Laboratory Approach To Chlamydia Trachomatis Conjunctivitis

Elham Ragab Abd El Samea [1], Eman Badr El Din El Hadidy[1], Sahar Mostafa El Tarshouby[2], Amina Mostafa Abd El Aal[1]

1. Clinical Pathology Department, Faculty of Medicine, Mansoura University, Egypt
2. Opthalmology Department, Faculty of Medicine, Mansoura University-Egypt

elhamelngar@yahoo.com

Abstract: It is a chronic follicular conjunctivitis. In Egypt, the high prevalence of trachoma represents a major cause of blindness especially in rural areas. The aim of this work is to confirm the clinical diagnosis and to evaluate the enzyme linked immunosorbent assay in direct antigen detection of C.trachomatis in conjunctival scrapings and C.trachomatis antibodies in the sera of patients. Two groups of patients; the first group included 20 active cases (group I) and the second group included 25 cicatricial cases (group II). Direct antigen detection by ELISA from conjunctival scrapping, trachoma IgG and IgM by ELISA. Evaluation of direct antigen detection of C.trachomatis in conjunctival scrapings by ELISA revealed that there were insignificant difference between active and cicatricial ($P>0.05$). There was insignificant higher titre in active than cicatricial cases. As regard, IgM detection of C.trachomatis there were insignificant difference between them ($P>0.05$). There was insignificant higher titre in active than cicatricial cases. Detection of C.trachoatis IgG revealed 20 positive cases (44.4%), all of them were cicatricial cases (80%) which were significantly higher than active cases ($P<0.001$). There was significantly higher titre in cicatricial than in active cases. All antigen positive cases in group I were bilaterally affected, while in group II, detection of C.trachomatis antigen was higher in unilateral than bilateral eye infection. There was insignificant difference between active and cicatricial cases in either affection ($P>0.05$) with insignificant higher titre in bilateral than unilateral positive cases. The sensitivity of ELISA IgM compared to direct antigen detection in cicatricial cases was 50%, the specificity was 100%. Direct antigen detection test and serodiagnosis of C.trachomatis IgM by ELISA are more reliable than ELISA IgG in diagnosis of active trachoma infection. ELISA IgG is a reliable method in the serodiagnosis of cicatricial phase of trachoma.

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Introduction

Six million people-most of whom live in crowded, unhygienic conditions in developing countries- are blind because of an infectious disease called trachoma. It is generally accepted that trachoma is caused by Chlamydia trachomatis, bacteria that pass easily between people on hands and clothing[(1)]. Infection usually occurs first during childhood, but people do not become blind until adulthood. Successive infections cause progressive scarring of the inside of the eyelid. Eventually, the eyelashes turn inward and rub painfully over the front of the cornea. This causes corneal scarring, loss of corneal transparency and, finally, irreversible blindness. However, C.trachomatis and other organisms appear to be developing drug resistance to antibiotics commonly used to treat these infections. In addition, early scarring and in-turned eyelashes can be treated surgically. The World Health Organization has been promoting these "SAFE" interventions (surgery, antibiotics, facial cleanliness, and environmental improvement) since 2001 with the aim of eliminating trachoma by 2020. However, these control measures have had limited success so far and it looks like a vaccine may also be needed. To develop an effective vaccine, scientists need to know whether all cases of human trachoma are caused by so-called ocular strains of C.trachomatis. Might C.trachomatis strains that are usually associated with sexually transmitted disease or different species in the family Chlamydiaceae also cause human trachoma[(2)]**.** It is chronic follicular conjunctivitis caused by infection with Chlamydia trachomatis serovars A, B, Ba and C**.** Trachoma remains a serious health problem despite of great advances in therapeutic regimen. In Egypt, the high prevalence of trachoma represents a major cause of ocular morbidity and blindness especially in rural areas. The disease is often neglected because of illiteracy and real diagnostic difficulty thus delaying therapeutic intervention. Pannus is a common ocular intrachoma and punctuate keratitis[(3)]**.** Variable methods for diagnostic of trachoma had been widely discussed in recent years. Until a decade ago, the complement fixation test measuring group specific antibody was the most widely applied technique. However, despite

showing high sensitivity in diagnosis of systemic chlamydial infections, it had a little value in diagnosis of localized infections such as trachoma or inclusion conjunctivitis[4]. The aim of this work is to diagnose Chlamydia trachomatis as an important cause of trachoma to confirm the clinical diagnosis and to evaluate the enzyme linked immunosorbent assay in direct detection of C.trachomatis antigen in conjunctival scrapings and C.trachomatis antibodies in sera of trachomatous patients.

