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Detection of Antichlamydial Antibody in Patients With Ectopic Pregnancy and Normal Pregnancy

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ABSTRACT

Aim of the current research is to assess the Chlamydia Trachomatis infection role in the development of early pregnancy complication including ectopic pregnancy and miscarriage in Sulaimanyia Maternity Teaching Hospital. It is a comparative study conducted in Gynecology Clinic and Emergency department of Sulaimanyia Maternity Teaching Hospital during the period from 1st of September 2018 to 31st of March 2019. The study groups included of 70 pregnant women; the first group included 35 ectopic pregnant women and the second group included 35 normal pregnant women that both groups had been selected randomly. Pregnant women with history of ectopic pregnancies, women used intrauterine device, in vitro fertilization, assisted reproduction and history of pelvic surgery. Blood sample (2 ml venous blood) collected to test for antibodies level for Chlamydia Trachomatis by Alegria test system for both studied groups and patients with ectopic pregnancy detected by beta human chorionic gonadotropin and ultrasound scanning. The collected data analyzed by SPSS program and for compare between means of two variables independent sample t-test was used while for comparison of categorical variables Chi square test was used with considering ≤ 0.05 P-value as significant level. The results shows that the mean age of normal pregnancy were (28.3±4.6) group compared with mean age ectopic pregnancy (29.5±4.9) group. The mean IgG (6.3±5.1) of patients with ectopic pregnancy was found to be significantly higher than mean IgG (2.8±1.1) for normal pregnant patients (P-value 0.01) and IgM mean (4.5±2.4) of patients with ectopic pregnancy was significantly higher than mean IgM (1.6±1.2) for

normal pregnant patients with P-value 0.01. In conclusion, infection of Chlamydia Trachomatis has a significant relationship with the development of ectopic pregnancy therefor screening and treatment of Chlamydia infection may reduce ectopic pregnancy rate with low cost.

1. INTRODUCTION

The implantation of the gestational sac outside of the uterine cavity known as Ectopic pregnancy. The major implantation locations in the orders are in the fallopian tubes (95.5%), followed by the ovaries (3.2%) and abdominal cavity (1.3%) [1]. globally, it is one of the main causes of maternal mortality, with incidence in developed countries as 1-2% and higher in developing countries [2, 3]. In the united kingdom (UK) around 10,000 ectopic pregnancies are diagnosed annually and ectopic pregnancy incidence rate is 11.1 in the UK for each 1000 pregnancies which causing 0.35 deaths per 1000 ectopic pregnancies in the period 2003 to 2005 [4]. Ectopic pregnancy have three types; cornual pregnancy is pregnancy that implant inside the rudimentary horn of a bicornuate uterus and interstitial pregnancy is the pregnancy in the interstitial part of the tube while heterotopic pregnancy is a rare type which is a fertilized egg implant inside and outside the uterus [5]. The mechanism and the cause of the implantation of gestational sac outside the uterine cavity is unknown. The four main possibilities are an anatomic obstruction, an abnormal zygote, abnormalities in the tubal motility and trans peritoneal migration of the zygote [6]. Multiple factors can leads to ectopic pregnancy; the causes may be impaired tubular motility, obstruction of the tubes by one or both functional and anatomical causes, cilliary dysfunction and tubal implantation promoted and stimulated by the molecular chemotactic factors [7]. The risk factors that may increase probability of ectopic pregnancy are history of ectopic pregnancy, infertility and it is treatments such as IVF. Maternal high age, smoking and tubal damages caused by surgery or infection [8, 9]. Also, another main risk factor that lead to ectopic pregnancy is a history of pelvic inflammatory disease which is rise the incidence of ectopic pregnancy in the United Kingdom including Chlamydia infection which is the main cause of pelvic inflammatory disease. Ectopic pregnancy will increase by seven fold after acute salpingitis; prevention and treatment of sexually transmitted diseases and pelvic inflammatory disease will decrease the rate of ectopic pregnancies also rate of heterotopic pregnancy increased after assisted reproductive techniques used [9-11].

