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Molecular detection for Trichomonas virginals in placenta tissue from women with BOD

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ABSTRACT

Trichomonas viginalis is a unicellular parasite, occur in one stage (Trophozoite) and transmitted to human by sexual activity. Trichomoniasis is common in sexually active women of all ages. Disease symptoms range from asymptomatic to severe inflammation. Also, studies indicated that trichomoniasis implicated in causing adverse pregnancy outcomes. In this study, we try to investigate this effect in women with BOD history by the molecular method. Among the selected pregnant women, two hundred placental tissues were collected with a history of "BOD" that is sent to outpatient gynaecology departments, units of the Al Alamad Al Kadhimain Medical City Crisis Unit and the Fatima Al-Zahraa University Hospital from December 2015 to May 2016 Baghdad, Iraq. The tissues were homogeneous, and the DNA was extracted. Two genes were used for the detection of Trichomonas by conventional PCR. The mean age for two hundred women enrolled in this study were 27 ± 6.6. One hundred fourth of them had abnormal pregnancy outcomes (62%). The main abnormal outcomes were abortion (36%), stillbirth (18%) and congenital abnormality (8%). By the conventional PCR, *Trichomonas viginalis* was detected in 24 /200 (12%) of the total placental tissues. 75 % were in age 20-29. Nine of infected women with *Trichomonas vaginalis* had Abnormal pregnancy, the majority of abnormal outcomes were abortion (29.17%), stillbirth (8.33%) and no congenital abnormalities were detected. 54.17% had 3-4 previous pregnancies. All the results were significantly important. *Trichomonas vaginalis* can reach the placental tissue, and there is a clear association with a woman who had a BOD.

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INTRODUCTION

Trichomonas vaginalis is a whipped and anaerobic protozoan parasite, which is a helpful specialist of trichomoniasis. Every year, there are an expected 180 million diseases around the world (Soper *et al.,* 2004). We realize that individuals are the best places where trophozoites are transmitted predominantly by vaginal sex and characterized by sex (WHO, 2010). *Trichomonas vaginalis* contaminates the genitourinary tract and causes sickness in explicitly dynamic ladies of all age gatherings (Cintia *et al.,* 2016; Andreia *et al.,* 2016; Sabrina *et al.,* Guilherme *et al.,* Siebel *et al.,* Marcos 2016). The commonness of trichomonas relies upon components, for example, age, sexual movement and some sexual accomplices. In ladies, the infection has an assortment of indications running from extreme aggravation and frothy bothering to a generally asymptomatic bearer state. However, the primary clinical appearance of trichomonas is vaginitis, yellow or green urethritis, and in some cases the stream of greeneries (Schwebke *et al.,* 2004; Burgess, 2004). BOH: Poor obstetric history involves a prior unfriendly guess in the hatchling as far as successive or back to back unconstrained

premature births, early neonatal passing, intrauterine fetal passing, intrauterine development hindrance, and inborn abnormalities. For a given pregnancy, the danger of loss of pregnancy revealed is 15% and the likelihood of three back to back misfortunes, which relates to the exemplary meaning of poor obstetrical history, would be 0.34% (Beigi *et al.,* 2008). Numerous Etiologies are viewed as the reason for Bad obstetric history, for example, Genetic, Endocrine, Immunologic, furthermore primary contaminations caused by TORCH disease which included *Toxoplasma gondii*, rubella infection, cytomegalovirus (CMV), and Herpes simplex infection (HSV) additionally blamed as one for the causes in BOH (Beigi et al. 2008). As parasitic contamination in pregnant ladies, trichomoniasis is related to antagonistic pregnancy results (Johnson et al. 2011). Ladies contaminated amid pregnancy frequently have untimely breaks, untimely birth, low birth weight, and expanded youngster mortality and bleakness (Rasti et al. 2011). The point of this examination: The current examination was intended to research the commonness and job of *Trichomonas vaginalis* disease in ladies with of terrible obstetric history by traditional polymerase chain response strategy (PCR).

