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RESEARCH ARTICLE

A STUDY OF CHLAMYDIA ANTIBODY STATUS IN INFERTILE WOMEN AND ITS CORRELATION WITH TUBAL FACTOR INFERTILITY

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ABSTRACT

Background: Persistent exposure to the chlamydia infection results in chronic inflammatory response and increases the risk of tubal damage. Although a few studies have been conducted correlating previous chlamydial infection with laparoscopic findings for TFI no such study has been done from the Indian subcontinent, **Methodology:** 150 women attending the Gynecology outpatient department of Lady Harding Medical College for female factor infertility were enrolled in the study. Estimation of chlamydial antibodies in blood was done for IgG, IgA, and IgM by commercial ELISA kit. Indirect ELISA technique was performed in house to detect IgG antibody against cHSP60. Laparoscopy was done in all these patients to determine the incidence of TFI and to relate it to seroprevalence of Chlamydia. Further the different chlamydial antibodies were related to the TFI Observations and results Out of 150 patients total of 62 patients were found chlamydia antibody positive. The type of Chlamydia antibody found in 62 Chlamydia positive patients, was IgM antibody in 28(45.1%), IgG antibody in 20(32.2%) and IgA antibody in 14(22.5%) patients. On studying the laparoscopic findings in 62 Chlamydia positive patients it was observed that there were 43(69.3%) with TFI as against 19(30.7%) patients who did not have TFI. When Chlamydia antibody negative patients were correlated with TFI it was found that out of 88 patients 44 had TFI and 44 had NTFI. When the prevalence of type of Chlamydia antibody was studied in TFI and NTFI it was observed that there was no significant difference in the IgM antibodies, which is a marker of recent infection, between TFI and NTFI being 16(57.1%) and 12(42.8%) respectively. On the other hand IgG and IgA antibodies which are observed to be marker of past and persistent infection were significantly higher in patients of TFI as compared to NTFI. IgG antibody against cHSP60 was found to be positive in 29 out of 62 Chlamydia positive patients. Only 1 of the patients who was positive for IgM antibody was also found to be positive for cHSP60 antibody. On the other hand, out of 25 IgG positive patients 19(79.2%) tested positive for cHSP60 antibody while 100% patients of IgA antibody were positive for cHSP60 antibody as well Conclusion In the present study the best antibody which could predict tubal damage accurately was observed to be IgA. Other antibodies IgG and cHSP60 also had strong association with TFI therefore they can also be considered good markers for predicting tubal damage in an infertile patient. These patients should have early diagnostic laparoscopy without delay for optimum management.

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INTRODUCTION

Chlamydia infection of female genital tract is an important cause of infertility and is gaining considerable attention. Chlamydia infection causes pelvic inflammatory infection which commonly involves the tubes causing tubal factor infertility. In upto 80% of women this infection is asymptomatic and often silent clinically, therefore majority of these women do not report any history of symptoms suggestive of pelvic inflammatory disease (PID) (Westrom).Persistent exposure to the micro-organism results in chronic inflammatory response and increases the risk of tubal damage (Morre, 2002; Patton, 1994). Persistent antigen synthesis and an ineffective immune response to Chlamydia contribute to chronic inflammation, tissue damageand pathology associated with infertility (Grayston, 1985). C. trachomatis has been detected in 56–79%of the tubes of women with tubal factor subfertility (Pal, 1998; Patton, 1994). Although acute chlamydial infection can be diagnosed by direct detection of microorganism from the infected site by DNA PCR, the organism is no longer detectable once the acute phase is over. Besides culture from cervical swab is generally not possible during infective phase as majority of the patients are asymptomatic. Chlamydia produces several antibodies like IgG, IgA, IgM and Chlamydia specific heat shock protein 60 (cHSP60). These antibodies in serum remain the only markers