Subject and Methods

The study was carried out on 45 cases having clinical signs of trachoma and included two groups of patients; the first group included 20 active cases ranging from 4 – 12 years (group 1) and the second group included 25 cicatricial cases ranging from 36 – 72 years. They were out patients in Mansoura ophthalmic center after establishment of clinical diagnosis. *Specimen collection for C. trachomatis antigen detection:* Vigorous scraping from the upper tarsal conjunctiva were taken and placed in the transport medium which is sucrose phosphate saline. Antibiotics were added aseptically; amphotricin B, Gentamycin and vancomycin. Specimens were vortexed or sonicated to help disrupting cell depris and releasing chalmydial elementary antibodies, and the swabs were removed under complete aseptic conditions. Then the specimens were stored at -70°C to preserve the viability of organism. *For C. trachomatis antibody detection (IgM & IgG):*Serological specimens were collected aseptically; 2ml blood were taken from each patient and were put in dry test tube and after separation of the serum, serum samples were stored at -20°C. *Screening test for diagnosis of Chlamydia trachomatis conjunctivitis:* Direct antigen detection in conjunctival scrapings by ELISA using Mastazyme-Chlamydia test method*;* Detection of Chlamydia trachomatis IgM and IgG in serum by ELISA using Genozyme Virotech GmbH test method.

Results

Table (1) shows detection of C. trachomatis antigen, IgM and IgG by ELISA in studied 45 cases. Antigen detection of C. trachomatis was positive in 7 cases; 3 active cases and 4 cicatricial cases with insignificant difference between them (P>0.05).IgM detection of C. trachomatis was positive in 5 cases; 3 active cases and 2 cicatricial cases with significant difference between them (P>0.05). While detection of C.trachomatis IgG revealed 20 positive cases, all of them were cicatricial cases which were significantly higher than active cases (P<0.001).

Table (2) shows positive ELISA titre of C.trachomatis antigen, IgM & IgG. C.trachomatis antigen detection showed insignificant higher titre in active than cicatricial cases (P>0.05). Also, C.trachomatis IgM showed insignificant higher titre in active than cicatricial cases (P>0.05). In contrary, ELISA IgG titre was significantly higher in cicatricial than active (P<0.001).

Table (3) shows comparison between ELISA IgM and direct antigen detection in active cases. The sensitivity of ELISA IgM compared to direct antigen detection was 66.7%, the specificity was 94.1%. The negative predictive value was higher (94.1%) than the positive predictive value (66.7%). The accuracy was 90%. The percentage of false positive and false negative results was 33.3% and 5.9% respectively.

Table (4) shows comparison between ELISA IgM and direct antigen detection in cicatricial cases. The Sensitivity of ELISA IgM compared to direct antigen detection was 50%, the specificity was 100%. The positive predictive value was higher (100%) than the negative predicitive value (91.3%). The accuracy was 92%. The percentage of false positive and false negative results was zero% and 8.7% respectively.

Table (5) shows comparison between ELISA IgG and direct antigen detection in active cases. The sensitivity of ELISA IgG compared to direct antigen detection was zero, the specificity was 100%. The positive and negative predictive values were zero, 85% respectively. The accuracy was 85%. The percentage of false positive and false negative results was 100% and 15% respectively.

Table (6) shows comparison between ELISA IgG and direct antigen detection in cicatricial cases. The sensitivity of ELISA IgG compared to direct antigen detection was 75%, the specificity was 19.1%. the negative predictive value was higher (80%) than positive predictive value (14.3%). The accuracy was 28%. The percentage of false positive and false negative results was 85.7% and 20% respectively.

