

# Appraisals of Sero-detection of Treponema pallidum Antibodies IgG and IgM and CBC Parameters among Spontaneous Recurrent Miscarriage in Women- Case-Control Study in Gezira State 2018

Alaa Abdelgadir Mohamed El-Hag<sup>1\*</sup>, Nadir Abuzeid<sup>2</sup>

<sup>1</sup>Department of Microbiology, Al-Neelain University, College of Postgraduate Studies, Faculty of Medical Laboratory Science, Khartoum, Sudan.

<sup>2</sup>Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Omdurman Islamic University, Omdurman, Sudan.

\*Correspondence to: Alaa Abdelgadir Mohamed El-Hag (E-mail: alsanosi.ahmed@gmail.com)

(Submitted: 18 November 2021 – Revised version received: 06 December 2021 – Accepted: 17 December 2021 – Published online: 26 March 2022)

## Abstract

**Objectives:** We determined Sero-detection of Treponema pallidum (IgG and IgM antibodies) using ELIZA techniques women with recurrent Miscarriage in Gezira state and appraisal of other risk factors.

**Methods:** In this case-control, a hospital-based study conducted at Wad Madani teaching hospital Department of Obstetrics & Gynecological, Gezira state, Sudan. Ninety subjects were involved, 45 were women with recurrent Miscarriage, and controls were healthy pregnant women (no miscarriage). Serum Treponema pallidum antibodies were estimated by the ELIZA method.

**Results:** Sero-detection of IgG and IgM antibodies by using ELIZA techniques, a total of 45 miscarriage women (cases) for IgM 6(13.3%) were positive, and 39(48.8%) were negative for Treponema pallidum by using ELISA techniques. A total of 45 non-miscarriage women (control) for IgM 4(8.9%) were positive, and 41(91.1%) were negative for Treponema pallidum by using ELISA techniques. A total of 45 miscarriage women (cases) for IgG 13(28.9%) were positive, 32(71.1%) were negative. Furthermore, IgG for non-miscarriage, like IgM 4(8.9%), was positive, and 41(91.1%) were negative.

**Conclusion:** Higher prevalence of Treponema pallidum IgG seropositivity among pregnant women who reported miscarriages compared to those who did not report miscarriages ( $P < 0.001$ ) while no association between IgM seropositivity and pregnant women who reported miscarriages.

**Keywords:** Treponema pallidum, antibodies, recurrent miscarriage women, ELIZA

## Introduction

Treponema pallidum (T.P.) is a spirochete bacteria and the major class of clinical importance which originates syphilis. Pathogenicity of treponemes is found in the injuries of syphilis.<sup>1</sup> Treponema is remarkably contagious, and saprophytic treponemes can be found on mucous layers in the lips, genital tract, and surface ulcers. Gained syphilis via communication congenitally or sexually has primary, secondary, or tertiary stages.<sup>2</sup> In congenital syphilis, an untreated mother with syphilis taints her embryonic fetus. The treponemes transfer through the placenta in the blood.

T.P. is a spirochete bacteria and the major class of clinical importance which originates syphilis. Pathogenicity of treponemes is found in the injuries of syphilis.<sup>1</sup> Treponema is remarkably contagious, and saprophytic treponemes can be found on mucous layers in the lips, genital tract, and surface ulcers. Gained syphilis via communication congenitally or sexually has primary, secondary, or tertiary stages.<sup>2</sup> In congenital syphilis, an untreated mother with syphilis taints her embryonic fetus. The treponemes transfer through the placenta in the blood. The embryo is infected with T.P. that can obscure early pregnancy. However, certain risk factors linked with a high prevalence of syphilis include maternal age, husband's occupation, late antenatal care, ignorance, lay-off, habitual drug use, husband's accustomed drug use, husband's extramarital relation, and un screened blood transfusion.<sup>3,4</sup> The true load of syphilis in our country is unknown. The main goals are that the mothers are mostly asymptomatic or have non-specific symptoms, lack of awareness about such diseases, inadequate facilities for screening tests or high cost, poor

access to a health facility and nonexistence of surveillance systems.<sup>5,6</sup> Several previous studies reported a high prevalence of Treponema pallidum and women miscarriage. Miscarriages or Spontaneous abortion (S.A.) is defined as the loss of fetal product before 20 weeks of gestation.<sup>7</sup> Miscarriage commonly occurs in about 25% of pregnancies, usually in the first 12 weeks of pregnancy. Ten percent to 15% of clinically recognized pregnancies end Recurrent in South Africa, and total pregnancy loss is estimated to 30% to 50% of all conception.<sup>8</sup> Recurrent Miscarriage is a critical problem, and it takes to increase during the current decade. The present study was conducted to estimate the prevalence and associated risk factors for Treponema pallidum infection in women with recurrent miscarriages attending Gyn & Obs Clinic of Wad Madani teaching hospital, Al-Gezira state, Sudan, with a history of recurrent miscarriages.