of previous Chlamydia involvement and are fairly predictive of chlamydial infection. Presence of IgG antibody indicates previous infection and is observed to be associated with high risk of tubal damage in 70% cases (Mol, 1997). Increased level of IgM antibodies is suggestive of recent infection. Elevated level of IgA antibodies is a marker of chronic persistent inflammation (Sharma, 2003). Enhanced antibody response to Chlamydia specific heat shock protein 60 (cHSP60) has been observed in subfertile women with tubal pathology. These antibodies have been studied and their presence may be indicative of increased degree of tubal damage (Falck, 2002). Their presence would help in detecting prognosis of immune-pathological sequel in C. trachomatis infected women (Claman, 1997). Although a few studies have been conducted correlating previous chlamydial infection with laparoscopic findings for TFI, no such study has been done from the Indian subcontinent. Besides, the association of chlamydial infective status of past, recent and persistent infection i.e. presence of IgG, IgM, and IgA antibody with degree of tubal damage needs further evaluation. Therefore the present study was undertaken to find out the seroprevalence of C. trachomatis infection in patients of infertility by measuring IgG, IgM and IgA antibodies against Chlamydia and to co-relate these with tubal factor infertility.

Aim: To evaluate the chlamydial antibody status in patients of infertility and correlate these with tubal factor infertility.

METHODOLOGY

This study was carried out in Department of Obstetrics and Gynecology in collaboration with Department of Microbiology at Lady Harding Medical College and Smt. Sucheta Kriplani Hospital, New Delhi. 150 women attending the Gynecology outpatient department and infertility clinic of the hospital for female factor infertility were enrolled in the study. Detailed history and examination was done with regards to infertility. Male partner underwent semen analysis. Females were subjected to routine investigations for infertility. Estimation of chlamydial antibodies in blood was done for IgG, IgA, and IgM by commercial ELISA kit. As commercial cHSP60 kit is not available so indirect ELISA technique was performed in house to detect IgG antibody against cHSP60. Women were divided on the basis of Chlamydia antibody into Chlamydia seropositive and Chlamydia seronegative patients. Patients in the Chlamydia positive group were divided according to type of antibody as past infection when IgG antibody was present, recent infection when IgM antibody was present and persistent infection when IgA antibody was present on basis of serological findings. Next, laparoscopy was done in all these patients to determine the incidence of TFI and to relate it to seroprevalence of C. trachomatis. Further different chlamydial antibodies were again correlated to TFI.

Data analysis: Clinical and laboratory data were analyzed using SPSS software. Statistical analysis included chi-square test, t test, and analysis of variance (ANOVA) was implemented to determine the ratio of discrepancies. P value of <0.05 was taken as significant. Sensitivity, specificity, positive predictive value, negative predictive value were calculated.

OBSERVATIONS AND RESULTS

The causes of infertility in 200 patients were analyzed. Out of 200 couples, 40 couples (20.0%) had male factor infertility, 87(43.5%) couples had TFI, and 22(11.0%) had ovulatory dysfunction, 4(2.0%) had uterine cause, and 47(23.5%) couples had no identified cause of infertility. (Table 1)

Table 1. Causes of infertility

Cause of Infertility	No. of patients	Percentage
Make factor	40	20.0
Tubal factor	87	43.5
Ovulatory dysfunction	22	11.0
Uterine factor	4	2.0
Unexplained	47	23.5
Total	200	100

Table 2. Demographic profile of patients in study

Demographic parameter	
Mean age	27.22 ± 3.41 yrs (range 21 to 37 yrs)
Type of infertility	Primary infertility- 109 (72.6%) Secondary infertility- 41(27.3%)
Mean Duration of infertility	4.94 ± 3.16 yrs (range-1 to 19 years)

Table 3. Distribution of type of antibodies in chlamydia positive patients

Type of antibody	No. of patients	Percentage
IgM	28*	45.3
IgG	20	32.2
IgA	14**	22.5
Total	62	100

