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Correlation between Leukocytospermia Detected by Cytochemical Peroxidase Staining and Chlamydia Trachomatis Infection in Iraqi infertile Males

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Abstract
Background and objective: To detect pus cells in semen by cytochemical peroxidase staining method and to study whether there is a difference in its distribution among infertile males with positive, negative titer of antichlamydial immunoglobulins compared with normal fertile males **Patients and Methods:** Semen samples were collected from 144 infertile male, 35 normal fertile. Seminal plasma was used for detection of chlamydia antibodies by Microimmunofluorescence test (MlF) using species-specific antigens (genital serotypes D-K)**Results:**Only 1/144(0.69%) was IgM positive at a titer (1:16) in secondary infertile. Only 3/144(2.08%) primary infertile males were IgG positive, the frequent titer was (1:64). Only 64/144 (44.44%) infertile were IgA positive. IgA was detected in (65.62%) among primary infertile at a titer (1:64) . Only 40/144 infertile (27.77%) were IgA and IgG positive .IgA and IgG were detected together in (52.5%) among secondary infertile males at a titer (1:64) and at a titer (1:8) in (12.5%) among primary infertiles. No classes of immunoglobulines were detected at any titer in seminal plasma among normal fertile males.Out of 144 infertile males 93(64.58%) with history of urogenital tract infection (UGI) . The majority of males with history of UGI were primary infertile with positive titer in seminal plasma. Only 4/108 (3.7%) azoospermic males with positive titer in seminal plasma have previous history of (UGI) .Peroxidase positive leukocytes with less than $1x10^6$ cell/ml of semen were detected in 108/179(60.33%) and (32.96%) were infertile with positive titer .Peroxidase positive leukocytes ≥ 1x106 cell/ml of semen detected in (24.58%) . The incidence of this count was high among infertile males with positive titer (16.2%) . The incidence of peroxidase positive leukocytes at the count more than 4x106 cell/ml of semen was high among infertile males with positive titer.**In conclusion:** There was no relationship between count of pus cells and antichlamydial antibodies in seminal plasma of infertile males. Antichlamydial immunoglobulins were detected in (32.96%) among infertile males with positive titer in seminal plasma and pus cell <$1x10^6$ cell/ml of semen. The cytochemical peroxidase method is cheap, fast and easily to perform and reliable to identifies granulocytes, which is the most prevalent WBC type in semen.
Keywords: Infertility**,** Antichlamydial immunoglobulins, pyospermia, peroxidase

Introduction

Sexually transmitted infections (STIs) are a major public health problem in most parts of the world, and are responsible for a number of acute illnesses, infertility, long-term disability, and premature death, in addition to contributing to an increase in the spread of HIV[1].C. trachomatis is the most prevalent bacterial cause of sexually transmitted infections in the world and can result in severe genital disease . Over 90 million chlamydial infections are detected annually worldwide and various studies have estimated that there are four to five million new cases of chlamydial infection each year in the USA alone [2]. However, the reported incidence rates of genital chlamydial infections in the population likely are an underestimate because of the highly asymptomatic nature of the pathogen. Approximately 50% of infected men have asymptomatic urogenital infections, which represents a huge population of untreated individuals who can transmit the organism [2].

Both acute and chronic C. trachomatis infection and/or inflammation can cause partial or complete obstruction of sperm transport with, respectively, oligozoospermia or azoospermia. Bilateral obstruction of the epididymis is common after recurrent infection with C. trachomatis [3]. On the other hand, chronic inflammatory changes in the seminiferous tubules observed in orchitis, would be expected to disrupt the normal process of spermatogenesis and cause alterations both in sperm number and quality [4].

The main objective of this study is to detect pus cells in semen by cytochemical peroxidase staining method and to study whether there is a difference in its distribution among infertile males with positive, negative titer of antichlamydial immunoglobulins compared with normal fertile males.

Materials and methods

One hundred seventy nine infertile patients were examined in Kamal Al-Samarae Hospital for infertility and In vitro Fertilization from November 2000 to November 2001. Ethics committee of Baghdad University, College of Medicine, approved the present research. At first the aim of study was explained for all participants and after obtaining their oral and signed consent they have been studied. The age of the patient ranged between 22-55 years and the duration of infertility problem ranged from 1-18 years.

