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IMMUNOLOGICAL AND MOLECULAR DETECTION OF *CHLAMYDIA TRACHOMATIS* AMONG WOMEN AT REPRODUCTIVE AGE ATTENDING OMDURMAN MATERNITY HOSPITAL

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ABSTRACT: *Chlamydia trachomatis* is the most common bacterial sexually transmitted disease. Over 100 million men and women worldwide are infected with Chlamydia at any point in time. The aim of this study to detect the presence of *C. trachomatis* antibodies and antigen among women on the reproductive age and to study the relationship between the presence of *C. trachomatis* and age, abortion and ectopic pregnancy. Five hundred women classify into three hundred pregnant women with a gestational age between 12 and 36 weeks and two hundred nonpregnant women. Two blood samples collected individually in two different containers, one EDTA container for PCR technique and plane container for ELISA testing during the period June 2011 to August 2013. Thirty-one samples (6.2%) were positive for *C. trachomatis* IgG only, while 8 samples (1.6%) were positive for both IgM and IgG. Out of 31 samples, 25 samples (5%) were positive by using the PCR technique. The results revealed statistically a significant relationship between PCR and previous abortion and ectopic pregnancy with P value, 0.001 and 0.002, respectively. The age range between (30-35) years is considered as a risk factor that exposes sexually –active women to *C. trachomatis* infection. *Chlamydia trachomatis* infection in women is a cause of abortion and ectopic pregnancy.

INTRODUCTION: Over 100 million men and women worldwide are infected with chlamydia at any point in time¹.

It is the most commonly reported bacterial sexually transmitted infection (STI) in developed countries, with over 1.4 million cases reported in the United States in 2011².

There are at least 18 serovars of *C. trachomatis*; the important medical serovars associated with endemic trachoma are A, B, Ba, and C while D-K serovars are associated with sexually transmitted disease and that cause lymphogranuloma venereum are L1, L2, and L3³.

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Left untreated, *Chlamydia* can ascend from the endocervix to the upper genital tract in women and cause pelvic inflammatory disease (PID) which can increase the risk of developing fallopian tube scarring, potentially leading to ectopic pregnancy, tubal infertility, and chronic pelvic pain^{4,5}. Also, genital infection in pregnant women increases the risk of preterm delivery, can be passed on to the baby during vaginal delivery and may result in eye and lung infections in the newborn^{4,6}.

MATERIAL AND METHOD:

Ethical Approval: The study was approved by the Al Neelain Medical Research Committee and Omdurman Maternity Hospital ethical committee. All participants signed informed consent forms before enrolment in the study.

Study Design: This is a descriptive cross-sectional laboratory and a hospital-based study conducted in Omdurman state, to determine the frequency of *C. trachomatis* among women at the reproductive age during the period June 2011 to August 2013.

Study participants were filled with a self-administered questionnaire, which included data on sociodemographic, reproductive, and medical history.

Samples Collection: The samples were collected from patients attending Omdurman maternity hospital antenatal Care unit and family planning unit. Three hundred blood samples from pregnant women with a gestational age between 12 and 36 weeks and two hundred samples from non-pregnant women. Two blood samples collected individually in two different containers one EDTA container for PCR technique and plane container for ELISA testing.

ELISA Test: The ELISA test is done by using NEUROIMMUNE kit, briefly, basis of antigen preparation was BGM- cells infected with the *C. trachomatis* of the serotype K. The microplate well were coated with purified MOMP antigen (major outer membrane protein), which is a transmembrane protein in the outer membrane of the elementary bodies. The test kit contains microtitre strips, each with eight break-off reagent wells coated with *C. trachomatis* antigens. In the first reaction step, diluted patient samples were incubated with the wells. In the case of positive

samples, specific IgG \ IgM antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labeled anti-human IgG/IgM (enzyme conjugate) which is capable of promoting a color reaction.

Photometric measurement of color intensity was made at a wavelength of between 620 nm and 650 nm within 30 minutes of adding the stop solution. The intensity of the formed color is proportional to the concentration of antibodies against *C. trachomatis* antigens.

