Whites Science
A Scientific Erudition

DEVELOPMENT OF A LATEX AGGLUTINATION TEST FOR RAPID DIFFERENTIAL DIAGNOSIS OF VAGINAL INFECTIONS

Morteza Yousefi[1], Seyedeh Maryam Sharafi[2], Hossein Yousofi Darani[2*]

[1]Shahrekord University of Medical Sciences, Shahrekord, Iran.

[2]Infectious Diseases and Tropical medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

ABSTRACT

Background: Vaginitis is a common problem among women. Appropriate laboratory diagnosis is important to succeed better treatment. Routine methods for laboratory diagnosis are not enough sensitive or time consuming. So construction of a latex agglutination kit for rapid diagnosis of infectious vaginitis has been investigated in this "study".

Methods: Anti *Trichomonas vaginalis* and anti *Candida albicans* sera raised in rabbits. Antibodies were purified from the antisera using salt precipitation method. The antibodies were then conjugated to latex particles and used to detect the relevant antigens.

Results: When anti trichomonas coated latex particles mixed with *Trichomonas vaginalis*, agglutination was appeared. Also agglutination was occurred when anti candida coated latex particles were mixed with *Candida albicans* antigen.

Conclusion: Latex agglutination test developed in this work could be a sensitive method for rapid differential diagnosis of infectious vaginitis.

Key words: latex agglutination, vaginal infection, laboratory diagnosis

INTRODUCTION

Vaginitis is a common problem in clinics, and it is considered as the main reason of visiting obstetricians and gynecologists. Candidiasis, trichomoniasis, and bacterial vaginosis are three major causes of vaginitis. *Trichomonas vaginalis* is responsible for about 174 million sexually transmitted infections annually worldwide. This infection has prevalence of about 3%-48% among women and causes about 5 million new cases in American women (1).

Vaginitis is a common manifestation of trichomoniasis. However this infection cause other complications such as preterm delivery, cervical intraepithelial neoplasis and pelvis inflammatory disease(2). In the united states the prevalence of bacterial vaginosis in 2004 was 29.2% . However only 15.7% of the infected women had vaginal symptoms (3).

Vulvovaginal candidiasis is the frequent infection of the female genital tract(4). Some laboratory investigations revealed that 15% of non-pregnant and 30% of pregnant women were infected with vulvovaginal candidiasis (5). And *Candida albicans* accounts for 80–90% of the infections(6).

Clinical differential diagnosis of vaginitis is difficult because Candidiasis, trichomoniasis, and bacterial vaginosis have some overlap manifestations (7, 8). Therefore proper laboratory diagnosis is very important to achieve better follow up and treatment. Different laboratory techniques such as wet smear and culture are usual methods for diagnosis of vaginitis. However wet smear is not enough sensitive and culture is time consuming. So in this study development of a latex agglutination kit for rapid and accurate differential diagnosis of vaginitis has been investigated.

*Corresponding author:
Email: Yousofidarani@gmail.com

http://dx.doi.org/10.20530/IJIBCS_7_8-10
ISSN 2047-9093 © 2016

MATERIAL AND METHODS

Trichomonas vsginalis was isolated from vaginal discharge of women refereed to Shaharekord Clinic and kept in TYIS culture medium. *Trichomonas vaginalis* kit was developed as we published before(9). *Candida albicans* (standard strain) was cultured in Sabouraud Dextrose medium. The yeasts was then harvested from the culture and suspended in isotonic saline. Candida was washed twice by sedimentation. The final sediment was suspended in isotonic saline and subjected to sonication and the mixture was kept at -20 as candida antigen. The antigen then was mixed with complete Freund's adjuvant and injected subcutaneously to a rabbit. Boosters were injected with the same antigens and incomplete Freund's adjuvant every fortnight. Following the forth injection blood sample was taken from the rabbit and its serum was checked for anti candida antibody using ELISA method as we published before(10). Following strong antibody the whole blood of the rabbit was collected and the antiserum was kept at -20.

Salt precipitation method was used for purification of antibodies from rabbit antiserum as we published before and SDS-PAGE was performed to confirm antibody purification(9). Polyesteren beads particles were purchased from SIGMA company and they were conjugated with antibodies as described by V. Hopwood et al(11).

