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Original Article **Open Access**

Seroprevalence of microbial organisms during routine infertility evaluation at University of Benin Teaching Hospital, Benin-City, Nigeria

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Abstract:

Background: The association of genital microorganism with infertility has been documented but no consensus exists. Understanding their prevalence amongst infertile clients may assist in facilitating better screening protocols. The objective of this study is to determine the prevalence of microorganisms routinely screened among women undergoing infertility evaluation at the University of Benin Teaching Hospital.
Methods: A three year (January 2015 to December 2017) retrospective survey of all patients evaluated for infertility at the assisted reproduction unit of the hospital was undertaken. *Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis*, cytomegalovirus (CMV), hepatitis B (HBV), hepatitis C (HCV) virus and the human immunodeficiency virus (HIV) were microorganisms serologically assayed at the unit. We analyzed data containing patients' demography and results of serological assay of these microorganisms.
Results: There were 576 patients (288 couples) who completed their microbiological evaluation during the study period. The mean age (years) of female partners was 38.2±5.7, while the mean age of the male partners was 42.7±6.1. The frequency of CMV positive assay for infertile couples was 129 (22.4%); *C. trachomatis* 125 (21.7%); *M. hominis* 92 (15.9%) and *U. urealyticum* 76 (13.2%). Overall, more women (50.7%) were seropositive compared to men (26%). HIV was positive in 10 patients (1.73%) with 60% being women. HBV was seropositive in 8 (1.4%) (women 62.5% and men 37.5%) while HCV was positive in only 2 (0.3%) patient. Majority (over 80%) of couples were sero-discordant with 20% (2) concordance rate for HIV and 12.5% (1) for hepatitis B.
Conclusion: Despite a relatively high seroprevalence rate of the studied microorganisms, the documented uncertainty on their association with infertility or its treatment limits justification for incorporation of routine screening of microbiological organisms into standard protocols for evaluation of infertile couples. A robust study on the impact of genital microorganism on specific infertility variables with comparison to fertile controls is recommended.

Keywords: microorganism, viruses, infertility, assisted reproduction, serological assay, screening

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Séroprévalence d'organismes microbiens au cours de l'évaluation de routine de l'infertilité à l'hôpital universitaire de Benin, Benin-City, Nigéria

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Abstrait:

Contexte: L'association d'un microorganisme génital à l'infertilité a été documentée mais il n'y a pas de consensus. Comprendre leur prévalence chez les clients infertiles peut aider à faciliter de meilleurs protocoles de dépistage. L'objectif de cette étude est de déterminer la prévalence des micro-organismes régulièrement dépistés chez les femmes subissant une évaluation de la stérilité à l'hôpital universitaire de Bénin.
Méthodes: Une enquête rétrospective de trois ans (de janvier 2015 à décembre 2017) sur tous les patients évalués pour l'infertilité dans l'unité de procréation assistée de l'hôpital a été entreprise. *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, cytomégalovirus (CMV), l'hépatite B (VHB), le virus de l'hépatite C (VHC) et le virus de l'immunodéficience humaine (VIH) étaient des microorganismes testés sérologiquement à l'unité. Nous avons analysé les données contenant la démographie des patients et les résultats du dosage sérologique de ces micro-organismes.
Résultats: 576 patients (288 couples) ont terminé leur évaluation microbiologique au cours de la période d'étude. L'âge moyen (en années) des partenaires féminins était de 38,2 ± 5,7 ans, tandis que l'âge moyen des partenaires masculins était de 42,7 ± 6,1. La fréquence du test CMV-positif pour les couples infertiles était de 129 (22,4%); *C. trachomatis* 125 (21,7%); *M. hominis* 92 (15,9%) et *U. urealyticum* 76 (13,2%). Dans l'ensemble, plus de femmes (50,7%) étaient séropositives que d'hommes (26%). Le VIH était positif chez 10 patients (1,73%), dont 60% de femmes. Le VHB était séropositif chez 8 (1,4%) (les femmes 62,5% et les hommes 37,5%), tandis que le VHC était positif chez seulement 2 patients (0,3%). La majorité (plus de 80%) des couples étaient sérodiscordants avec un taux de concordance de 20% pour le VIH et de 12,5% (1) pour l'hépatite B.
Conclusion: Malgré un taux de séroprévalence relativement élevé des microorganismes étudiés, l'incertitude documentée de leur l'association à l'infertilité ou à son traitement limite la justification de l'incorporation du dépistage systématique des organismes microbiologiques dans les protocoles standard d'évaluation des couples infertiles. Une étude robuste sur l'impact des microorganismes génitaux sur des variables spécifiques d'infertilité comparées aux témoins fertiles est