The color intensity obtained from the automated ELISA reader was interpreted by qualitative analysis by the formula extinction value of the control extinction of calibrator 2 interpretation:

Ratio < 0.8: negative

Ratio \geq 0.8 to > 1.1: borderline

Ratio \geq 1.1 positive

DNA Amplification: Confirmation of *Chlamydia trachomatis* isolates was carried out by Amplification of the major outer membrane protein (copB) target gene using GenePack DNA PCR tests (Geneon-Germany). A master mix reagent was prepared. 10 μ l specific dilution buffer (Gene On), 2 μ l bovine serum albumin (BSA), 7 μ l sterile water, and 1 μ l template DNA were added separately to each lyophilized DNA-PCR test tube to give a final volume of 20 μ l. The amplification was conducted using TC-312 Techne thermal cycle (Germany). The PCR products were analyzed by 1.5 agarose gel electrophoresis **Fig. 1**.

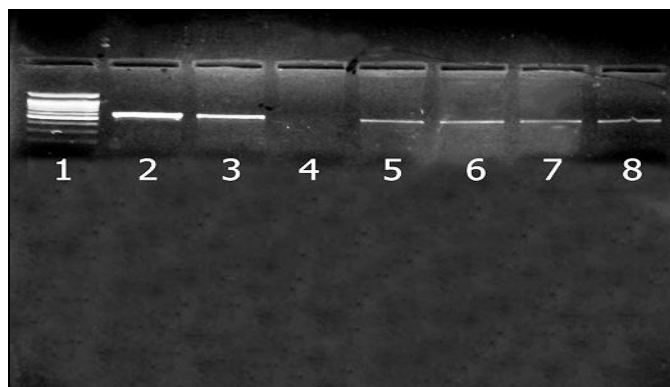


FIG. 1: CHLAMYDIA TRACHOMTIS PCR AMPLICON ON 2% AGAROSE GEL

Lane 1= Molecular weight marker; Lane 2 and Lane 3 = Positive control; Lane 4= Negative control; Lane 5 – Lane 8 = Positive bands for *Chlamydia trachomitis* strains (550 bp).

RESULT AND DISCUSSION: Chlamydia IgG and IgM ELISA were done for all the subjects enrolled in the study. The mean age of the women enrolled in the study was 30-35 years. Out of 500 women enrolled in the study, 31 samples (6.2%) were positive for *C. trachomatis* IgG only, while eight samples (1.6%) were positive for both IgM and IgG. Out of 31 samples, 25 samples (5%) were positive by using PCR technique **Fig. 1**.

TABLE 1: DETECTION OF CHLAMYDIA TRACHOMITIS AMONG PREGNANT AND NON-PREGNANT USING DIFFERENT TECHNIQUES

Test	Pregnant	Nonpregnant
IgG (ELISA)	17	14
IgM (ELISA)	4	5
PCR	13	12

Out of the 500 women participated in current study, 339 (62.8) women complained from the symptom of infection, 21 women (6.2%) were positive for *C. trachomatis*, while 161 (32.2%) were said they had not complained from the symptom of the infection, (6.2%) were positive for *C. trachomatis* antibodies **Fig. 2**.

