Microbiology Research Journal International

22(5): 1-12, 2017; Article no.MRJI.35552
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Chlamydiasis and Human Heat Shock Protein 60-KDa Expression among Women with Miscarriages in Sokoto Metropolis, North-Western Nigeria

T. H. I. Spencer[1], U. Yahaya[1], K. Mohammed[1*], D. E. Argbonlahor[2], A. E. J. Okwori[3], M. K. Garba[1] and S. U. Nataala[1]

[1]Department of Medical Microbiology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria.
[2]Lahor Research and Molecular Diagnostic Laboratory, Benin City, Nigeria.
[3]College of Veterinary and Medical Laboratory Technology, NVRI, Vom, Plateau State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors THIS and UY designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KM and DEA managed the analyses of the study. Authors AEJO, MKG and SUN managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2017/35552
Editor(s):
(1) Dharampal G. Dambhare, Associate Professor, Department of Community Medicine, Mahatma Gandhi Institute of Medical Sciences Sevagram, Wardha, India.
(2) Eliton da Silva Vasconcelos, Department of Physiological Sciences, Federal University of São Carlos – UFSCar, Rod. Washington Luiz, São Carlos, SP, Brazil.
(3) Giuseppe Blaiotta, Professor, Department of Agriculture, Division of "Grape and Wine Sciences", University of Naples Federico II, Via Universita' 100 – Palazzo Mascabruno 80055 Portici, Italy.
Reviewers:
(1) Anonymous, Jaypee University of Information Technology, India.
(2) Bernard China, Belgium.
(3) Balbina Plotkin, Midwestern University, USA.
(4) Kulvinder Kochar Kaur, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/22880

Original Research Article

Received 18th July 2017
Accepted 20th November 2017
Published 26th January 2018

ABSTRACT

Aims: The aim of this study was to determine the role of *C. trachomatis* and expression of human heat shock protein-60 in pregnancy loss among women with miscarriages in Sokoto Metropolis. North-western, Nigeria.
Study Design: Case-control study.
Place and Duration of Study: This study was conducted in Specialists Hospital and Maryam Abacha women and children hospital, Sokoto between June, 2015 to August, 2016.

**Corresponding author: E-mail: kmohd1970@yahoo.co.uk;*

Methodology: A total of ninety (90) subjects comprising of fourty five women with miscarriages and fourty five women without any history of miscarriage served as control were involved in the study. Antibodies to *C. trachomatis* (IgG) and human heat shock protein-60 were estimated using enzyme linked immunosorbent assay.
Results: The overall seroprevalence of *C. trachomatis* was 7.7%. The seroprevalence in the cases and control subjects were 11% and 4.4% respectively ($\chi 2$=0.6196, df=1, OR=2.688 (95% CI: 0.4930 to 14.65); p= 0.4312). The mean and standard error of mean of the Human heat shock protein 60 among the cases and control subject were 20.9 ± 0.8 and 23.2 ± 1.6(ng/mL).
Conclusion: In this present study, it was conclude that antibodies to *C. trachomatis* was detected more 5/45 (11.0%) in women with miscarriage than 2/45 (4.4%) in women without any history of miscarriage. Estimation of human heat shock protein-60 could serve as a marker to complement the laboratory diagnosis *C. trachomatis* including pregnancy loss.It was also suggested that women with previous exposure to *C. trachomatis* have a risk of miscarriage with an odd ratio of 2.688.Public awareness and preconception screening is advocated.

Keywords: Chlamydiasis; women with miscarriage; human heat shock protein 60-KDa; Sokoto metropolis; North-western Nigeria.

1. INTRODUCTION

Chlamydia trachomatis is one of the most common bacterial sexually transmitted infections worldwide and women are responsible for the majority of the disease burden. Miscarriages are a major public health problem affecting all regions of the world. According to the World Health Organization (WHO), 101 million chlamydial infections are detected annually worldwide [1].*Chlamydia trachomatis* is a gram-negative obligate intracellular bacterium of the genus Chlamydia. They have a unique biphasic development cycle, multiplying by binary fission. They contain both DNA and RNA, they have ribosome and contain enzyme system required for metabolism and they are inhibited in varying degree by antibiotics [2] *C. trachomatis* was first isolated from the eyes of babies suffering from Ophthalmianeonatorum by [3] and from the cervix by Collier in 1960 [2].

Chlamydia*. trachomatis* has a worldwide distribution, affecting both sexes, but has a much greater impact on females than on males [4]. It particularly affects young women and sexually active adolescents. Unfortunately, Chlamydial infections may, in many cases, remain silent [2]. Erythema, edema, and mucopurulent discharge can be seen by physical examination during acute infection [2]. Fifteen Serovars of *C. trachomatis* have been recognized*.* Serovars A, B, Ba and C infect mucosal epithelial cells in the conjunctivae and cause trachoma, the leading cause of infectious blindness worldwide [5]. Serovars D through K infects mucosal epithelial cells in the urogenital tract and the leading cause of sexually transmitted bacterial infections in the World. Serovars L1, L2, L2a, and L3 infect the genital epithelium as well as monocytes and cause a systemic disease called lympho granuloma venereum [6].

During its unique developmental cycle, two different forms were observed; elementary bodies which are infectious but not able to divide, and reticulate bodies which are metabolically active and able to multiply [5]. The elementary body of *C. trachomatis* attaches to the epithelial cell surface and incorporates into phagosomes that migrate to the distal region of the Golgi complex. Lysosome fusion is prevented, and chlamydial infection averts immediate destruction. The elementary body then differentiates into the non-infectious but replicative reticulate body, which further divides by binary fission [6].