Mots-clés: microorganisme, virus, infertilité, procréation assistée, test sérologique, dépistage

Introduction:

Genital microbial infections can influence the fertility potential of a couple. Common pathogenic species implicated in both genital infections and infertility are *Chlamydia trachomatis*, Genital ureaplasmas (*Ureaplasma urealyticum* and *Ureaplasma parvum*) and mycoplasmas (*Mycoplasma genitalium* and *Mycoplasma hominis*) as well as viruses such as cytomegalovirus, human immunodeficiency virus and hepatitis viruses. The causal role of *C. trachomatis* in infertility is reasonably defined, as it may cause pelvic inflammatory disease with resultant tubal factor infertility and ectopic pregnancy (1, 2).

Chronic viral infections have been found to be a risk during infertility treatment (3, 4). In a previous work, hepatitis B was reported as the most prevalent of the viral infection detected amongst infertile couple (5). Another research showed that hepatitis C virus (HCV) seropositive women had decreased response during ovarian stimulation at assisted reproduction treatment cycles (4). Mycoplasma and Ureaplasma species

have been associated with increased risk of genitourinary tract infections and recurrent pregnancy losses (6, 7). Their role in the aetiology of infertility is still speculative but they may impair fertilization or implantation. *Ureaplasma urealyticum* and *M. hominis* have been demonstrated to affect semen quality of infertile men, with recovery of the organisms in 7–14% of semen samples of infertile men (8, 9).

Human Cytomegalovirus (CMV) also known as human herpesvirus-5 (HHV-5) is the most studied of all CMVs. Human CMV infection is typically non pathogenic but can be life-threatening for the immuno-compromised patients such as organ transplants recipients, newborn, infants or HIV-infected persons. Some studies have shown that CMV may play a significant role in male infertility, and its early detection will permit successful antiviral therapy, to increase the fertility potential of the individual (10, 11).

It is with this foregoing that, some guidelines have recommended that women should be screened for *C. trachomatis* or given appropriate antibiotic prophylaxis, before any uterine instrumentation takes place with regard to infertility patients receiving *invitro* fertilization (IVF) treatment (12, 13). For this reason many centres with assisted reproduction units have evolved guidelines for evaluation of the infertile couple to include detailed history, physical examination and laboratory analyses. However the genital microbial infection screening protocol still varies for different institutions. Hence the prevalence of these infections in the couples undergoing infertility evaluation is still uncertain.

The University of Benin Teaching Hospital (UBTH) is a public tertiary healthcare centre offering assisted reproduction services in a population characterized by deepening economic and resource constraints, and poor health seeking behavior. The benefit of screening for the aforementioned micro-organisms while evaluating infertile couple in the overall interest of their reproductive health is underscored in the goal of improving success of infertility treatment as well as opportunistic detection of these infections in apparently healthy individuals.

In sub-Saharan Africa, reports of prevalence and significance of these key micro-organisms that may influence overall reproductive health of the infertile couple are limited in the literature. This study aims to determine the prevalence of *C. trachomatis*, *U. urealyticum*, *M. hominis*, cytomegalovirus (CMV), hepatitis B (HBV) and C (HCV) virus and the human immunodeficiency virus (HIV) among women undergoing infertility evaluation.

Methods:

Study design and setting:

A retrospective survey of all patients evaluated for infertility at Human Reproduction and Research Programme (HRRP) unit of University of Benin Teaching Hospital (UBTH) was undertaken between January 2015 and December 2017. The HRRP is a dedicated infertility treatment unit of the hospital with services for *Invitro* Fertilization (IVF) and other assisted reproduction techniques (ART). Evaluation of infertility at the unit is in line with global standard guideline on the investigation of infertile couples.(12) In addition routine screening for micro-organism is done specifically for *C. trachomatis*, *U. urealyticum*, *M. hominis*, cytomegalovirus (CMV), hepatitis B (HBV) and C (HCV), and human immuno-deficiency virus (HIV) infections.