## Methods

This case-control study was conducted at the Gyn & Obs clinic of Wad Madani teaching hospital, Al-Gezira state, Sudan. Ninety women in each arm of the study have over 80% power to detect a difference of 5% at  $\alpha = 0.05$ . We assumed that 10% of the women might have incomplete data or samples.

## Data Collection

The patients were interviewed using a structured questionnaire to collect the bio-data and history of patients. General physical examination was made with the help of a lady doctor, and blood samples were obtained for serological analysis.

## Blood Sample

Approximately 5 ml Blood samples were drawn using a disposable syringe through vein puncture technique from vein. Approximately 2.5 ml of blood was transferred into an aliquot containing EDTA immediately after collecting a blood sample for hematological analysis. The remaining blood was transferred into the plain container, allowed to clot, centrifuged, and kept at  $-20$  until serological analyses in the Central Research laboratory. Complete blood count (CBC) was calculated by using hematological analyzer (Sysmex-XP 300) Manufacturer Company. The specimens were analyzed to detect *Treponema pallidum* IgG and IgM antibodies by commercially available enzyme-linked immune sorbent assay *Treponema pallidum* IgG and IgM ELISA kit chemux Bioscience, INC America this company can use Euro immune. The tests were performed as instructed by the manufacturer. The reagents have positive and negative controls were already used solution that specific for *Treponema pallidum*. Results of cutoff of *Treponema pallidum* index more than 1.0 IU/ml considered as positive result and cutoff of *Treponema pallidum* indexes less than 1.0 IU/ml considered a negative result.

The collected data were analyzed using SPSS and double-checked before analysis. Means and proportions of the socio-demographic and clinical characteristics were calculated for *Treponema pallidum* seropositive groups. Univariate and multivariate analyses were used for *Treponema pallidum* IgG and IgM seropositive groups as dependent and socio-demographic and obstetrics variables as independent variables. Odds ratio (OR) with 95% confidence interval was calculated, and statistical significance was defined as  $P$ -value  $<0.05$ . This study was approved form Department of medical microbiology, Medical Laboratory, Al-Neelain University, Khartoum, Sudan.

## Results

Total 90 women were enrolled with a history of recurrent miscarriages and tested in this study, according to Socio-demographical and clinical characteristic of case and control in Al-Gazeera Hospital our results found significant difference in the age ( $30.89 \pm 0.9504$  vs.  $26.02 \pm 0.8531$   $P = 0.0003$ ), biomass index ( $27.85 \pm 0.5751$  vs.  $25.66 \pm 0.6089$   $P = 0.0104$ ), MCV ( $84.22 \pm 1.010$  vs  $90.72 \pm 1.057$   $P = 0.0001$ ), MCHC ( $33.16 \pm 0.3316$  vs  $31.91 \pm 0.3579$   $P = 0.0125$ ), MPV ( $9.593 \pm 0.2327$  vs  $8.687 \pm 0.1015$   $P = 0.0006$ ), RDWCV ( $14.59 \pm 0.3397$  vs  $15.88 \pm 0.2821$   $P = 0.0044$ ), RWDS (  $44.98 \pm 0.8974$  vs  $52.48 \pm 0.8195$   $P = 0.0001$ ) while there was no significant difference between case and control include RBCs ( $3.843 \pm 0.1349$  vs  $10.65 \pm 6.849$   $P = 0.3235$ ) presented in [Table 1](#).

Sero-detection of IgG and IgM antibodies by using ELISA techniques, a total of 45 miscarriage women (cases) for IgM 6(13.3%) were positive, and 39(48.8%) were negative for *Treponema pallidum* by using ELISA techniques. A total of 45 non-miscarriage women (control) for IgM 4(8.9%) were positive, and 41(91.1%) were negative for *Treponema pallidum* by using ELISA techniques. A total of 45 miscarriage women (cases) for IgG 13(28.9%) were positive, 32(71.1%) were negative. Moreover, IgG for non-miscarriage, like IgM 4(8.9%), was positive and 41(91.1%) were negative by ELISA techniques presented in [Table 2](#).

