Glycogen Assay for Diagnosis of Female Genital Chlamydia trachomatis Infection

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Received 23 October 1997/Returned for modification 17 November 1997/Accepted 15 December 1997

Chlamydia trachomatis can synthesize glycogen at various stages in its developmental cycle. The glycogen content of female genital epithelial cells was detected by anthrone, and the results were compared with those from PCR. A total of 320 cervical samples were examined. Of 92 specimens that were positive by PCR, 78 were positive and 14 were negative by the glycogen assay. Of 228 specimens that were negative by PCR, 220 were negative and 8 were positive by the glycogen assay. The specificity and sensitivity of the glycogen assay obtained from these data were 84.8% (78 of 92) and 96.5% (220 of 228), respectively. Use of the glycogen assay to detect the glycogen content in genital epithelial cells may be helpful in the diagnosis of C. trachomatis infection. This is an easy, fast, and inexpensive assay and can be done in less-sophisticated labs.

Materials and Methods

Clinical specimens. A total of 320 specimens were collected from the Outpatient Department of No. 1 Affiliated Hospital of our university between March 1996 and June 1997 by exposing the cervix, wiping away cervical secretions with swabs, scraping epithelial cells, and placing them into sterilized saline in duplicate.

PCR. Primers of plasmid PCTT1 of C. trachomatis were synthesized by Shanghai Shisheng Bio-technological Corporation (Shanghai, People’s Republic of China). The nucleotide sequences of primer set 5’-GTG TAA GTG TTC CCA TCA TAA AAA CAT ATTC-3’ and 5’-ATC CTT GTA TCC TGT TGG GAA GCC ATC AAAG-3’ covered a 503-base portion of plasmid PCTT1 of C. trachomatis (4). Clinical specimens were washed, and the cells were resuspended in 250 ml of Tris-HCl buffer containing 50 µmol of proteinase K per ml, 0.45% Nonidet P-40, 0.45% Tween 20, and 1 mmol of EDTA per liter. The mixture was kept at 56°C for 1 h, followed by boiling for 10 min. A 25-µl reactive mixture contained 5 µl of sample DNA; 100 mmol of deoxynucleoside triphosphate, 2 mmol of MgCl2, and 0.1 mmol of each primer per liter; and 10 × Tris-HCl buffer. After addition of 30 µl of paraffin oil to cover the surface, the reactive mixture was incubated at 94°C for 10 min and 1.5 U of Taq DNA polymerase (Promega, Shanghai, People’s Republic of China) was added. Then, 35 cycles consisting of 50 s at 94°C, 50 s at 50°C, and 50 s at 72°C followed by 10 min at 72°C after the last cycle were completed on a PTC351B DNA Thermocycler. Each product was run on a 2% agarose gel and stained with ethidium bromide to determine the size of the amplified product according to the standard molecular weight of PBR322/BSTNI.

Glycogen detection. Clinical specimens were washed twice, and the volume of them was restored to 0.5 ml. Cells in the samples were numerated with a hemocytometer and adjusted to a concentration of approximately 106 cells per ml. A 0.5-ml volume of 30% KOH was added, and the vials were kept at 100°C for 20 min. After addition of 1.5 ml of anhydrous ethanol, the vials were centrifuged at 4,000 × g for 15 min and the supernatants were discarded. A 0.5-ml volume of distilled water and 1 ml of 0.2% anthrone (0.2 g of anthrone in 100 ml of 98% H2SO4 [g/ml], prepared freshly in 1 h) were added, and the vials were placed in boiling water for 20 min. The optical density (OD) at 620 nm of the solution in vials was determined by photometry.

The ODs of glucose at different concentrations (50, 25, 12.5, 6.25, 3.12, and 1.6 μg/ml) were 0.88, 0.4, 0.26, 0.10, and 0.05, respectively. This method could detect 1.6 µg of glucose per ml, which was equivalent to 1.44 µg of glycogen per ml (7).

Because glycogen is insoluble in ethanol, it can be precipitated in anhydrous ethonal. Glycogen is dehydrated by 98% H2SO4 in order to produce furfural derivatives, which then produce a blue compound after reacting with anthrone. The OD of the color compound can be determined by photometry (7).

Glucose curve. A fixed weight of glucose was diluted in series, and anthrone was added to determine the ODs of glucose at different concentrations.