In the developing countries, out of all the pregnant women who diagnosed with the ectopic pregnancy, near 10% of them die by this condition. Also, ectopic pregnancy causing many morbidity for pregnant women such as; sever pain in the pelvic area, vaginal bleeding and if the condition not treated may lead to infertility, because of those complications ectopic pregnancy condition is a major cause of maternal morbidity [12]. Therefore, early diagnose and treatment will prevent development of this life-threatening conditions [13].

Chlamydia is a gram-negative microorganism that infect the epithelium of the genital tract. It is one of the main sexually transmitted infections worldwide and it is mostly affects sexually active adolescents and young adults. 80% of women are asymptomatic, there for diagnosis will be missed in most of the cases [14]. Chlamydial infection complications are chronic pelvic pain, pelvic inflammatory disease (PID), Ectopic pregnancy (EP) and infertility [15] also it may cause neonatal conjunctivitis and pneumonia if infection occurs during vaginal delivery [16]. Peritonitis caused by *Chlamydia Trachomatis* leads to fibrin exudate on the surface of the ovary and adhesion formation.

Infection occurs because of *Chlamydia* cells by specific receptors that entered the columnar cells. Inclusion bodies form inside columnar cells and divide by binary fission rapidly. These divided cells reform new infection particles and released from the infected cells. The infected cells die and the body immune system response which leads to more cell damages [17].The adhesions leads to pelvic pain and tube obstructions, with Subsequent episodes of PID more damage occur so may leads to ectopic pregnancy and infertility [18].

There are different diagnosis methods to diagnose chlamydial infection such as; nucleic acid amplification test for chlamydia bacteria DNA with high sensitivity but need log duration after exposure and have three methods; urine, swab and direct fluorescent antibody. Urine samples need to collect first-catch urine avoid dilution. Direct Fluorescent Antibody (DFA) for swab culture from Endocervical, for antibodies (IgM) that present in the blood and lymph fluid Enzyme-linked Immunosorbent assay (ELISA) test used, Enzyme immunoassay (EIA) for antibodies (IgG) present in the every body fluids and the last method is fluorescent antibody test for culture of the *Chlamydia* cells which is need around 5 to 7 days get result [19].

The goal of the current study is to assess the role of the *Chlamydia Trachomatis* infection in the pregnancy complications including ectopic pregnancy and early pregnancy miscarriages in comparison to normal pregnancy in Sulaimanyia Maternity Teaching Hospital.

2. METHODS AND MATERIALS

This is a comparative study carried out from 1st September 2018 to 31th March 2019 in the Gynecology department of Maternity Teaching Hospital in Sulaimanyia city. For this study 70 pregnant women selected randomly which divided into two groups; first group 35 patients with normal pregnancy and second group included 35 women with ectopic pregnancy that their age ranges from 18-38 years old in the first trimester of pregnancy and those who had history of ectopic pregnancies, Intrauterine device (IUD), In vitro fertilization (IVF) and assisted reproduction, previous pelvic surgery and patients who refuse to participate have been excluded from the study.

A sample of 2 ml venous blood was drawn from women in both groups and centrifuged, all sera aliquots were kept at -20c until evaluation for anti-chlamydial antibody IgG and IgM in the hospital's laboratory has been performed. Patients with ectopic pregnancy were admitted to emergency ward and diagnosed by beta Human chorionic gonadotropin (HCG) blood test and ultrasound examination. *Chlamydia Trachomatis* antibodies (IgG & IgM) were assessed by the Alegria test system.

The Alegria test are used for the differentiation of various immunoglobulin subtypes of the antibodies for both acute (IgM) and previous (IgG) bacterial infections, it is very useful test for the asess of the patient's immune status.

This test was used for both women with first and second groups. Normal value for serum Anti Chlamydia Antibody by ELISA for IgG level were < 20u/ml and for IgM level <20u/ml.

The data entered and analyzed by SPSS software program version 20. Descriptive statistics presented in frequency and percentage while continuous variables presented in the mean and standard deviation also SPSS soft wear program used to generate tables and figures. In addition, for the relationship between two categorical variables Chi square test was used and if 20% of the expected variables were less than 5 of total variables Fishers exact test was used. However, for comparison between two means independent sample t-test used with consider P-value less and equal to 0.05 as significant level for all tests.