* 2 also had IgG antibody
** 5 also hadIgG antibody

Table 4. Type of antibodies

Type of antibody	Number of patients
IgM alone	26
IgG alone	20
IgA alone	9
IgM and IgG	2
IgG and IgA	5

In the present study, the 40 patients who were identified to have only male factor infertility were excluded from study. Rest of patients underwent further investigations, 10 patients had obvious ovulatory dysfunction and were managed medically. These patients were also excluded from study. Thus a total of and 150 patients were enrolled into the study.87 patients (58%), out of these 150 had TFI. Of these 6 patients also had associated endometriosis. 63(42%) patients had non tubal factor infertility, these patients had ovulatory dysfunction, uterine anomaly, or unexplained infertility. The mean age of patients in the present study was 27.22 ± 3.41 years. Primary infertility was seen in 109 (72.6%) woman which was much higher than secondary infertility seen in only 41(27.3%) cases. The mean duration of infertility in patients was 4.94 ± 3.16 years and ranged from 1 to 19 years. Demographic profile of patients is depicted in Table 2. Inspite of presenting with infertility, majority of patients had no history suggestive of PID both in TFI and NTFI. Only 13(14.9%) out of 87 in TFI and 10 (15.8%) out of 63 in NTFI had symptoms of PID like acute pelvic pain, chronic lower abdominal pain or discharge per vaginum. Even in patients who were Chlamydia positive only 12(19.4%) out of 62 patients reported history of PID. A total of 62 patients were found chlamydia antibody positive (Table 3). The type of Chlamydia antibody found in 62 Chlamydia positive patients, were IgM antibody in 28(45.1%), IgG antibody in 20(32.2%) and IgA antibody in 14(22.5%) patients. When seroprevalence of different antibodies was estimated, it was observed that a few patients had more than one type of antibody (Table 4). 28(18.6%) patients were positive for IgM antibody and of these 2 patients were also positive for IgG. 14(22.6%) patients

Table 5. Prevalence of chlamydia antibodies in tubal factor infertility and non tubal factor infertility

Type of infertility	Chlamydia positive patients		Chlamydia negative patients		Total
	Number	Percentage	Number	Percentage	
TFI	43	69.3	44	50	87
NTFI	19	30.7	44	50	63
Total	62	100	88	100	150

Table 6. Illustrates the incidence of different chlamydial antibodies in tubal factor infertility and non tubal factor infertility

Type of antibody	TFI		NTFI		Total		p value
	No	%	No	%	No	%	
IgM	16	57.1	12	42.8	28	100	0.149
IgG	15	75	5	25	20	100	0.048
IgA	12	85.7	2	14.2	14	100	0.047
Total	43	69.3	19	30.7	62	100	

Table 7. Performance of each antibody testing in predicting tubal factor infertility

Antibody	Sensitivity	Specificity	PPV	NPV	P Value
IgG	21.3%	92.0%	79.2%	46.03%	0.022
IgA	13.0%	96.0%	85.7%	44.8%	0.027
IgM	28.7%	84.1%	71.4%	46.1%	0.066

Table 8. Comparision of tubal factor infertility in different types of chlamydia antibody in different studies

Author	Type of antibody	No of patients with tubal factor infertility	No. of seropositive patients	Percentage of seropositive patients with tubal factor infertility
Present study	IgM	16	28	57.1
	IgG	15	20	75.0
	IgA	12	14	85.0
Hartog	IgG	32	52	61.5
	IgA	21	44	50
Tanikawa	IgA& IgG	24	51	49.0
Akande	IgG	356	550	64.7
Peivandi	IgG	22	27	81.5