Seminal plasma was collected from 144 male. The criteria for patients' selection were those having abnormal of seminal fluid parameters during examination with history of primary and secondary infertility. Thirty-five normal fertile volunteers were examined as a control group. Patients were provided with clearly written or oral instructions as appropriate concerning the collection of semen sample .Each sample was collected after a minimum of 48 hours but not longer than seven days of sexual abstinence[5] .

The sample was obtained by masturbation and ejaculated into clean, wide mouth container . The name of the man, the period of abstinence, the date and time of collection and the interval between collection and analysis were recorded. Seminal plasma was separated by centrifugation of semen samples at 300-500 g which is yet enough to remove the seminal plasma from cellular components. Seminal plasma was dispensed in aliquots and was kept at – 20^{o}C until used [5].

Blood samples

Ten ml of blood sample was collected from each infertile and normal fertile volunteer. Freshly isolated blood sample was incubated at 37^{o}C in water bath or incubator and centrifuged at 2000g for 30 minute and then after that serum was very carefully aspirated from the cells, blood clot, and aliguoted as required then kept at-20^{o}C until used[6].

Microimmunofluorescence test (MIF)

MIF test was used to examine the seminal plasma of infertile and fertile males.

Preparation of antigen smears:

Ten µl of *C. trachomatis* antigen was applied to each slide circle, slide was air dried for at least 30 minute, it was important that all antigen dots were completely dried and fixed in acetone for 10-15 minute at room temperature. Then the fixed slides were wrapped with clean papers and kept in airtight container at -20^{o}C until used[7].

Fluorescein labeled conjugate dilution:

Two folds dilution of immunoglobulins conjugate were made in Phosphate buffer saline(PBS), starting with 1:5 to 1:80. These were tested by MIF to demonstrate a dilution, which gives best fluorescence. Thus a dilution 1:10 was chosen for use in the test. Each immunoglobulin conjugate was reconstituted according to labeled directions and dispensed in to 50µl aliquots and were kept at -20^{o}C until used.

Microimmunofluorescence test procedure:

The thawed slides were incubated for 30 min. at 37^{o}C with the appropriate antiserum or seminal plasma dilution (two-fold dilution from 1:2 to 1:256) diluted in PBS, pH7.2. Three circles of each slide were used for positive serum, negative serum and antigen controls. Before being washed by dipping in two PBS Jar each for 5min. and in distilled water for 5min.; 10µl fluorescein isothiocyanate conjugate at a predetermined working dilution was added to each well which was freshly diluted 1:10 and stained with 1% Evan's blue (50µl conjugate + 50µl of Evan's blue +400µl PBS). The slides were incubated for 30min. at 37oC in moist chamber and dark place. Then the slides were washed with PBS and distilled water as previously described. Glycerin buffer was used in mounting step by adding small drop on each circle of the slide and then a cover slip was applied over the slide for examination by fluorescent microscope with a 40X Lens and exciter filter No.3 and barrier filter No. 3[8]. The highest dilution giving specific fluorescence associated with elementary bodies was regarded as end point [7].

Leukospermia : leukocyte count of ≥ 1000 000 / ml of semen was considered as an indication for presence of infection [9].

Peroxidase activity stock solution

This solution used for detection of polymorphonuclear cells in semen. The solution consist of the following:- Ethanol (50ml),Distilled water(50ml),Benzidine(125mg),H_2O_2 30%(5µl).A stock solution was prepared by mixing 50ml distilled water with 50ml of 96% ethanol, then 125mg benzidine was added to previous mixture and finally the working solution was obtained by addition of 5µl of 30% H2O2 to 4ml of stock solution. The stock solution could be stable for more than a year if stored at 4oC[10] [11].

Detection of leukocytes in semen By cytochemical peroxidase method

The method is based on chemical visualization of peroxidase activity within polymorphonuclear granulocytes. Twenty µl Peroxidase activity stock solution were mixed with 20µl of ejaculate in a small test tube. After incubation for 5 minutes at room temperature, intensely brown cells were counted in a haemocytometer and rated as peroxidase granulocytes[10, 12].

