Assessment of Humoral Immune Response as a Guide for Chlamydial Eye Infection Diagnosis Using Enzyme-Linked Immunosorbent and Micro-Immunofluorescence Assays

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Abstract: In Egypt, the high prevalence of trachoma represents a major cause of blindness especially in rural areas. The aim of this study is to assess the humoral immune response in trachoma patients and to observe the change in immune response in acute and chronic stage to confirm the clinical diagnosis. This study was performed on 80 patients attending the outpatient clinic of Research Institute of Ophthalmology starting from December 2012 to December 2013; the first group included 50 patients clinically diagnosed as trachoma either in the acute or chronic stage of the disease, while the second group included 10 patients defined as controls in which they are diagnosed with conjunctivitis due to other cause than Chlamydia. Lastly, the third group included 20 patients in which were investigated as non-affected controls. Two tests were done for the detection of specific IgA and IgG, these are enzyme-linked Immunosorbent Assay (ELISA) and Micro Immuno-Fluorescence (MIF).The results demonstrated an increase in both specific anti-*Chlamydia trachomatis* (*C.trachomatis*) IgG and IgA. IgG was recorded to be more prevalent in patient's sera than IgA, accordingly, IgG show more sensitivity to the ELISA and MIF detection tests than for specific IgA (sensitivity by ELISA was 90% v.72% and by MIF was 92%v. 48% for IgG and IgA respectively). A significant agreement between ELISA and MIF for detection of specific IgG was also observed, however, this conformity was not significant for IgA. The combination of the two tests ELISA and MIF offer an absolute accuracy for the specific detection of IgG and IgA, when done, the sensitivity of the combined tests for specific IgG was higher than that for specific IgA (86% v.38% respectively). The combined two tested indicate the high prevalence and chronicity of the disease. Therefore it is suitable for screening clinically diagnosed trachoma patients in Egypt as both tests are rapid, simple and can be used to test large number of samples at once.

Key words: Chlamydia Trachomatis · Conjunctivitis · Trachoma · ELISA · MIF

INTRODUCTION

"Trachoma is both an ancient and a stubborn disease, slow to blind and obviously hard to cure in a public health context [1]. Ocular infection with *C. trachomatis*, especially trachoma, continues to be a major public health problem in many parts of the world [2, 3]. Six million people-most of whom live in crowded, unhygienic conditions in developing countries- are blind because of a trachoma. It is generally accepted that trachoma is caused by *C. trachomatis*, bacteria that pass easily between people on hands and clothing [4]. Infection usually occurs first during childhood, but

people do not become blind until adulthood [5]. Children younger than ten years—primarily pre-school aged children-are most susceptible to active infection and thus the target of preventive activities, most notably face-washing to eliminate the ocular and nasal discharge that attracts flies to the face and allows the transmission of Chlamydia from person to person [6]. Recurrences often cause follicular/intense trachoma that may lead to trichiasis, corneal opacity and eventually blindness [7]. The World Health Organization (WHO) has been promoting these "SAFE" interventions (surgery, antibiotics, facial cleanliness and environmental improvement) since 2001 with the aim of eliminating

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trachoma by 2020, however, these intervention have had limited success [8]. The programme could truly be implemented only in 10 out of 55 trachoma endemic countries [9, 10], due to lack of insufficient information from the densely populated countries [11]. Although the disease has been eliminated from many developed countries, a large population in Africa, Middle-East, South America, Asia, The Pacific and Australia still suffer from this blinding disease [12].

In rural Egypt, The disease is hyper endemic where more than 75% of children show signs of having at least one episode of infectious trachoma during the first year of life [13]. Researches from the 1960s and earlier demonstrated a high prevalence of trachoma in selected governorates of Egypt. The Ministry of Health embarked upon a large scale antibiotic (tetracycline eye ointment) treatment programme in the 1960s and it was assumed that this programme led to a substantial decline in active trachoma in the targeted areas; soon thereafter the Ministry of Health declared that trachoma was no longer a public health problem in the country [14].

