Prevalence of *Chlamydia Trachomatis* in the Serum Sample of Individuals in Akpakpa, Cotonou

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Research Article

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Abstract

Background

Chlamydia is one of the most common sexually transmitted diseases in the world with severe complications. This study aimed to determine the prevalence of *Chlamydia trachomatis* infection in the serum samples of individuals in Akpakpa, Cotonou.

Study design:

A cross-sectional study, using serological testing to perform the analysis in Akpakpa, Cotonou. The sample used was whole blood. The survey was carried out using a questionnaire.

Method

A random sample of about 11 adults aged 15–30 years was taken. The separated sera were subjected to anti-Chlamydia trachomatis (IgG) and anti-Chlamydia trachomatis (IgA) using ELISA techniques. Consenting men and women were issued questionnaires to collect information related to the study such as age, sex, lifestyle, symptoms associated with Chlamydia, family history, and past genital tract infection. Risk factor analyses were carried out for chlamydial infection

Results

Chlamydia antibodies were found in 27.27% of the individuals tested. The presence of Chlamydia antibodies was associated with unprotected sex, a history of genital tract infection, having more than one sexual partner, and a young age for both men and women. At the population level, the prevalence of chlamydial infection correlated well with reported rates of partner change.

Conclusion

The present study revealed the prevalence of chlamydia among women, individuals with a past history of genital tract infection, individuals associated with unprotected sex, individuals who have more than one sexual partner, and young people. A high percentage of individuals were unaware of the infection and its complications which was also associated with the prevalence of the infection.

Introduction

Chlamydia trachomatis is a gram-negative bacterium that infects the columnar epithelium of the cervix, urethra, and rectum, as well as nongenital sites such as the lungs and eyes. The bacterium is the cause of

the most frequently reported sexually transmitted disease, which is responsible for more than 1 million infections annually [1] and is the leading cause of infectious blindness in the world [2].

A research program aimed at female sex workers (FSWs) and their male clients in Benin was conducted to estimate the prevalence of Human Immunodeficiency Virus (HIV), *Neisseria gonorrhoeae* (NG), and *Chlamydia trachomatis* (CT) and to determine the social, demographic and behavioral factors associated with these infections. The study provided a follow-up of the epidemiological situation in Cotonou, Benin's largest city, where the intervention began in 1993 with the collection of baseline data; it also collects such data for three other cities in Benin, to which the intervention was extended in 2002. The prevalence of *chlamydia trachomatis* (CT) was found to be 4.8% [3].

The prevalence of chlamydial infection was 1.3% in women in Cotonou, in research carried out to study the epidemiology of gonorrhea, chlamydial infection, and syphilis in four African cities. Chlamydial infection was associated with the rate of partner change for both men and women, and with a young age for women [4].

In men, the infection usually is asymptomatic. Most women with chlamydial infection have minimal or no symptoms. Chlamydial infection in newborns can cause ophthalmia neonatorum [5]. In women, untreated *Chlamydia trachomatis* infection can cause pelvic inflammatory disease (PID). PID can cause infertility, chronic pelvic pain, tubal pregnancies, and the continued spread of the disease. In men, untreated *chlamydia* can spread to the epididymis causing urethral infection and complications such as swollen and tender testicles. The study findings showed a statistically significant association between current *C. trachomatis* infection with infertility [6].

The aim of this research work is to identify risk factors that are associated with *chlamydia trachomatis* infection and to find out the prevalence of *Chlamydia* among sexually active individuals in Akpakpa, Cotonou.

Materials And Methods

This work was carried out in the Regional Laboratory of Sanitary Safety and Analytical Expertise of IRGIB-AFRICA UNICOMER Akpakpa, Cotonou, established and officially launched in 2016 in the Republic of Benin. The IRGIB-Africa laboratory is equipped with standard technologies capable of various analyses. It is organized such that it has different departments which in turn have units and are managed by engineers and researchers who work under the supervision of the Director of the laboratory, technical director, interns' supervisor, and personnel in charge of quality control.