More than 10^6 peroxidase positive cells per ml semen are indicative of genital tract inflammation[9] .The

concentration of leukocytes were calculated relatively to known number of spermatozoa by mean of the following formula:-

$$C = N/S$$

C: concentration of leukocytes in million /ml of semen
N: number of leukocytes counted in the field (s) per 100 spermatozoa
S: the sperm count in millions /ml

Results

Out of 144 seminal plasma of infertile males, only 1(0.69%) was IgM positive at a titer (1:16) detected in secondary infertile male (Table 1). IgM not detected at any titer in seminal plasma among normal fertile males. Only 3/144(2.08%) infertile males were IgG positive (Table 2). IgG was detected only among primary infertiles and more frequent titer was (1:64). IgG was not detected at any titer in seminal plasma among normal fertile males.

Only 64/144 (44.44%) seminal plasma samples of infertile males were IgA positive (Table 3 and figure1). IgA was detected more frequently in (65.62%) among primary infertile males at a titer (1:64) than in secondary infertile males . IgA at a titer (1:8) was detected least frequently in (1.56%) among primary infertile males. IgA was not detected at any titer in seminal plasma among normal fertile males.

Only 40/144 seminal plasma samples of infertile males (27.77%) were IgA and IgG positive (Table 4). IgA and IgG were detected together more frequently in (52.5%) among secondary infertile males at a titer (1:64) than in secondary infertile males IgA and IgG were detected together least frequently at a titer (1:8) in (12.5%) among primary infertiles. IgA and IgG were not detected at any titer in seminal plasma among normal fertile males.

Out of 144 infertile males 93(64.58%) with history of urogenital tract infection (UGI) and the remaining 51(35.41%) without history of UGI. The majority of males with history of UGI were primary infertile with positive titer in seminal plasma while only (5.37%) were primary infertile with history of UGI and negative titer. Four (3.7%) azoospermic males with positive titer in seminal plasma have previous history of (UGI) in which 3 (2.78%) were primary infertiles and only 1(0.93) was secondary infertiles. Thirty six Patients with negative titer in seminal plasma with positive history of UGI represent. Azoospermic patients in current study 9/36 (25%). Those with negative titer in seminal plasma and positive history for UGI represent 2/36(5.56%) azoospermic males .Only 7/36(19.44%) azoospermic males with negative history of UGI(Table 5).

A total of 179 males were investigated, 108(60.33%) were infertile with positive titer, 36(20.11%) were infertile with negative titer and 35(19.55%) were normal fertile with negative titer (Table 6). Peroxidase positive leukocytes with less than 1x106 cell/ml of semen were detected more frequently in 108(60.33%) out of 179 (figure2).The majority of males (32.96%) were infertile with positive titer in seminal plasma. (Figure 2) .Peroxidase positive leukocytes ≥ 1x106 cell/ml of semen were detected in (24.58%) and the incidence of this count was high among infertile males with positive titer (16.2%) and least frequently detected among fertile males with negative titer (2.79%). The incidence of peroxidase positive leukocytes at the count more than 4x106 cell/ml of semen was high among infertile males with positive titer.

Table (1): Distribution of (1) C.trachomatis positive seminal plasma IgM according to titer

No. of semen samples for infertile patients	No. of anti C.trachomatis IgM positive seminal plasma	IgM Seminal plasma dilution /No. of reactor / percent								No.(%) of IgM positive cases
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	
144	Primary infertile	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Secondary infertile	0(0%)	0(0%)	0(0%)	1 (0.69%)	0(0%)	0(0%)	0(0%)	0(0%)	1(0.69%)
	Total No. (%)	0(0)	0(0%)	0(0%)	0(0%)	0(0)	0(0%)	0(0%)	0(0%)	0(0%)
No. of seminal plasma for control group	No(%) of anti C.trachomatis IgM positive in Fertile males	IgM Seminal plasma dilution /No. of reactor / percent								
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	No.(%) of IgM positive cases
35	Total No. (%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

Table (2): Distribution of (3) positive seminal plasma IgG according to titer

No. of semen samples for infertile patients	No. of IgG positive seminal plasma	IgG Seminal plasma dilution /No. of reactor / percent								No. (%)of IgG positive cases
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	(%)
144	Primary infertile	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	2(1.39 %)	0(0%)	1 (0.69%)	3 (2.08 %)
	Secondary infertile	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
		0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1 (33.33%)	3 (2.08%)
No. of seminal plasma for control group	No. of positive seminal plasma in Fertile males	IgG Seminal plasma dilution /No. of reactor / percent								No. (%)of IgG positive cases
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	
35	Total No. (%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