MATERIALS AND METHODS

From December 2015 to May 2016, collected in 200 maternities and vaginal discharges in obstetrics and gynaecology clinics, services and emergency rooms of Al Imamain Al Kadhimain Medical Center and Fatima Al-Zahraa University Hospital Sample of placental tissue. Samples were collected from women aged 15 to 54 years. All pertinent data was gotten from all cases utilizing an extraordinary poll. An assent shape got from all patients. This investigation was endorsed by the Research Ethics Board (REC) of the Faculty of Medicine/AL-Nahrain University. The investigation was led at the Department of Microbiology of the Medical College - University of Alena. Patients with a person with diabetes through the HbA1c level, Anti-phospholipid disorder through the enemy of cardiolipin test and Rh and ABO contradiction were avoided from this study, all females with BOH and vaginal release were incorporated.

SAMPLE PROCESSING

Preparation of tissue homogenate

The total number of 25 gm placental tissue homogenized with PBS using a homogenizing tissue for about 1 minute at 4°C is "10 ml". The subsequent suspension was exposed to 2 cycles of defrosting and solidifying to additionally separate cell layers. The homogenate was then centrifuged for around 15 minutes. At 5000 rpm and temperature (2-8)°C. The supernatant was then deliberately collected and put away at (- 20°C) until DNA extraction.

DNA extraction and gene amplification:

DNA separated from the tissue, i.e., placental tissue utilizing DNA seclusion unit ((DNA-sorb-B (Sacace)/Italy) Kit). One hundred µl of tests had been lysed by included 300µl of lysis arrangement and hatched at 65ºC for 5 min. DNA purging was conveyed utilizing sorbent material. After washing for two times, DNA eluted by including 100 µl of DNA eluent. DNA was put away at - 30 ºC until downstream application. The explicit of preliminary oligonucleotide successions were utilized in traditional PCR to identify the presence of *Trichomonas vaginalis* geneses appeared in table (1) The ace blend substance were defrosted at room temperature before utilize, and the PCR ace blend was made on a different biohazard security bureau with wearing hand gloves consistently to stay away from pollution. PCR response was made in a 25 µl response containing 12.5 µl of Green Master Mix (Promega, US). 1 µl of 10 pmol/µlof each primer (Alpha DNA, Canada), two µl of DNA templet and the volume was completed to 25 µl utilizing without nuclease water. Thermocycling conditions were as per the following: Initialed maturational 95°C for v4min, followed by 30 cycles of denaturation at 95°C for 30 sec; annealing at 55°C for 30 sec; extension at 72°C for 1 min, and a final extension cycle at 72°C for 10 min.than the program was held at 4°C. 10 µl of each PCR item was exposed to electrophoresis on agarose gel at 1% (w/v) with ethidium bromide (0.5 µg/ml, Sigma). Items electrophoresis amplicon perception was performed utilizing a UV light transilluminator and afterwards shot utilizing computerized camera (Sony-Japan).

Statistical Analysis

Test information was introduced as far as watched numbers and rate frequencies, and after that dissected by utilizing the Chi-square ($\chi 2$) test. P esteem ≤ 0.05 was considered measurably critical.

RESULTS

Demographic and clinical data of the patient.

The study was attended by a total of 200 pregnant women between the ages of 27 and 6.6, including 110 (55%) aged 20-29 and 60 (30%) 30 to 39 years of age. 19 (9.5%) were less than 20 years, and 11 (5.5%) 40 40 years, Table (2).

Of the 200 (62%) pregnant women interviewed, 122 were the aftereffect of an anomalous preg-

Table 1: Oligonucleotide primers used for *T. vaginalis* Gene

Locus (abbreviation)	Primer		Size(bp)		Reference
	Orientation	Sequences(5´-3´)	Gene	Amplicon	
Tryptophanase (P1)	Forward	CGTCAACATCGGTGGCTTCA	1,451	489	
	Revers	GCGACAGCGACGACATTCAT			
Family T2 aspara-ginase-like threo-nine peptidase (P6)	Forward	GAACAGGAGCACCAGCAGAA			
	Revers	TCTCTAGCAACGCAGCCAAC	990	412	Saiki *et al.*, 1988 (7)
β-globin	Forward	GAAGAGCCAAGGACAGGTAC		408	
	Revers	GGAAAATAGACCAATAGGCAG			