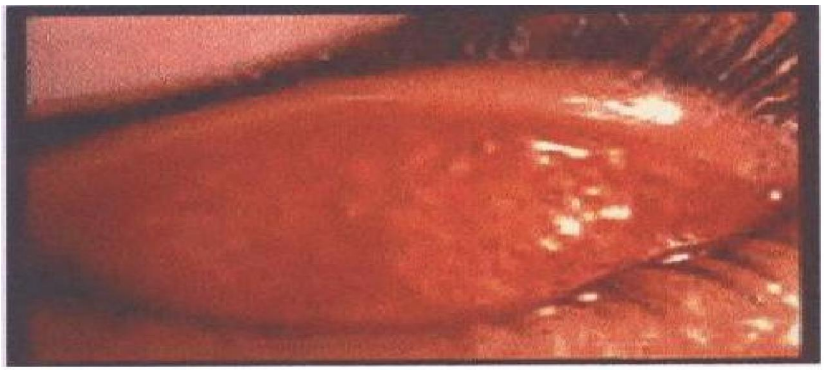

Photo (1): Active phase of trachoma (Grade I)

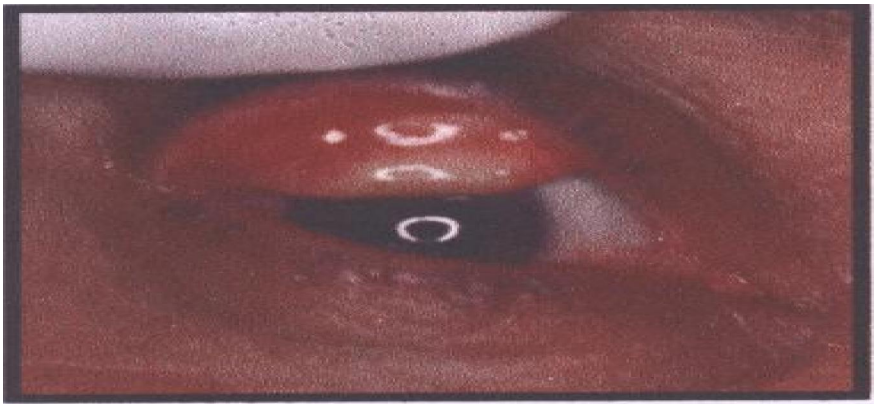

Photo (2): Cicatricial phase of trachoma (Grade III)

Table (1): Detection of C. trachomatis antigen, IgM & IgG by ELISA in studied cases (45).

Test	Total (n=45)				Active cases Group I (n=20)				Cicatricial cases Group II (n=25)				P
	Positive		Negative		Positive		Negative		Positive		Negative		
	No	%	No	%	No	%	No	%	No	%	No	%	
ELISA test: Antigen detection	7	15.6	38	84.4	3	15	17	85	4	16	21	84	>0.05
IgM	5	11.1	40	88.9	3	15	17	85	2	8	23	92	>0.05
IgG	20	44.4	25	55.6	0	0	20	100	20	80	5	20	<0.001

Table (2): Positive ELISA titre of C.trachomatis antigen detection, IgM & IgG.

Test	Active Cases Group I (n=20)				Cicatricial cases Group II (n=25)				P
	NO	%	Mean±SD	Range	NO	%	Mean±SD	Range	

ELISA test: Antigen detection	3	15	0.091±0.058	0- 0.22	4	16	0.062±0.182	0.01-0.68	>0.05
IgM	3	15	0.177±0.093	0.01-0.37	2	8	0.140±0.063	0.01-0.3	>0.05
IgG	0	0	0.036±0.029	0.01-0.13	20	80	0.192±0.147	0.01-0.43	<0.001

Table (3): Comparison between ELISA IgM and antigen detection in active cases

	ELISA antigen Detection				Sensitivity	Specificity	Accuracy	P.P Value	False +ve	N.P Value	False -ve
	positive		negative								
	No	%	No	%							
ELISA IgM:											
+ve	2	66.7	1	5.9	66.7%	94.1%	90%	66.7%	33.3 %	94.1 %	5.9%
-ve	1	33.3	16	94.1							

Table (4): Comparison between ELISA IgM and antigen detectin in cicatricial cases

	ELISA antigen Detection				Sensitivity	Specificity	Accuracy	P.P Value	False +ve	N.P Value	False -ve
	positive		negative								
	no	%	No	%							
ELISA IgM:											
+ve	2	50	0	0	50%	100%	92%	100%	zero	91.3%	8.7%
-ve	2	50	21	100							