Table (3): Distribution of (64) positive seminal plasma IgA according to titer

No. of semen samples for infertile patients	No. of IgA positive seminal plasma	IgA Seminal plasma dilution /No. of reactor / percent								No. (%) of IgA positive cases
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	
144	Primary infertile	0(0%)	0(0%)	1 (1.56%)	6 (9.37%)	3 (4.68%)	20 (31.25%)	5 (7.81%)	7 (10.93%)	42 (65.62%)
	Secondary infertile	0(0%)	0(0%)	0(0%)	1 (1.56%)	4 (6.25%)	9 (14.06%)	6 (9.37%)	2 (3.12%)	22 (34.37%)
	Total No. (%)	0(0%)	0(0%)	1 (1.56%)	7 (10.93%)	7 (10.93%)	29 (45.31%)	11 (17.18%)	9 (14.06%)	64 (44.44%)
No. of seminal plasma for control group	No. of IgA positive seminal plasma in Fertile males	IgA Seminal plasma dilution /No. of reactor / percent								No. (%) of IgA positive cases
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	
35	Total No. (%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

Table (4): Distribution of (40) positive seminal plasma IgA and IgG according to titer

No. of semen samples for infertile patients	No.(%) of IgA IgG positive seminal plasm	IgA, IgG Seminal plasma dilution /No. of reactor / percent								No. (%) of IgA, IgG positive cases
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	(%)
144	Primary infertile	0(0%)	0(0%)	5(12.5%)	2(5%)	3(7.5%)	4(10%)	1(2.5%)	4(10%)	19(47.5%)
	Secondary infertile	0(%)	0(0%)	0(0%)	3(7.5%)	7(17.5%)	8(20%)	2(5%)	1(2.5%)	21(52.5%)
		0(0%)	0(0%)	5 (12.5%)	5(12.5%)	10(25%)	12(30%)	3(7.5%)	5(12.5%)	40(27.77%)
No. of seminal plasma for control group	No.(%) of IgA, IgG positive seminal plasma in Fertile males	IgA, IgG Seminal plasma dilution /No. of reactor / percent								
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	IgA ,IgG positive cases No. (%)
35	Total No. (%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

Table (5): Relationship between seminal antichlamydial immunoglobulins and history of urogenital tract infection

History of UGI* among Infertiles No.(%)		Patients with positive titer in seminal plasma No. (%)	Patients with negative titer in seminal plasma No. (%)
Positive history of UGI* 93 (64.58%)	Primary infertile	41(44.08)**	5(5.37)
	Secondary infertile	37(39.78)**	10(10.75) $
	Total No. (%)	78(83.87)	15(16.12)
Negative history of UGI 51 (35.41%)	Primary infertile	22(43.13)	18(35.29)$$
	Secondary infertile	8(15.68)	3(5.88)
	Total No. (%)	30(58.82)	21(41.17)
Total No. 144			

*** UGI Urogenital infection**

** four (3.7%) azoospermic males with previous history of (UGI) in which 3(2.78%) of them were primary infertiles and only 1(0.93) was secondary infertiles

$: 9/36 (25%) azoospermic males in which only 2/36(5.56%) azoospermic males with previous history of UGI

$$:7/36(19.44%) azoospermic males with negative history of UGI

Table (6): Relationship between antichlamydial immunoglobulins in seminal plasma and the presence of leukocytes in semen

Parameters	No. of leukocytes x 10^6 cell/ml semen										Total No.(%)
	<1	≥1	2	3	4	5	6	7	8	9	
No.(%) of Infertile males with positive titer	59 (32.96%)	29 (16.2%)	7(3.91%)	2(1.11%)	2(1.11%)	2(1.11%)	2(1.11%)	1 (0.55%)	1 (0.55%)	3 (1.67%)	108(60.33%)
No.(%) Infertile males with negative titer	20 (11.17%)	10 (5.58%)	4 (2.23%)	1 (1.11%)	1 (1.11%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	36 (20.11%)
No.(%) fertile males with negative titer	29 (16.2%)	5 (2.79%)	1 (1.11%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	35 (19.55%)
Total No.(%)	108(60.33%)	44(24.58%)	12(6.70%)	3(1.67%)	3(1.67%)	2(1.11%)	2(1.11%)	1(0.55%)	1(0.55%)	3(1.67%)	179(100%)

