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Study the relation between *Chlamydia trachomatis* infection and poor semen quality that cause infertility in Iraqi male

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Abstract

Chlamydia trachomatis (C.T.) infection is considered to be one of the most common sexually transmitted diseases it is associated with a wide clinical spectrum causing infertility. The study involves two groups, the first group included 64 males as a patient group (they failing to conceive a child with their partner after at least 1-3 years of marriage), while the second group included 10 males as control group. (They had children). Serum and semen antichlamydial antibodies using Enzyme linked Immune Sorbent Assay (ELISA), and standard semen analyses in both studios group were investigated. Results showed seropositive association between C.T. infections with poor semen quality. The prevalence of C.T. IgG was significantly higher in patient group (53.1%) as compared with control group. ($p \leq 0.05$) , while the prevalence of IgA in patient group was (12.5%). Standard semen analysis showed abnormalities in various semen parameters of the seropositive male patient. The results showed that the prevalence of anti-chlamydial IgG, IgA in semen sample were (6.6%, 18%).

Keywords: *Chlamydia trachomatis*, Infection, Semen quality, Men, Iraq.

Introduction

Chlamydia trachomatis an obligate intracellular parasite, has a biphasic life cycle characterized by an elementary body (EB) with infective capacity and reticular body that in able to replicate with in eukaryotic cells (Annalisa *et al.*, 2006). *Chlamydia trachomatis* (C.T) is the most prevalent bacterial cause of sexually transmitted infections moreover World Health Organization (WHO) estimates that more than 92 million all C.T infection occurred worldwide in that last year's (WHO 2001), this high diffusion is probably due also to the fact that approximately 75% of C.T infections in women and up to 50% of those in men are asymptomatic (Cai *et al.*, 2009). In men, Chlamydial infection is associated with epididymitis and/ or prostatitis that can lead to stenosis of the duct system, orchitis or an impairment of male sexual accessory gland function (Annalisa *et al.*, 2006).

Recent reports showed a clear correlation between poor semen quality and C.T. infection in male patient (Mazzoli *et al.,* 2009). These reports suggest that it may cause male infertility by acting directly on sperm; indeed incubation with C.T. has been reported to decrease sperm motility and to increase the number of nonviable sperm (Hossein *et al.,* 2001). The importance of methodology in screening C.T. as asymptomatic infection of male genital tract are difficult to detect. A number of studies have based their diagnosis on an attempt to culture C.T. from ejaculates or urethral swabs however seminal plasma in known to be inhibitory to the effective culture of C.T. other studies have attempted overcome these problems by attempted to use serological method although there is some differs between individuals in the immune response more recently, authors have measured chlamydia specific immunoglobulin (IgG, IgA) either in serum or seminal plasma (Gdoura *et al.,* 2007) .

The aim of this study is to determine the seroprevalence of *Chlamydia_trachomatis* infection and its association with semen quality in Iraq infertile males.

Materials and methods

Seventy four males attending to the Institute of embryo and infertility treatment in Al-Nahrain University were enrolled in this study, those male divided in to two groups, the first group included 64 male as patient group (they failing to conceive a child with their partner after at least 1-3 years of marriage), while the second group included 10 meals as control group (they had children). Samples of blood and seminal fluid were taken from patients and control this study:

1- Blood samples were taken and sera were used for :
   a- Determination of IgG, antibodies against *Chlamydia trachomatis*_was done by using Enzyme linked Immune Sorbent Assay ELISA kit (human, Germany) .
   b- Determination of IgA. Antibodies against *Chlamydia trachomatis* was done by ELISA kit.

All assays and calculations were preformed according to the manufacturer's instructions.

2- Seminal fluid used for :-
   a- Routine semen analysis: Prior to semen analysis, the male patients were asked to abstain from sexual intercourse for 3-5 days before the seminal fluid analysis. All samples collected in to standard containers that had previously been shown not to have any cytotoxic effect on human spermatozoa .The semen was allowed to stay up to 30 minutes to liquefy at 37^{c} before the analysis. Semen analysis was performed according to (WHO 2010) guidelines with all the measures of semen quality parameters i.e. sperm morphology, sperm motility, sperm vitality, concentration and leucocyte enumeration. Eosin – negrosin stain was used to performed sperm morphology.
   b- Determination of IgG, IgA antibodies against *Chlamydia trachomatis* were done by using ELISA technique.

Results and Discussion

A. Serum analysis : In this study we showed seropositive association between *Chlamydia trachomatis*_infection with poor semen quality. The prevalence of *Chlamydia trachomatis* (C.T.) IgG antibodies were significantly higher in patient group (53.1%) as compared with control group (20%). (P≤0.05, chi – square =3.79, DF=1) (Tale 1).