Study design

A cross-sectional study, using serological testing to perform the analysis in Akpakpa, Cotonou. The participants were randomly selected in July 2016. The sample used was whole blood. A survey was carried out using a questionnaire to gather data from the study participants.

Sample Population & Size

The sample population of this particular research is focused on individuals in Akpakpa, Cotonou, Benin Republic between the age range of 15-35 years and sexually active. 11 blood samples were collected and analyzed.

Materials

For Blood Sample Collection

- Syringes 2ml
- Blood Collection Tubes 5ml
- Tourniquets
- Alcohol Wipes – 70% isopropyl alcohol
- Gloves
- 2×2 Gauze or cotton balls
- Sharps Disposal Container
- Bandages or Tape
- Marker

For ELISA Techniques

- Disposable plastic vial for dilution of the concentrated
- HRP- conjugated anti-human IgA.
- Adjustable micropipettes, or multichannel pipettes (5-50, 50-200, and 200-1000µl ranges) and disposable tips.
- One 50 ml volumetric cylinder.
- Wash bottle.
- Absorbent paper.
- Vortex mixer.
- A 37°C water bath with a lid, or a moisture chamber placed in an incubator at 37°C.
- ELISA reader with 450nm filter.
- Distilled or double deionized water

Table 1: Reagent Composition

REAGENT	COMPOSITION
MICROTITERPLATE WELLS	ELEMENTARY BODIES
NEGATIVE CONTROLS	HUMAN IgG/IgA/IgM BUFFER: 0.05M PBS
POSITIVE CONTROL	HUMAN IgG/IgM/IgA 0.05M PBS TWEEN 20
SAMPLE DILUENT	0.05M PBS-TWEEN 20
SUBSTRATE B	Urea peroxidase
SUBSTRATE A	TMB in DMSO
STOP SOLUTION	Sulfuric acid
WASHING SOLUTION	200g/liter sodium chloride

Table 1 shows the different reagents used and their composition respectively.

The reagents are stable up to the expiration date stated on the label when stored at 2...8 ° C. Figure 1 shows the chlamydia kits, its reagents, and the ELISA microtiter plate wells.

Sample Collection and Processing

Sterile disposable vacutainer tubes were used to collect 2 ml of blood from the antecubital vein under aseptic conditions, the blood samples were allowed to clot at room temperature. The clotted samples were centrifuged at 5000 rpm for 10 minutes to separate the sera; the obtained sera were frozen at -20°C until used. The separated sera were subjected to anti- *Chlamydia trachomatis* (IgG) and anti-*Chlamydia trachomatis* (IgA) using ELISA techniques.

The type of specimen depends on the clinical picture, the diagnosis conditions, and the laboratory technique used for detection, with the transport and storage being adapted to the particular technique. The data obtained from the survey will be compiled and analyzed using Excel Spreadsheet.

Principle of the ELISA Test (Chlamydia)

The ELISA test provides a semiquantitative in vitro assay for human antibodies of class IgG or IgA against *Chlamydia trachomatis* in serum. The test kit contains microtiter strips each with 8-break- off reagent wells coated with *Chlamydia trachomatis* antigens. ELISA offers a relatively simple and rapid test for the diagnosis of *C. trachomatis* infections in high-risk populations. The technique (ELISA) is the most reliable and common method used in the diagnosis of *C. trachomatis*. Contrary to the ordinary

immunological principle where IgM is used to detect current infection, in this study IgA was used to detect the recent infection. Multi studies suggested that the presence of serum IgA may be a useful marker for active *C. trachomatis* infection [7].

