cannonballs and large number of neutrophils found in subjects with Mh infections. The result is unconsistent with another previous study using a ratio of leukocytes to epithelial cells quantified from liquid cytology specimens which significantly high in Mycoplasma infection [25]. As well as another study that showed positively cervical polymorphonuclear lymphocytes in subjects with M. genitalium infection [11] [26].

This study finding was relevan with other previous studies that found Uu infection was detected significantly more often than Mh which conducted of females with bacterial vaginosis (BV) or asymptomatic [27] [28]. The subjects with negative Uu or Mh infections in this study suggests had bacterial vaginosis (BV) or asymptomatic according to cannonballs feature in their Pap smear.
Predominance of coccoid bacteria and the present of clue cells have been known associated with bacterial vaginosis that is marked by disappearance of lactobacilli which produces hydrogen peroxide and lead to massive growth of anaerobic species [29]. Several previous studies also found that predominance of coccoid bacteria occured by increased pH level due to ammonia production and might have a causal relationship with the onset of bacterial vaginosis [17]. This study found that subjects with negative Uu or Mh infections had predominance of coccoid bacteria higher (50.0%) than subjects with Uu infections (33.3%), while the present of clue cells almost similar by number of subjects in both two groups. None of that features found in subjects with Mh infections. This results relevan with previous study that found a large number of coccoid bacteria were present in Uu-infected Pap smears [17]. Several studies have been investigated the role of epithelial damage due to Uu infection and found the relationship of occuring squamous intraepithelial lesions (SIL)[17] [21] [22].

Otherwise previous study found that bacterial vaginosis was diagnosed by culture significantly more in the group with inflammation on Pap smear compared to the group without inflammation, suggest that inflammatory changes on the cervical Pap smear cannot be used to reliably predict the presence of a genital tract infection, especially in asymptomatic women [30].

4. Conclusion
This study showed that presence of cannonballs, predominance of coccoid bacteria, the present of clue cells and large number of neutrophils were cytological features that found in Pap smears of Uu infection and negative infection of Mh and Uu. Reactive cellular change was found in Pap smears of negative infection of Mh and Uu. Non-specific cytological features were found in Mh infection. It need to be traced to the infection of other organisms in subjects with negative infection of Mh and Uu.

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