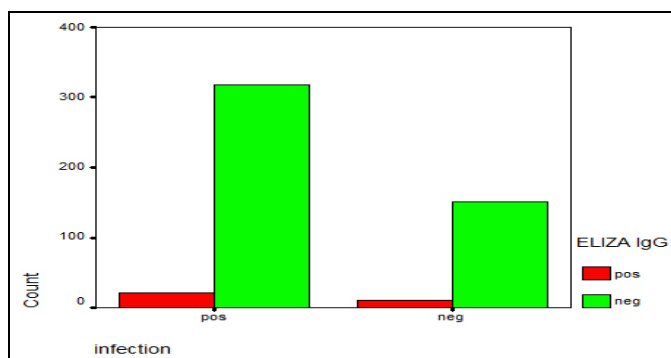


FIG. 2: THE PERCENTAGE OF WOMEN WHO HAD A HISTORY OF INFECTION COMPARING WITH WOMEN WHO HAD NOT A HISTORY OF INFECTION

TABLE 2: CORRELATION BETWEEN IGG AND NUMBER OF ABORTION

No. of abortion	IgG		PCR	
	Negative	Positive	Negative	Positive
Zero	49(98.8%)	3(1.2%)	250(99.2)	2(0.8)
1	126(93.3)	9(6.7%)	128(94.8)	7(5.2)
2	8(86.1%)	11(13.9%)	70(88.6)	9(11.4)
3	1(75.0%)	7(25.0%)	22(78.6)	6(21.4)
4	5(83.3%)	1(16.7%)	5(83.3)	1(16.7)

P value 0.001

TABLE 3: CORRELATION BETWEEN IGG AND NUMBER OF ECTOPIC PREGNANCY

No of ectopic pregnancy	IgG		Total number
	Negative	Positive	
non	458(94.6%)	26(5.4%)	484
1	11(68.8)	5(31.3%)	16

There were several studies conducted in Sudan that reported the prevalence of *C. trachomatis*. The percentage of *C. trachomatis* reported in this study was 5% using the PCR technique and 6.2% by ELISA technique, and this not a surprising result because Sudan is a traditional Islamic society, and according to the customs and traditions of Islamic societies, free sexuality is prohibited, but this result is lower in comparison with study done by Mohamed *et al.*, who found that percentage was 60 (47.2%) and this may be due high selection criteria for the symptomatic participate ⁷.

Another Sudanese's study found that the percentage was 14.1 ⁸, this result was relatively near to finding in IRAN and Saudi Arabia 15.8%, 10.5% respectively ^{9, 10} and greatly less than that reported in Brazil and Quter 25.7 %, 37.5% respectively ^{11, 12}.

Our findings indicated that *C. trachomatis* infections common among age group (30-35 year), this result was in alignment with study done in Saudi Arabia ¹⁰ where they reported that the common age was above 34 years, and in contrary with Eltaybe *et al.*, ⁸ results whose stated that *C. trachomatis* infections common in the age group between 24-30 years. Other reported that 25 years as suspected age group ¹³.

However, Kusano and his team mentioned that junior school graduates had the highest frequency of positive cases, followed by graduates of high schools, vocational schools junior colleges, and university graduates had the lowest frequency ¹⁴, also Elia *et al.*, reported that the lower age range of 18-25 had the highest prevalence of *C. trachomatis* infection ¹⁵.

In the current study the presence of *C. trachomatis* was not affected by past genital tract infection, out of 339 (62.8%) women were complained from the symptom of infection only 21(6.2%) women were positive for *C. trachomatis*, while out of 161 (32.2%) women were said they had no symptom of the infection only (6.2%) were positive for *C. trachomatis*. This result is in line with that of Fatholahzadeh *et al.*, who stated that there was no association between genital tract infection and presence of Chlamydial infection ¹⁶. In the present analysis, there is a correlation between detection of *C. trachomatis* antigen and abortion p-Value 0.001,

this result similar to the finding of Wilkowska *et al.*, who reported that the percentage of *C. trachomatis* infection in cervical swabs was 44.4% of women with previous miscarriages¹⁷.

Recent Polish and Iranian studies suggested that there was a significant association between the number of abortions and abnormal vaginal discharge with *C. trachomatis* infection^{18, 19}. This study detected a relationship between several ectopic pregnancy and presence of *C. trachomatis* antigen whereas the p-value was (0.008) this was agreed with Abdolreza *et al.*, who observed that presence of *C. trachomatis* antibodies with an ectopic pregnancy patients²⁰.

CONCLUSION: The rang age between 30-35 years is considered as a risk factor that exposes sexually-active women to *Chlamydia trachomatis* infection. *C. trachomatis* infection in women is a cause of abortion and ectopic pregnancy.

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CONFLICT OF INTEREST: Nil

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