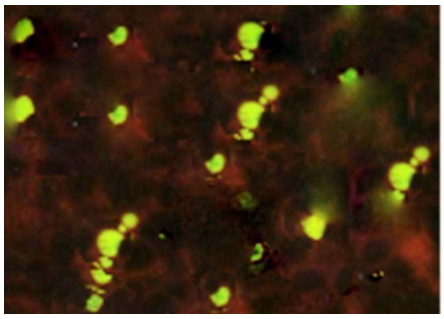

Figure (1): Specific fluorescence of *C.trachomatis* (100X) by microimmunofluorescence test in seminal plasma of infertile males

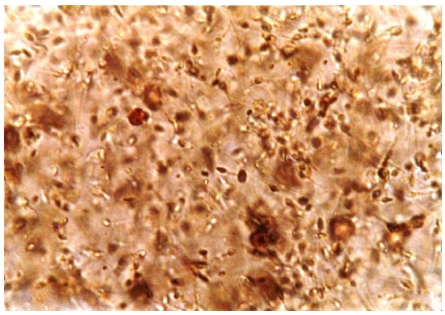

Figure (2): Appearance of peroxidase positive polymorphonuclear cells in semen (40x*)*

Discussion

In current study, only 1/144(0.69%) seminal plasma of infertile males was IgM positive at a titer (1:16) detected in secondary infertile male . IgM not detected at any titer in seminal plasma among normal fertile males. This

result reflect the cause of limited diagnostic value of anti C.trachomatis IgM depending on the fact that chlamydial infection tend to be chronic [13].

In current study, IgG was detected in (2.08%) seminal plasma of primary infertiles with a titer (1:64) to (1:256) . IgG was not detected in secondary infertiles . this result come in line with [13] and [14] disagree with this result, they found that antichlamydial IgG was detected in (8.33%) seminal plasma with a titer ≥ 1:32. In current Regarding to the titer of antichlamydial immunoglobulins, IgA was the highest class that detected in (44.44%) seminal plasma with a titer from (1:8 to 1:256). IgA was detected in (65.62%) seminal plasma of primary infertile more than that of secondary infertile males. The predominant titer was (1:64) that detected in (31.25%) seminal plasma and the least frequent titer was (1:8) that detected in (1.56%) seminal plasma. This result come in line with [13] and in contrary with [15], reported that Genus specific anti-chlamydia-IgA was found in (9%) of the seminal plasmas of infertile males. While [16] reported that seminal antichlamydial IgA was detected in 23% of primary infertile males at a titer ≥1:64. The higher incidence of both IgA and IgG in seminal plasma using MIF support the fact that infertile males had previously encountered with sexually transmitted *C.trachomatis* in the form of chronic asymptomatic infection within genital tract [17]. It is clear from this work that the presence of specific antichlamydial IgA in seminal plasma of infertile males that detected by MIF at a titer ≥1:8 consider a true indicator of local, chronic, chlamydial infection and suggest an ongoing inflammation in male genital tract caused by *C. trachomatis* genital serotypes [15].

This study show that both IgA and IgG were detected in (52.5%) seminal plasma of secondary infertile males and in (47.5%) seminal plasma of primary infertile males . the predominant titer in seminal plasma was (1:64) which was detected in (30%) of positive samples. This result come in line with [13]reported that 27.77% of infertile males have both IgA and IgG .

This finding support the fact that local IgA and IgG that detected in seminal plasma are significantly associated with *C.trachomatis* specific antibodies in serum and the presence of both IgA and IgG in seminal plasma consider as indicator for the presence of *C.trachomatis* that may reside in accessory glands and act as immunostimulator for the continuous production of antibodies. The differences in detectable titer in seminal plasma may be attributed to the differences in host ability to develop an immune response to chlamydial antigens, which may be related to human leukocyte antigen (HLA) class II [18].