Chlamydia genital infection is the most common bacterial infection sexually transmitted in the world [7]. The highest age-specific rate was reported in females aged 15 - 35 years. The majority (80%), of the infected women are asymptomatic, thus, providing a continuous reservoir for the infection [8].The risk factors for infection include amongst others, being young or adolescent, having new or frequent sexual contacts, inconsistent use of barrier contraception, history of prior sexual transmitted infection, and low educational and socioeco-nomic levels [7]. Thus, the prevalence of *C. trachomatis* may differ among racial groups because of differences in sexual risk behavior and cultural backgrounds [9].

Untreated Chlamydial infections may lead to Pelvic Inflammatory Disease (PID), ectopic pregnancy, premature delivery, spontaneous

abortion, low birth weight, tubal infertility and subsequent scarring of the fallopian tubes [10]. During pregnancy, Chlamydial infection can cause miscarriage, premature rupture of membranes, preterm labor, low birth weight, infant mortality, neonatal Chlamydial infection, and postpartum endometritis [11]. Screening, early diagnosis and treatment is considered the main policy to prevent complications and further transmission of the infection and women with abnormal results of screening but with normal karyotype are at risk for adverse subsequent pregnancy loss [12-14].

Miscarriage (pregnancy loss) is the spontaneous loss of a pregnancy before 12 weeks (early miscarriage) or from 12 to 24 weeks (late miscarriage) of gestation [15]. It is one of the most common yet under-studied adverse pregnancy outcomes. In the majority of cases the effects of a miscarriage on women's health are not serious and may be unreported. However, in the most serious cases, symptoms can include pain, bleeding and a risk of hemorrhage. Feelings of loss and grief are also common and the psychology and mental health of those affected can suffer [16].

Heat shock proteins (HSP) are a family of proteins that are produced by cells in response to exposure to stressful conditions. They were first described in relation to heat shock, and are named according to their molecular weight [17] and [50]. HSPs are found in virtually all living organisms, from bacteria to humans [50] and [51]. The 60 kilo-dalton (kDa) heat shock protein (HSP60) functions as a molecular intracellular chaperone, transporting peptides within the cell and preventing incorrect polypeptide folding and denaturation [17]. The *Chlamydia trachomatis* and human proteins share almost a 50% amino acid sequence homology [17]. Heat shock proteins 60 are among the first groups of proteins produced by the zygote after fertilization [18]. In addition, the maternal decidua also expresses heat shock proteins during the early stages of pregnancy [19]. Autoimmunity to heat shock proteins is not typically evident in healthy women of reproductive age. However, chronic microbial infection, such as asymptomatic *Chlamydia trachomatis* and upper genital tract infection results to prolonged exposure of the immune system to the microbial-derived 60 kDa heat shock protein (HSP60). This may result in immunity to conserved HSP60 epitopes and subsequent autoimmunity to self HSP60 [19].

Sensitization to this conserved region in CHSP60 can result in reactivation of previously tolerized HSP60-specific lymphocytes. This may, in turn, compromise fetal or maternal cell viability via the direct activity of anti-HSP60 antibodies and/or the accompanying pro-inflammatory response [20,19]. The chlamydial HSP60 shows an amazing analogy to human proteins. Thus, there is high possibility of cross-reaction between the human HSP60 and the bacterial HSP60. This reaction leads to the formation of antibodies against the HSP60 in the serum and follicular fluid of women exposed to *C. trachomatis* [21]. These antibodies exhibit negative impact on embryonic growth, and increase the likelihood of adverse pregnancy outcomes [22].

Laboratory diagnosis of *Chlamydia trachomatis* includes; isolation in appropriate cell lines, serology and nucleic acid or molecular detection. Serological investigation is the recommended method of diagnosing sequelae of the infection [13]. Treatment requires the use of antibiotics. Because of the unique intracellular nature of *C. trachomatis*, only certain antibiotics are effective in its treatment. Examples of such drugs are azithromycin, tetracycline and doxycycline. Clinically cure rate for doxycycline and azithromycin are 96-99% and 97% respectively. Doxycycline is contradicted in pregnancy [14]. The control and prevention measures against *C. trachomatis* infection include prompt diagnosis and treatment. More so, The USA Centre for Disease Control and Prevention (CDC) recommends condom use to reduce the spread of the infection [12].

2. MATERIALS AND METHODS

2.1 Sampling Technique

Purposive sampling technique was used in recruiting the subjects until the desired sample sized was attained [24].

2.2 Blood Sample Collection and Processing

Five (5) ml of blood samples were collected from subjects using standard veni-puncture phlebotomy. The blood was carefully and gently dispensed into sterile plain sample container. The tubes were labeled appropriately with participant's identification number. Sera from these blood samples were separated by allowing the blood to clot at room temperature then by centrifugation at 2500 rpm for 10 minutes.

Thereafter transferred into serum aliquot containers and stored at - 20℃ in chemical pathology laboratory, Usmanu Danfodiyo University Teaching Hospital, pending laboratory analyses. During these procedures, all form of work areal contamination, spills and aerosols was essentially minimized.

2.3 Ethical Issues for the Study

Ethical approval was sought and given from the Sokoto State Ministry of Health (SMH/1580/V.IV. Informed consent was obtained from all the subjects in accordance with the standards of human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. A structured questionnaire was used in sourcing relevant information. Phlebotomy did not pose any form of risk during and after sample collection. Study subjects personality and privacy were fully secured. All data was be analyzed anonymously throughout the study and Study participants bears no financial burden.

