Short Communication

Differences in *Chlamydia trachomatis* Cryptic Plasmid Loads in Two Types of Female Commercial Sex Workers in Korea

Youngmin Byun, Hee Yoon Park, Haekung Kim and Gilho Lee*

Department of Urology, Dankook University College of Medicine, Cheonan, and

Department of Obstetrics and Gynecology, Gwonseon Gu Health Centre, Suwon, Korea

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**SUMMARY**: Female sex workers in Korea can generally be divided into one of two categories, namely, historically rooted brothel-based prostitutes (BBPs) and a newly developed category of freelance prostitutes (FLPs). Though some social differences are known, there have been few reports of distinguishing biological characteristics between the two groups. We examined the chlamydial plasmid loads (CPLs) and the numbers of detached host cells (NDCs) from these two groups with different approaches to selling sex. Chlamydia plasmid and human genomic DNA were extracted from endocervical swabs of the subjects, and we determined the CPLs and the NDCs using real-time PCR analysis. Forty-six women in the FLP group and 21 women in the BBP group had a chlamydial infection. The CPL and NDC values were higher in the FLP than in the BBP group ($P = 0.000$, $P = 0.0001$ respectively). In the FLP group, younger-aged women had higher CPLs and more detached cells than did older women ($P = 0.02$, $P = 0.01$ respectively). However, the BBP group did not show any such age-related differences in CPL and NDC values. There was a statistically significant positive correlation between the NDC and CPL values in the BBPs ($P < 0.001$) as well as in the FLPs ($P < 0.001$). In conclusion, different biological characteristics were observed between FLPs and BBPs, as based on different CPL and NDC values. The high plasmid loads among the sex workers were isolated from large numbers of scraped cells within a cotton swab. Thus, minor injury may render the endocervical epithelium of FLPs more easily detachable than that of BBPs.

There are two socially distinct types of sex worker in Korea, i.e., the traditional brothel-based prostitutes (BBPs) in red-light districts, and a newly developed category of freelance prostitutes (FLPs). The social characteristics of the two groups are well known. BBPs make a living selling sex, and have little bargaining power with respect to their working conditions; they are forced to sell sex for several hours per day. In contrast, FLPs spend time entertaining customers (e.g., drinking alcohol and singing in a saloon), and they may serve as a bar hostess on a part-time basis. It is generally accepted that different social characteristics in these two groups of sex workers are associated with different phenotypes (1). However, there is little known about which biological responses differ between FLPs and those BBPs in a historically rooted profession.

Almost all strains of *Chlamydia trachomatis* harbor 4 to 10 copies of cryptic plasmid per genome, which is a good target for the detection of chlamydial infection (2). Because the development of a functional study to observe cryptic plasmid has been hampered by the lack of a gene transfer system in *Chlamydiaceae*, a patient phenotype database will be crucial for inferring the biologic function of cryptic plasmid.

We hypothesized that plasmid loads between these two groups may differ, as the plasmid copy number can be altered by various host factors (3,4). In addition, we evaluated the number of detached host cells (NDCs), which may be in-exfoliated with minor trauma. Finally, we attempted to establish a correlation between plasmid load and the NDCs per swab.

*Corresponding author: Mailing address: Department of Urology, Dankook University College of Medicine, 16-5 Anseo-dong, Cheonan, Chungnam, 330-715, South Korea. Tel: +82-41-550-6630, Fax: +82-41-550-6167, E-mail: multitoonins@yahoo.com