3. RESULTS

The current study included 70 pregnant women separated into two groups; the first group (normal pregnancy) consist of 35 pregnant women and the second group consisted of 35 pregnant women with ectopic pregnancy condition. The age mean and standard deviation of the normal pregnancy group were 28.3±4.6 lower than ectopic pregnancy group 29.5±4.9 and there was only 1 (2.9%) women at lower than 20 years old in the normal pregnancy women group compare with ectopic pregnancy was a none. For other age groups, there were 18

(51.4%) at the 20-29 years old for normal pregnancy group compare with ectopic pregnancy group were 17 (48.6%) and women in the 30-38 years old were 16 (45.7%) in the normal pregnancy group while 18 (51.4%) were in the ectopic pregnancy group. In addition, according to the age between first and second groups there was a non-significant relationship with P-value 0.2.

For the pregnant women address in the current study. Results show that, pregnant women in the urban areas were 31 (88.6%) for the normal pregnancy group compare to ectopic pregnancy group were 29 (82.9%) women and pregnant women in the rural areas were 4 (11.4%) and 6 (17.1%) for the normal pregnancy group and ectopic pregnancy group respectively. Furthermore, there was a non-significant relationship (P-value 0.4) between normal pregnancy group and ectopic pregnancy groups according to the pregnant women living location.

The gravidity mean and standard deviation of the ectopic pregnancy group were higher 3.2±1.7 compared with normal pregnancy group 2.9±1.3 and primigravida were higher in the normal pregnancy group 4 (11.4%) compare with ectopic pregnancy group were 3 (8.6%) while multigravida were higher among ectopic pregnancy group 32 (91.4%) compare with normal pregnancy group were 30 (85.7%) women. Also, there was a non-significant relationship (P-value 0.3) between normal pregnancy group and ectopic pregnancy group according to the women gravidity. In addition, for the parity the current study shows that mean and standard deviation were approximately similar for the normal pregnancy (1.6±1) and ectopic pregnancy group (1.6±1.4) also were similar for the nulliparous 5 (14.3%) and multipara 30 (85.7%) for both normal pregnancy group and ectopic pregnancy group and between the first and second groups there was a non-significant relationship with P-value 0.9.

Finally, for the miscarriage the study reveals that; out of the 35 pregnant women in the ectopic pregnancy group 14 (40%) had abortion and 21 (60%) were higher compare to normal pregnancy group 6 (17.1%) women had abortion and 29 (82.9%) did not have abortion. Also, there was a significant relationship for women with previous miscarriage and did not between normal pregnancy group and ectopic pregnancy group with P-value 0.03. Table 1

Table 1: Demographic variables between the two studied groups

Variable		Normal Pregnancy No (%)	Ectopic Pregnancy No (%)	P-value
Age	**(Mean±SD)**	28.3±4.6	29.5±4.9	
	<20 years	1 (2.9)	0 (0)	0.2
	20-29 years	18 (51.4)	17 (48.6)	
	30-38 years	16 (45.7)	18 (51.4)	
Residence	**Urban**	31 (88.6)	29 (82.9)	0.4
	Rural	4 (11.4)	6 (17.1)	
Gravidity	**(Mean±SD)**	2.9±1.3	3.2±1.7	
	Primigravida	4 (11.4)	3 (8.6)	0.3
	Multigravida	31 (88.6)	32 (91.4)	
Parity	**(Mean±SD)**	1.6±1	1.6±1.4	
	Nulliparous	5 (14.3)	5 (14.3)	0.9
	Multipara	30 (85.7)	30 (85.7)	
Miscarriage	**Yes**	6 (17.1)	14 (40)	0.03
	No	29 (82.9)	21 (60)	

To reveal the presence of chlamydial infection in the second group (ectopic pregnancy group) the tests for the presence of IgG and/or IgM antibodies combined of chlamydial infection show that out of 35 pregnant women in the ectopic pregnancy group 82.9% of them had a positive IgG and/or IgM antibodies in their blood at 1st trimester of pregnancy period and reaming pregnant women in the ectopic pregnancy women group 19.1% had negative results for presence of IgG and/or IgM antibodies of chlamydial infection in their blood samples at 1st trimester of pregnancy period. Figure 1