Table (5): Comparison between ELISA IgG and antigen detection in active cases

	ELISA antigen Detection				Sensitivity	Specificity	Accuracy	P.P Value	False +ve	N.P Value	False -ve
	positive		negative								
	No	%	No	%							
ELISA IgM:											
+ve	0	0	0	0	Zero	100%	85%	Zero	100%	85%	15%
-ve	3	100	17	100							

Table (6): Comparison between ELISA IgG and antigen detection in cicatricial cases

	ELISA antigen Detection				Sensitivity	Specificity	Accuracy	P.P Value	False +ve	N.P Value	False -ve
	positive		negative								
	no	%	No	%							
ELISA IgM:											
+ve	3	75	17	80.9	75%	19.1%	28%	14.3 %	85.7%	80%	20%
-ve	1	25	4	19.1							

Discussion

Chlamydia trachomatis is a unique obligate intracellular bacterium that is the leading cause of bacterial sexually transmitted and blinding disease worldwide. Trachoma is a chronic disease of the conjunctival mucosa that can lead to blindness 10-40 years after infection. The estimated number of people with trachoma who will develop blindness by the year 2020 is 12 million[(2)]. Postoperative rates of trichiasis recurrence are high even with treatment for C.trachomatis at the time of surgery[(5)]**,** and C.trachomatis infection rates return within a year or two following cessation of mass or targeted antibiotic treatment programs. The latter may in part be due to an accelerated rate of reinfection following azithromycin treatment, which may blunt the immune response to the organism and lead to a population with increased susceptibility to infection[(6)]**.** Trachoma is one of the earliest recorded disease, it is a chronic follicular conjunctivitis caused by Chlamydia trachomatis serovars A, B, Ba and C[(7)]**.** Trachoma remains serious health problem despite of great advances in therapeutic regimen. The disease affects about 500 million people worldwide and is considered to be among the most important human chronic infections and most common cause of preventable blindness today[(8)]. In Egypt, the high prevalence of trachoma represents a major cause of ocular morbidity and blindness especially in rural areas. The disease is often neglected because of illiteracy and real diagnostic difficulty thus delaying therapeutic intervention[(9)]. The Nile Delta of Egypt represents a unique environment for trachoma to persist. Although economic improvements in last decade have affected even the poorest rural environments, the poor hygienic conditions still the primary factor in trachoma transmission**.** Enzyme immunoassays had been evaluated as rapid screening tests for diagnosing Chlamydia trachomatis conjunctivitis. They had the potential advantages of simplicity and objectivity; they are also easy, inexpensive and allow for large scale screening in endemic populations. Moreover, they do not depend on the presence of viable Chlamydiae during handling, transportation or storage of specimens[(10)]**.** This study was carried out on 45 cases having clinical signs of trachoma and including two groups of patients; the first group included 20 active cases, and the second group included 25 cicatricial cases. All cases were subjected to conjunctival scrapings for direct antigen detection of C.trachomatis by ELISA test, serum sample were also taken to detect antichlamydia trachomatis antibodies (IgM & IgG) by ELISA. The prevalence of trachoma was totally higher in female patients than in male patients. In cicatricial cases, trachoma was found in 16 cases female patients compared to 9 cases male patients. This was in agreement with ***Mabey et al.,***[(11)] who found a high prevalence of active trachoma in young female children. They also stated that trichiasis and blindness due to cicatricial trachoma may be 2-4 times more common in adult women than in men due to prolonged contact of women with children in active infection during child bearing age. Active trachoma was found in 65% of rural setting. ***Shehmann et al.,***[(12)] noticed a high relationship between spread of trachoma among children in rural area of Burkina Faso with the presence of flies and poor community hygiene. They observed that flies were present on 80% of children faces who had active infection. ***Broman AT, et al.,***[(13)] detected a low prevalence of active trachoma (15.6%) from total of 178 cases in rural Tanzania. ***Lansingh et al.,***[(14)] found that grade I; trachomatous follicles was higher (79%) than grade II; trachomatous inflammation (37%) on studying a trachoma survey in school children less than 10 years of age. Another study conducted by ***Madani et al.,***[(15)] revealed lower prevalence of active trachoma in the same age group; 31.5% trachomatous follicles (grades I) and 16.7% trachomatous inflammation (grade II). ***Katz et al.,***[(16)] studied the prevalence and severity of trachoma in school children and found that cicatricial trachoma was not present among this age group. However, ***Lansingh et al.,***[(14)] detected trachomatous scarring in 23% of