had IgA antibody out of which 5(3.3%) had both IgA and IgG antibodies, 20(13.3%) had IgG antibody alone, 9 had IgA antibody alone. On studying the laparoscopic findings in 62 Chlamydia positive patients it was observed that there were 43(69.3%) with TFI as against 19(30.7%) patients who did not have TFI, a difference that was statistically significant (p=0.018). When Chlamydia antibody negative patients were correlated with TFI it was found that out of 88 patients 44 had TFI and 44 had NTFI (Table 5). Similarly, out of 87 patients of TFI almost half of the patients i.e. 43(49.4%) were Chlamydia antibody positive and other half were Chlamydia negative. When however, the prevalence of Chlamydia antibody was observed in patients of NTFI, only 19 patients out of 63 i.e. (30.1%) were Chlamydia positive as against 70 % who were Chlamydia negative, a difference which was significant (p=0.018) (Figure 1). When the prevalence of type of Chlamydia antibody was studied in TFI and NTFI it was observed that there was no significant difference in the IgM antibodies, which is a marker of recent infection, between TFI and NTFI being 16(57.1%) and 12(42.8%) respectively.

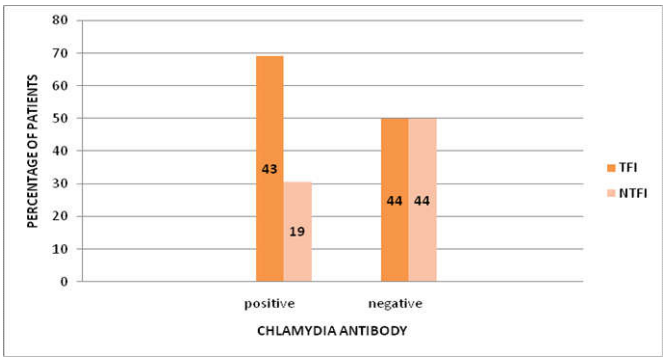

Figure 1. Prevalence of chlamydia antibody in patients of tubal factor infertility and non tubal factor infertility

On the other hand IgG and IgA antibodies which are observed to be marker of past and persistent infection were significantly higher in patients of TFI as compared to NTFI (Table 6). IgG antibody against cHSP60 a marker of chronic chlamydial infection, was studied in those patients who were positive for any of the three chlamydial antibodies. It was found to be positive in 29 out of 62 Chlamydia positive patients. Only 1of the patients who was positive for IgM antibody was also found to be positive for cHSP60 antibody. On the other hand, out of 25 IgG positive patients 19(79.2%) tested positive for cHSP60 antibody while 100% patients of IgA antibody were positive for cHSP60 antibody as well. Table 7 shows performance of each antibody testing inpredicting TFI.

DISCUSSION

In the present study, Chlamydia antibody was positive in 41.3% of cases and negative in 58.7% in all 150 cases of infertility who were enrolled. Prevalence of Chlamydia seropositivity was almost similar to that of Tanikawa et al who also reported that39% of their patients were Chlamydia antibody positive out of 131 infertile women in their study (Tanikawa 1996). On the other hand some authors have reported a low incidence like the study by Peivandi *et al.* where incidence was 25.4% (Peivandi, 2009). Some studies have reported a higher seroprevalence of Chlamydia like study by Akande et al who reported an incidence of 54.6% (Akande 2013). In the present study, when seroprevalance of different antibodies was estimated it was found that 28(18.6%) of patients were positive for IgM antibody, 20(13.3%) had IgG antibody alone and 9(6%) had IgA antibody alone, whereas 5(3.3%) had both IgA and IgG antibodies. Tanikawa *et al.* observed that of the 39% patients who had Chlamydia antibodies, 27.5% were positive for both IgG and IgA