Table 2: Age distribution of pregnant women enrolled in the study

Variable	No.	%
Age means (27±6.6 years)		
<20 years	19	9.5
20-29 years	110	55.0
30-39 years	60	30.0
≥40 years	11	5.5
Total	200	100.0

Table 3: Distribution of pregnant women in relation to pregnancy outcome

Variable	No.	%
Pregnancy outcome		
Normal	76	38.0
Abnormal	124	62.0
Total	200	100.0
Pregnancy outcome types		
Normal	76	38.0
Stillbirth	36	18.0
Abortion	72	36.0
Congenital abnormality	16	8.0
Total	200	100.0

nancy and 76 out of 200 (38%) had typical pregnancy results. The principal variations from the

norm was: premature birth 72/200 (36%), dead fetal birth 36/200 (18%), inherent peculiarities 16/200 (8%) (3).

Molecular detection

The results of the amplification of both genes by conventional PCR with using of a specific set of primers sequences showed that both genes present in 24 (12%) out of 200 placental tissue (Figure 1 &2).

Table 4 presented that 18 (75%) out 24 of infected women with Trichomoniasis was in age gruop20-29 years. The association between age and Trichomoniasis was highly significant.

The Analysis of patients which did in a study that was subsequently proved that to be positive for

the trichomoniasis in association with adverse pregnancy outcome was studied and the results showed a highly significant association as shown in the table (5). From nine infected pregnant women seven of them had an abortion and two stillbirths. All abortion cases were occurring during the first trimester, and there is a noncongenital abnormality.

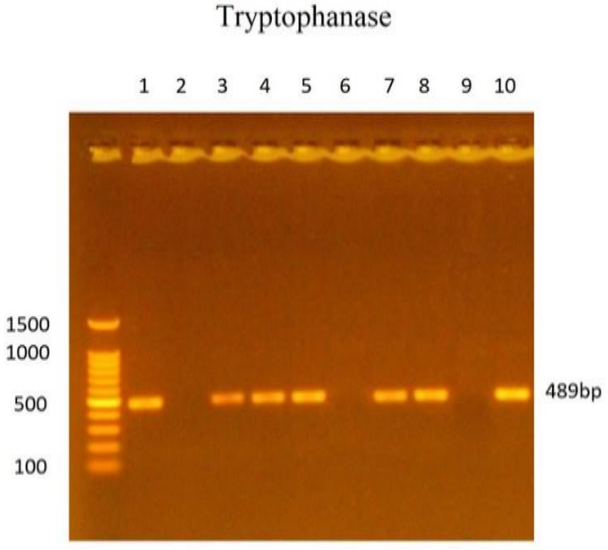

Figure 1: Gel electrophoresis (1% agarose) of PCR products for tryptophanase, lane M,

Table 4: Distribution of *Trichomonas viginalis* according to age group in the placental tissue

Age group (year)	No.	Percentage (%)
Less than 20	2	8.33
20-29	18	75.00
30-40	4	16.67
More than40	0	0.00
Total	24	100%
Chi-Square-χ^2	---	12.371 **

** (P<0.01).

Table 5: Pregnancy outcome and adverse pregnancy outcome type in *trichomoniasis* positive patients

Pregnancy outcome	No.	Percentage (%)
Normal	15	62.50
Abnormal	9	37.50
Total	24	100 %
Chi-Square-χ^2	----	9.481 **
Adverse pregnancy outcome type		
Normal	15	62.5
Stillbirth	2	8.33
Abortion	7	29.17
Congenital abnormality	0	0
Total	24	100 %
Chi-Square-χ^2	----	10.636 **

** (P<0.01).

Table 6: Distribution of sample study according to Pregnancies in trichomonas's patients

Pregnancies	No.	Percentage (%)
1-2	7	29.17
3-4	13	54.17
More than4	4	16.67
Total	24	100%
Chi-Square-χ^2	---	11.026 **

** (P<0.01).

1500pb DNA ladder, lane (1,3,4, 5,7,8,10): Positive sample for *Trichomonas vaginalis*