Univariate and multivariate analysis showed that preclampsia, microcytic hypochromic anemia, vaginal bleeding, and menstruation cycle and biomass index were significantly associated with Miscarriage in both univariate and multivariate. While diabetic patient, age, and family history were significant associated with Miscarriage in univariate analysis [Table 3](#).

Table 1. Socio-demographical and clinical characteristic of case and control in Al-Gazeera Hospital

Items	Control N = 45 Mean $\pm$ SEM	Case N = 45 Mean $\pm$ SEM	P-value (95% confidence interval)
Age	26.02 $\pm$ 0.8531	30.89 $\pm$ 0.9504	0.0003 "-7.409 to -2.324"
Biomass index	25.66 $\pm$ 0.6089	27.85 $\pm$ 0.5751	0.0104 "-3.860 to -0.5250"
RBCs	10.65 $\pm$ 6.849	3.843 $\pm$ 0.1349	0.3235 "-6.836 to 20.44"
Hb	10.93 $\pm$ 0.2420	10.58 $\pm$ 0.3481	0.4187 "-0.4995 to 1.188"
TWBCs	9.109 $\pm$ 0.4661	7.907 $\pm$ 1.214	0.3577 "-1.386 to 3.790"
Platelates	251.7 $\pm$ 12.61	243.8 $\pm$ 14.61	0.6803 "-30.43 to 46.39"
PCV	33.94 $\pm$ 0.6871	31.84 $\pm$ 1.053	0.0984 "-0.4025 to 4.602"
MCV	90.72 $\pm$ 1.057	84.22 $\pm$ 1.010	0.0001 "3.590 to 9.410"
MCH	29.00 $\pm$ 0.5027	28.11 $\pm$ 0.5391	0.2311 "-0.5784 to 2.356"
MCHC	31.91 $\pm$ 0.3579	33.16 $\pm$ 0.3316	0.0125 "-2.216 to -0.2733"
MPV	8.687 $\pm$ 0.1015	9.593 $\pm$ 0.2327	0.0006 "-1.412 to -0.4012"
PCT	0.2115 $\pm$ 0.01113	0.2579 $\pm$ 0.03219	0.1762 "-0.1143 to 0.02136"
RDWCV	15.88 $\pm$ 0.2821	14.59 $\pm$ 0.3397	0.0044 "0.4121 to 2.170"
RWDS	52.48 $\pm$ 0.8195	44.98 $\pm$ 0.8974	0.0001 "5.078 to 9.917"
Neutrophil	65.34 $\pm$ 1.864	66.43 $\pm$ 1.908	0.6829 "-6.403 to 4.216"
Lymphocyte	27.42 $\pm$ 1.617	32.19 $\pm$ 4.928	0.3599 "-15.10 to 5.551"
Monocyte	4.627 $\pm$ 0.3153	5.324 $\pm$ 0.3098	0.1180 "-1.578 to 0.1822"
Eosinophil	2.553 $\pm$ 0.1767	2.267 $\pm$ 0.14	0.2108 "-0.1660 to 0.7394"
Basophil	00.00	00.00	Constant

Table 2. Assessment of Sero-detection of IgM and IgG antibodies of Treponema by using ELISA

Item	Number	IgM		IgG	
		Techniques	ELISA	ELISA	ELISA
		Positive	Negative	Positive	Negative
Miscarriage	45	6(13.3%)	39(48.8%)	13(28.9%)	32(71.1%)
No miscarriage	45	4(8.9%)	41(91.1%)	4(8.9%)	41(91.1%)
Total	90	10(11.1%)	80(88.9%)	17(18.9%)	73(81.1%)