Early diagnosis is mandatory to avoid serious complications especially with the development of effective treatment. Confirmation of Chlamydia infection usually depends on taking an appropriate specimen and a suitable laboratory-based diagnostic test [15]. As *C. trachomatis* is an obligate intracellular pathogen, cell culture remains the reference method and it has 100% specificity but it is not recommended for routine use because of its technical complexity, the long turn-around time and it is unsuitable in developing countries. Accordingly, many commercial non-culture-based assays are now available for diagnosis [16, 17].

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 [5, 18].

The aim of this study is to assess the humoral immune response through the demonstration of specific anti-*C. Trachomatis* immunoglobulins A&G in the sera of trachoma patients using ELISA and MIF tests. These tests aim to evaluate the immunoglobulins A&G sensitivity and reliability in diagnosis of trachoma infections, in addition to observe the change in immune response in the acute and chronic stage; in order to confirm the clinical diagnosis.

MATERIALS AND METHODS

A total of 80 persons attending the outpatient clinic of Research Institute of Ophthalmology divided into three groups; the first group of whom 50 patients clinically diagnosed as trachoma includes 10 school children in which they attend a primary school. They were 3 males and 7 females and their age ranging from 6 to 10 years; while the 40 adult patients were 8 males and 32 females and their age ranging from 19 to 50 years (Table 1). All patients were screened for conjunctival follicles and papillary hypertrophy as signs of active trachoma; or for conjunctival scarring indicating chronicity. Among the 50 trachoma patients, 33 were diagnosed in the acute stage and 17 in the chronic stage. The intensity of trachoma was graded according to the scheme recommended by the WHO programme for the prevention of blindness, assessment of trachoma. The main presenting symptoms were foreign body sensation, watering, with or without mucopurulent discharge, redness and pain. The second group includes 10 control patients having eye infections other than *C.trachomatis,* they were 4 males and 6 females; and their age ranging from 6 to 36 years.. The main presenting symptoms were edema of the eye lids, redness, pain and mucopurulent discharge. The third group comprise 20 control subjects with apparently normal eyes, they included 10 males and 10 females, their age ranging from 6 to 55 years.

Blood samples were taken under complete aseptic conditions. Two cc blood was withdrawn and dispended in a sterile tube devoid of heparin and allowed to stand at room temperature for 1 to 2 hours for clot formation, then centrifuged and the serum was aseptically separated from the clot and stored at -70 until tested by ELISA technique (The Eurogenetic diagnostics Kit- Belgium) and micro-immunofluorescence techniques which is indirect fluorescent antibody technique for the sero-diagnosis of human *C. trachomatis* infection (BioMerieux Laboratory reagents and instruments).

The tests done in this study were performed on the clinically affected patients as well as on the normal and non-Chlamydial conjunctivitis.

Statistical Methods: Statistical Analysis System (SAS) was used for data management and analysis, while Harvard Graphics package was used for the figures.

Data were summarized as percentages, comparisons of the differences in positivity between the different studied groups and the relation between positivity of the tests and t he stage of the disease was done using the

Table 1: Age distribution of studied group.

Age (years)	Patients n (%)	Normal controls n (%)	Other controls n (%)
≤20	13(26)	12(60)	9(90)
21-40	25(50)	5(25)	1(10)
>40	12(24)	3(15)	0(0)
Total	50(100)	20(100)	10(100)

Fisher's exact test. Kappa measure of agreement was used to measure the strength of the agreement between the tests. The significance of the association between the tests was tested by the McNemar's Chi square [19].

P- Values≤0.05 is considered significant. All reported p-values are two-sided.

The clinical diagnosis of trachoma according to the categories of Dawson *et al.*[20] and Thylefors *et al.* [21] was taken as the gold "standard" for the statistical evaluation of all tests done in this study.

RESULTS

In this study the prevalence of trachoma was totally higher in female patients than in male patients, trachoma was found in 39 female patients compared to 11 male patients (Table 2).