Serological methods:

Rapid test strip was the principal method for microorganism detection in this study. Colloidal Gold Immuno-filtration (GIFA) Assay was used to qualitatively detect *U. urealyticum*, *M. genitalium* and *C. trachomatis* antibody in human serum. Qualitatitive detection of viral antibodies in serum or plasma was with Rapid test chromatographic immuno-assay. Those with positive serological results were counseled and managed as appropriate. Approval for the study was obtained from the institutions Ethics and

Research Committee.

Case files of patients who presented for initial infertility evaluation during the selected study period were retrieved. All clients with completed laboratory work-up results were included for analysis. The data extracted were analyzed using InStat Graph Pad and presented in the form of frequency tables and descriptive statistics. Chi square test was used to test for statistical difference where appropriate and *p* value of less than 0.05 was considered significant.

Results:

Overall, 576 patients (288 couples) completed their microbiological evaluation during the study period and these were extracted for analysis. The age (years) of female partners ranged from 23 to 56 and the mean was 38.2±5.7, while the mean age of the male partners was 42.7±6.1 and a range of 31 to 63. On the whole seropositive detection rate was 76.7% (442/576) with 292 (50.7%) being seropositive female and 150 (26.0%) for male. Overall sero-concordance rate was 10.8% (62/576). It is pertinent to note that some patients were seropositive for more than one microbial organism.

Cytomegalovirus was the commonest organism detected amongst infertile couples (Table 1) with frequency of seropositivity for immunoglobulin G of 129 (22.4%); female 88 (68.2%) and male 41 (31.8%). One hundred and twenty-five (21.7%) were seropositive for *C. trachomatis*; 81 (64.8%) female and 44 (35.2%) male. Ninety-two (15.9%) were seropositive for *M. hominis*; female 64.1% and male 35.9%, and 76 (13.2%) were seropositive for *U. urealyticum* (female 68.4% and male 31.6%).

Also shown in Table 1 is the frequency of couples with concordant positive test; 5.3%, 5.4%, 19.4% and 20.0% for ureaplasma, mycoplasma, cytomegalovirus, and chlamydia resp-ectively. Other organisms screened for routinely are shown; HIV was positive in 10 patients (1.73%) with 6 (60%) female and 4 (40%) male. Hepatitis B was seropositive in 8 (1.4%) with females 62.5% (5) and male 37.5% (3); hepatitis C was positive in only 2 (0.3%) patients. Majority of couples were sero-discordant with 20% (2) concordance rate for HIV and 12.5% (1) for hepatitis B.

Comparison of microorganism seroprevalence rate between female and male patients (Table 2) showed that for Chlamydia, Ureaplasma, Mycoplasma and Cytomegalovirus, significantly more females were infected compared to males ($p<0.05$), however there was no statistical difference between female and male seroprevalence for HIV, HBV and HCV ($p>0.05$).

Table 1: Seroprevalence of microorganisms in subjects evaluated for infertility at UBTH Benin between January 2015 and December 2017

Microorganism	Subjects (%) (n=576)	Women (%) (n=288)	Men (%) (n=288)	Sero-concordance No of couples
Ureaplasma urealyticum	76 (13.2)	64 (69.6)	28 (30.4)	4 (5.3)
Mycoplasma hominis	92 (15.9)	56 (73.7)	20 (26.3)	5 (5.4)
Chlamydia trachomatis	125 (21.7)	88 (66.7)	44 (33.3)	25 (20.0)
Cytomegalovirus	129 (22.4)	116 (69.1)	52 (30.9)	25 (19.4)
Human immunodeficiency virus	10 (1.7)	6 (2.1)	4 (1.4)	2 (20)
Hepatitis B Virus	8 (1.4)	5 (1.7)	3 (1.0)	1 (12.5)
Hepatitis C Virus	2 (0.3)	1 (0.3)	1 (0.3)	0
Total	442 (76.7)	*292 (50.7)	150 (26.0)	62 (10.8)

n = number of subjects screened, * = some subjects were seropositive for more than one organism

Table 2: Comparison of microorganism seroprevalence rate between female and male patients