2.4 Study Population

The research was a hospital based study of women attending the study sites. Women with miscarriages served as the case subjects and women without any history of miscarriage served as control subjects. The study was conducted between June, 2015 to August, 2016.

2.5 Control Subjects

Women going to the study sites for post-natal clinic that have at least given birth were enrolled as controls. Subject with history of ectopic pregnancy, preterm birth, with chronic diseases (cancer, diabetes and hypertension), HIV and tuberculosis were excluded as control subjects. The control subjects were recruited From June, 2015 to August, 2016.

2.6 Inclusion and Exclusion Criteria

Inclusion criteria are as follows:

A. Patients presented with previous miscarriages.
B. Those that will agree to voluntary participation.

Exclusion criteria for the study subjects:

The exclusion criteria are as follows:

A. Those that will not consent to voluntary participation.
B. Subjects with chronic diseases (Cancer, diabetes, hypertension, atherosclerosis, leukaemia, malaria)
C. Patients with Tuberculosis and Human Immunodeficiency Virus infection.

2.7 Sample Size Determination

The minimum number of subjects that participated in the study was determined by the formulae below according to [24].

$$n = \frac{r+ 1P^* (1- P^*) (Z_{\beta} + Z_{\alpha/2})^2}{r (P_1 - P_2)^2}$$

According to a study by [22], the prevalence of *Chlamydia trachomatis* exposed among women with miscarriage and those without miscarriage is 67.3% and 36% respectively

Z_{β}= Power 80% = 0.84
$Z_{\alpha/2}$= For 0.05 significance level, for 95%= 1.96
P_1= Proportion exposed in the cases=67.3%= 0.673
P_2= Proportion exposed in the controls=36.0%= 0.36

Effect size = $P_1 - P_2$
=0.67.3-0.36
=0.313

$$n = \frac{(1+1)}{1} \frac{(0.313)(1-0.313) (0.84+1.96)^2}{(0.313)^2}$$
= 34

The minimum Number of subjects in the cases is 34.

Since the ratio of control to cases is 1, the minimum number of controls will be 34 also.

However, a total of fourty five (45) each of cases and control was enroll in to the study making a total of ninety (90) samples or participants.

2.8 Principle and Laboratory Procedures

2.8.1 Determination of serum *Chlamydia trachomatis* IgG antibodies

2.8.1.1 Principle of test

Purified *Chlamydia Trachomatis* antigen is coated on the surface of micro-wells. Diluted patient serum is added to wells, and the

Chlamydia trachomatis IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB Chromogenic Substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG specific antibody present in the sample. The results are read by a standardized micro-well reader compared in a parallel manner with calibrator and controls.

2.9 Procedure

One in ten (1:10) dilutions of the sera was prepared by adding 10 µl of the test samples to 1.0 ml of sample diluents. 100 µl of diluted sera, calibrator, and controls was dispensed into the appropriate wells. The mixture was incubated for 30 minutes at room temperature. the mixture was washed three times with washing buffer. 100 µl of enzyme conjugate (peroxidase-labelled antihuman IgG) was dispense to each well and incubate for 30 minutes at room temperature. Excess conjugate was washed three times with washing buffer. TMB Chromogenic Substrate (100 µl) was dispence to each well and incubated for 15 minutes at room temperature. Stop solution (100 µl) was added to stop the reaction. The absorbance or Optical Density was read at 450 nm with a standardized micro-well reader.

2.10 Interpretation of Results

<10RU/ml: negative, ≥10 to <12 RU/ml: borderline, ≥13 RU/ml: positive [49].

2.11 Quantification of Human Heat Shock Protein-60

2.11.1 Source of the kits

E-EL-H1862–HSP60 Elabscience Biotechnology Company, Limited

2.12 Principle of the Assay

This ELISA uses sandwich-ELISA. The microtitre plate was pre-coated with an antibody to specific to HSP-60. Standards or samples added to the appropriate wells and combined with the specific antibody, then the biolanated detection antibody specific for HSP-60 and avidin-Horseradish peroxidase conjugate is added to each well and incubated. Free components are wash away. The substrate solution is added to each well. Only those wells that contain HSP-60, biolanated detection antibody and avidin-HRP will appear blue in colour. The enzyme reaction is terminated by the addition of a sulphuric acid solution and colour turns yellow. The optical density is measured spectrophometically at a wavelength of 450 nm [25].

2.13 Procedure

All reagents were allowed to attain room temperature before used. The assay was performed at room temperature (20-25℃). 100 µl of Human HSP60 Standard, blank and sample was added in to their respective wells. It was incubated at 37° for 90 minutes by covering the wells with a sealing tape. After incubation the liquid was removed. Biotinylated antibody was added (100 µl) and incubated at 37^{o} for one hour. It was Washed five times with 200 µl of Wash Buffer manually. 100 ul of enzyme conjugate working solution was added to the wells and incubated for 30 minutes at 37°. it was washed five times. 90 µl of substrate was added to the well-sand incubated for 15 minutes. The reaction was stop by adding 50µl of stop solution to each well. The colour changed from blue to yellow. The absorbance was read on a microplate reader at a wavelength of 450 nm immediately. The absorbance and concentration was recorded from the microtiter plate reader in ng/ml [25].