None of the trachoma patients gave a negative results with the two tests performed in the present study.

The results of positivity of specific serum anti- *C.trachomatis* IgA and IgG, were detected by ELISA technique, showed that out of the 50 patients, 36(72%) had specific IgA compared to 45(90%) who had specific IgG.

From the 20 normal controls and 10 non Chlamydial conjunctivitis controls, 10% of each group had specific IgA, while 25% and 40% respectively had specific IgG.

Also the positivity of specific anti- *C.trachomatis* IgA and IgG antibodies were detected by MIF test. The results showed that specific IgA was detected in 24(48%) out of the 50 trachoma patients, while specific IgG was detected in 46(92%).

Among the 20 normal control subjects 3(15%) and 7(35%) had specific IgA and IgG respectively. While for the non-Chlamydial controls 2(20%) and 3(30%) had specific IgA and IgG respectively (Table 3).

Table (4) shows the positivity of ELISA and MIF in relation to stage of trachoma; among the 50 trachoma patients, 33 were diagnosed in the acute stage and 17 were in the chronic stage. The positivity of ELISA shows out of the 33 acute patients, specific IgA and IgG was detected in 22 (66.7%) and 29 (87.9%) respectively; while out of the 17 chronic patients, specific IgA and IgG was

detected in 14(82.4%) and 16(94.1) respectively. The table also shows the positivity of the MIF test in patients in relation to stage of the disease. Specific IgA and IgG were detected in 14(42.4%) and 29(87.9) respectively in acute patients, compared to 10(58.8%) and 17 (100%) respectively for chronic patients. There was no significant difference between acute and chronic stages using ELISA or MIF tests (The P values are non-significant).

Table (Table 5(5) shows the agreement between ELISA and MIF tests for IgA and IgG, the percentage of positive and negative agreement between the two tests for IgA was 38% and 18% respectively. Kappa was non-significant (=0.14).The percentage of positive and negative agreement between both tests for specific IgG was 86% and 4% respectively. Kappa was significant (=0.39). In addition, 10% of patients were ELISA negative and MIF positive while 34% were ELISA positive and MIF negative for IgA. The McNemar's (Paired Chi-Square) test was significant (P=0.019). In comparison for IgG, 6% were ELISA negative and the MIF test positive, while 4% were ELISA positive and MIF negative. The McNemar'stest was non- significant (P=1.000).

Table (6) shows the agreement between the ELISA and MIF tests for IgA by disease stage. The percentage of positive and negative agreement between the two tests in the acute stage was 30.3% and 21.1% respectively; while the percentage of positive and negative agreement between the two tests in the chronic stage was 52.9% and 11.8% respectively. The difference between the results of the two tests in the acute stage was non -significant, similar to that in the chronic stage which was also non-significant by McNemar's (P= 0.080 and 0.221 respectively).

The agreement between the ELISA and MIF tests for IgG by disease stage is demonstrated within Table (7). The percentage of positive and negative agreement between the two tests in the acute stage was 81.7% and 6.1respectively; while that in the chronic stage was 94.1% and0% respectively. There were no significant differences between the results of the two tests; either in the acute or chronic stages by McNemar's test.

The mean absorbance values for the ELISA specific IgA and IgG in trachoma patients was significantly different than that for normal and non-Chlamydial conjunctivitis control groups (p-value for ANOVA<0.001 and 0.001 respectively) (Table 8).

The combined ELISA and MIF tests for specific IgA and IgG gave absolute specificity 100% and absolute positive predictive value (PPV) and negative predictive

Table 2: Sex distribution of studied groups.