Test Procedure

- In the first reaction step, diluted patient samples were incubated in the wells. In the case of positive sample-specific IgG or IgA antibodies bound to the antigens.
- To detect the bound antibodies, a second incubation was carried out using an enzyme-labeled anti-human IgG or IgA (enzyme conjugate) catalyzing a color reaction. The test was carried out according to the manufacturer’s instructions.
- Dilute serum sample and take 100µl from each of calibrators, positive control, negative control, and diluted samples were transferred into proper microtitre wells, and incubated for 30 minutes at room temperature.
- Subsequently, they were washed three times using 300 µl of working wash buffer for each microplate.
- 100µl of the enzyme conjugate was added into each of the microplate wells and incubated for 30 minutes at room temperature, then after washed as described above.
- 100µl of TMB chromogenic/ substrate solution was added to each of the microplate wells and incubated for 15 minutes at room temperature.
- 100µl of the stop solution was pipetted into each of the microplate wells to end the reaction.
- Finally, the color intensity was measured at a wavelength of 450 nm using an ELISA micro-well plate reader.

Results

Table 2: The summary of the result derived from the 11 samples.

SAMPLES	ABSORBANCE IgA	CONCENTRATION IgA index	CONCENTRATION IgG index
1	0.018	1.161	03
2	0.130	8.387	09
3	0.023	1.484	23
4	0.000	0.000	02
5	0.016	1.032	06
6	0.013	0.839	04
7	0.227	14.65	11
8	0.117	7.55	14
9	0.015	0.968	07
10	0.017	1.097	05
11	0.006	0.387	03
Positive control	0.656	42.32	
Negative control	0.057	3.677	
Cut-off control 1	0.017	1.097	
Cut-off control 2	0.014	0.903	

Table 2 shows the absorbance of IgA, the concentration of IgA, and the concentration of IgG for the 11 blood samples analyzed and also for the positive controls, negative controls, cut-off 1 control, and cut-off 2 controls.

Table 3: Summary of the results gotten after the analysis

CHARACTERISTICS	TOTAL NUMBERS	POSITIVE n (%)	NEGATIVE n (%)
SEX			
MALE	06	01(16.67)	05(83.33)
FEMALE	05	02(40.00)	03(60.00)
AGE			
15-20	04	01(25.00)	03(75.00)
21-25	06	02(33.33)	04(66.67)
25-30	01	00(0)	01(100.00)
INVOLVED IN UNPROTECTED SEX	02	02(100.00)	00(0)
INVOLVED IN PROTECTED SEX	05	00(0)	05(100.00)
DIAGNOSED OF STD	02	02(100.00)	00(0)
HAVING MORE THAN ONE SEXUAL PARTNER	03	03(100.00)	00(0)
JUST ONE SEXUAL PARTNER	07	00(0)	07(100.00)
DIFFERENT CONTRACEPTIVES USED			
CONDOM	03	00(0)	03(100.00)
PILLS	06	02(33.33)	04(66.67)
IMPLANTS	03	01(33.33)	02(66.67)
SYMPTOMS			
ITCHING AROUND OR WITHIN GENITAL ORGAN	01	01(100.00)	00(0)
YES	10	00(0)	10(100.00)

NO			
DISCHARGE AFTER SEX			
YES	01	01(100.00)	00(0)
NO	10	02(20.00)	08(80.00)
PAINFUL URINATION			
YES	02	02(100.00)	00(0)
NO	09	00(0)	09(100.00)
PAIN DURING SEX			
YES	02	02(100.00)	00(0)
NO	09	00(0)	09(100.00)
FAMILY HISTORY			
YES	00	00(0)	00(0)
NO	11	00(0)	11(100.00)

Table 3 shows the overall results obtained from the analysis for the quantitative determination of *Chlamydia trachomatis* among the Akpakpa populace, Cotonou. A total of 11 blood samples were collected with different data which include; age, gender, lifestyle, symptoms, and family history.