2.14 Statistical Analysis

Results generated from study was analyzed using Statistical Package for Social Sciences SPSS version 20, California Inc.USA. Data was reported as mean ± SD (for continuous variables). Numbers and percentages (for categorical variables). Continuous variables was compared between case and control subjects using Student's t test, while categorical variables was compared using chi-square test .One way analysis of variance was used for continuous variable of the mean and standard error of mean of the MDA, TAC and HSP-60 at 95% confidence interval (95% CI) between study groups. Odd ratio (OR) was determined using two by two contingency table. The various tests were carried out as two-tailed and outcomes with probability value below 0.05 was considered to be statistically significant.

3. RESULTS

Results of the IgG antibody testing for *Chlamydia trachomatis* on the 45 cases with miscarriage

and 45 control subjects without miscarriages are summarized in Table 1. The study findings shows that the overall sero-prevalence of *Chlamydia trachomatis* was 7.7% while the Antibodies to *C. trachomatis* was detected in 5/45 (11%) of the cases and in 2/45(4.4%) in controls subjects ($\chi 2$=0.6196, df=1, OR=2.688 (95% CI: 0.4930 to 14.65); p= 0.4312) as shown in (Table 1).

Fig. 1, shows the distribution of scatter plot Heat shock protein-60 KDa among group of women with miscarriage and control group without miscarriage. The result of the scatter plot shows no statistically significance (p= 0.2057) result of HSP 60 KDa values among the study population and control group.

Fig. 2, shows the distribution of scatter plot Heat shock protein-60 KDa among group of women with miscarriage and control group without miscarriage with respect to the presence or absence of antibodies to *Chlamydia trachomatis*. An error bar and mean showing HSP 60 values among the cases and control group. The result of the scatter plot shows no statistically significance (p= 0.1425) result of HSP 60 values among the study subjects and control group.

The mean age and standard deviation of the cases and control subjects were 27.2 ± 7.5 and 29.0 ± 5.7 and student t test shows no significant difference between the ages of the study subject (p>0.05), this indicates that both the cases and control subjects in terms of age was not associated with the presence of antibodies to *C. trachomatis* infection (Table 2).

The mean and standard deviation of their body mass index (BMI) were 23.3 ± 1.9 and 25.3 ± 2.0 respectively. Statistical analysis shows a significant decrease in BMI of the women with miscarriage compared to control subjects (p<0.05). Based on educational status majority of women with miscarriages had only Quranic education 27 (60%) and 2 (26%) had no any form of education. 13 women in the control group had tertiary and secondary education had 13 each (29%) and 9 had primary education. The chi-square of trend shows a significant relationship between socioeconomic status and miscarriage (Table 2).

The mean and standard error of mean concentration of the human heat shock protein-60KDa was 20.9 ± 0.8 and 23.2 ± 1.6 (ng/mL) among the cases and controls respectively. Higher in the controls than in the cases but not statistically significant (p>0.05) as shown in (Fig. 1).

The mean and standard error of mean of HSP-60 among women with miscarriage and controls with previous exposure to *Chlamydia trachomatis* and without antibodies to *Chlamydia trachomatis* are 23.8 ± 3.2, 20.6 ± 0.8, 13.0 ± 5.0 and 23.6 ± 1.6 respectively. The HSP-60 was higher in the in the miscarriage group with positive antibodies to *Chlamydia trachomatis* followed by control group without antibodies to *Chlamydia trachomatis* and was lowest among the control group with positive antibodies to *Chlamydia trachomatis*, however, despite this differences the one way analysis of variance (one way ANOVA) shows no significance P>0.05 as shown in (Fig. 2).

Table 1. Prevalence of antibodies to *Chlamydia trachomatis* in cases compared with control subjects

	Cases n %	Control n %	Total N %
Positive	5 (11.1%)	40 (88%)	45 (50.0)
Negative	2 (4.44%)	43 (95%)	45 (50.0)
Total	7 (7.77%)	83 (92%)	90 (100)

$\chi 2$=0.6196, df=1, OR=2.688 (95% CI: 0.4930 to 14.65); p= 0.4312

Table 2. Prevalence of mean age and standard deviation of their body mass index BMI and socio-economic status to *Chlamydia trachomatis* in cases compared with control subjects

	Mean age (yrs) n %	BMI n %	P-value	Socio-economic	
				Education n %	No Educ. n %
Cases	27.25 ± 7.5	233 ± 1.9	>0.05	27 (60.0)	2(26.0)
Control	29.0 ± 5.7	25.3 ± 5.7	>0.05	13 (29.0)	9(20.0)
Total	56.5 ± 13.2	48.6 ± 7.6		40(89.0)	11(46.0)

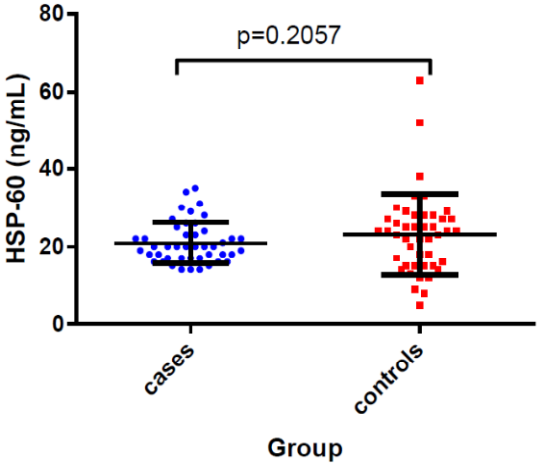

Fig. 1. Heat Shock Protein-60 distributions among group of women with miscarriage and control group (without miscarriage). A scatter plot showing HSP-60 values among the study population and control subjects

