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Research Article

Detection of Chlamydia Trachomatis from Endocervical Swabs by Direct Antigens Detection Comparing to Cytological Findings in Sudanese Women

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Abstract

Background: *Chlamydia trachomatis* are obligate intracellular bacteria that infect genital and ocular tissue. *Chlamydial* infections and their complications are costly and present a significant public health problem in both developed and developing countries. The management of those detected case is very easy, however the difficulty of detect the cases in their early stage as most cases are A symptomatic and the rate of progression is very high.

Objective: The aim of this study was to diagnose endocervical swabs taken from *Chlamydia trachomatis* suspected female patients using direct antigen detection and Papanicolaou stain.

Methods: A prospective, analytical, hospital based, case control study was carried out in Khartoum Teaching Hospital, Omdurman Maternity Hospital & Alzahra center at Ahfad University. 100 suspected *Chlamydia* females with cervical abnormalities compared to 20 healthy control women from the period from May 2011 to October 2012. *Chlamydia trachomatis* was diagnosed using direct antigen detection immunochromatography kit (Kwik test kit).

Results: The mean age of the selected patients was 30.4 ± 9.03 years and most of them were having a history of abortion. For endocervical swabs Pap smearing showed that almost [50%]of the suspected patients were cytologicaly positive for *chlamydial* infection, however DAD test showed that 74%of cervical swabs were positive for *chlamydial* antigen.

Conclusion: DAD is a simple, quick cost-effective diagnostic tool for *Chlamydia trachomatis* STI Infection

Key words *Chlamydia trachomatis, sexually transmitted infections, direct antigen detection, Papanicolaou stain, Sudan.*

1. INTRODUCTION

It clearly found that *Chlamydia* is one of the most isolated bacteria in cervical screening program's and it relationship with infertility in women's.

The *chlamydiae* are small groups of non-motile coccoid bacteria that are obligate intracellular parasites of eukaryotic cells. Because of their

dependence on host biosynthetic machinery, it was originally thought to be viruses; however, they have a cell wall and contain *DNA, RNA*, and ribosomes and therefore are now classified as bacteria[1]. *Chlamydia trachomatis* is a Gram-negative bacteria, therefore its cell wall components retain the counter-stain safranin and appear pink under a light microscope[2]. Identified in 1907 *C trachomatis* was the first *chlamydial* agent discovered in humans[3]. *C trachomatis* is the most prevalent sexually transmitted pathogen worldwide. It is common among sexually active young women[4]. Several important complications can result from *C trachomatis* infection in women, the most serious of which include pelvic inflammatory disease, ectopic pregnancy, infertility, and adverse pregnancy outcomes[5]. In addition, *C trachomatis* is also one of the most common causes of eye infections and pneumonia in neonates. *Chlamydia* is often known as a "silent" disease because the majority of infected patients are asymptomatic[5,6].*Chlamydial* genital infection is the most frequently reported infectious disease in the United States, and prevalence is highest in persons aged ≤25 years[7].

C trachomatis is a dimorphic bacterium with a two-phase life cycle. It exists as either an elementary body or a reticulate body. The dangerous communicable form is the smaller elementary body, and attaches and enters the host cell, while the reticulate body is the larger, intracellular form, which promotes metabolic activity and reproduction through binary fission[8,9]. This bacterium is parasitic and heavily depends on the host in order to perform its metabolic as well as reproductive processes[10]. Traditionally, the diagnosis of *C trachomatis* infections has relied on the isolation of the organism in tissue culture which is time-consuming and requires considerable technical expertise and a suitable cell culture facility. Polymerase chain reaction [PCR] and ligase chain reaction [LCR], are now established as sensitive and specific techniques for detecting *chlamydia* in clinical specimens[11]. However, the advent of direct antigen detection methods has provided more rapid and less expensive alternatives to these molecular biology approaches. An important advantage of the antigen detection assay is that it can greatly increase the availability of *chlamydia* diagnostic service. Nucleic Acid Amplification Test [NAAT] has replaced culture as the new "gold standard". NAAT is currently too expensive for use in limited resource settings. Rapid tests provide results within 30 minutes of testing and are less expensive to perform and simple to interpret since testing results are reflected in a test strip color change[11] Therefore many commercial non-culture-based assays are now available for diagnosis[12].

There is no previous data about prevalence of *C Trachomatis* infection in Sudanese women. In this work we tried to evaluate the diagnostic efficacy of direct antigen detection methods in Sudanese women compared to cytological finding in Pap smear.

2. MATERIAL AND METHODS

One hundred suspected *Chlamydia* females patients who reported to the above mentioned hospitals compared to 20 healthy control women were enrolled in the study during the period from May 2011 to October 2012. Enrollment was done after the patient been characterized by several signs and symptoms [mucopurulent cervical discharge, erythema, and unusual vaginal bleeding or discharge, pain in the abdomen, painful sexual intercourse, fever and painful urination], all the 100 selected patients were referred to gynaecological clinics for further examinations and confirm the infection. The gynecologists performed a speculum examination of the cervix, bimanual pelvic examination. An endocervical swab for *C trachomatis* detection was obtained from all patients.