In order to test reaction of antigens and antibody conjugated latex beads were mixed with different concentration of either *Trichomonas vaginalis* or *Candida albicans* on latex agglutinatin slide and rotated for 5-15 minutes agglutination appeared as visible clumping.

RESULTS

A drop (about 50µl) of Latex particles coated with anti *Trichomonas vaginalis* antibody mixed with a drop of isotoni saline containing about 100 *Trichomonas vaginalis* and rotated for 5-15min. Negative control was consisted of a drop of buffer which mixed with a drop of latex conjugated to antibody. Visible clumping (agglutination) was apperared only on slide containing latex particles coated with antibody and *Trichomonas vaginalis*.

For *Candida albicans* also a drop of Latex particles coated with anti *Candida albicans* antibody mixed with a drop of isotonic saline containing about 200 *Candida albicans* and rotated for 5-15min. In negative control a drop of buffer mixed with a drop of latex conjugated to antibody. Again visible clumping (agglutination) was apperared only on slide containing latex particles coated with antibody and *Candida albicans* (Figure1).

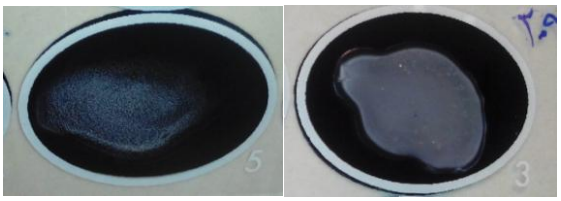

Figure 1: Drops of latex particles coated with antibody against *Candida albicans* mixed with a drop of Candid albicans antigen (left) or buffer (right).

To evaluate the Kit for detection of vaginal infection, five vaginal samples which were positive for *Trichomonas vaginalis* by wet smear, five vaginal samples which were positive for *Candida albicans* by wet smear were tested by the kit. For this purpose a drop of each sample was put on the slide. Then a drop of latex particles coated with anti *Trichomonas vaginalis* antibody was added to samples which were positive for *Trichomonas vaginalis* in wet smear. Also a drop of latex particles which were coated with anti *Candida albicans* antibody was added to samples which were positive for *Candida albicans* in wet smear. In positive control solution containing either *Trichomonas vaginalis* or *Candida albicans* were used instead of patients samples. For negative control a drop of buffer mixed with latex conjugated to antibody. All samples which positive with wet smear were also positive in latex agglutination Kit constructed in this study.

DISCUSSIONS

In this investigation a latex agglutination test for rapid differential diagnosis of vaginal infection was developed. Vaginitis is a common problem among women referred to obstetricians and gynecologists. So it would be very worthwhile to have a simple test that be able to differentiate rapidly candidiasis, trichomoniasis, and bacterial vaginosis which are major causes of vaginitis.

Latex agglutination tests are used in medicine for the detection of antibody or antigen in clinical samples. Some advantages of these assays are that the procedures are usually simple, they are safe without hazardous, and the results of these tests are obtained in a very short time (12).

Latex agglutination test has been used for diagnosis of acute candida vaginitis and was showed

to have sensitivity of 72.7% (13). This test has also been used for diagnosis of *Trichomonas vaginalis* infection(14, 15) . Carney reported sensitivity of about 95% for the test(14) . Also Adu-Sarkodie reported sensitivity of 98.8% and specificity of 92.1% for this test (15).

Although latex agglutination test has been used for detection of either *Trichomonas vaginalis* or *Candida albicans* infections, to our knowledge there is no kit available for differential diagnosis of three major causes of vaginal infections.

Azole cream and Fluconazole are used for treatment of vaginal candidiasis. However both trichomonal and bacterial vaginitis are treated with metronidazole (7). So it will be very important to differentiate candida vaginitis from trichomonal or bacterial vaginitis. The kit which has been developed in this study is able to differentiate *Candida albicans* and *Trichomonas vaginalis* infections from bacterial vaginitis. So with using this kit for examination of vaginal discharge from symptomatic women, the clinicians will be able to decide about prescription of either metronidazole or Fluconazole. Although trichomonal and bacterial vaginitis are treated with the same drug (metronidazole), still differential diagnosis of these two type of vaginitis is necessary because Simultaneous treatment of the male sexual partner is recommended in cases of trichomonal vaginitis.(8)

The other advantage of the kit which developed in this work is that it is simple and quick. This kit dose not need any equipment and can be performed in all rural area labs and even in obstetricians and gynecologists clinics. Also following taking vaginal sample the result will be ready for the physician in about 15 minutes.