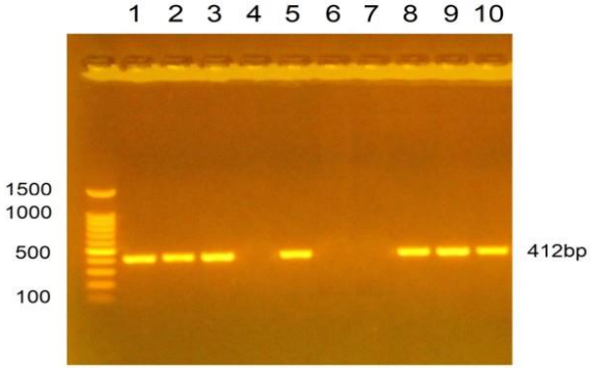

Figure 2: Gel electrophoresis (1% agarose) of PCR products for family T2aparaginase-like threonine peptidase, lane M, 1500pb DNA ladder, lane (1,2,3, 5,7,8,9,10): Positive sample for *Trichomonas vaginalis*

The highest number of infected women were in women who had 3-4 previous pregnancies as shown in table 5.

According to the delivery number, 58.33% had 1-2previous delivery, and 29.17% had 3-4, and the relation was highly significant.

DISCUSSION

Trichomonas vaginalis was first portrayed in 1836 as a safe microorganism commensal until 1916 that Hoèhne attested that *T. vaginalis* was the etiological specialist now and again of vaginitis (prin), since thane, there are numerous investigations a session parasite the study of disease transmission, clinical picture, analysis thus on (Jae-Sook *et al.,* Duk-Young 2006). Genuine well-being results were related with trichomoniasis, for example, barrenness, helplessness to cervical malignant growth, provocative pelvic ailment, and antagonistic pregnancy results, for example, low birth weight and untimely birth (Grodstein *et al.,* 1993; Viikki *et al.,* 2000). What's more, forceful prostate malignancies have been connected to trichomonas malady (Sutcliffe *et al.,* 2012). The consequences of the present investigation uncovered that the greater part of pregnant ladies be-

tween the ages of 20 and 29 years, trailed by ladies between the ages of 30 and 39 (Table 2), can be clarified by the way that different components impact ladies' reflexes (social, instructive, monetary. Ends upheld by different investigations of Enas Talib Abdul Karim (2012) (Enas, 2012). The present examination of helpful found that the odd pregnancy result was unmitigated higher among ladies with a diminished number of pregnancies, history of no past transport and history of no past troublesome births ($p<0.001$) (Table 3). This resembles appropriated by Danish and others (2012) (Danish *et al.,* 2010) that women with prim gravida or women with some past pregnancies had a higher threat of sporadic birth results. Prim gravida is imperative in association with later obstetric execution.

BOH-Bad Obstetric History could be a direct result of the ecological variables like as:

- Infections,
- Radiation,
- Occupational risks,
- Addictions
- Habits.

Rema *et al.,* (2002) in his study has affirmed the critical relationship of irresistible causes, particularly BOH and TORCH, his decision was the TORCH diseases are viewed as a known as the causal factor, Abdulhassan *et al.,* (2017) found that the parvovirus is normal and exceedingly appropriated among ladies with BOH.also, Herpes Simplex-2 Virus may assume a noteworthy job in pregnancy misfortune and can be conveyance result related factor as finished up by Lina et al. (2017). Trichomoniasis has been associated with causing threatening pregnancy results. Women who are defiled in the midst of pregnancy are slanted to preterm break, preterm work, low birth weight infant kids and extended infant mortality and bleakness (Rezaie *et al.,* 2009, Al-Hajjiah *et al.,* 2018). So they go for this exploration is recognition *Trichomonas vaginalis* in placental tissue in the gathering of ladies with BOH utilizing atomic strategy. Nucleic corrosive intensification based tests have for the most part been more delicate than conventional tests in the finding of *T.vaginalis*, yet their staggering expense and need of framework has restricted them across the board use in research facilities (Maraghi *et al.,* 2008). From 200 examples of placenta tissue, just 24 (12%) were positive for trichomoniasis the frequency of 'trichomonas's' that find out in this investigation is more than that recorded and gotten by different examinations and this due to the technique utilized for determination, the example size and kind of patients incorporated into this examination.