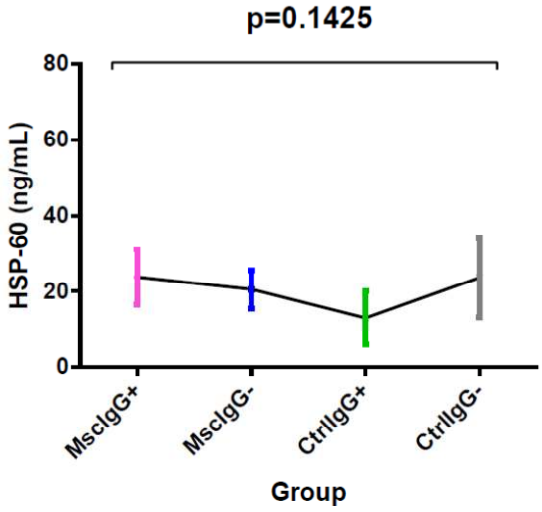

Fig. 2. Heat Shock Protein-60 distributions among group of women with miscarriage and control group (without miscarriage) with respect to the presence or absence of antibodies to *C. trachomatis*. An error bar and mean connected showing HSP-60 values among the cases and control subjects

4. DISCUSSION

This study aimed to determine the role of *C. trachomatis* and expression of human heat shock protein-60 in pregnancy loss among women with miscarriages in Sokoto Metropolis. North-western, Nigeria. Findings from this study shows no significant difference between the ages of the study participants and this implies that the subjects were sampled from the same area. However, there was significant difference between Body Mass Index of participants. This implies that high body mass index is a significant risk factor for miscarriage [26]. Absence of formal education and lower socioeconomic status were associated with spontaneous abortion ($p<0.05$). This could be due to fact that formal education and lower socioeconomic status are indicators that determine the health status of individuals [27].

4.1 Association of *C. trachomatis* Antibodies (IgG) and Miscarriage

Findings from this study revealed an overall seroprevalence of Chlamydia *trachomatis* was 7.7%, while the prevalence in the cases and controls subject were 11.0%and 4.4% respectively. The presence of IgG antibodies against *C. trachomatis* seen in the control subject of this study may be attributed to the genetic make up of such women because it has been shown that women with functional polymorphisms in the gene coding for Human Leucocytes Antigen DQ, mannose-binding lectin, an innate immune system antimicrobial component as well as other genes with immune, are at increased likelihood to develop sequelae after being infected with *C. trachomatis* [28,29]. Previous reports demonstrated seroprevalence of *Chlamydia trachomatis* in case-control studies to be 22.9% and 11.9% by [30] 25.4% and 5.20% by [31] and 21.3% and 6.5% by Arsonic in Serbia respectively [32-34]. The lower prevalence obtained in this study compared to previous studies may be due to the relatively lower sample size used. However, another outcome was obtained by Baud et al. [35] who showed higher anti-*Chlamydia trachomatis* IgG prevalence in the miscarriage group of patient suffering from first trimester miscarriage (15.2%) and lower prevalence in the control subjects (7.3%, $p<0.018$) [29]. The association between *Chlamydia trachomatis*-positive serology and miscarriage remained significant after adjustment for age, origin, education level, and number of sexual partners (OR 2.3, 95% CI 1.1-4.9). *Chlamydia trachomatis* DNA was more frequently amplified from products of conception or placentas taken from women who had suffered miscarriages (4%) than from controls (0.7%, $p<0.026$) [36]. Visnovsky et al. [36] reported from a study 131 (41.46%) pregnancies ended with miscarriage in the first trimester, in which 37 (11.7%) in the group with positive cultivation of *C. trachomatis*.

However, several studies failed to document an association between *C. trachomatis* and spontaneous or recurrent miscarriage Nevertheless; [37] reported a contrasting association i.e. anti-chlamydia IgG positivity was not significantly associated patients with recurrent abortions (24.5%) and the control group (20.3%) [38]. Paukku et al. [38] also described the absence of correlation between IgG antibodies to *C. trachomatis* and recurrent pregnancy loss. Antibodies were detected in women who experienced abortions as often as in those in the control group and as often as in women with 1 or 2 abortions in the past. IgG anti-chlamydial antibodies were also detected with similar frequency in the study group and the comparative group in a study conducted by [39]. In women who experienced miscarriage due to *C. trachomatis* infection was confirmed in 137/349 (39.3%) and in 116/349 (33.2%) respectively. Similarly, [40,41] also did not support the relationship between cervical Chlamydial infection and the development of spontaneous abortion. The infection was detected by Ligase Chain Reaction method in women with abortion in anamnesis more rarely than in the control group in 3.8% and 8.5% respectively. However, these studies were conducted more than 20 years ago, before the recent dramatic increase in the prevalence and incidence of *C. trachomatis* infection. Because of improved statistical power, such increased prevalence might indicate an association between *C. trachomatis* infection and adverse pregnancy outcomes. Secondly, sensitivity and specificity of diagnostic methods have also improved during the past decade. We use serology in this study because from the literature reported no significance difference between the use of culture, molecular methods and serology in determining association of *C. trachomatis* and spontaneous abortion (55%, 42% and 56% respectively). And the best diagnostic methods in assessing the sequelae of *C. trachomatis* is the use of serology. This is because *C. trachomatis* is an obligate intracellular organism that affect the urogenital tract and 75% of the infection is asymptomatic. Even when the symptoms appear they often go unnoticed, hence undiagnosed and no proper treatment. So at this stage diagnosing *C. trachomatis* DNA will be difficult [42]. Another reason for the differences observed may be due to immunogenetics, genetic studies of individual immunopathogenetic factors have suggested that a single nucleotide polymorphism in inflammation-associated NLRP3 is related to the severity of *C. trachomatis* infection [43]. Early pregnancy loss or recurrent pregnancy loss may be induced by asymptomatic Chlamydial infection through the operation of immune mechanism since both epidemiologic and experimental studies have suggested that Chlamydial infection during pregnancy poses a risk for adverse outcomes. The mechanism by which Chlamydial infection may lead to adverse outcomes in pregnancy is not well understood. It is thought that *C. trachomatis* may infect the fetus, triggering a harmful inflammatory response with cytokine release leading to miscarriage, premature rupture of membranes, or preterm labor or possibly causing a maternal inflammatory response that induces embryonic rejection due to homology of the chlamydial and human 60 kDa heat shock proteins [44];[35] ; [38].