antibodies, 6% were positive for only IgG antibody and 5.5% for onlyIgA antibody (Tanikawa,1996).These authors observed a higher incidence of 27.5% for both IgG and IgA antibodies in their patients as against only 3.3% found in present study. They however, reported a lower incidence of 6% for IgG antibodyalone as against 13.3% in the present study. The percentage of patients with IgA antibody was almost equal being 5% in the above author's study and 6% in the present study. They had not analysed IgM antibody. When only chlamydial IgG antibody was analyzed, its prevalence was found to be 16.6% in the present study, 24.5% in study by Peivandi *et al.* and 54.6% by Akande *et al.* In the present study, IgG antibody against cHSP60 was estimated in Chlamydia antibody positive patients. 29(46.7%) out of 62 Chlamydia antibody positive patients were cHSP60 antibody positive also. All patients who were IgA positive in the study were also positive for cHSP60 antibody. Whereas 19(79.2%) out of 25 patients who were IgG antibody positive had cHSP60 antibody. Patients of recent infection who were IgM positive were all negative for cHSP60 antibody except for 1 patient. From this it appears that cHSP60 antibody correlated best with IgA Chlamydia antibody. Dutta *et al.* studied 255 Indian women with complains of cervicitis, PID, cervical erosion, ectopic, infertility etc. Of these 255 patients, Chlamydia trachomatis infection in endocervical specimens was confirmed by direct fluorescence assay (DFA) and the polymerase chain reaction (PCR) in 75 (29.4%) women. Out of these 75 women, 48 (64.0%) were positive for antibody against cHSP60 (Dutta *et al.,* 2007). As Dutta et al did not study the correlation of cHSP60 and other Chlamydial antibodies, no inference can be drawn on the correlation of cHSP60 antibody and other Chlamydia antibodies i.e. IgM, IgG and IgA. In the present study a statistically significant association was found between C. trachomatis antibody and the presence of tubal damage. TFI was seen in 69.3% of Chlamydia positive as against 50% in Chlamydia negative patients. It was noted that half of the Chlamydia seronegative patients too had TFI which may be due to other known causes of TFI. When the incidence of TFI in Chlamydia seropositive patients of the present study and that of the other authors was compared it was observed that Akande et al reported an almost similar incidence of TFI of 64% in their study done to explore the relationship between Chlamydia antibody titers and tubal damage on 1006 infertile women as against 69.3% in the present study. On the other hand Tanikawa *et al* reported a lower incidence of 49% in their study done to determine the ability of Chlamydia antibody titers to predict severity of tubal adhesions in 131 infertile women (Tanikawa 1996). Peivandi *et al.* however reported an incidence of 81.5% in their study on 150 infertile women to determine the role of Chlamydia IgG antibody testing in predicting TFI which is higher than that of present study (Peivandi, 2009).

On analyzing the reason for difference in prevalence of Chlamydia antibody and its types amongst the patients of infertility in the various studies, it may be due to estimation of different type of Chlamydial antibodies, using different techniques for their estimation, different cut-off values used and different population under study. In present study, the incidence of tubal damage was analyzed in Chlamydia antibody negative patients. It was found to be 50% i.e. in half the seronegative population. This was in contrast to the studies by Tanikawa et al, Peivandi et al and Akande et al where 24%, 13.2 % and 17% seronegative patients had tubal damage respectively (Tanikawa, 1996; akande,2003;Pievandi,2009) which is much lower than that of present study. This might be perhaps due to the fact that the etiology of tubal damage and population studied may be different than the present study as compared to the other authors mentioned above. In the present study, on analysing the other causes of TFI in Chlamydia seronegative patients it was found that 25 patients showed association with tuberculosis either in form of history of pulmonary/ abdominal/ endometrial tuberculosis or presence of tubercles/ beading of tubes on laparoscopy. The cause of TFI in most of these seronegative patients was genital tuberculosis and endometriosis. When incidence of TFI was related to the type of Chlamydia antibody it was observed that amongst 28 IgM antibody positive patients, 57.1% had TFI. On the other hand, 75% ofpatients with IgG antibody, 85% of IgA antibody and 79.3% patients with cHSP60 antibody had TFI. A significant difference in incidence of TFI was evident with regard to different chlamydial antibodies. When the ability of different chlamydial antibodies to predict TFI was analysed, it was observed that IgA antibody was best marker, IgG and cHSP60 were intermediate, whereas IgM was the poorest marker for prediction of TFI.

