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MEASUREMENT OF INTERLEUKIN-2 AND INTERLEUKIN-8 IN SERA OF PATIENTS INFECTED WITH ADENOVIRAL KERATOCONJUNCTIVITIS IN IRAQ

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Abstract

Seventy conjunctival swabs and sera sample were collected from patients suffering from keratoconjunctivitis who were suspected to be infected with Human Adenovirus, whom were aged 1 to 68 years including both sexes. Samples were taken from patients admitted to Ibn Al-Haitham Hospital for Ophthalmology/ Baghdad, Al-Hilla Teaching Hospital/ Babylon, Al-Imam Al-Sadiq Hospital/ Babylon and Al Garaawi Ophthalmology Specialist Center/ Babylon. The sampling period lasted from January to April 2019. Samples of conjunctival swabs were collected and placed in viral transport medium (VTM) and transported to the laboratory inside cool box to be stored at (-80°C). Blood samples were also collected from the patients and each sample were evacuated inside Gel and Clot activator vacuum tubes. The serum samples were separated and stored in the freezer at -20°C. The samples were analyzed in vitro using the enzyme-linked immunosorbent assay (ELISA) to measure interleukins levels including IL-2 and IL-8. Real-time PCR results showed that 64 positive samples (91.4%) out of 70 samples tested represented different types of Human Adenovirus, while 6 samples were negative (8.6%) for this virus. The virus load was measured and its values were found to be range from (1.32×10^3 -6.62×10^{23}). Whereas the results of control samples were negative for Human Adenovirus. The results showed that the different age groups are susceptible to infection with Adenoviral keratoconjunctivitis and a slight increase in the number of infections in the age group (20-40) years. The results also showed that males were more susceptible to infection with the Adenovirus than females. In terms of residential areas, it was found that the rate of infection with Human Adenovirus in urban areas more than those in rural areas. The study also found that individuals with diabetes, chronic illnesses, or obesity, as well as individuals with other types of Human Adenovirus infection, had the same chance of infection with the Adenovirus in comparison with individuals of healthy control group. ELISA test for serum samples also showed that the levels of IL-2 and IL-8 in samples infected with Human Adenovirus were significantly higher than those of healthy control groups. Besides, there was highly significant positive correlation between log IL-2 and log IL-8 ($r = 0.500$; $P \leq 0.001$). Analytical results of the study showed that there was no correlation between IL-2 and IL-8 levels in sera of patients suffering from Adenoviral keratoconjunctivitis with sex, age, BMI and Residency in urban or rural as well as patients with diabetes and patients who have previously infected with other types Human Adenovirus keratoconjunctivitis. The study also showed a significant and positive correlation of log IL-2 with the log viral load ($r = 0.268$; $P = 0.032$). Whereas, there was not significantly correlated of log IL-8 with the log viral load ($r = 0.076$; $P = 0.550$). Finally, current results showed an increased level of IL-2 in sera sample taken from patients in Baghdad governorate compared to samples taken from patients in Babylon province with very significant differences ($P \leq 0.001$). Interleukin 8 showed a significant difference in females sera sample compared with male sera ($P = 0.024$) and significant increase in sera of patients with chronic disease ($P \leq 0.014$).

Key words: Adenovirus, Keratoconjunctivitis, Interleukin-2, Interleukin-8, PCR, Real-time PCR, Eye Swabs, Serum.

Introduction

Epidemic keratoconjunctivitis (EKC) is the most severe type of infection that is caused by Human Adenovirus (HAdV) belong to genus *Mastadenovirus* of family *Adenoviridae*. (Prusinkiewicz and Mymryk, 2019).

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To date, Human Adenoviruses were classified into seven groups (A-G) based on phylogenetic analysis, genomic organization, growth characteristics and oncogenicity, including 52 serotypes and 90 Human Adenovirus genotypes, which were classified by Human Adenovirus Working Group by using serology methods (by viral neutralization) but are now classified using of

genomic sequence analysis (Ghebremedhin, 2014; Radke and Cook, 2018; Prusinkiewicz and Mymryk, 2019; Zhang and Huang, 2019).

HAdVs are non-enveloped double-stranded linear DNA and an icosahedral nucleocapsid. Seven members of the HAdVs species D types HAdV-8, 19 (reclassification as type 64), 37, 53, 54, 56 and 85 are the main causes of epidemic keratoconjunctivitis, a highly contagious infection that can cause severe inflammatory disease of the conjunctiva and cornea (Zhou *et al.,* 2012; Hashimoto *et al.,* 2018; Radke and Cook, 2018).