As to the age (Table 4), the investigation examination demonstrated that all patients in a conceptive (same) age amass 29-39 years this might be credited that the Women in regenerative matured gathering were at an expanded hazard for "vaginitis". in this examination the gathering was higher than past investigations in Iraq this may be because no signify the chance is given to the part of ladies' wellbeing. Central point behind this is the absence of instruction particularly wellbeing training, the absence of arrangement of wellbeing administrations, the absence of mindfulness, the absence of a legitimate referral framework bringing about the bungle of patients. The as of now accessible investigation found the exceedingly huge relationship in concentrated the gathering with antagonistic pregnancy results with various sorts are as per the following

- Stillbirth,
- Abortion
- Congenital abnormality (Table5)

The disagreeable pregnancy results are any event which reduces the likelihood of having a strong newborn child [16] this agree with, in any case, a couple of examinations demonstrated opposing revelations. (Silver *et al.,* 2014) demonstrated that *T. vaginalis* in pregnancy is connected with an extended peril of preterm birth and critical augmentations in the threat of preterm less than an ideal burst of movies and little for gestational age infant kids. Only nine from 24 trichomoniasis patients had irregularity in consequence of their pregnancy's, and 7 of them had untimely birth, 2 stillbirths and there is on congenital abnormalities). These results are in simultaneousness with earlier examinations which prescribed a connection between *T. vaginalis is* sullying and veritable threatening outcomes including unproductiveness and low birth weight infant kids. Previous thinks about exhibited that *T.vaginalis* disease in pregnancy is fundamentally associated with a hoisted danger of PROM (Silver *et al.,* .2014, Al-Hajjah and Almkhadree, 2018) which is upheld by a case-control ponder among pregnant ladies of Uganda. Another investigation, a companion directed on 13,816 cases, underlined on the critical probability of a low birth weight newborn child and preterm conveyance in *T.vaginalis* - contaminated pregnant ladies. In table 6, the outcome indicted increment the impact of contamination with the expansion of pregnancy number and this may have prosecuted the presence of asymptomatic past contamination the knot will be diagnosed in light of the fact that most gynaecologists rely upon the clinical picture and higher chance to get sickness through degraded rigging or may be through spoiled restorative articles and specialist's gloves during gynaecological examination. In one exami-

nation in South Carolina, relying upon the maternal, infant, and child record for 134,596 Medicaid-protected the outcome arraigning that ladies with trichomoniasis were altogether bound to have a youngster with a scholarly handicap (ID) (Avni *et al.,*2018). For a situation announced by A. sealed al, (2018). *Trichomonas vaginalis* was available in the amniotic liquid. The patient was treated for chorioamnionitis and trichomonas, Labor was prompted, and an unconstrained vaginal conveyance happened. The neonate had burst vesicular injuries on the stomach area, back, and penis and was treated for assumed sepsis and trichomonas contamination and. The instrument by which trichomonads entered the amnion and chorion are dark. This may illuminate the delayed consequence of this examination by demonstrating the parasite in the placenta and plasma. This charged the parasite (or parts) could reach to the placenta and the plasma. And this needs further examination to upgrade the frameworks used by the parasite to associate or cross the placenta. Regardless of the way that pathogenesis and ruinous tendency in human trichomoniasis isn't appreciated, the advance has been made in perceiving parasite things that can hurt have cells and tissues. Adhesion is thought to expect basic employment in the pathogenesis of trichomoniasis, and Cysteine proteinases give off an impression of being central for the capable Ad-mediated obligation of parasites to targets, little is considered the host cell. These parasites degenerate proteins, such as lamina, vitronectin and various extracellular manifestations, the flu. *T. vaginalis* can produce particles that are transmitted to the target cells and promote cytotoxicity in the event of destruction of the target cell's plasma film. A form of particles in the erythrocyte layers, as recognized by electron microscopy (Cappuccinelli 1993), long lasting by a similar activity to that of performing (Fiori *et al.,* 1999).

CONCLUSION

Trichomonas viganils is an important cause associated with BOH by the ability of the parasite to reach the placenta that means the parasite can escape and across the host defense, which needs further studies.

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