RESEARCH NOTE

Detection of Anti-pili Antibodies of Gonococcus Using an Enzyme-Linked Immunosorbent Assay

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Neisseria gonorrhoeae genital infection are an important cause of morbidity and infertility in the Caribbean. The frequent lack of obvious symptoms in persons affected by this pathogen is a major reason for introducing screening programs for its detection. Most significantly, a high prevalence of infection with *N. gonorrhoeae* has been observed in females presenting tubal infertility, ectopic pregnancy, and pelvic inflammatory disease (A Miettinen et al. 1990 *Sex Transm Dis 17*:10-4), indicating that *N. gonorrhoeae* infection is a risk factor for these conditions.

Several studies of epidemiological interest (EC Gotschilch 1984 *Bull WHO* 63: 671-680) have used serological methods to determine the cumulative history of previous expositions to gonococcus in population groups. This serological methods could be performed by detecting anti-pili antibodies that are exposed on the gonococcus surface (BG Welch et al. 1973 *J Infect Dis 125*: 69-83). Although gonococcal pili are highly variable, the conserved portion of the N-terminal region of pili allows for the detection of antibodies directed against different strains. Based on this approach, we attempted to set up an immunoezymatic assay (ELISA) using a pili antigen to detect gonococcal anti-pili antibodies in the Cuban population.

A total of 567 sera of fertile women (group I) and 225 sera of women with antecedents of sexually transmitted diseases (STDs) (Group II) were studied. The former were clinically healthy and without antecedents of STDs. The clinical and epidemiological data were collected through a detailed inquiry in each case, which allowed to know clinical picture and response to treatment. Polystyrene microtitre plates for ELISA (Titertek) were coated with pili antigen of reference strain P9 2 WHO at concentration of 1 mg/ml (donated by Dr Heckles, University of Southampton, UK). An initial blocking step was carried out using skimmed milk in phosphate buffer solution. Subsequently, the sera plate were incubated with a 1/3000 dilution of the test sera for 4 hr. After washing three times, antibody binding was detected using a 1/8000 dilution of anti-human immunoglobulin G peroxidase conjugate (Camppel) in saline-phosphate-skimmed milk buffer (JN Robertson et al. 1987 *J Clin Pathol 8740*: 377-383). An analysis of frequency distribution of sera from people who did not report STDs was carried out to obtain the cut off value (0.443). Serum from a patient with acute gonococcal disease was used as positive control (*N. gonorrhoeae* was isolated from urethra smear), and the sera of clinically healthy children as negative control.

We observed 3% of seropositivity for Group I. This result can reflect either, the presence of meningococcal pili or the existence of asymptomatic carriers of *N. gonorrhoeae* in that group (K Reiman et al. 1980 *Acta Path Microbiol Scand C 88*: 155 - 162). Our observation is consistent with previous reports using pili or outer membrane proteins as antigens for detection of antibodies, with methods such as indirect immunofluorescence and hemagglutination: in all cases specificity values that ranged from 89 to 98% were consistently obtained (Welch *loc. cit.*, Reiman *loc. cit.*, AA Glynn & C Ison 1978 *J Vener Dis 54*: 97-102).

A seropositivity rate of 28% was observed among women with antecedents of STDs (Group II). This rate is significantly higher than the one determined for healthy women (Group I). This observation confirms a higher level of exposition to the gonococcus and points to its microorganism being one of the main causal agents of STD in that group of woman.

Several reports have documented the use of serological assays to determine the seroprevalence of gonococcal infection and its relation to various clinical and epidemiological aspects in specific population groups from Europe and Africa (Welch *loc. cit.*, Reiman *loc. cit.*, Glynn& Ison *loc. cit.*). This is the first time that a serological assay has been used to determine exposure to *N. gonorrhoeae* in Cuban population, which will contribute to understand the behavior of the gonococcal disease in Caribbean region.

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