REFERENCES

1. Sena AC, Miller WC, Hobbs MM, Schwebke JR, Leone PA, Swygard H, et al. Trichomonas vaginalis Infection in Male Sexual Partners: Implications for Diagnosis, Treatment, and Prevention . CLIN INFECT DIS ; 2007 Jan;44(1):13–22. Available from: http://dx.doi.org/10.1086/511144
2. Schwebke JR, Hook III EW. High Rates of Trichomonas vaginalis among Men Attending a Sexually Transmitted Diseases Clinic: Implications for Screening and Urethritis Management . J INFECT DIS; 2003 Aug;188(3):465–8. Available from: http://dx.doi.org/10.1086/376558
3. Koumans EH, Sternberg M, Bruce C, McQuillan G, Kendrick J, Sutton M, et al. The Prevalence of Bacterial Vaginosis in the United States, 2001???2004; Associations With Symptoms, Sexual Behaviors, and Reproductive Health. Sexually Transmitted Diseases; 2007 Nov;34(11):864–9. Available from: http://dx.doi.org/10.1097/olq.0b013e318074e565
4. Sobel JD. Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis. American Journal of Obstetrics and Gynecology [Internet]. Elsevier BV; 1985 Aug;152(7):924–35. Available from: http://dx.doi.org/10.1016/s0002-9378(85)80003-x.
5. Kubota T. Chronic and recurrent vulvovaginal candidiasis. Nihon Ishinkin Gakkai zasshi= Japanese journal of medical mycology. 1997;39(4):213-8.
6. Boselli F, Chiossi G, Garutti P, Matteelli A, Montagna MT, Spinillo A. [Preliminary results of the Italian epidemiological study on vulvo-vaginitis]. Minerva Ginecol. 2004 Apr;56(2):149-53. Italian. PubMed PMID: 15258544.
7. Wiener C, Fauci A, Braunwald E, Kasper D, Hauser S, Longo D, et al. Harrisons Principles of Internal Medicine Self-Assessment and Board Review. 18th Edition: McGraw Hill Professional; 2012.
8. Danforth D, Scott J. Danforth's obstetrics and gynecology. Philadelphia, Pa.: Lippincott Williams & Wilkins; 2003.
9. Darani HY, Ahmadi F, Zabardast N, Yousefi HA, Shirzad H. Development of a Latex Agglutination Test as a Simple and Rapid Method for Diagnosis of Trichomonas vaginalis Infection. Avicenna J Med Biotechnol. 2010 Jan;2(1):63-6. PubMed PMID: 23408769; PubMed Central PMCID: PMC3558142.10. Darani H, Doenhoff M. Anomalous immunogenic properties of serine proteases. Scand J Immunol. 2009;70(4):384-8.
11. Hopwood V, Evans EG, Carney JA. Rapid diagnosis of vaginal candidosis by latex particle agglutination. J Clin Pathol. 1985 Apr;38(4):455-8. PubMed PMID:3988958; PubMed Central PMCID: PMC499178.
12. Molina-Bolívar JA, Galisteo-González F. Latex Immunoagglutination Assays. Journal of Macromolecular Science, Part C: Polymer Reviews; 2005 Jan;45(1):59–98. Available from: http://dx.doi.org/10.1081/mc-20004581913.
13. Sobel J, Schmitt C, Meriwether C. A new slide latex agglutination test for the diagnosis of acute Candida vaginitis. Am J Clin Pathol . 1990;94(3):323-5.
14. Carney JA, Unadkat P, Yule A, Rajakumar R, Lacey CJ, Ackers JP. New rapid latex agglutination test for diagnosing Trichomonas vaginalis infection. Journal of Clinical Pathology; 1988 Jul 1;41(7):806–8. Available from: http://dx.doi.org/10.1136/jcp.41.7.806.
15. Adu-Sarkodie Y. Comparison of latex agglutination, wet preparation, and culture for the detection of Trichomonas vaginalis. Sexually Transmitted Infections; 2004 Jun 1;80(3):201–3. Available from: http://dx.doi.org/10.1136/sti.2003.007864