Infection was confirmed by cervicitis accompanied by erosions. Patients were divided into 2 groups; group 1 included those who have positive cervicitis accompanied by erosions (49 patients) and patients with cervicitis alone (65) were allocated as group 2. Twenty healthy individuals were also included as control subjects who have no clinical symptoms of *chlamydial* infection.

Patients were subjected to cervical swab sampling [cervical swab for direct antigen detection "*DAD*" and endocervical cells for cytology]

For the endocervical cells for cytology Papanicolaou staining method was performed according to Drijver and Boon[13].

For the cervical swab for direct antigen detection (DAD) *Chlamydia* kwik-chek rapid immuno-chromatography was used [*Chlamydia* Kwik-Chek cassette was allowed to come at room temperature prior to testing, after that, 20 drops of the extraction reagent were added to the plastic extraction tube, then the swab was inserted and squeezed for 10-15 seconds at the upper internal surface of the extraction tube, the swab was then left inside the tube for further 2–5 minutes. 3-5 drops of the incubated material were taken by Pasteur pipette and added to the test strip. The results were read visually after 15 minutes]

2.1. Inclusion criteria
All married women with cervical abnormalities consenting to take part in the study were irrespective of age

2.2. Exclusion criteria
Patients who had received antimicrobial chemotherapy during the previous four weeks.
Unmarried women were excluded from testing

2.3. Statistical analysis
Data entry and analysis were performed using the statistical package SPSS version 17. Data were expressed as mean ± standard deviation (SD). The means were compared using Independent sample t.test.

3. RESULTS
The mean age of the selected patients was 30.4 ± 9.03 years and most of them were having a history of abortion. Endocervical swabs were subjected to cytological examination to detect the *chlamydial* inclusion bodies and confirm the infection as well. Positive swabs were confirmed by blue/black color of the nucleus. The cytoplasm of non-keratinizing squamous cells appeared with blue/green, whereas keratinized cells showed pink/orange colors. *Chlamydia*-infected cells appeared inflamed with pre nuclear hallow (Figures 1, 2).
Table 1 showed that DAD test were positive in 74 of cervical swabs for *chlamydial* antigen while Pap smearing showed that almost half of the suspected patients were cytologically- positive for *chlamydial* infection 49%, The sensitivity, specificity, PPV and NPV of DAD method was summarized in table 1.
By comparing DAD method to the cytological method [Figure 3], 49/100 of cases were positive by both techniques and 25/100 was negative cases by them.
DAD showd higher diagnostic detection potentils. On the other hand cytological test was less in detecting chalmydial bodies in cervical swabs [Figure 4]

5. DISCUSSION
C trachomatis infection is a worldwide-distributed sexually transmitted infection. Prevalence of infection is difficult to estimate without screening, as most of the cases are asymptomatic. Although 929,462 cases of *chlamydia* infection were reported in the USA, according to the Centers for Disease Control and Prevention, in 2004,[14] the actual number of cases is thought to be more than 2.8 million per year[15]. According to the World Health Organization, new cases of *chlamydia* infection have been estimated globally to be 92 million[16]. Both health and economic consequences of infection are serious; thus, prevention and early detection are essential.
Although *Chlamydia* infection is widely spread among a considerable number of people in the Sudan, scanty studies have been done to assess the prevalence of *Chlamydia* in the Sudan. The only available data is about the prevalence of antibodies to *C. trachomatis* and other *chlamydial* species[17,18].
The results of this study showed that the DAD diagnosis has sensitivity relative to cytopathological diagnosis which has an absolute sensitivity. These findings were supported by a number of studies[19] ; the obtained results were significantly relevant to these studies with regard to simplicity of methods of detection, quality improvement, laboratory judgments, follow-up and ancillary testing. The advantage of DAD that it is quick observing results, cheapness, and it is easiness of performing, the test doesn't require the high experiences that require in cytology.

5. CONCLUSION
This study concluded that sensitivity and specificity of the DAD in detecting of *Chlamydia trachomatis* infectious agent is higher, comparing to cytological finding. Furthermore, the reliability is depends on various factors including the proper collection of specimen and quality of the sample, preparation method and experience in interpretation. DAD is a simple, quick cost-effective diagnostic tool for *Chlamydia trachomatis* STI Infection. Different cell types can be detected in the endocervical cytology in where high experience is required, unlike DAD where such experiences are not that necessary. This is one of the few reports on the *Chlamydia* infection in women from Sudan, due to cultural and social constraints this study excluded a large proportion of women aged less than 19 years of age. Hence no direct comparisons on prevalence could be made with studies from the West, which all included younger women at high risk of *Chlamydia*.