The study population included 159 BBPs and 454 FLPs who attended one regional healthcare center to be evaluated for the carriage of sexually transmitted infection from March 2004 to September 2004. After the subject gave informed consent, two nurses removed extraneous discharge and introduced a cotton swab into the endocervix and rotated the swab for 15 to 30 s. Gonorrhea-infected patients were excluded from this study. DNA extraction from the samples was performed as previously reported (5). In-house polymerase chain reaction (PCR) revealed that 46 women in the FLP group and 21 women in the BBP group had cryptic plasmids. All 67 samples were used for quantitative real-time PCR amplification (qRT-PCR). To evaluate the samples for cryptic plasmid, 2 μl of extracted DNA were added to a 25-μl reaction mixture containing 400 nM of each forward primer (5'-CAG CTT GTA GTC CTG CCTT GAG AGA-3') and reverse primer (5'-CAA GAG TAC ATC GGT CAA CGA AGA-3'), 200 μM of probe (FAM-CCC CAC CAT TTT TCC GGA GCG A-BHQ1a-Q) (Operon, Cologne, Germany), and 2X QuantiTech Probe PCR Master Mix (Qiagen GmbH, Hilden, Germany). qRT-PCR was performed in a Rotor-Gene (Rotor Gene 6.0; Corbett, Sydney, Australia). Amplification consisted of 15 min at 95°C, 10 s at 95°C, and 56°C for 45 s for 40 cycles. Amplification plots showing the relative change in fluorescence (ΔRn) during the PCR reaction were assessed; the arbitrary threshold cycle (Ct) for each sample was the PCR cycle number at which the threshold level of ΔRn was achieved (2). To determine the NDCs, 2 μl of extracted DNA were added to a 25-μl reaction mixture containing 300 nM of each forward primer in human β-globin (5'-GAA GAG CCA AGG ACA GGT AC-3'), reverse primer (5'-CAA CTT CAT CCA CGT TCA CCA CC-3'), 0.5 unit of uracil-N-glycosylase (Sigma, St. Louis, Mo., USA), and 2X QuantiTech SYBR Green PCR Master Mix (Qiagen). Amplification consisted...
years and $25.2 \pm 5.0$ years, respectively, and the two groups had a similar age distribution. The $C_t$ values in chlamydial plasmid loads (CPLs) of the FLPs and the BBPs were $16.78 \pm 3.27$ and $22.95 \pm 5.11$, respectively. The FLPs had statically higher plasmid loads than the BBPs ($P = 0.001$). Age was a good predictor for estimating the plasmid load in the FLPs (correlation coefficient, $r = 0.33$, $P = 0.02$), whereas this was not the case in the BBP group ($P = 0.37$) (Fig. 1). A copy of $\beta$-globin located on chromosome showed a single peak in the melting curve analysis (temperature of $85^\circ$C). The $C_t$ values of detached cells of the FLPs and the BBPs were $17.82 \pm 1.59$ and $20.03 \pm 2.28$, respectively. A higher number of detached cells from the endocervix were obtained from the FLPs than from the BBPs ($P = 0.0001$). In addition, age was also a good predictor for estimating the NDCs in the FLPs ($r = 0.35$, $P = 0.01$), whereas this was not the case in the BBP group ($P = 0.155$) (Fig. 2). There was a very good correlation between the CPL and the NDCs in both groups ($P = 0.0001$ FLPs, $P = 0.001$ BBPs) (Fig. 3).

We have demonstrated that FLPs and BBPs in Korea differ in terms of cryptic plasmid loads and the number of cells detached from the endocervix. However, it remains difficult to ascertain which biologic factors are involved in producing such differences. The sex industry in Korea is rapidly changing. Older sex-related businesses are fading out as new sex industries are invented or imported from Japan. Evaluation of biological features such as plasmid loads and detached cells may be helpful for characterizing the newly developing class of prostitutes as distinct from the more well-known class of prostitutes in terms of health. Moreover, we can trace them in terms of a “balloon effect.” The finding of a higher NDCs in the FLPs than in the BBPs was noteworthy. As the two nurses collected all samples in a blinded fashion, they were unaware of the prostitute’s social characteristics. Moreover, the CPL and NDC values correlated well with age distribution in the FLPs. It is reasonable to infer that the high loads of plasmid in both groups were primarily due to the large NDCs on the sampling swabs. It is also likely that infected host cells in the FLPs were more easily exfoliated from the cells of the endocervical lining with mild trauma (e.g., sexual intercourse) than those of the BBP, a mechanism which may be involved in the spreading of chlamydial infection to sexual partners.

**REFERENCES**