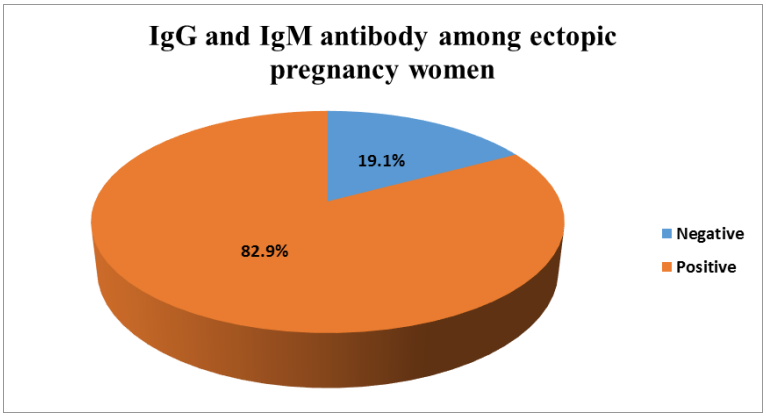

Figure 1: The Distribution of positive IgG and IgM antibody among patients with ectopic pregnancy.

Table 2 shows the demographic and previous pregnancy characteristics among ectopic pregnant group separately and the relationship between these variables. For the age, majority 18 (51.4%) of the ectopic pregnant women were between 30-38 years old followed by 17 (48.6%) between 20-29 years old and there was a significant relationship for women in the ectopic pregnancy group between age groups with P-value <0.001. In addition, most of ectopic pregnant women 29 (82.9%) were from urban areas rather than rural area 6 (17.1%) and there was a significant relationship between ectopic pregnant women from different living areas with P-value <0.001.

For the ectopic women gravidity, majority of women had multigravida 32 (91.4%) compare with primigravida 3 (8.6) with a significant relationship (P-value <0.001) between multigravida and primigravida among women with ectopic pregnancy and for the women parity among ectopic pregnancy group, most of the women 30 (85.7%) had multipara rather than nulliparous were minor 5 (14.3%) with a significant co-relationship (P-value <0.001) between multipara and nulliparous cases among ectopic pregnancy women group.

Finally, for the miscarriage history among ectopic pregnant women group, out of 35 women 14 (40%) of them had abortion while rest of them 21 (60%) women did not have history of miscarriage and there was a significant relationship (P-value <0.001) between women with history of the miscarriage and not among women in the ectopic pregnancy group.

Table 2: Distribution of demographic and previous pregnancy characteristics among the ectopic pregnancy group.

Variable		Ectopic Pregnancy group No (%)	P-value
Age	<20 years	0 (0)	<0.001
	20-29 years	17 (48.6)	
	30-38 years	18 (51.4)	
Residence	Urban	29 (82.9)	<0.001
	Rural	6 (17.1)	
Gravidity	Primigravida	3 (8.6)	<0.001

	Multigravida	32 (91.4)	
Parity	Nulliparous	5 (14.3)	<0.001
	Multipara	30 (85.7)	
Miscarriage	Yes	14 (40)	<0.001
	No	21 (60)	

Table 3 illustrate the mean and standard deviation of IgG and IgM Chlamydia antibodies rats in the blood for both groups; normal pregnancy and ectopic pregnancy. The mean IgG in the ectopic pregnancy group 6.3 (U/ml) were higher compare to the IgG Chlamydia antibodies mean rate 2.8 (U/ml) in the normal pregnancy group and there was a significant relationship between IgG Chlamydia antibodies means between normal pregnancy group and ectopic pregnancy group with P-value 0.01. Also, the mean IgM Chlamydia antibodies in the ectopic pregnancy group 4.5 (U/ml) were higher in comparison with the IgM Chlamydia antibodies mean rate 1.6 (U/ml) in the normal pregnancy group and there was a significant relationship (P-value 0.01) between IgM Chlamydia antibodies means between normal pregnancy group and ectopic pregnancy group. Table 3

Table 3: Distribution of anti-Chlamydia immunoglobulin according to ectopic pregnancy and normal women.