children in the same age group. Evaluation of direct antigen detection of C.trachomatis in conjunctival scrapings by ELISA revealed 7 positive cases; 3 active cases and 4 cicatricial cases with insignificant difference between them (P>0.05). There was significant higher titre in active than cicatricial cases, similar results were reported by ***Mabey et al.,***[17]. Also, ***Adenis et al.,***[18] found that out of 73% trachoma cases, 19.2% had a positive ELISA results for C.trachomatis antigen. A national survey was conducted by ***Saal et al.,***[19] to determine the prevalence of trachoma in children under the age of 10 years and the estimated prevalence of active infection by ELISA was 10.8%. ***Zhang et al., 1995***[20] studied 63 patients with severe active trachoma using enzyme linked immunosorbent assay for chlamydial antigenicity and detected a positive result in 97% of studied cases. This high prevalence of C.trachomatis antigen could be attributed to the severity of the disease. The appearance of antichlamydial antibodies in sera and tears of patients with trachoma after experimental eye infections is well documented, specific antibodies of IgM, IgG and IgA classes were detected[21]**.** Following an initial infection with C.trachomatis, the serum antibody response is usually of the IgM type that appears in two weeks and persists for a period of four to eight weeks. The IgG antibody usually appears late and persists for longer periods [22]**.** IgM detection of C.trachomatis was positive in 5 cases (11.1%); 3 active cases (15%) and 2 cicatricial cases (8%) with insignificant difference (P>0.05). There was insignificant higher titre in active than cicatricial cases. Our results were in harmony with ***Garg et al.,***[23] who detected antichlamydial IgM antibodies in 17% of patients suffering from active trachoma eye infection. Also, ***Abdel Rahman et al.,***[24] found serum IgM antibodies in 16.6% of patients with active infection. These data were also consistent with ***Numazaki et al.,***[25] who detected serum IgM in 13.2% of Japanese children with active inflammatory trachoma. The presence of IgM antibodies by ELISA may facilitate the diagnosis of an early infection and is particular helpful in infants. Reinfection with homologous trachoma serovar results only in anamnestic response in IgG antibodies without stimulating the IgM type, whereas reinfection with a new serovar results in an IgM antibody response to the new type as well as an anamnestic IgG rise to the previous one. Since most ocular infections in endemic areas are caused by the closely related serovars, it is not surprising that IgM antibody could be found only in a small percentage of patients from which the organism was isolated in cell culture[26]. Tear IgG usually exeeds the IgA titres and both are lower than serum titres in the same patient. Also IgG antibody was detectable in conjunctival secretions only when it was also present in serum, suggesting the possibility of transudation from the serum to conjunctival secretions through inflamed conjunctiva[27]. In the current study, detection of C.trachomatis IgG revealed cicatricial cases (80%) which were significantly higher than active cases (P<0.001). ELISA IgG titre was significantly higher in cicatricial than active cases. Our results were parallel with ***Hermann et al.,***[28] who found high prevalence of IgG antibodies (88%) in adult sera with cicatricial trachoma. Moreover, ***Numazaki et al.,***[25] reported that by means of ELISA, 71.3% of cicatricial cases aged more than 50 years had significantly elevated levels of IgG antibodies. ***Hessel et al.,***[29] stated that antichlamydial IgG antibodies were reactive in 90% of patients with follicular trachoma and 89% with inflammatory trachoma on examination of tear samples from Nepali villagers. The sensitivity of ELISA IgM in active cases was 66.7%, the specificity was 94.1%. The negative predictive value was higher (94.1%) than the positive predictive value (66.7%), the accuracy was 90%, ***Haller et al.,***[30] reported similar ELISA IgM sensitivity (70%) and lower specificity (78.9%). Also, ***Numazaki et al.,***[25] found a higher ELISA IgM sensitivity (81%) and similar specificity (92%). The sensitivity of ELISA IgM in cicatricial cases was 50%, the specificity was 100%. The positive predictive value was higher (100%) than the negative predictive value (91.3%). The accuracy was 92%, this result was previously reported by ***Pearce and Gaston,***[26] who stated that ELISA IgM had a sensitivity of 41.7% in diagnosis of cicatricial trachoma. They concluded that the role of ELISA IgM can be restricted to the detection of early cases and usually not detected in chronic infections. The sensitivity of ELISA IgG in active cases was zero, the specificity was 100%. The positive and negative predictive values were zero and 85% respectively. The accuracy was 85% . ELISA IgG in cicatricial cases had a sensitivity of 75% and a specificity of 19.1%. The negative predictive value was higher (80%) than the positive predictive value (14.3%), the accuracy was 28%. ***Haller et al.,***[30] found that ELISA IgG had a higher sensitivity (92%) and specificity (100%), the positive predictive value was 96% and the negative predictive value was 94%. ***Schachter et al.,***[31] stated that ELISA IgG had a higher sensitivity (90%) and specificity (98.9%). The high specificity in the previous studies may be attributed the difference in the antigen utilized which was the major outer membrane protein of C.trachomatis compared to LPS used in this study. Reevaluation of treatment regimens and approaches to vaccine development may be required as over II genomes of the order chlamydials have been sequenced[32]**,** infection with multiple species as C. psittaci and C.pneumoniae in addition to C.trachomatis in trachoma may explain also failure to detect Chlamydia among active trachoma

