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Poster No 99: Prevalence of 7 sexually transmitted organisms detected simultaneously in Fallopian tube bearing an ectopic pregnancy: The significance of multiplex Taqman real-time PCR in infertility workup

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BACKGROUND

Ectopic pregnancy (EP) is associated with maternal morbidity and occasionally mortality during the first trimester. A history of sexually transmitted infection and pelvic inflammatory disease have been implicated as major risk factors for EP [1,2].

OBJECTIVES

Our aim was To measure the prevalence of *Chlamydia Trachomatis* (CT), *Neisseria Gonorrhoeae*, *Mycoplasma Genitalium* (MG), *Ureaplasma Parvum/Urealyticum*, *Gardnerella Vaginalis*, *Trichomonas Vaginalis* and herpes simplex virus (HSV)-1&2 in Fallopian tubes collected from EP and the results were compared with those obtained from total abdominal hysterectomy (TAH) or tubal ligation.

METHODS

Study design

This was a prospective case-control study conducted from 2012 to 2015 and, fresh Fallopian tubes were collected from 84 EPs and 51 controls as follow: 20 total abdominal hysterectomy (TAH) during the midluteal phase and 31 tubal ligations. The patients were enrolled from 2 Maternity hospitals in the Western region of Saudi Arabia.

Sampling and processing

The collected tubes were immediately cut using RNase/DNase-free equipment (baked at 200 °C for 4 h) into small pieces of 1 cm each. These samples were then transferred in 10 ml of sterile RNA*Later* solution (Ambion, Warrington, UK) for preservation. All the tissues were stored at -80 °C until processed for DNA extraction.

DNA was extracted from a random piece from each tubal specimen using a DNA extraction kit (Qiagen, CA, USA) according to the manufacturer's instructions and following homogenisation using electrical homogeniser and sterile plastic probes (Omni International, GA, USA). The quality and quantity of extracted DNA were assessed with the BioSpec-nano (Shimadzu Corporation, Tokyo, Japan) and typically had an A260/A280 ratio of 1.7 to 1.9. Extracted DNA from all tubes were diluted to a final concentration of 50 ng/µl and aliquots of the diluted samples were stored at -20°C until used

Multiplex-PCR for simultaneous detection of the 7 pathogens in tubal tissues

Multiplex PCR amplification was performed on ABI® 7500 platform (Thermo Fisher Scientific, Warrington, UK) and using the FTD STD9 kit (Fast-track diagnostics, Junglinster, Luxembourg) according to the manufacturer's protocol. The kit is IVD CE certified and contained sets of primers and TaqMan probes that were specifically designed from highly conserved regions of genetic sequences for the 7 pathogens.

RESULTS

Table 1: Details of recruitment of the 135 study participants according to study groups and study centres during the 3 years period of the study.

	Maternity Hospital-Makkah				Maternity Hospital-Jeddah				Althagar General Hospital-Jeddah			
	Year 1	Year 2	Year 3	Total	Year 1	Year 2	Year 3	Total	Year 1	Year 2	Year 3	Total
Ectopic pregnancy (n = 84)	10	11	9	30	7	16	13	36	5	8	5	18
Total TAH (n = 20)	4	3	3	10	3	2	4	9	0	1	0	1
Tubal Ligation (n = 31)	5	5	3	13	4	5	4	13	2	3	0	5
Total (n = 135)	19	19	15	53	14	23	21	58	7	12	5	24

Table 2: Distribution of positive cases of C. trachomatis (CT), N. gonorrhoeae (NG), M. genitalium (MG), U. urealyticum/parvum (U. Urea/Parv), G. vaginalis (GV), T. vaginalis (TV) and herpes simplex virus (HSV)-1/2 in ectopic pregnancy (EP) and control (total abdominal hysterectomy and tubal ligation) groups according to single and co-infections.

		Control (n = 51)	EP (n = 84)	Total (n = 135)
CT	Single infection	1 (1.9%)	6 (7.2%)*	7 (5.2%)
	Co-infections	2 (3.9%)	17 (20.2%)*,‡	19 (14.1%)‡
	Total	3 (5.9%)	23 (27.4%)*	26 (19.2%)
NG	Single infection	ND	ND	ND
	Co-infections	1 (1.9%)	5 (5.9%)	6 (4.5%)
	Total	1 (1.9%)	5 (5.9%)	6 (4.5%)
MG	Single infection	ND	3 (3.6%)	3 (2.2%)
	Co-infections	2 (3.9%)	14 (16.7%)*,‡	16 (11.8%)‡
	Total	2 (3.9%)	17 (20.2%)*	19 (14.1%)
U. Urea/Parv	Single infection	1 (1.9%)	2 (2.4%)	3 (69.2%)
	Co-infections	3 (5.9%)	7 (8.4%)‡	10 (30.8%)‡
	Total	4 (7.8%)	9 (10.7%)	13 (9.6%)
GV	Single infection	ND	ND	ND
	Co-infections	1 (1.9%)	6 (7.2%)*	7 (5.2%)
	Total	1 (1.9%)	6 (7.2%)*	7 (5.2%)
TV	Single infection	ND	ND	ND
	Co-infections	ND	4 (4.7%)	4 (2.9%)
	Total	ND	4 (4.7%)	4 (2.9%)
HSV-1/2	Single infection	ND	1 (1.2%)	1 (0.75%)
	Co-infections	3 (5.9%)	17 (20.2%)*,‡	20 (14.8%)‡
	Total	3 (5.9%)	18 (21.4%)*	21 (15.6%)

(ND = Not detected; * = P < 0.05 compared with control and ‡ = P < 0.05 compared with single infection).