6. COMPETING INTERESTS
The authors declare that there are no conflicts of interests.

7. ACKNOWLEDGMENTS
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Table1: Comparison of DAD to Cytological smears for diagnosis of *C. trachomatis*.

Test	No of positive cases	Sensitivity%	Specificity%	PPV %	NPV%
Direct Antigen detection	74%	93	84	93	83
Cytological Smear	49%	54	83	90	65

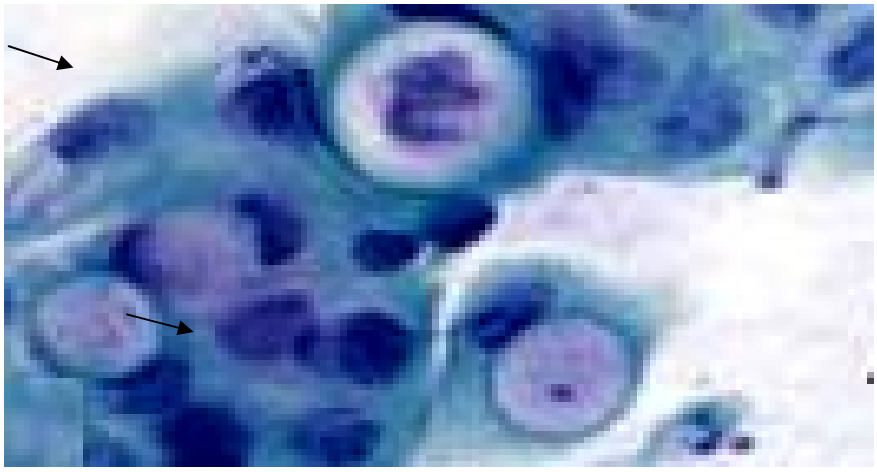

Figure 1: Pap smear taken from Endocervical samples showing inflamed cytoplasm with large prenuclear hallows (arrows) which reflect *chlamydial* inclusion bodies.

Figure 2: Magnified inflamed *chlamydial*-infected cell

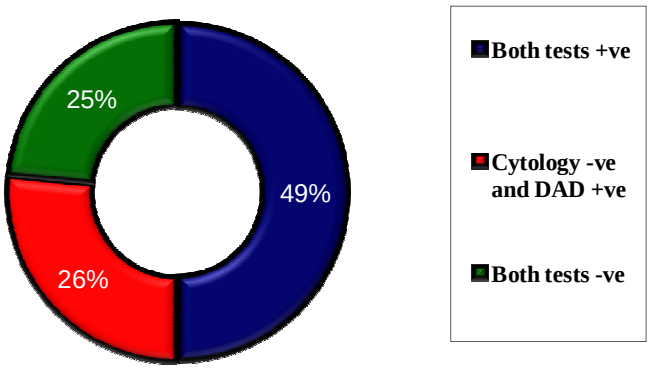

Figure 3: Positivity of endocervical swab samples by cytology and DAD

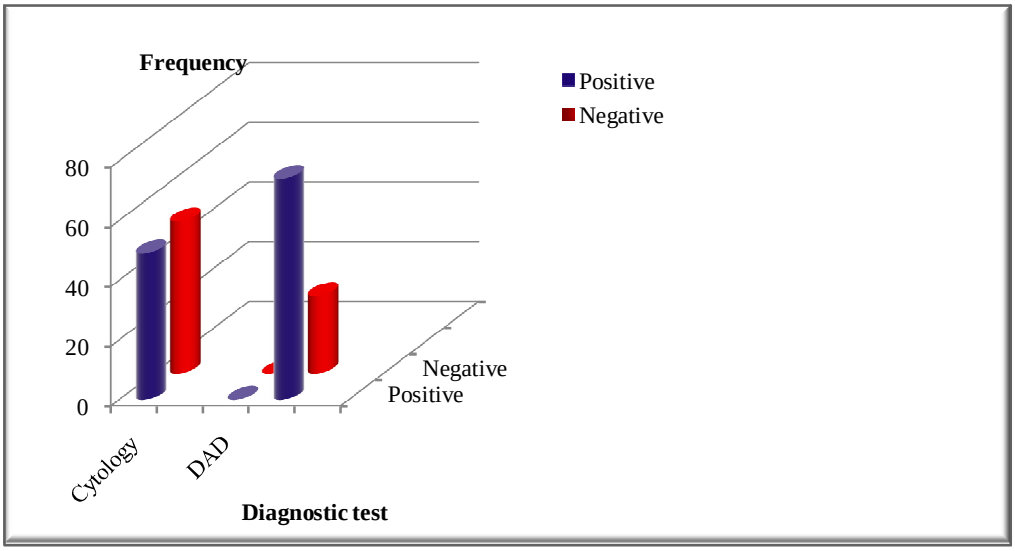

Figure 4:Comparative diagnostic results of cytology and DAD tests of cervical swabs collected from *Chlamydia* suspected patients in Khartoum area

8. REFERENCES

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