4.2 Human Heat Protein 60-KDa, *C. trachomatis* and Miscarriage

The idea that humoral immunity to *C. trachomatis* heat shock protein lead to miscarriage came from a study in which Women who are infertile due to occluded fallopian tubes could be pregnant by undergoing in vitro fertilization and embryo transfer, a process that bypasses the need for the ovum to be fertilized in the fallopian tube and pass through to the uterus. However, several studies have demonstrated that the human HSP60 is expressed during early embryo development and that an immune response that recognizes the human HSP60 is detrimental to successful pregnancy [19]. From this study, the human heat shock protein 60 KDa was just a preliminary study to determine the expression of HSP 60 in cases and control and in women who have antibodies to *C. trachomatis* in cases and control group whose HSP60 was little bit higher in control subjects that in the cases but is not statistically significant this may result other confounding variables since many conditions such as elevated temperature, stress, radiations, infections inflammations, toxins, are known to induce the expression of HSP60 [17]. However, other studies demonstrated the expression of Human HSP60 on the surface of epithelial cells in the human decidua during early pregnancy [45]. Interestingly, human HSP60 is expressed more in miscarriage group with positive antibodies to *C. trachomatis*, in the miscarriage group. A murine hybridoma specific for HSP60 was shown to react with human trophoblast, suggesting a cell surface location for HSP60 on these cells [46]. A monoclonal antibody generated against the human HSP60 blocked the

in vitro development of mouse embryos [47]. Thus, prolonged exposure to the chlamydial HSP60 as a consequence of a persistent asymptomatic upper *C. trachomatis* infection may result in sensitization to conserved HSP60 epitopes that are also expressed in the human HSP60. Subsequent expression of the human HSP60 during early pregnancy, by the embryo and/or maternal decidua, will lead to reactivation of HSP60-sensitized lymphocytes. Pregnancy outcome may subsequently be compromised by the direct impairment of fetal or maternal cell viability by anti-HSP60 antibodies and/or induction of a pro inflammatory response by HSP60-sensitized lymphocytes. An additional potential mechanism of HSP60-related early stage pregnancy loss has recently been identified. Chlamydial HSP60 binds to Toll-like receptor 4 on trophoblasts and induces apoptosis of the embryo and subsequently leading to spontaneous abortion [48]. We observed a higher expression of HSP-60KDa in *Chlamydi*a positive miscarriage patients and lowest in the control group with antibodies to *C. trachomatis*, followed by women in the control group without antibodies to *C. trachomatis* observed in this study could be probably be due to other confounding variables that may triggered their expression since our exclusion criteria cannot guaranteed total elimination of confounders [49]. Thus, immune sensitization to HSP60 as a consequence of a previous genital tract infection by *C. trachomatis* as well as possibly by other microorganisms may result in induction of anti-HSP60 antibody production in response to placental HSP60 expression [50], Antibody binding to human HSP60 in placenta may activate a pro-inflammatory immune response thereby triggering the sequence of events that induce myometrial contractions and preterm labor and delivery [19,51]..

5. CONCLUSION

In this present study, it was conclude that antibodies to *C. trachomatis* was detected more 5/45 (11.0%) in women with miscarriage than 2/45 (4.4%) in women without any history of miscarriage. Estimation of human heat shock protein60 could serve as a marker to complement the laboratory diagnosis of *Chlamydiasis.* Including pregnancy loss.

6. RECOMMENDATION

Findings from this study suggest that all women experiencing a miscarriage should be screened for *C. trachomatis* infection and, if positive, adequately treated to prevent recurrent miscarriages. Moreover, preconceptional screening might be proposed to reduce the prevalence of this adverse pregnancy outcome. Public awareness of the possible risk of *C. trachomatis* infection to a future pregnancy might be helpful. Intending couples are advice to be screen for *C. trachomatis* and treat accordingly to prevent possible development of adverse pregnancy outcome.

7. STUDY LIMITATION

This work was restricted to *Chlamydia trachomatis*, human heat shock protein 60 and biomarkers of oxidative stress as other microbial aetiologies of miscarriage e.g *Herpes, Cytomegalo virus*, *Toxoplasma, Mycoplasmas, Bacterial Vaginosis*e. t.c were not determined due to limited resources. Also our exclusion criteria might have not eliminated all the confounding variables.