Sex	Patients n (%)	Normal controls n (%)	Other controls n (%)
Male	11(22%)	10(50)	4(40)
Female	39(78%)	10(50)	6(60)
Total	50(100%)	20(100)	10(100)

Table 3: Positivity of ELISA and MIF

		ELISA		MIF	
Group	Total number tested	IgA n (%)	IgG n(%)	IgA n (%)	IgG n(%)
Patients	50	36(72.0)	45(90.0)	24(48.0)	46(92.0)
Normal controls	20	2(10.0)	5(25.0)	3(15.0)	7(35.0)
Other controls	10	1(10.0)	4(40.0)	2(20.0)	3(30.0)

Table 4: Positivity of ELISA and MIF in relation to stage among patients

		ELISA		MIF	
Stage	Total number tested	IgA n (%)	IgG n(%)	IgA n (%)	IgG n(%)
Acute	33	22(66.7)	29(87.9)	14(42.4%)	29(87.9%)
Chronic	17	14(82.4)	16(94.1)	10(58.8%)	17(100%)
Total	50	36(72.0)	45(90.0)	24(48.0%)	46(92.0%)
P-Value		0.327	0.650	0.373	0.285

*P-value-<0.05 is considered significant.

Table 5: Agreement between ELISA and MIF test among patients

ELISA	MIF	IgA	IgG
Positive	Positive	19(38.0%)	43(86.0%)
Negative	Positive	5(10.0%)	3(6.0%)
Positive	Negative	17(34.0%)	2(4.0%)
Negative	Negative	9(18.0%)	2(4.0%)
Total number tested		50	50
Kappa measure of agreement		0.14	0.39*
P-value for McNemarm's test#		0.019	1.000

*kappa significantly different than zero, p<0.05

#P-value<0.05 is considered significant

Table 6: Agreement between ELISA and MIF test for IgA among patients by stage

ELISA	MIF	Acute	Chronic
Positive	Positive	10(30.3%)	9(52.9%)
Negative	Positive	4(12.1%)	1(5.9%)
Positive	Negative	12(36.4%)	5(29.4%)
Negative	Negative	7(21.1%)	2(11.8%)
Total number tested		33	17
Kappa measure agreement		0.08	0.20
P-value for McNemar's test#		0.080	0.221

#P-value<0.05 is considered significant

Table 7: Agreement between ELISA and MIF test for IgG among patients by stage

ELISA	MIF	Acute	Chronic
Positive	Positive	27(81.7%)	16(94.1%)
Negative	Positive	2(6.1%)	1(5.9%)
Positive	Negative	2(6.1%)	0(0.0%)
Negative	Negative	2(6.1%)	0(0.0%)
Total number tested		33	17
Kappa measure of agreement		0.43*	--
P-Value for McNemar's test#		0.617	--

*Kappa significantly different than zero<0.05

#P-value<0.05 is considered significant

Table 8: Mean Absorbance for ELISA (IgA and IgG) in the different groups.

Group	Number	IgA	IgG
Patients	50	0.996±.561a	1.424±0.947a
Normal controls	20	0.466±0.148b	0.444±0.185b
Other controls	10	0.486±0.159b	0.790±0.599b
P-value for ANOVA#		<0.001	<0.001

#P-value<0.05 is considered significant, group means sharing same letter are not significantly different than each other.

value (NPV). The MIF test for the detection of specific IgG gave the highest sensitivity 92%, but with low specificity 65%.

The combined ELISA and MIF test for IgA and IgG gave absolute specificity (100%) and absolute PPV (100%), whereas the combined tests for IgA had a lower sensitivity than that for IgG 38% for the former and 86%for the latter (Table 9).

Table 9: Sensitivity, Specificity, Positive and Negative predictive values for each test

Test	Sensitivity	Specificity	Positive predictive value	Negative predictive value
ELISA IgA	72.0	90.0	94.7	56.3
ELISA IgG	90.0	75.0	90.0	75.0
MIF Ig A	48.0	85.0	88.9	39.5
MIF Ig G	92.0	65.0	86.8	76.5
ELISA&MIF				
IgA	38.0	100.0	100.0	39.2
IgG	86.0	100.0	100.0	74.1

DISCUSSION

Trachoma is one of the earliest recorded disease, it is a chronic follicular conjunctivitis caused by *C. trachomatis* serovars A, B, Ba and C [22]. 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 [23]. In 2000 trachoma was responsible for an estimated 6.75 million cases of blindness, which corresponds to 15% of all cases of blindness in the world [24].The estimated number of people with trachoma in which they are expected to develop blindness by the year 2020 is 12 million [8, 25].