Table 3. Logistic regression analyses of the predictors for miscarriage

Variables	Univariate			Multivariate		
	OR	95% CI	P-value	OR	95% CI	P-value
Tribes	0.93	0.865–1.004	0.065	1.000	0.000–1.000	1.000
Education	1.107	0.698–1.756	0.667	2.639	0.369–18.859	0.333
Jobs	0.712	0.420–1.206	0.206	1.000	0.000–1.000	1.000
Rate of Miscarriage	0.000	0.000–0.000	0.993	1.000E-013	0.000–1.000	0.998734
Family history	2.94	2.946–948	0.000	1.000	0.000–1.000	1.000
Menstruation Cycle	3.775	1.2–11.5	0.02	2.59	0.078–8.61	0.028
Vaginal disease	0.230	0.211–1.453	0.230	0.689	0.239–1.987	0.491
Vaginal Bleeding	6.353	2.1–19.2	0.001	1.39	0.043–4.47	0.001
Normochromic anemia	0.29	0.030–2.723	0.1	0.554	0.170–1.801	0.326
Macrocytic anemia	2.1	0.723–5.846	0.176	0.554	0.170–1.801	0.326
Microcytic hypochromic anemia	11	1.086–110.2	0.04	2.9	1.3–6.7	0.000
Sero-positivity of Anti-Treponema pallidum IgG*	4.164	1.239–13.99	0.021	3.606	0.921–14.123	0.012
Sero-positivity of Anti-Treponema pallidum IgM*	1.577	0.413–6.016	0.505	2.506	0.569–11.032	0.224
MMR vaccine	0.389	0.130–1.166	0.1	3.919	0.758–20.268	0.103
Tetanus vaccine	9.649E8	0.000–1.166	1.1	1.7	0.000–0.000	0.997
All the vaccine MMR+TT	0.339	0.109–1.058	0.1	3.375	0.845–13.473	0.085
Diabetic patient	11.1	11–11.38	0.000	10	0.10–10.3	0.476
Thyroid	8.9	8.1–8.9	0.00	8.739E-008	8.739E-8.739E-	0.476
Hypertension	1.08	0.065–17.8	0.96	0.972	0.057–15.741	0.951
Preeclampsia	16.1	1.9–131.1	0.01	2.983E-009	1.314E-010–6.776E-008	0.000
Blood group	0.000	0.010–1.722	0.1	7.2	5.4–8.42	0.997
Age	5	2–13	0.001	0.336	0.090–1.250	0.104
Biomass index	5	2–12	0.001	1.73	0.062–4.79	0.001
HB	1.3	0.56–3.1	0.4	0.574	0.126–2.615	0.473
RBCs	2.3	0.85–6.2	0.1	0.494	0.156–1.564	0.230
Platelets	0.7	0.21–2.2	0.52	1.208	0.343–4.251	0.768
TWBCS	0.7	0.254–1.97	0.5	1.928	0.635–5.855	0.246
Vaccination	0.6	0.23–1.4	0.23	1.277	0.280–5.831	0.753
PCV	0.432	0.162–1.157	0.095	0.594	0.180–1.959	0.392
MCV	1.000	0.234–4.271	1.000	1.571	0.327–7.549	0.573
MCH	0.577	0.248–1.343	0.202	0.442	0.165–1.188	0.106
MHC	1.545	0.616–3.878	0.354	2.112	0.759–5.881	0.152
MPV	0.302	0.058–1.587	0.157	0.677	0.085–5.401	0.713

(Continued)

Table 3. **Logistic regression analyses of the predictors for miscarriage** —(Continued)

Variables	Univariate			Multivariate		
	OR	95% CI	P-value	OR	95% CI	P-value
PCT	1.000	0.269–3.724	1.000	0.811	0.144–4.576	0.813
DOC	4.375	1.750–10.9	0.002	3.531	1.190–10.472	0.023
RDWSD	19.158	5.158–71.1	0.000	17.019	4.187–69.179	0.000
Neutrophil	0.518	0.044–6.037	0.599	0.309	0.012–4.033	0.309
Monocyte	2.098	0.364–12.1	0.407	4.718	0.256–87.032	0.297
Eosinophil	1.400	0.295–6.651	0.672	0.633	0.043–9.426	0.740
Basophil	1.400	0.295–6.651	0.672	0.356	0.054–2.322	0.280
Lymphocyte	1.680	0.405–6.962	0.474	1.036	0.154–6.989	0.971

## Discussion

The present study was conducted Sero-detection of *Treponema pallidum* (IgG and IgM antibodies) using ELISA techniques among women with habitual Miscarriage in Gezira state and appraisal of other risk factors to the questionnaire. The main finding was a much higher prevalence of *Treponema pallidum* IgG seropositivity among pregnant women who reported miscarriages (28.9%), compared to those who did not report miscarriages (8.9%) ( $P < 0.001$ ). In contrast, there was no association between IgM seropositivity and pregnant women who reported miscarriages. Our results were consistent with many other studies that noted *Treponema pallidum* IgG seropositivity is a major challenge to public health and is responsible for a large number of miscarriages.<sup>9,10</sup> Also, the study by Abreu et al. reported that miscarriage rates increase from two to three times among pregnant women with *Treponema pallidum*.<sup>11</sup> Another study disagrees with our study carried by Magalhães et al., who confirmed that syphilis not only affects specific groups at risk but that prevention should be of paramount importance for the general population.<sup>12</sup>