ACKNOWLEDGEMENTS

The authors wish to thank Ministry of Health Sokoto State for granting permission to carry out this study. Specialist Hospital Sokoto and Maryam Abacha Women and Children Hospital are gratefully acknowledged. Special thanks are due to Dr. T.H.I Spencer, Dr. Kabiru Mohammed PG Co-ordinator, School of Medical Laboratory Sciences, UDUS as well as staff of the specialist Hospital, Sokoto and Maryam Abacha for excellent technical assistance. We are also grateful to all subjects and control participants for their collaborations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. Global prevalence and incidence of selected curable sexually transmitted diseases: Overview and estimates; 2011.
2. Manavi K. A review on infection with *Chlamydia trachomatis*. Best practice Res. Clinical Infectious in Obstetrics and Gynecology. 2006;120:941-951.
3. Jauniaux E, Watson AL, Hempstock J, Bao YP, Skepper JN, Burton GJ. Onset ofmaternal arterial blood flow and placental

oxidative stress. A possible factor in human early pregnancy failure. American Journal of Pathology. 2000;157:2111–2122.
4. Malhotra M, Seema S, Anjan M, Sumathi M.andManju B. Genital *Chlamydia trachomatis*: An update. Indian Journal of Medical Research. 2013;138:303-316.
5. Peipert JF. Clinical practice. Genital Chlamydial infections. New England Journal of Medicine. 2003;349(24):24-2430.
6. Linhares MI, Witkin SS. Immunopathogenic consequences of *Chlamydia trachomatis* 60 kDa heat shock protein expression in the female reproductive tract. Cell Stress and Chaperones. 2010;15(5):467–473.
7. Kalwij S, Macintosh M, Baraitser P. Screening and treatment of *Chlamydia trachomatis* infections. British Medical Journal. 2010;340:1915-1918.
8. Malhotra M, Seema S, Anjan M, Sumathi M, Manju B. Genital *Chlamydia trachomatis*: An update. Indian Journal of Medical Research. 2013;138: 303-316.
9. Shima A, Rezvan M, Mansoreh S, Ahmad, K, Seyyed G, Abbas Mousavi. The prevalence of endocervical *Chlamydia trachomatis* infection among young females in Kashan. Iran Jundishapur Journal of Microbiology. *2015;*8(4):15576-15580.
10. Malik J, Adow M, Marnette LJ. Generation of mutagens duringarachidonic acid metabolism. Cancer Metastastics Review, 2006;13:303-308.
11. Manavi K. A review on infection with *Chlamydia trachomatis*. Best practice Res. Clinical Infectious in Obstetrics and Gynecology. 2006;120:941-951.
12. Mardh PA. Influence of infection with *Chlamydia trachomatis* on pregnancy outcome, infant health and life-long sequelae in infected offspring. Best Practice. Res. Clinical. Obstetrics and Gynaecology. 2002;16:847-864.
13. Max A, Chernersky S. The laboratory diagnosis of *Chlamydia trachomatis* infections. Canadian Journal of Infectious Disease and Medical Microbiologyl. 2005; 16(1):39-44.
14. Low M, Allanson B, Jennings B, Jacques A, Charles AK, Keil AD, Dickson JE. Infection and fatal loss in the mid-second trimester of pregnancy. Australian New Zealand Journal of Obstestrics and Gynaecology. 2009;50:221-225.
15. Regan L, Rai R. Epidemiology and the medical causes of miscarriage. Baillieres Best. Pract. Res. Clin. Obstet Gynaecol. 2000;14:839-854.
16. Engelhard IM, Vanden-Hout MA, Arntz A. Posttraumatic stress disorder after pregnancy loss. Psychiatry. 2001;23:62–66.
17. Chandra A, Surti N, KesavanSh AA. Significance of oxidative stress in human reproduction. Archieves Medical Science. 2009;5(1A):S28-S42.
18. Witkin SS. Immunity to heat shock proteins and pregnancy outcome. Infectious Diseases in Obstetrics and Gynecology. 1999;7(1):35–38.
19. Linhares MI, Witkin SS. Immunopathogenic consequences of *Chlamydia trachomatis* 60 kDa heat shock protein expression in the female reproductive tract. Cell Stress and Chaperones. 2010;15(5):467–473.
20. Wilkowska T, Kuo CC, Barnes RC, Stephens RS, Grayston JT. Immunotyping of Chlamydia *trachomatis* with monoclonal antibodies. Journal of Infectious Disease. 2009;152:791-800.
21. David B, Vincent T, Aliaa A, Lesley R, Gilbert G. Arole for *Chlamydia trachomatis* in miscarriages. Clinical Microbiology. 2012;17:1321-1322.
22. Jozef V, Kristina Biskupska B, Barbora C, Erik K, Karol D. Early fetal loss and chlamydia *trachomatis* infection. Gynecology and Obstetrics. 2013; 3(5):181-188.
23. United nation population found. Sokoto state population projection, UNFPA, Nigeria; 2013.
24. Charan J, Biswas T. How to calculate sample size for different study designs in medical research. Indian Journal Psychology and Medicine. 2013;35(2): 121–126.
25. Magen D. Estimation of antibodies to human heat shock protein-60 in human serum. American Journal of Human Genetics. 2008;83(1):30-42.
26. Sevi G, Nick W, Kate C, Gary E, Sarah EM, Howie, Andrew WH. The role of infection in miscarriage human reproduction. Update. 2015;1(3):1–18.
27. Chen MY, Fairley CK, De-Guingand D, Hocking JS, Tabrizi S, Wallace EM. Screening pregnant women for Chlamydia:

What are the predictors of infection? Sexually Transmitted Infections. 2008; 85(1):31-35.