Data Analysis

Table 4: Percentage result based on age range

AGE RANGE	TOTAL NO OF SAMPLE	POSITIVE	NEGATIVE	POSITIVE (%)	NEGATIVE (%)
15-20	04	01	03	25.00	75.00
21-25	06	02	04	33.33	66.67
26-30	01	00	01	0.00	100.00

Table 4 illustrates the percentage result based on the recorded age range of 15-20, 21-25, and 26-30, respectively. For the age range of 15-20, the percentage value for positive is 25.0% while the percentage value for negative is 75.0%. For 21-25, the percentage value for positive is 33.33% and 66.67% for negative, and for the age range of 26-30, the percentage value for positive is 0% while the percentage value for negative is 100%.

Table 5: Percentage result based on gender

GENDER	TOTAL NO OF SAMPLE	POSITIVE	NEGATIVE	POSITIVE (%)	NEGATIVE (%)
MALE	06	01	05	16.67	83.33
FEMALE	05	02	03	40.00	60.00

Table 5 indicates the percentage of the result based on their gender. The total number of samples recorded for males is six (06), where 16.67% are positive while 83.33% are negative. Also, the total number of the sample recorded for women is five (05) where 40% is positive and 60% are negative, these analyses show the prevalence of *chlamydia* infection in women than in males.

Interpretation of the Result

Table 6: Interpretation of Results

Antibodies against C.trachomatis			Interpretation	Recommendation
IgM	IgA	IgG		
-	-	-	Seronegativity. Infection not proven.	In case of justified clinical suspicion direct antigen detection should be performed and repetition of the serology testing within 2-3 weeks.
+	-	-	Suspect acute infection	
-	+	-		
+	+	-		
+	+	+	Suspect acute or recent infection	Control tests within 2 to 3 weeks are recommended.
-	+	+	Suspect reinfection or recent infection	
-	-	+	Infection in history	

The probability to assign a diagnostic significance to a given reactivity increases with increasing absorption value, or increasing index value, or increasing value of reactive units.

It is recommended to test serum samples for all three antibody classes (IgG, IgM, and IgA) for the optimal result interpretation. In the cases of acute *chlamydial* infections, serological results may be negative despite clinical symptoms and positive antigen detection. If serological confirmation of a positive antigen result is desired, we recommend testing after 10-14 days to find seroconversion. (Note to the table: – Negative, + Positive). Table 6 above, shows the interpretation of IgM, IgA, and IgG antibodies detection and also shows the possible recommendation.

Colorimetric Analysis

The coloration in the well that occurred after the addition of enzyme-substrate (blue coloration) shows that the samples are positive, while the clear wells show that the samples are negative, as shown in Fig 2. At the addition of the stop solution, the reactive specimen turned from blue to yellow. The intensity of the coloration is directly proportional to the severity of the infection; this mechanism makes ELISA a semi-quantitative technique.

Discussion

The prevalence of CTI in this study is 27.27% in Akpakpa, Cotonou was found to be 27.27%, which is quite high. This figure is higher than the prevalence rate of 4.8% recorded by Ahoyo AB et al. [3]. And 1.3% obtained in epidemiological research carried out by Buvé A et al. [4]. This calls for caution among the individuals in Akpakpa, Cotonou, the high rate was related to the increase in unprotected sex, having more than one sexual partner, and coinfection. In this study, we assume there is a higher percentage of infection observed among the age group 21-25years.

The increasing number of *chlamydial* infections in Akpapa, Cotonou, is related to the fact that these infections frequently cause asymptomatic or silent diseases and do not motivate patients to seek medical care, resulting in a prolonged period of infectivity and a high risk of developing complications.

The technique applied in this study (ELISA) is the most reliable and common method used in the diagnosis of *C. trachomatis*. Contrary to the ordinary immunological principle where IgM is used to detect current infection, in this study IgA was used to detect the recent infection. Multi studies suggested that the presence of serum IgA may be a useful marker for active *C. trachomatis* infection [7] [8] [9].

Finally, conclusions can't be made on the age range 26-30 due to the lower number of samples obtained which cannot determine the prevalence of *Chlamydia*.