Table 3: Distribution of positive cases of C. trachomatis (CT), N. gonorrhoeae (NG), M. genitalium (MG), U. urealyticum/parvum (U. Urea/Parv), G. vaginalis (GV), T. vaginalis (TV) and herpes simplex virus (HSV)-1/2 in ectopic pregnancy (EP) and control (total abdominal hysterectomy and tubal ligation) groups according to single/co-infections and the city of recruitment.

		Makkah centre		Jeddah centres	
		Control (n = 23)	EP (n = 30)	Control (n = 28)	EP (n = 54)
CT	Single infection	1 (4.3%)	2 (6.7%)	ND	4 (7.4%)
	Co-infections	1 (4.3%)	7 (23.3%)a,*	1 (3.6%)	10 (18.5%)c,*
	Total	2 (8.7%)	9 (30%)a	1 (3.6%)	14 (25.9%)c
NG	Single infection	ND	ND	ND	ND
	Co-infections	ND	1 (4.3%)	1 (3.6%)	4 (7.4%)
	Total	ND	1 (4.3%)	1 (3.6%)	4 (7.4%)
MG	Single infection	ND	1 (4.3%)	ND	2 (3.7%)
	Co-infections	1 (4.3%)	5 (16.7%)a,*	1 (3.6%)	9 (16.7%)c,*
	Total	1 (4.3%)	6 (20%)a	1 (3.6%)	11 (20.4%)c
U. Urea/Parv	Single infection	ND	1 (3.3%)	1 (3.6%)	1 (1.9%)
	Co-infections	1 (4.3%)	2 (6.6%)	2 (7.2%)	5 (9.2%)*
	Total	1 (4.3%)	3 (10%)	3 (10.7%)	6 (11.1%)
GV	Single infection	ND	ND	ND	ND
	Co-infections	ND	3 (10%)	1 (3.6%)	3 (5.6%)
	Total	ND	3 (10%)a,*	1 (3.6%)	3 (5.6%)b
TV	Single infection	ND	ND	ND	ND
	Co-infections	ND	1 (3.3%)	ND	3 (5.6%)
	Total	ND	1 (3.3%)	ND	3 (5.6%)
HSV-1/2	Single infection	ND	ND	ND	1 (1.9%)
	Co-infections	1 (4.3%)	6 (20%)a,*	2 (7.1%)	11 (20.4%)
	Total	1 (4.3%)	6 (20%)a	2 (7.1%)	12 (22.2%)c

(ND = Not detected; a = P < 0.05 compared to control from Makkah city, b = P < 0.05 compared to EP from Makkah centre, c = P < 0.05 compared with control from Jeddah city and * = P < 0.05 compared with single infection).

Table 4: The values of gene copies/ml of positive cases of C. trachomatis (CT), N. gonorrhoeae (NG), M. genitalium (MG), U. urealyticum/parvum (U. Urea/Parv), G. vaginalis (GV), T. vaginalis (TV) and herpes simplex virus (HSV)-1/2 in ectopic pregnancy (EP) and control (total abdominal hysterectomy and tubal ligation) groups according to single and co-infections.

		Control	EP
CT (copies/ml)	*Single infection*	(n = 1) 6.06×10^6	(n = 6) $2.45 \times 10^7 \pm 1.43 \times 10^5$
	Co-infections	(n = 2) $4.19 \times 10^6 \pm 2.98 \times 10^6$	(n = 17) $4.22 \times 10^7 \pm 2.1 \times 10^7$
NG (copies/ml)	*Single infection*	ND	ND
	Co-infections	(n = 1) 6.24×10^4	(n = 5) $7.9 \times 10^5 \pm 2.08 \times 10^5$
MG (copies/ml)	*Single infection*	ND	(n = 2) $5.23 \times 10^6 \pm 2.13 \times 10^6$
	Co-infections	(n = 2) $3.1 \times 10^6 \pm 1.98 \times 10^5$	(n = 14) $9.67 \times 10^6 \pm 3.35 \times 10^7$
U. Urea/Parv (copies/ml)	*Single infection*	(n = 1) 2.2×10^7	(n = 2) $7.54 \times 10^7 \pm 3.54 \times 10^7$
	Co-infections	(n = 3) $1.58 \times 10^7 \pm 0.88 \times 10^7$	(n = 7) $9.87 \times 10^7 \pm 2.15 \times 10^7$
GV (copies/ml)	*Single infection*	ND	ND
	Co-infections	(n = 1) 3.89×10^6	(n = 6) $11.95 \times 10^7 \pm 5.38 \times 10^7$
TV (copies/ml)	*Single infection*	ND	ND
	Co-infections	ND	(n = 3) $1.38 \times 10^6 \pm 0.71 \times 10^6$
HSV-1/2 (copies/ml)	*Single infection*	ND	(n = 1) 3.05×10^4
	Co-infections	(n = 3) $3.34 \times 10^4 \pm 1.41 \times 10^4$	(n = 17) $2.90 \times 10^5 \pm 9.41 \times 10^4$

(n = number of positive cases and ND = Not detected).

CONCLUSIONS

In conclusion, the prevalence *of C. trachomatis, M. genitalium* and HSV-1/2 was significantly higher in the EP group.

Hence, future studies on the effect of these microorganisms on tubal ciliary beat frequency and the expression of implantation markers by the tubal epithelial are required to establish whether they play a role in the pathogenesis of EP.

Additionally, the observed high rates of co-infection advocate the necessity of establishing national guidelines and/or screening program in the kingdom that could probably adopt syndromic approach using multiplex PCR technique for the simultaneous detection of the common sexually transmitted pathogens among high risk groups.

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