Active trachoma is largely a disease of children, yet its sequelae are mainly seen in adults. In view of the fact that villages in the rural Egyptian Delta have a preponderance of children already exposed to trachoma, it is estimate that by 2020 the number of blind cases in the Egyptian Delta will be 868,000, a crude blindness rate of 3.2% [26]. It should be noted that the disease is often neglected because of illiteracy and real diagnostic difficulty thus delaying therapeutic intervention [27].

Enzyme immunoassays had been evaluated as rapid screening tests for diagnosing *C. trachomatis* conjunctivitis. These tests have 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 Chlamydia during handling, transportation or storage of specimens [25].

In our present study work, the immunoglobulins IgA and IgG were the class antibodies selected to diagnose trachoma infection using the ELISA and MIF.

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 re-infection 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. Given that 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 [28], however, Schachter [29] found that IgM antibody responses tend to be markedly specific and together with the use of single

broadly reacting antigens, will miss at least15 to 25% of the infections that can be proven to be due to Chlamydia by other procedures.

This study was carried out on 80 cases, in which serum samples were also taken from all cases to detect specific anti- *C.trachomatis* antibodies (IgA &IgG) by ELISA and MIF aiming to evaluate their sensitivity and reliability in diagnosis of trachoma infections. In addition, changes in immune responses between acute and chronic stages of the disease were studied by comparing the diagnostic value of serological assays in each stage. Classification into acute or chronic stages was done according to Dawson *et al*. [20].

The culture is till now the "gold standard" to confirm the diagnosis of Chlamydial conjunctivitis. However, in this study, it was decided to consider the clinical diagnosis by the ophthalmologist, to be a good basis to evaluate correctly each test used and was considered as the "gold standard "of our statistical calculations. This was decided, relying on the fact that every patient examined clinically and diagnosed as trachoma case, gave at least one positive result of the two tests performed in this study.

The aforementioned choice was supported by Rapoza *et al*. [30] who claimed that despite the general acceptance of the culture technique as standard, most clinicians recognize patients whom they diagnose to have Chlamydial conjunctivitis despite a negative Chlamydial culture. Moreover, Peter *et al*. [31] did not recommended a routine culture because of its failure to detect organisms in chronic conjunctivitis. In addition, Schachter *et al*. [29] reported that trachoma is essentially a clinical one, especially in developing countries, where microbiological diagnosis is rarely obtained.

During this study, the prevalence of trachoma was totally higher in female patients than in male patients, trachoma was found in 39 female patients compared to 11 male patients. This was in agreement with Elham Ragab *et al*. [5], who found in cicatricial cases, trachoma was found in 16 female patients compared to 9 male patients. This also was in agreement with Mabey *et al*. [32] 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. Furthermore, it is widely recognized that women carry an increased burden of trachoma trichiasis compared with men [33].

MIF test for the assessment of IgG and IgA antibodies, as seen from table (3) in the different groups have shown that trachoma patients had higher prevalence of *C.trachomatis* IgG seropositivity than IgA (92% and 48% respectively), indicating that the MIF test for IgG was more sensitive than that for IgA.

In addition, patients had higher prevalence of *C.trachomatis* IgG than normal healthy controls (92% and 35% respectively), whereas this difference of prevalence was not significant for IgA (48% and 15% respectively)

The MIF test was slightly more specific for IgA than for IgG (85% vs. 65% respectively). This latter low value (65%) indicates the moderate incapacity of the test for correct diagnosis in confirmed negative trachoma cases (healthy and non-Chlamydial control subjects). Thus it was not uncommon to have a moderately high negative predictive value (76.5%) denoting the incorrectness of negative results as an index of negative accuracy (Table 9).