The incidence of positive titer in seminal plasma among infertile males with previous history of urogenital infection (UGI) was (83.87%) in which (44.08%) were primary infertile and (39.78%) were secondary infertile. While the incidence among those without previous history of UGI was (58.82%) in which (43.13%) were primary infertile and (15.68%) were secondary infertile males. in contrary to this result[16] reported that the incidence of seminal antichlamydial immunoglobulins among primary infertile males with history of UGI was (51.1%) while among those without history of UGI the incidence was (26.9%). Others [19]reported that seminal antichlamydial immunoglobulins were detected in (38%) among primary infertile males with previous history of UGI . In contrary,[20] reported that incidence of antichlamydial immunoglobulins in seminal plasma of primary infertile was (26%) among infertile males with previous history of UGI.

Highest detection of antichlamydial immunoglobulins in seminal plasma of infertile males with previous history of UGI, support the possibility that C. trachomatis was harbored in the epididymis, seminal vesicle or prostate which lead to obvious alterations in semen parameters and subsequently causing infertility [19]. Detection of antichlamydial immunoglobulins in seminal plasma of infertile males without history of UGI, support the fact that one of the most important clinical features of chlamydial infection is the long latent period between the time of exposure and the onset of symptoms which ranges from weeks to months. For this reason chlamydial infection is most often asymptomatic and frequently not identified until overt infection occur beside incorrect administration of antibiotics which may be leads to suppression of chlamydial infection without complete eradication accompanied by absence of clinical signs.

The higher incidence of abnormal forms among infertile males with positive titer observed in current study in semen analysis may attributed to the presence of chlamydial genital infection in which elementary bodies adhere and enter in to spermatozoan head or tail leading to abnormal morphology of spermatozoa beside the fact that the majority of infertile males with positive titer in seminal plasma have significant elevation in the number of white blood cells (WBCs) in semen more than $1x10^6$ cell/ml and this will be stimulate the production of hydrogen peroxide, oxygen radical and reactive nitrogen intermediate by activated macrophage and granulocytes. This products have a highly deleterious effect on spermatozoa and the degree of sperm damage by WBCs products depends on the location of inflammatory reaction, the duration of exposure of sperm to these products, beside the possibility of genetic defects in the formation of sperms during spermatogenesis also must be keep in mind [1, 17]

This comes in agree with [16, 17]where they found that percentage of nonmotile spermatozoa was higher among those with positive evidence of chlamydial infection either by direct or indirect detection method, when compared with infertile with negative evidence or normal fertile males. This could be due to direct effect of chlamydial elementary bodies (EBs) that impaired motility of sperms by its adherence and entry to head or

tail of spermatozoa or as a result of indirect effect of chlamydial infection that cause elevation of leukocytes in semen even in the absence of signs which reflect an inflammatory response .

Leukocytes play an important role in impairment of sperm motility by producing reactive oxygen species (ROS) which have a deleterious effect on spermatozoa . Impairment of sperm motility may be due to presence of anti-sperm antibodies as a result of chlamydial infection or other genital infections [1, 21] .

This study revealed that among infertile males with positive titer in seminal plasma there was four (3.7%) azoospermic males with previous history of urogenital infection (UGI) in which 3 of them were primary infertiles and only one was secondary infertiles. Among infertile males with negative titer in seminal plasma there was 9/36 (25%) azoospermic males in which only two azoospermic males with previous history of UGI. This coincide with [13, 17]found that azoospermic males with positive antichlamydial antibodies in seminal plasma were more observed among primary infertile males with positive seminal plasma.

In this study, the presence of four azoospermic males with positive titer in seminal plasma and previous history of UGI may be attributed in part to chronic genital infection with C. trachomatis which may be reside in epididymis and subsequently lead to tubal occlusion of the duct system in testis or may be due to disturbance in sexual hormone levels or as a result of congenital factors[1, 13]

This work shows that incidence of peroxidase positive leukocytes (granulocytes) with less than $1x10^6$ cell/ml of semen was detected among infertile males with positive titer in seminal plasma more than that of infertile males with negative titer and normal fertile. This result comes in agree with that reported by [22]where they reported that even if leukocytospermia is not observed in semen of asymptomatic infertile males, C. trachomatis can be detected in semen samples.