cases when only C.trachomatis is assayed ***Dean et al.,***[(2)]

It could be concluded that, application of ELISA IgM method in the serodiagnosis of active trachoma infection while IgG method is recommended during the cicatricial phase of the disease. In bilaterally affected patients it is better to obtain bilateral scrapings to provide more chance for antigen detection. Detection of secretory IgA in tear would be helpful in measuring the prevalence and intensity of active trachoma infection. Further evaluation of other techniques for C.trachomatis antigen detection such as culture method and polymerase chain reaction is recommended.

Correspondent:
Elham Ragab Abdul Samea
Assistant professor in Clinical Pathology
Department of Clinical Pathology
Mansoura University, Egypt
Tel. 0114571726

References:

1. ***Numazaki K:*** Current problems of perinatal chlamydia trachomatis infections. Journal of Immune Based Therapies and Vaccines.2004;2;4
2. ***Dean D, Kendal RP, Adhikari HK, Hessel T:*** Multiple Chlamydiaceae species in trachoma: implication for disease pethogenesis and control. Plus Med 2008;5(1): e14 doi:10.1371/journal.pmed.0050014
3. ***Holmes KK, Sparling FP, Stamm WE, Piot P, Wasserheit JN, et al:*** Sexuallly Transmitted disease. New York: McGraw-Hill. 2007;1440p
4. ***Zhang H, Kandel RP, Atakari HK, Dean D:*** Prospective impact of oral azithomycin on postoperative recurrence of trachomatous trichiasis in Nepal over one year. Br J Opthalmol. 2006;90:943-948
5. ***West SK, West ES, Alemayehu W, Melese M, Munoz B et al:*** Single-dose azithomycin prevents trichiasis recurrence following surgery: randomized trial in Ethiopia. Arch Opthalmol. 2006;142:309-314
6. ***Atik B, Thanh TT, Luong VQ, Lagree S, Dean D:*** Impact of annual targeted treatment on infectious trachoma and susceptibility to reinfection. JAMA. 2006;296:1488-1497
7. ***Burton MJ, Bowman RJ, Faal H, Aryee EA, Ikumapayi UN, et al:*** Long term outcome of trichiasis surgery in Gambia. Br J opthalmol.2005;89: 575-579
8. ***Zhang H, Kandel RP, Sharma B, Dean D:*** Risk factors for recurrence of postoperative trichiasis: implications for trachoma blindness prevention. Arch Opthalmol.2004;122:511-516
9. ***Kumar S, Kutlin A, Roblin P, Kohlhoff S, Bodetti T, et al:*** Isolation and antimicrobial susceptibilities of chlamydia isolates from western barred bandicoots. J Clin Microbiol. 2007;45:392-394
10. ***Taylor HR and Dax Em:*** New precision in measuring trachoma infection. Lancet; 2003;362(9379): 198-204
11. ***Mabey DC, Solomon AW and Foster A:*** Trachoma. Lancet; 2003;362 (9379): 223-9
12. ***Shemann JF, Guinot C, Ilboudo L, Momo C and Ko B:*** Trachoma, flies and environmental factors in Burkina Faso. Trans. T. Soc. Trop. Med. Hyg.; 2003;97(1):63
13. ***Broman AT, Shum K, Munoz B, Duncan DD, West SK:*** spatial clustering of ocular chlamydial infection over time following treatment, among households in a village in Tanzania. Invest Opthalmol Vis Sci. 2006;47:99-104
14. ***Lansingh VC, Weih LM, Keeffe JE and Taylor HR:*** Assessment of trachoma prevalence in mobile population in central Australia. Opthalmic Epidemiol.; 2001;8(2-3):97-108
15. ***Madani MO, Huget P, Mariotti SP and Negrel AD:*** Trachoma in Northern Chad. Sante.; 2003;13(3): 9-15
16. ***Katz J, West KP, Khatry SK and Thapa MD:*** Prevalence and risk factors for trachoma in Sarlahi district. Nepal. Br. J. Opthalm.; 1996;80(12): 1033-4
17. ***Mabey DC, Robertson JN and Ward ME:*** Detection of Chlamydia trachomatis by enzyme immunoassay in patients with trachoma. Lancet; 1987;2(8574): 1491-2
18. ***Adenis JP, Saint- Blancat P, Ranger S and Martin P:*** Chlamydia trachomatis in adults, study of diagnostic techniques. J. Fr. Opthalmol.; 1993;16(3):178-83
19. ***Saal MB, Schemann JF, Saar B, Faye M and Negrel AD:*** In process citation. Med. Trop.; 2003;63(1):53-9
20. ***Zhang W, Wu Y and Zhao J:*** Rapid diagnosis and treatment of chlamydial conjunctivitis. Chin. Med. J.; 1995;108(2):138-145
21. ***Tabbara KF and Hyndiuk RA:*** Infections of the eye. 2nd ed. Little, Brown and Company, Boston, Toronto. 1996;P:433-451
22. ***Mahon CR and Manuselis G:*** Diagnostic microbiology. 2nd ed.The W.B. Saunders Comp., Toronto. 2000;P:636-655
23. ***Garg SP, Bajaj MS, Jaffery NF and Mahajan VM:*** Chlamydial serology in uveitis. Rev. Int. Rach. Pathol. Occul. Trop. Subtrop. Sante.; 1989;66(1-2):7-20