The specificity of our IgG by MIF test was in close agreement with that reported by Darougar *et al.* [34]. They detected anti- Chlamydial IgG in the blood of 14 patients among 28 with no obvious ocular Chlamydial infection, with a specificity of 50%. They attributed this low specificity to the fact that IgG in the blood may remain for some months after recovery from Chlamydial ocular infection.

Cevenini *et al*. [35], using MIF test for detection of anti-Chlamydial IgG and IgA in the sera of Chlamydial infected patients, have reported a sensitivity of 97% and 45.2% respectively. Their results are in agreement with ours (92 and 48%, respectively).

In addition, Patel *et al*. [36] have shown that patients with clinically diagnosed Chlamydial ocular infection had a sensitivity of 94% of specific IgG using the MIF test. Their results are in agreement with ours (92%) whereas the specificity for the IgG by MIF test reported by them was high (97.7%). This was much higher than our results (65%). This discrepancy of results could be explained by the endemicity of trachoma in our country.

The lower sensitivity obtained by the MIF test for anti-Chlamydial IgA in our current study can be tentatively explained on the basis that specific IgA antibodies are short lived thus limiting their detection to a certain extent [37].

On the other hand, there was no significant difference between the prevalence of *C.trachomatis* IgA in patients and healthy controls obtained in our studies (48% and 15% respectively). This disagreed with the results obtained by Cevinini *et al*. [35], who reported

that only 2 (5.9%) of 34 healthy controls had serum IgA to *C. trachomatis*. This could be attributed to differences in geographical locality and the persistence of infection (sub-clinically) and endemicity in our country.

Table (4) shows the positivity of MIF test in relation to stage of the disease. Among the 33 patients in the acute stage anti- Chlamydial IgA and IgG were present in 42.4 and 87.9%, respectively, while among the 17 trachoma patients in the chronic (cicatricial stage), the MIF for IgG test was positive in all patients 100% and IgA was positive in 58.8%.

The difference between positive MIF test for IgG and IgA in acute and chronic stages, using the Chi-square test for statistical analysis was found to be insignificant (p=0.285 and 0.373 respectively). Our results are in agreement with those obtained by El Ghobashy and Sehsah [38].

Other workers have reported that the presence of specific IgA antibodies in a single sample may serve as a diagnostic tool for monitoring of active Chlamydial infection [39].

This discrepancy in agreement between our results and those of other workers may be attributed to the low number of affected subjects in each stage.

The ELISA was used in the current study for the detection of specific anti-*C.trachomatis* IgG and IgA antibodies. Jones *et al*. [40] have studied the *C.trachomatis* immunodominant MOMP which is a target of neutralizing antibodies and the serotyping antigen. They have reported that the MIF test uses whole organisms as antigen and thus it is incapable of revealing the molecular specificity of the humoral response to infection. These limitations were resolved by using synthetic peptides corresponding to serovar specific antigenic regions of MOMP in an ELISA to analyze the serospecificity of sera from trachoma cases.

In the current study Table (3) shows that anti *C.trachomatis* IgA antibody was found by ELISA in (72%) of 50 clinically diagnosed patients and in (10%) of 20 normal healthy controls, while of the 10 other control group, 1 (10%) subject only showed a positive test.

Thus it is seen that trachoma patients had a significantly higher prevalence of *C.trachomatis* IgA antibodies compared with controls (72%vs. 10%). This moderate sensitivity obtained the ELISA result (72%) was probably due to the high cut-off value used. Some authors who worked on trachoma patients in a high risk population reported that they could have obtained more positive results if a lower cut-off has been used in an endemic area [41].

The mean absorbance values of specific serum IgA by ELISA were significantly higher in patients than in controls (p 0.001). However the values reached for normal controls and other controls were not significantly different Table (8). These results point to the fairly high specificity of the test (90%) with a low number of false positive results obtained.