Limitations

The study used a very low amount of samples. This was justified by the lack of financial resources and the minimal cooperation of the defined population. Despite these limitations, we were able to achieve the pre-set goals.

Conclusion

Although carried out with a very low amount of sample amount, this study has revealed *the* prevalence among individuals who are sexually active, diagnosed with other STDs and those involve in unprotected sex. Also, the study showed the prevalence of CTI been common among females than males.

The research also revealed that the causative organism of CTI is *chlamydia trachomatis* which is usually transmitted upon the exchange of sexual bodily fluid, although *chlamydia* is asymptomatic the study also pointed out symptoms that are associated with the presence of CTI since it's a very common STD.

It could be suggested that in the face of clear CTI symptoms and the absence of a physician or clinician, a single dose of Antibiotics such as Azithromycin and Doxycycline could be procured with an experienced doctor seen for confirmation.

Recommendation

Education of the masses that emphasize the relevance of abstinence or the retention of a single sexual partner and the use of barrier contraceptives in the form of seminars, brochures, posters, radio and television messages, or cluster group discussions should be encouraged in African communities to help curb infection rates, as several studies have highlighted the increased prevalence of the pathogen as a result of ignorance and unawareness.

Also, sexual intercourse is still a very hard relationship to tackle among the unmarried and adolescents. Hence, governments should focus on how to create awareness, as well as the utilization of barrier contraceptives, to be used as recommended by the World Health Organization (WHO) which will be a step towards control of the transmission of *chlamydia trachomatis*

Furthermore, further studies and research should be made on the other geographical parts of the Republic of Benin, taking into account age, sex, family history, socioeconomic status, lifestyle, and other factors to have a better understanding of the prevalence, transmission, and conditions associated with this disease.

List Of Abbreviations

FSWs- Female sex workers

CT- *Chlamydia trachomatis*

NG- Neisseria gonorrhoeae

HIV- Human immunodeficiency virus

GTI- Genitourinary tract

STD- Sexually transmitted disease

NAAT- Nucleic acid amplification tests

PCR- Polymerase chain reaction

TMA- Transcription-mediated amplification

SDA- DNA strand displacement amplification

LCR- Ligase Chain reaction

IgM- Immunoglobulin M

IgA- Immunoglobulin A

IgG- Immunoglobulin G

ELISA- Enzyme-linked immunosorbent assay

PID- pelvic inflammatory disease

CTI- *Chlamydia trachomatis* infection

IRGIB- Institut Régional du Génie Industriel, des Biotechnologies et Sciences Appliquées

Declarations

The work presented in this manuscript is the result of our original research work. Where we have used the works of other persons, due acknowledgements are clearly stated. This work has not been submitted for publication in any journal before.

Ethics approval and consent to participation

The research was carried out in IRGIB-Africa University. The Institution obtained its ethical approval for research studies from the MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH. PROTOCOL N°31/05RIT. All methods were performed in accordance with the relevant guidelines and regulations.

AUTHORISATIONS : MESRS/CAB/SGM/DPP/DESUP/SP/2006/2007/2008

Consent for publication

Not applicable.

Availability of data and materials

All data generated during this study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

EDP and AGM contributed to the design and implementation of the research, analysis of the results, and writing of the manuscript. All authors have reviewed and approved the manuscript.

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Supplementary Material

The supplementary file contains the filled STROBE statement checklist for the study.

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Figures

Figure 1

The chlamydia trachomatis kit

The reagents are stable up to the expiration date stated on the label when stored at 2...8 ° C. Figure 1 shows the chlamydia kits, its reagents, and the ELISA microtiter plate wells.

Figure 2

Colorimetric analysis of ELISA

The coloration in the well that occurred after the addition of enzyme-substrate (blue coloration) shows that the samples are positive, while the clear wells show that the samples are negative, as shown in Fig 2. At the addition of stop solution, the reactive specimen turned from blue to yellow. The intensity of the coloration is directly proportional to the severity of the infection; this mechanism makes ELISA a semi-quantitative technique.