Based on our study, the chance of Miscarriage among pregnant women with *Treponema pallidum* increases with age that similar to the previous study by Abreu et al. and Magalhães et al. those found that women with *Treponema pallidum* at risk of miscarriage increases with age; natural fertility and pregnancy rates decline, and the rate of intrauterine insemination also declines.<sup>11,12</sup>

Our study predictors for Miscarriage exhibited that women with preeclampsia, microcytic hypochromic anemia, and seropositivity of anti-*Treponema pallidum* IgG have a high risk for Miscarriage as univariate multivariate factor significant effects. At the same time, women with thyroid diabetic patients, vaginal bleeding, menstruation cycle, and family history have reasonable as a univariate risk for Miscarriage. These factors may increase the risk of Miscarriage.

The findings presented here concluded a higher prevalence of *Treponema pallidum* IgG seropositivity among

pregnant women who reported miscarriages (28.9%), compared to those who did not report miscarriages (8.9%) ( $P < 0.001$ ), while no association between IgM seropositivity and pregnant women who reported miscarriages.

Routine screening of *Treponema pallidum* antibodies among pregnant women with recurrent Miscarriage is highly recommended due to its high prevalence and the significant clinical impacts. Further studies with more advanced techniques like using PCR should be carried to confirm the result by ELISA.

## Acknowledgment

We thank all the study participants, all staff members at the medical laboratory sciences-Al-Neelain University faculty for their help and support, and colleagues at the Laboratory and Research Unit. Moreover, heartfelt gratitude to Dr. Amel Ibrahim from the Central Laboratory for helping with the practice tests.

## Conflict of Interest

Authors declare no conflict of interest.

## Authors Contribution

All authors participated in study design, performing surgeries, follow up of patients, data interpretation and manuscript organization and editing.

## Funding Source

Funding: Self-funding.

## List of Abbreviation

T.P., *Treponema pallidum*; S.A., Spontaneous abortion; CBC, Complete blood count. ■

## References

1. Rahman M, Akhtar GN, Yasmin L. Seroprevalence of syphilis in the blood donors in Lahore. *Pak J Med Sci.* 2002;18:284–2864.
2. WHO Office of HIV/AIDS and STDs. An overview of selected curable STDs. Syphilis estimates, 1995. Geneva, Switzerland: World Health Organization; 1995.
3. Ameeta ES, Romanowski B. Syphilis: a review with emphasis on clinical, epidemiologic, and some biologic features. *Clin Microbiol Rev.* 1999;12:187–209.
4. Robert B, Stroube MD. Infectious syphilis: the return of an epidemic. *Medscape Infect Dis.* (serial online) Medscape; 2008.
5. Fonck K, Claeys P, Bashir F, Bwayo J, Fransen L, Temmerman M. Syphilis control during pregnancy; effectiveness and sustainability of the decentralized program. *Am J Public Health.* 2001;91:705–707.
6. Kustner HG, Swanevelder JP, Van MK. The South Africa HIV epidemic, reflected by nine provincial epidemics, 1990–1996. *South Africa Med J.* 1998;88(1):1235–1320.
7. Speroff L, Glass RH, Kase NG. *Clinical gynecologic endocrinology and infertility.* 6th ed. Philadelphia: Lippincott Williams & Wilkins; 1999.
8. Zinaman MJ, Clegg ED, Brown CC, O'Connor J, Selevan SG. Estimates of human fertility and pregnancy loss. *Fertil Steril.* 1996;65:503–9.
9. Saab F. Syphilis prevalence in pregnant women who miscarried served by the maternity protection program - PPG Sergipe State, 2005 to 2007;2016.
10. World Health Organization. Global guidance on criteria and processes for validation: elimination of mother-to-child transmission (EMTCT) of HIV and syphilis, Geneva. 2014.
11. Abreu LG, Santana LF, Navarro PAAS, Reis RM, Ferriani RA, et al. The pregnancy rate in women undergoing assisted reproductive techniques is less from 30 years. *Rev Bras Gynecol Obstet* 2006;28:32–37.
12. Magalhães DMS, Kawaguchi IAL, Dias A, Calderon IMP. Maternal and congenital syphilis: still a challenge. *Cad Public Health* 2013;29:1109–1120.

This work is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported License which allows users to read, copy, distribute and make derivative works for non-commercial purposes from the material, as long as the author of the original work is cited properly.