28. Nester EW, Denise GA, Evans CR, Nancy NP, Martha TN. Microbiology: A human perspective. 4th ed. New York: McGraw-Hill companies, Inc. 2010;646-658.
29. Baud D, Greub G. Role of *Chlamydia trachomatis* in miscarriage. Emerging infectious diseases. 2011;17:1630–1635.
30. Amjad A, Khodabandehloo M, Ramazanzadeh R, Farhadifar F, Roshani D, Ghaderi E. The relationship between *Chlamydia trachomatis* genital infection and spontaneous abortion. Journal of Reproduction and Infertility. 2016; 17(2):110-116.
31. Abdolreza SJ, Farjam M, Mogharrab F, Amiryan M, Makiani J. *Chlamydia trachomatis* and Rubella antibodies in women with full term deliveries and women with abortions. American Journal of Infectious Diseases. 2010;6(3):66-69.
32. Arsonic A, Nikolov A, Sazdanomic P, Popovic S, Baskic D. Prevalence and diagnostic significance of specific of specific IgA and anti-heat shockprotein-60 *Chlamydia trachomatis* antibodies in subfertile women. European Journal of Clinical Microbiology and Infectious Diseases. 2014;33:761-766.
33. Baud D, Greub G. Role of *Chlamydia trachomatis* in miscarriage. Emerging Infectious Diseases. 2011;17:1630–1635.
34. Geisler A, Seafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: *A* critical review and experimental data. Free Radical Biology and Medicine. 2004;2(1):1106-1114.
35. Baud D, Regan L, Greub G. Emerging role of chlamydia and chlamydia-like organisms in adverse pregnancy outcomes. Current Opinion in Infectious Diseases. 2008; 21:70–76.
36. Vinowsky P, Tapia A, Zacharias S, Riquelme R, Salgado AM, Varleta J. First-trimester pregnancy loss and active *Chlamydia trachomatis* infection: correlation and ultrastructural evidence. Andrologia. 2013;34(6):373-378.
37. Rae R, Smith IW, Liston WI, Kilpatrick D C. Chlamydial serologic studies and recurrent spontaneous abortion. American Journal of Obstetrics and Gyneacology. 1994;170: 782-785.
38. Paukku M, Tulppala M, Puolakkainen M, Ohashi J, Naka I. Lack of association between serum antibodies to Chlamydia *trachomatis* and a history of recurrent pregnancy loss. Fertility and Sterility. 1999;72:427-430.
39. Osser S, Persson K. Chlamydial antibodies in women who suffer miscarriage. Brazillian Journal of Obstetrics and Gyneacology. 1996;103:137-141.
40. Sozio J, Ness RB. Chlamydial lower genital tract infection and spontaneous abortion. Infectious diseases in Obstetrics and Gyneacology. 1998;6(1):8-12.
41. Ness RB, Grisso JA, Hirschinger N, Markovic N, Shaw LM, Day NL, Kline J. Cocaine and tobacco use and the risk of spontaneous abortion. New England Journal of Medicine. 2006;340(5):333–339.
42. Max A, Chernersky S. The laboratory diagnosis of *Chlamydia trachomatis* infections. Canadian Journal of Infectious Disease and Medical Microbiologyl. 2005; 16(1):39-44.
43. Wang SP, Kuo CC, Barnes RC, Stephens RS, Grayston JT. Immunotyping of *Chlamydia trachomatis* with monoclonal antibodies. Journal of Infectious Diseases. 2009;152:791-800.
44. Witkin SS, Zdrodowska S, Ostaszewska PR. Immunity to heat shock proteins and pregnancy outcome. Infectious Diseases in Obstetrics and Gynecology. 1997;7(1)2: 35–38.
45. Neur A, Spandorfer SD, Giraldo F, Dieterle S, Rosenwaks Z, Witkin SS. Role of heat shock proteins in reproduction. Human Reproduction Update. 2000; 6(2):149-159.
46. Haggerty CL, Gottlieb SL, Taylor BD, Low N, Xu F, Ness RB. Risk of sequelae after *Chlamydia trachomatis* genital infection in women. Journal of Infectious Diseases. 2010;201(2):S134-155.
47. Neuer AS, Spandorfer P, Giraldo C, Mele HC, Liu K, Marzusch D, Kneissl, Witkin SS. Immune sensitization to the 60 kD heat shock protein and pregnancy outcome. Infectious Diseases in Obstetrics and Gynecology. 1998;5:154-157.
48. Equils O, Danning LM, Mary GS, Witkin CB. *Chlamydia trachomatis* heat shock protein 60 induces trophoblast apoptosis through toll likr receptor 4. Journal of Immunology. 2000;177:1257-1263.
49. Linhares MI, Witkin SS. Immunopathogenic consequences of *Chlamydia trachomatis* 60 kDa heat shock

protein expression in the female reproductive tract. Cell Stress and Chaperones. 2010;15(5):467–473.
50. Lindguist S, Craig EA. The heat shock proteins. Annual Review of Genetics. 1988;22:1:631-677.
51. Tiwari S, Thakur R, Shankar J. The role of heat shock proteins in cellular function and in the biology of fungi. Biotechnology Research International. 2015;Article ID 132636,11:2015.
DOI: 10,1155/2015/132635

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/22880