The ability of prediction of different antibodies for TFI, appears logical as IgM signifies recent infection which may not damage the tubes in the first attack of chlamydial infection and IgG and IgA signify past and persistent infection with greater likelihood of tubal damage. Hartog et al in their study on 313 sub fertile women studied the serological markers of persistent Chlamydia Trachomatis infection (Hartog 2005). They found that 54.2% of IgG antibody positive patients had TFI as against 75% in present study and 35.6% of IgA antibody positive patients had TFI in their study as against 85% in present study. These percentages in the above author's studies were much lower than that found in present study. Tanikawa et al (1996) reported 49% of TFI in their seropositive patients of IgG and IgA antibody whereas Akande et al(2003) reported 64.7% of TFI in their IgG positive patients which was again lower than that of present study. Peivandi et al who estimated only IgG antibody reported 81.5% of TFI in their seropositive patients which was close to that of present study (Peivandi 2009) (Table 8). These differences in the results between present study and that of other authors may again be due to same reasons as stated before like use of different techniques for antibody estimation, different cut-off values used and different population groups studied. In the present study, the predictive efficiency of Chlamydia antibody for TFI were determined by calculating various parameters like sensitivity, specificity , negative predictive value (NPV), positive predictive value(PPV)for each of the IgM, IgG and IgA Chlamydia antibodies. The sensitivity of IgG antibody in predicting TFI was found to be 21.3% which was much lower in comparison to study by Akande *et al*, Tanikawa *et al,* Peivandi *et al* and Hartog *et al.* who reported a sensitivity of 82% ,68.2%, 61.7% and 54% respectively(Tanikawa, 1996; akande,2003;Pievandi,2009). The specificity of IgG in detecting TFI of the present study was high being 92.0% and was comparable to the specificity reported by Hartog *et al.* of 92% and Peivandi *et al.* of 93.5% (Hartog 2005; Peivandi 2009). The specificity reported by Tanikawa *et al.* and Akande *et al.* was 78.8% and 66% respectively which was much lower than that in present study (Tanikawa, 1996; Akande, 2003). When the sensitivity of IgA antibody to determine TFI was analyzed, in the present study it was found that it was much lower (13%) than that reported by Tanikawa et al (68.2%) and Hartog *et al.* (36.0%). The specificity of IgA antibody to detect TFI was 96% which was close to Hartog et al (92%) and much higher than Tanikawa et al (82.7%).

CONCLUSION

Women in study were divided on the basis of Chlamydia antibody serostatus into Chlamydia seropositive and Chlamydia seronegative patients. Out of 150 patients, 62(41.3%) were chlamydia seropositive and 88(58.6%) were chlamydia seronegative. Out of 62 chlamydia seropositive patients 20 (32.2%) had past infection (when IgG was positive), 28(45.1%) had recent infection (when IgM was positive) and 14 had persistent infection(when IgA antibody was positive). Those patients who were chlamydia seropositive were also tested for cHSP60, another marker for chronic chlamydial infection. It was found positive in 29 out of 62 chlamydia positive patients. 19(79.2%) out of 27 IgG positive patients were also positive for cHSP60. 100% patients who were IgA positive were cHSP60 positive. 75% of patients with IgG antibody (marker of past infection), 85% of IgA antibody(marker of persistent infection) and 79.3% patients with cHSP60 antibody (marker of persistent infection)had TFI. Amongst those patients with IgM antibody (marker of recent infection) 57.1% had TFI. Thus, from the above study it is concluded that chlamydial antibody status of past and persistent infection in the patients of infertility correlated well with tubal factor infertility. These calculated parameters of the antibody tests can be used to categorize the patients into low risks and high risk groups. In the present study the best antibody which could predict tubal damage accurately was observed to be IgA, in Chlamydia seropositive patients. Other antibodies IgG and cHSP60 also had strong association with TFI therefore they can also be considered good markers for predicting tubal damage in an infertile patient. These patients should have early diagnostic laparoscopy without delay for optimum management.

Conflict of interest: None

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