24. ***Abdel Rahmna MM, Abdel Dayem SI, Eid SA, Badie OA and Kotb NA:*** Immunoflorescence diagnosis of Chlamydia trachomatis infection of the respiratory tract and the eye. J. Egypt Soc. Parasitol.1993;23(3):659-65
25. ***Numazaki K, Chiba S and Aoki K:*** Evaluation of serological tests for screening of chlamydial eye disease. In Vivo.1999;13(3):235-7
26. ***Pearce JH and Gaston JS:*** Chlamydia. In: Molecular Medical Microbiology. Sussman M (ed). The Academic Press, New York. 2002;P: 1825-1864
27. ***Bailey RL, Kajbaf M and White:*** The influence of local anti-chlamydial antibody on the acquisition and persistence of human ocular chlamydial infection. Epidem. Infec.1993;11(2);315-324
28. ***Hermann B, Stenberg K and Mardh PA:*** Immune response in chlamydial conjunctivitis among neonates and adults with special reference to tear IgA. APMIS.1991;99(1): 66-74
29. ***Hessel T, Dhital Sp, Plank R and Dean D:*** Immune response to chlamydial 60-kilodalton heat shock protein in tears from Nepali trachoma patients. Infect. Immuno. 2001;69(8): 4996-5000
30. ***Haller Em, Auer-Grumbach P, Stuenzner D and Muellner MA:*** Evaluation of two non culture antigen test and three serotests for detection of antichalmydial antibodies in the diagnosis of ocular chlamydial antibodies in the diagnosis of ocular chlamydial infections. Grafes Arch. Clin. Exp. Opthalmol.1996;234(8):510-4
31. ***Schachter J, Ridgway GL and Collier L (1998):*** Chlamydial disease. In: Topley & wilson's Microbiology and Microbial Infections. Collier L, SUssman M, Balows A and Hausler WJ (ed). 9th ed. Vol. 3 Oxford University Press Inc., New York. 1998;P: 977-991
32. ***Thomson NR, Holden MT, Carder C, Lennard N, Lockey SJ, et al,(2007):*** Chlamydia trachomatis:genome sequence analysis of lymphogranuloma venereum isolates. Genome Res.2007;Epub ahead of print.doi:10.1101/gr.7020108

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