The presence of low number of leukocytes in semen samples among infertile males with positive titer may attributed to continuous administration of antibiotics without seeking medical advice there by eradication or diminishing of the presence of C. trachomatis in the genital tract will be happen and subsequently the inflammatory response will be weak and for this reason chlamydial genital infection must be not excluded when leukocytes were detected below the threshold of pyospermia.

It is clear that incidence of peroxidase positive granulocytes $\geq 1x10^6$ to $9x10^6$ cell/ml of semen was higher among infertile males with positive titer (27.32%).This come in agree with [23, 24] they reported that infertile males with positive evidence of infection with C. trachomatis either by direct or indirect detection methods have peroxidase positive cell $\geq 1x10^6$ cell/ml.

Among infertile males with negative titer, peroxidase positive cell $\geq 1x10^6$ to $4X10^6$ cell/ml was (10.03%) while among normal fertile it was detected in (3.9%).Genital tract infections other than Chlamydial infection were postulated, also leukocytospermia may derived from environmental and toxic factors. Consumption of cigarettes may result in significant increase of leukocyte count also intake of alcohol causing marginal increase of leukocyte concentration. Other factors that could be contribute to increased seminal leukocyte include, sexual behavior, frequency of ejaculation and hygiene practices [25, 26] .

It is obvious that infertile males with positive titer in seminal plasma which accompanied by leukocytospermia in semen have abnormal semen parameters. This agree with the finding of [1, 27] reported that C. trachomatis can bring changes in semen of infertile males either by direct effect in number of spermatozoa themselves and diminished as well as their ability to fertilize or by indirect effects in so far as that infection should change in constituent quantities of seminal fluid and thus have a secondary effect on spermatozoa as for example the presence of large number of white blood cells (WBCs). Sperm damage by WBCs can be mediated by ROS, protease and cytokines, furthermore genital tract infection facilitate the formation of sperm antibodies . conventional light microscopy or conventional sperm staining techniques not differentiate WBC from immature germ cells in semen [28]. In contrast, the cytochemical peroxidase method reliably identifies granulocytes which is the most prevalent WBC type in semen. This method is cheap, fast and easily to perform.

In conclusion: There was no relationship between count of pus cells and antichlamydial antibodies in seminal plasma of infertile males. Antichlamydial immunoglobulins were detected in (32.96%) among infertile males with positive titer in seminal plasma and pus cell $<1x10^6$ cell/ml of semen. The cytochemical peroxidase method is cheap, fast and easily to perform and reliable to identifies granulocytes, which is the most prevalent WBC type in semen.

References:

1. Redgrove KA, McLaughlin EA: The role of the immune response in Chlamydia trachomatis infection of the male genital tract: a double-edged sword. *Frontiers in immunology* 2014, 5.
2. Dean D: Chlamydia trachomatis today: treatment, detection, immunogenetics and the need for a greater global understanding of chlamydial disease pathogenesis. *Drugs of today (Barcelona, Spain: 1998)* 2009, 45(Suppl B):25.
3. Depuydt C, Mahmoud A, Everaert K: Infection/inflammation of the male genital tract as cause of