Variable	Normal Pregnancy Group (Mean±SD)	Ectopic Pregnancy Group (Mean±SD)	P-value
IgG (U/ml)	2.8±1.1	6.3±5.1	0.01
IgM (U/ml)	1.6±1.2	4.5±2.4	0.01

4. DISCUSSION

Chlamydia affects reproductive age of both males and females, the rate are 2.8% among males in comparison with females are 4.0% [20]. About 10%–30% of females will get chlamydia infection one or more time [21, 22]. Majority of *Chlamydia* infections up to 70% are asymptomatic cases, because that many cases are remain untreated and are vulnerable to complications of chlamydia infection like ectopic pregnancy, pelvic inflammatory disease (PID), chronic pelvic pain and tubal factor infertility [23].

The current study showed that anti-Chlamydia immunoglobulin mean (IgG and IgM) were significantly higher among women with ectopic pregnancy in comparison to women with normal pregnancy. This relationship is similar to results of many studies such Abdullah study in Iraq [24] and Mpiima study in Uganda which revealed a significant positivity of IgG and IgM antibodies for *Chlamydia Trachomatis* among ectopic pregnancy group compare to women with normal pregnancy [25]. Also, a study carried out in Nigeria by Agholor [26] on 90 women with ectopic pregnancy and 98 women with normal pregnancy; they found that chlamydial infection has a limited effect in the pathogenesis of ectopic pregnancy with elevation of serum IgG and IgM antibodies for Chlamydia among women with ectopic pregnancy.

A recent study conducted in Pakistan found that rate of IgG antibodies was much higher (25%) in ectopic pregnancy women in comparison with control group (11.3%). Pelvic inflammatory disease (PID) are main cause of ectopic pregnancy which is caused by bacteria such as *Chlamydia Trachomatis* and gonorrhea and other causes of EP include the use of contraceptive, surgery and smoking [27]. In addition, many recent reports indicate that *Chlamydia Trachomatis* is the major causative agent in the progress of PID and EP compared to gonoccocal infection [28]. The genital *Chlamydia Trachomatis* infection relationship with EP has been established mostly from seroepidemiologic researches which are recruiting and comparing normal pregnant women group with ectopic pregnancy group women that have

Chlamydia Trachomatis infection which will lead to detectable formation of IgG antibody in the chronically infected women's serum [2]. Many report indicated that the serological test of *Chlamydia Trachomatis* antibodies are not cost effective and beneficial, while isolation of this agent by cell culture has not been applied to date [2, 29].

A prospective case-control study done in Saudi Arabia by Ashshi [30] showed a prevalence of 31.8% of sexually transmitted disease among 135 participants and the frequencies were higher in case of ectopic pregnancy. Out of this, *Chlamydia Trachomatis* infection had a higher rate 27.4%, of frequency in ectopic pregnancy compared to other sexually transmitted disease. But the technique used for testing the Chlamydia infection was by NAAT and multiplex PCR. Also, study in Egypt stated that ELISA screening test which is noninvasive and simple method might be used with a high predictive value for detection of *Chlamydia Trachomatis* infection antibodies IgG among infertile tubal occlusion Egyptian women [31].

In the current study, history of positive abortion factor have a significant effect (P-value 0.03) with the ectopic pregnancy. This finding coincides with results of Park [32] study concluded that abortion history was correlated significantly with ectopic pregnancy in the South Korea by analyzing data of six years from 2009 to 2015. Ectopic pregnancy is result of pregnancy a complication instead of abortion medical treatment complications. Because of some unknown reasons it is remain unclear the ectopic pregnancy rate among early aborted pregnant women is much lower compare to all pregnancies rate1.9% in the United States [33].

5. CONCLUSION

Chlamydia Trachomatis has a significant relationship in the development of ectopic pregnancy and it is obviously elevated in women with ectopic pregnancy. Also, previous miscarriages are also more in patients with ectopic pregnancy therefore screening and treatment of Chlamydia infection may decrease the rate of ectopic pregnancy with low cost.

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