The anti-Chlamydial IgG antibody by ELISA was positive in (90%) of 50 trachoma patients and 25% of normal healthy controls, while of the 10 other control group IgG was positive in 40% (Table (3). Patients with trachoma had a higher prevalence of *C.trachomatis* IgG than healthy normal controls.

Our results are in agreement with those obtained by Hallsworth *et al*. [42] where they reported a sensitivity of 88% by ELISA for IgG antibody to *C.trachomatis*. Similarly our results are in close agreement with those reported by Steinberg *et al.* [43] where they detected specific IgG antibody in 80% of cases.

A lower sensitivity result by ELISA for IgG than those obtained in our study was given by Numazaki *et al*. [18] with a sensitivity of 12.5%, whereas, Jenum [44] detected IgG anti *C.trachomatis* in patients with an ELISA sensitivity of 48%.

Evans and Taylor -Robinson in 1982 [45] detected IgG antibody to *C.trachomatis* using ELISA in healthy controls with a specificity of 80%, which was close to our results (75%). They attributed this low specificity to the study of different groups of persons and to the age factor which may influence the outcome of antibody levels.

The specificity obtained in our results 75%, was somewhat limited with comparison to that of IgA (90%) due to the subclinical, persistent nature of the organism or due to the possibility of a previous mild genital Chlamydial infection and hence the high prevalence of specific IgG [46].

The mean absorbance values of specific IgG by ELISA were significantly higher in patients than controls (p< 0.001). However, the mean absorbance values reached for the non -Chlamydial conjunctivitis control group were somewhat higher than those for normal healthy controls and this was expected and indicated some prevalence of IgG antibody level in their sera, indicating a very recent exposure or subclinical exposure to *C.trachomatis* infection on top of the non-Chlamydial conjunctivitis present (Table 8).

The positivity of the ELISA in relation to the clinical staging (Table 4) shows that in the acute stage anti *C.trachomatis* IgG was positive in 87.9% in comparison to anti *C.trachomatis* IgA (66.7%), while in the chronic

stage, the anti- Chlamydial IgG and IgA were 94.1% and 82.4% respectively, indicating a nearly close similarity in percentage. This was not expected for IgA, although the possibility of re-infection of these chronic cases and the reappearance of specific IgA cannot be ruled out [47].

Thus serum specific IgG to *C.trachomatis* is more likely associated with acute Chlamydial infection, especially in endemic areas in comparison to anti *C.trachomatis* IgA which although showed a higher specificity (90%), yet had somehow low sensitivity in acute cases. This could be due to the nature of trachoma infection in Egypt where it is a multicyclic disease with remissions and exacerbations and patients presenting with acute infections could have re-exposure to a previous infection or an exacerbation of a subclinical infection. By the time the patient is diagnosed as having acute trachoma, the specific IgA antibodies would have decreased to a lower rate beyond the threshold of detection and many patients would thus go undetected. In addition IgA antibody is short lived immunoglobulin. [36].

The chi-square test detected that the results of the ELISA for specific IgA and IgG *C.tracomatis* antibody performed on trachoma patients did not differ significantly with different clinical stages i.e. acute and chronic (p=0.327 and 0.650 respectively), although specific IgG antibody became somewhat higher in the chronic stage. This agrees with the study done by Holland *et al*. [47] who reported that IgG level remained high in the chronic stages of Chlamydial infection. This little correlation between clinical stage and Chlamydial antibody titer was attributed to the persistence of infection and hence of antibodies.

In the present study we tried to evaluate the agreement between the two assays (ELISA and MIF) performed on the trachoma patients using the kappa measurement of agreement (Table 5). In addition the difference between the results of the two tests performed on the same group of patients was evaluated using the McNemar's (Paired chi-square test). The percentage of positive and negative agreement for the ELISA and MIF specific IgA was 38% and 18% respectively. The measurement of agreement between the two tests was not significant (Kappa=0.14). However the percentage of positive and negative agreement for the ELISA and MIF specific IgG was significant 86% and 4% respectively, (Kappa=0.39)

In addition, the ELISA for the detection of specific IgA in trachoma patients was more sensitive than the MIF since there was a significant difference between the

results of the two tests (p=0.019) when compared together and performed on the same group of patients. On the other hand, the difference between the results of the two tests for specific IgG was not significant (p=1.00).