Microorganism	Female n=288	Male n=288	*p* value	Relative risk (confidence interval)
Ureaplasma urealyticum	52 (18.1)	24 (8.3)	0.001*	1.45 (1.21-1.73)
Mycoplasma hominis	59 (20.5)	33(11.5)	0.004*	1.36 (1.13-1.62)
Chlamydia trachomatis	81 (28.1)	44 (15.3)	0.001*	1.41 (1.19-1.66)
Cytomegalovirus	88 (30.6)	41 (14.2)	0.0001*	1.52 (1.30-1.78)
Human immunodeficiency virus	6 (2.1)	4 (1.4)	0.749	1.2 (0.72-2.01)
Hepatitis B Virus	5 (1.7)	3 (1.0)	0.721	1.25 (0.72-2.16)
Hepatitis C Virus	1 (0.3)	1 (0.3)	1	1 (0.25-4.12)

n = number of subjects screened, * = significant difference $p < 0.05$

Discussion:

The association of genital microbial infection and infertility is still not explicit. Hence there is no clear consensus guideline with regard to screening for these microorganisms during infertility evaluation. In this study, the high seroprevalence rate of microorganisms of over 75% apparently supports the call for routine screening for genital microbial infection at the infertility clinic. However, this finding cannot be generalized or equated to individual patients as some were seropositive for multiple micro-organisms. With regards to specific genital microorganism in this study, the most frequent organisms (cytomegalovirus and chlamydia) had seroprevalence rate of about 22%. This finding is similar to those of Rodriguez and co workers (14) who reported a seroprevalence rate of 23.5% for the most common microorganism detected (ureaplasma) although the organism was different from those from this study.

Our findings are also comparable to reported seroprevalence rate of 19.2% and 15.2% respectively for genital mycoplasma and ureaplasma among infertile male patients (15). Similar studies in southern Nigeria have reported prevalent rates among patients with infertility (16, 17) but while the rate in our study is higher than some previously reported in this environment, it is lower than others (8, 18, 19). Although, the exact role of genital microbial infection in infertility is not clearly defined, previous researchers have documented microbial infections of the genital tract or semen as causes of male infertility (16, 20). Another study that investigated the association between genital ureaplasmas and myco-plasmas and risk of infertility showed a significantly higher positive rate of *U. urealyticum* and *M. hominis* in the infertile compared to control group but there was no significant causal association with male infertility (21).

The seroprevalence was similar to that observed in this study although we did not compare with fertile controls. Evidently *C. trachomatis* infection in women has been implicated in tubal factor infertility and tubal ectopic pregnancy but its sequelae in men, is still unclear (2). Similarly, the role of cytomegalovirus in infertility is also doubtful. Some researchers reported that while sero-prevalence rate and shedding of cytomegalovirus in the genital tracts of infertile patients are relatively high, this seem to have no significant role on infertility (11). Also there was no difference in sperm count and motility between those with and without cytomegalovirus infections (11, 24). Notwithstanding, the high seroprevalence rate reported is consistent with the finding of cytomegalovirus being the commonest detected microorganism in our study.

The seroprevalence of other viruses (HIV, HBV and HCV) studied were low and comparable to previous research on viral infections amongst infertile couples (25). HCV seroprevalence in our study is lower compared to a previous study in our centre which investigated HCV prevalence amongst antenatal clinic attendees (26). A higher HCV rate of 3.2% amongst infertile couples has been reported in Brazil (27). The implications of these transmissible viral infections (HIV, HBV and HCV) on infertility management at assisted reproduction are quite est-ablished and unambiguous. These include issues of cross-contamination in the laboratory and vertical transmission with possible deleterious consequences. Also, HCV infection has been reported to negatively affect ovarian response during stimulation (3, 13, 27).

From the foregoing screening for genital microbial infections at infertility evaluation is important. Our finding of a relatively high seroprevalence and the negative influence of these micro organisms on an individuals' fertility potential supports the consideration for incorporating genital microorganism screening into routine infertility evaluation protocol. This may facilitate better screening protocols and improve infertility treatment outcome. In addition it will afford the opportunity for patients to be referred to appropriate caregivers for other treatment and follow-up. Although guidelines for routine screening for transmissible viruses such as HIV, HBV and HCV are well established, there is still no consensus recommendation for routine screening for other genital micro organisms during infertility evaluation. This is largely due to the uncertainty regarding the role and association of these microorganisms and infertility.

In conclusion the observed sero-prevalence of microbial organisms amongst infertile couples in this study is in line with global average. However while the utility of routine screening of a population seeking infertility treatment is not in doubt, there is no consensus on relevance and effect of these genital microorganisms on infertility or its treatment. Furthermore since this study focused on prevalence and not the effect of the organisms, justification and generalization on screening protocols for the aforementioned organisms during infertility evaluation is limited.

Conflict of interest:

No conflict of interest is declared

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