Methods for calculating the sensitivity and specificity of the glycogen assay. The sensitivity of the glycogen assay was calculated as the number of samples positive by the glycogen assay as well as positive by PCR divided by the number of samples positive by PCR. The specificity of the glycogen assay was calculated as the number of samples negative by the glycogen assay as well as negative by PCR divided by the number of samples negative by PCR.

Results

Of 320 cervical samples, 92 were positive and 228 were negative by PCR. The rate of positivity was 28.8%. The distribution of ODs in the glycogen assay was from 0 to 1.0 (Fig. 1). The mean OD of specimens positive by PCR was 0.6, and the mean OD of the specimens negative by PCR was 0.09. The results showed that the mean OD of the specimens positive by the glycogen assay was remarkably higher than that of the negative group (P < 0.0005). A result by the glycogen assay was considered positive when the ODs of specimens were equal to or higher than 0.3. Of 92 specimens positive by PCR, 78 were positive and 14 were negative by the glycogen assay. Of 228 specimens negative by PCR, 220 were negative and 8 were positive by the glycogen assay. The sensitivity and specificity of the glycogen assay obtained from these data were 84.8% (78 of 92) and 96.5% (220 of 228), respectively.

Discussion

C. trachomatis not only can cause male and female urogenital inflammations but can also endanger fetal and infant health by vertical transmissions of infections during pregnancy, resulting in abortion, infants with abnormally low weight, newborn pneumonia, or conjunctivitis, etc. (7). There are two kinds
confirmed that glycogen in inclusions originated from other chlamydial species (1). Studies by Chiappino et al. showed that the glycogen content of the pathogens is an important feature to distinguish the pathogen from other microbes. When cells are stained with iodine for light microscopy, glycogen accumulates in the cytoplasm, especially in elementary bodies. When cells are infected, including in elementary bodies, reticulate bodies, and intermediates (especially in elementary bodies), they are complicated, time-consuming, and limited by equipment and difficult to be applied to basic medical units.

They are the major methods to diagnose Chlamydia trachomatis infections. Iodine staining to detect Chlamydia trachomatis can synthesize glycogen in its developmental cycle, including in elementary bodies, reticulate bodies, and intermediates (especially in elementary bodies). When cells are stained with iodine for light microscopy, glycogen accumulation is an important feature to distinguish Chlamydia trachomatis from other chlamydial species (1). Studies by Chiappino et al. confirmed that glycogen in inclusions originated from Chlamydia trachomatis itself instead of host cells (1). No report on the synthesis of glycogen in host cells by other microbes has yet been found. Therefore, detection of the glycogen content in genital epithelial cells may be helpful in the diagnosis of Chlamydia trachomatis infections.

The amounts of serially diluted glucose were determined to evaluate the sensitivity of the glycogen assay. The results showed that this assay could determine glycogen to a concentration of as much as 1.44 μg/ml and that it had relatively high sensitivity. The plasmid primers used in PCR had very high sensitivity and specificity, and could be regarded as a reference method for the glycogen assay. When the critical point is set to 0.3 according to the distribution of ODs for tested glycogen contents, the sensitivity and specificity of the glycogen assay for diagnosis of Chlamydia trachomatis infections are 84.8 and 96.5%, respectively, compared with PCR. Of 92 samples positive for PCR, 78 were positive by the glycogen assay and 14 were false negative. This was probably owing to the very high sensitivity of PCR, which can detect elementary bodies even in a very small quantity, whereas the glycogen assay can detect glycogen only in a relatively large quantity (1). Therefore, when Chlamydia trachomatis is in a period of latent infection, it may give positive results by PCR and negative results by the glycogen assay. From another point of view, the results obtained by the glycogen assay can better represent symptomatic infections of Chlamydia trachomatis. Of 228 specimens negative by PCR, 220 were negative by the glycogen assay. The reason why some were false positive probably is that the cervical secretions of a few patients contained other types of carbohydrates.

The glycogen assay is a quick, convenient, and simple method to diagnose Chlamydia trachomatis infections compared with other methods. Iodine staining to detect Chlamydia trachomatis is adopted for cell culture instead of for detection of the organism in clinical samples. The sensitivity of Giemsa staining is very low. Its sensitivities for cervical and urethral specimens are 40 and 15%, respectively (3). The sensitivity and specificity of immunofluorescence and PCR, etc., which have been adopted in recent years, are relatively high. However, the costs of these are also high, and their application is limited by equipment. The sensitivity and specificity of the glycogen assay are relatively high, and the assay’s cost is low. If the test can be further refined, the need for photometry may be replaced by visual inspection alone.

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