Cevinini *et al*. (35) reported a good agreement for anti- Chlamydial IgA with the two methods, 98%. On the other hand our results for agreement of IgA by ELISA and MIF (56% agreement) was relatively similar to that obtained by Mahony *et al*. [48] where they reported a 63% correlation for IgA.

The high percentage of agreement for the IgG ELISA and MIF (90%) obtained in our study was similar to those obtained by Haalsworth *et al*. [42], which was a 93% agreement. Similarly, Raymond *et al*. [49] claimed that a 76% agreement was considered a good correlation between the two tests.

Evans and Taylor-Robinson [45] stated that the results obtained by the ELISA and MIF did not always correlate. They explained that this could be that the MIF measures antibody to various antigens on the Chlamydial surface, whereas the ELISA detects antibody to the group and species specific antigen which may be expressed to a lesser extent on the organism surface.

The MIF test was more sensitive than the ELISA in the acute stage than in the chronic stage because IgA ant *C.trachomatis* antibody was detected in 12 sera by MIF but not by ELISA, whereas in the chronic stage only 5 sera was positive by MIF and not by ELISA. Thus the McNemar's paired chi-square test showed a significant difference of results for both tests in the acute stage rather in the chronic stage (p=0.080 and 0.221 respectively). Thus *C.trachomatis* IgA antibodies detected by MIF were a better marker for acute *C.trachomatis* infection.

Similarly, the agreement between ELISA and MIF for IgG in relation to stage among trachoma patients showed that there was a 94% agreement in the chronic stage compared to an 88% agreement in the acute stage Table (7) Thus the IgG *C.trachomatis* antibody detected by both tests were in good correlation in both stages.

Our results were in close agreement with those obtained by Cevinini *et al*. [35], Ran and Aoki [50]. And Mattila *et al*. [37], who indicated a high degree of correlation between the ELISA and MIF test for IgG in all stages of trachoma, in addition their studies suggested that the MIF test for IgA ant *C.trachomatis* antibody detection was a potential marker of active infection.

Another approach in our study was carried out by evaluation of the combined ELISA and MIF test for the detection of anti-Chlamydial IgA and IgG antibody (Table 9).

The combined tests for IgA had an absolute specificity and PPV (100%) and a lower NPV than that for IgG (39.2% vs. 74.1% respectively). Yet this combined IgA test is not of practical value in an endemic country like Egypt as the sensitivity of the test is lower than that for IgG (38% and 86% respectively) and thus the diagnosis of many positive sera will be missed and many false negative results will be misinterpreted.

Thus it is clear from the current study that the performance of these two combined tests for the detection of anti-Chlamydial IgG was more efficient and will diagnose most of the patients who were clinically diagnosed for trachoma either in the acute or chronic stage. Each test is supplementary to the other, avoiding the possibility of false negative results. This combination offers a relatively high sensitivity (86%) and an absolute specificity (100%).

Ehgartner *et al*. [51], reported that in chronic cases the serologic test is of greater value since it is usually difficult to isolate the organism and for a secure diagnosis, a combination of two tests should be used, in addition to always taking the clinical picture into consideration.

Thus a diagnostic test may be suitable for the detection or confirmation of a disease, but it is rare for a single test to be suitable for both of these objectives. To achieve maximum sensitivity and specificity, it is often useful to use two tests in tandem fashion. Therefore, although isolation tests are highly sensitive, confirmation of disease requires a test of high specificity and sensitivity together with a high PPV and a low NPV. It is in this capacity that serological tests have an important role to play in the diagnosis of *C.trachomatis* infections [52].

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