Table(1): Classification of infertile male patient group and control group infected with *Chlamydia trachomatis* in semen and seminal fluids sample

	Patient group				Control group				
Fluids	No. +ve	%	No. -ve	Total no.	No. +ve	%	No. -ve	Total no.	
Serum:									
IgG	34	53.1	30	64	2	20	8	10	p≤0.05
IgA	8	12.5	56	64	0	0	10	10	p>0.05
Seminal fluid:									
IgG	4	6.6	60	64	0	0	10	10	p>0.05
IgA	12	18	52	64	1	10	9	10	p>0.05

These result were in agreement with previous studies that reported the prevalence of IgG against C.T. were (46%) (Mania *et al.,* 2001). But our results were disagreement with other studies those found high prevalence rate of IgG against C.T. (85, 4%) (Ali *et al.,* 2003), while other studies found low prevalence of IgG against C.T. (24%, 17%) (Ibadin *et al.,* 2009; Ochsendorf *et al.,* 1999). The prevalence of serum IgA antibodies against C.T. in patient group was (12.5%) while the prevalence of IgA antibodies in control group was (0%) . there is no significant difference between patient group and control group (p>0.05, chi–square=1.40, df=1). The immune pathogenesis of C.T. disease is current unclear . It been proposed that persistent infection leading to chronic inflammation and tissue damage can result from interleukin -10 (IL-10) down regelation of chlamydial – specific T-cell response (Hafner *et al.,* 2008).

B: Seminal fluid analysis: We found a good correlation between C.T. infection and poor in some semen parameters such as presence of white blood cells, dead sperms and anti-chlamydial antibodies in semen, reduce in sperm motility and sperm count (Table 2). Many recent studies were in agreement with our result they found that the infection with C.T. cause abnormalities in semen quality (WHO, 2001; Hossein *et al.,* 2001; Ibadin *et al.,* 2009). The infection cause physical blockage created by obstruct the movement of sperm, C.T. can also cause epithelial damage that reduce spermatogenesis induce the female partners fertility (Okoror *et al.,* 2012), reports have it that C.T. may attach to spermatozoa there by

immobilizing them (Okoro *et al.,* 2010). Recently many studies demonstrated that C.T. can have a direct and negative effect on sperm motility which was reflected is in corresponding increase in sperm death (Hossein *et al.,* 2001). The same group have also demonstrated that C.T. induced death of human sperm is primarily caused by lipopolysaccharide (LPS), It is the immune dominant antigen of most Gram – negative bacteria. LPS of C.T. is known to be particularly spermicidal (Hossein zadeh *et al.,* 2003). In particular, LPS of C.T. interacts with CD14 on the sperm surface leading to increased production of reactive oxygen species resulting in apoptosis (Eley *et al.,* 2005).

In only 4/64 (6.6%) of seminal fluid samples of patient group, IgG antibodies against C.T. was detected. there is no significant difference between patient group and control group ($P>0.05$, chi square=0.66, DF=1). These result was similar to result obtained in other studies which found the prevalence of IgG in semen of patient was 5.3% (Weidner *et al.,* 1996), while other studies obtained more than this percentage like (8.1%) (Waltraud *et al.,* 1996). In our study we found the percentage of IgA in seminal fluid were (18%). While the percentage of IgA in control group was 10% ($p>0.05$, chi-square 0.5, dfs1). These result were supported by other studies recorded the percentage of IgA against C.T. in semen was (18%, 19.9%) (Weidner *et al.,* 1996; Waltraud *et al.,* 1996). Some authors showed the level of IgA against C.T. is more higher than this level they recorded (26.7%, 26.9%) (Ochsendorf *et al.,* 1999; El-feky *et al.,* 2009). It is assumed that bacterial infections of genital tract ,in particular with C.T., may stimulate the Immune response ,perhaps via vasoepididymitis with unilateral obstruction or exposures of spermatozoa to immunologically competent cells in inflammatory conditions this infection may cause occultation in the canalicular system of male genital tract, may damage the epithelial cell involved in the spermatogenesis (Mania *et al.,* 2001).

Table (2): Semen parameters of seropositive (IgG against *Chlamydia trachomatis*) infertile male patient.

Age (years)	No. of seropositive IgG	TC.	TM.	MOR.	VIT.	WBC.	Liq. Time
20-24	4	8.3	3.2	8	20	9	90
25-29	8	9	5.1	10	14	7	55
30-34	10	7.4	2.9	16	17	11	80
35-34	6	6.2	4.2	11	13	8	75
35-39	5	9.5	3.7	10	15	14	60
40-44	3	8	5.5	14	20	13	85
>45							

Keys:	WHO criteria (2001)
TC. Total sperm count * 10^6/ml	≥15 million / ml
TM. Total motility (%)	≥ 7.2 million / ejaculate
MOR. Morphology (%)	≥ 30 %
ViT. Vitality (%)	>75 % (live)
WBC. Count * 10^6 / ml	≤ 5 cell / hpf
Liquefaction time	Within 60 minutes

Conclusions

This study thereby concludes that C.T. is serious sequelae in male infertility and could actually be the cause of low sperm counts as observed in this study and it is also suggested that during semen analysis for sperm count, pathogens like C.T. should be also tested for and further enhance treatment.

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