abnormal spermatozoa. In: *Andrology for the Clinician.* Springer; 2006: 322-327.
4. Jaramillo-Rangel G: The Role of Chlamydia trachomatis in Male Infertility. *Edited by Mihai Mares* 2012:245.
5. organization Wh: WHO Laboratory manual for the examination of human semen and sperm-cervical mucus interaction *(3'd ed) Cambridge University Press* 1992.
6. Suto K, Kojima Y: Blood serum or blood plasma separating material and blood-collecting tube using same. In.: Google Patents; 2013.
7. Collee JG, Fraser AG, Marmion BP, Simmons A: Practical medical microbiology. In.: Churchill Livingstone; 1996: 621-633.
8. Renshaw S: Immunochemical staining techniques. *Immunohistochemistry* 2007, 1.
9. Organization WH: WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction: Cambridge university press; 1999.
10. Endtz-AW: A rapid staining method for differentiating granulocytes from germinal cells in papanicolaou stained semen. . *Acta cytol* 1974, 18:2-7.
11. Cooper TG, Noonan E, Von Eckardstein S, Auger J, Baker HG, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT: World Health Organization reference values for human semen characteristics. *Human reproduction update* 2009:dmp048.
12. Wolff-H: Methods for detections of males genital tract inflammation. *Andrologia* 1998, 30(Supp.1):35-39.
13. Al-Ezzy AIA: Performance of Microimmunofluorescence Test in Detection of anti-ChlamydiaTrachomatis Immunog-lobulinsin Iraqi Infertile Males with Special Refer to Age Group Distribution. *International Journal of Science and Research (IJSR)* 2015, 4(2):3-7.
14. Munoz M, Witkin S: Immunology: Autoimmunity to spermatozoa, asymptomatic Chlamydia trachomatis genital tract infection and T lymphocytes in seminal fluid from the male partners of couples with unexplained infertility. *Human reproduction* 1995, 10(5):1070-1074.
15. Ochsendorf F, İ–zdemir K, Rabenau H, Fenner T, Oremek R, Milbradt R, Doerr H: Chlamydia trachomatis and male infertility: chlamydiaâ€ŞIgA antibodies in seminal plasma are C. trachomatis specific and associated with an inflammatory response1. *Journal of the European Academy of Dermatology and Venereology* 1999, 12(2):143-152.
16. Videau SP, Vivas JC, Salazar N: IgA antibodies to Chlamydia trachomatis and seminal parameters in asymptomatic infertile males. *Systems Biology in Reproductive Medicine* 2001, 46(3):189-195.
17. Al-Ezzy AIA: Detection Of Chlamydia trachomatis Immunogloblins In Seminal Plasma By Microimmunofluorescence Test And Their Effect On Semen Parameters Of Infertile Males In Iraq. *Journal of Biology, Agriculture and Healthcare* 2014, 4(26):48-53.
18. Bas S, Muzzin P, Ninet B, Bornand J, Scieux C, Vischer T: Chlamydial serology: comparative diagnostic value of immunoblotting, microimmunofluorescence test, and immunoassays using different recombinant proteins as antigens. *Journal of clinical microbiology* 2001, 39(4):1368-1377.
19. Bollmann R, Engel S, Petzoldt R, Gbel U: Chlamydia trachomatis in andrologic patientsâ€“direct and indirect detection. *Infection* 2001, 29(3):113-118.
20. Waltraud E, Nicola B, Gerhard R: Antibodies tochlamydia trachomatis in semen and relationship with parameters of fertily. *Human Reprod* 1996, 11(7):1408-1417.
21. Mazzoli S, Cai T, Addonisio P, Bechi A, Mondaini N, Bartoletti R: Chlamydia trachomatis infection is related to poor semen quality in young prostatitis patients. *European urology* 2010, 57(4):708-714.
22. Takahashi S, Takeyama K, Kunishima Y, Takeda K, Suzuki N, Nishimura M, Furuya R, Tsukamoto T: Analysis of clinical manifestations of male patients with urethritis. *Journal of infection and chemotherapy* 2006, 12(5):283-286.
23. Tokuda-N;, Kumazawa-T, S. N-: Chamydia trachomatis infection in male infertility. The clinical usefulness of the detection of antibodies against chlamydia trachomatis. . *Nippon-Hinyokika-Gakkai-Zasshi* 1999, 90(6):608-601 603.
24. Solis E, Gatti V, Bouvet B, Brufman A, Catalina PO, Feldman R: [Round cells in semen and genital infections]. *Archivos espanoles de urologia* 2000, 53(2):101-105.
25. Centola GM, Ginsburg KA: Evaluation and treatment of the infertile male: Cambridge University Press; 2004.
26. Keck C, Gerber-Schfer C, Clad A, Wilhelm C, Breckwoldt M: Seminal tract infections: impact on male fertility and treatment options. *Human Reproduction Update* 1998, 4(6):891-903.
27. Fujisawa M: Chlamydia trachomatis detected by ligase chain reaction in the semen of asymptomatic patients without pyospermia or pyuria. *Systems Biology in Reproductive Medicine* 1999, 42(1):41-44.
28. Wolff H: Methods for the detection of male genital tract inflammation. *Andrologia* 1998, 30(S1):35-39.