Human Adenovirus (HAdV) infections represent a significant source of morbidity and mortality, worldwide and at all ages, through highly transmittable infections at mucosal sites, including the eye, and urinary, respiratory, and gastrointestinal tracts. HAdV causes fatal acute respiratory distress syndrome in healthy adults and is especially lethal in infants and the immune compromised (Ismail *et al.,* 2018).

Symptoms of EKC appear within 14 day after exposure to virus and commonly include discomfort or pain in eye, redness, watery discharge, photophobia and. corneal involvement, including keratitis and subepithelial infiltrates, often develops in patients within days and can persist for months, decreased in visual acuity. Clinical illness typically lasts 7–21 days and it is usually self-limited. Transmission is predominately by contact with secretions of infected eye through contaminated surfaces, instruments, eye drops, or hands. An individual can be infectious a few days before developing symptoms to approximately 14 days after symptoms onset onset (Aplander *et al.,* 2011; King *et al.,* 2013).

The diagnosis is primarily based on clinical features including the detection of Adenoviral nucleic acid in conjunctival specimens of patients suffering from keratoconjunctivitis such as using technique of Real-time PCR because of its rapidity, high sensitivity and specificity, but cell culture method was not routinely used in clinics (King *et al.,* 2013; Gulati *et al.,* 2019).

Human Adenoviruses initiate innate and adaptive immune response. The cell-mediated immunity concerns as the most important host defense against Adenoviruses. Fatal Adenovirus infections occurred in immunocompromised patients (Al-Rubaey *et al.,* 2015; Joffe *et al.,* 2018).

The immune response of corneal epithelium utilizes different substances to increase the attack on viral invasion of ocular surface. Both of cytokines and chemokines are produced and endogenously released by corneal epithelium cells to direct and indirect recruit and immune cells activation of innate and adaptive immunity (Dartt *et al.,* 2011).

Interlukein-2 (IL-2) is a proinflammatory cytokine with immune modulation functions (Bachmann and Oxenius, 2007). Cytokines IL-2 can be induced by various resident corneal cells, and antigen presented cells can incur destructive effects by viral keratitis on the stroma. IL-2 knockout mice can be ameliorated by treat with recombinant IL-2 (Song *et al.,* 2016).

Interleukin-8 (IL-8) is a member of the CXC chemokines subfamily and is produced by blood cells and many types of tissues. Neutrophil are major specific target for IL-8 action. Many pathophysiological actions of IL-8 depend on activation of neutrophils (Shahzad *et al.,* 2010). Keratocytes: the capacity to amplify acute inflammation in the presence of infection. Indeed, keratocytes secrete proinflammatory chemokines, such as the neutrophil chemotactants IL-8 (Chodosh *et al.,* 2000).

Materials and Methods

Conjunctival swabs were taken from superior and inferior fornices with sterile dacron swabs of viral transport media without using topical anesthesia from infected eye or both infected eyes if bilateral infection of the patients noticed. The dacron swabs inserted inside tubes of transport media were transported in cold box and stored at -80! till they were used in qPCR technique.

Whole venous blood samples were aseptically collected by venous puncture using sterile 5ml disposable syringe. Blood were evacuated in Gel & Clot activator vacuum tubes that were centrifuged at 1500 rpm for 10 minutes. Sera were separate and transferred to sterile Eppendorf tubes which were labeled and stored at deep freezer at (-20!) till they were used for measurement of Interleukin-2 and Interleukin-8 for comparative study.

Viral DNA Extraction

Human Adenovirus DNA was extracted for each suspected patients' sample using Accuprep® Genomic DNA extraction kit (Bioneer, Korea). Extraction was carried according to instruction manual of this company.

Genomic DNA Profile

The extracted DNA samples were checked by using Nanodrop spectrophotometer (THERMO, USA), for checking and measurement of the purity of DNA by reading the absorbance at wave length (260 / 280 nm).

Real-time PCR

Real-time PCR was carried out for detection of Human Adenovirus in DNA from eye swab samples by using the primers and probe specific for hexon gene and

this technique was executed according to method described by Choi and Jiang, (2005). Real-time PCR master mix was prepared and well done according to the instruction manual of manufacturing company. Real-time PCR thermocycler requirements was set according to primer annealing temperature and instruction manual of RT-PCR TaqMan kit using Miniopticon Real-time thermocycler system.

Real-time PCR data analysis was performed by account the threshold cycle number (Ct value) that offered the positive amplification of Human Adenovirus hexon gene in Real-time PCR cycle number.

ELISA Method

Both human Interleukin-2 and Interleukin-8 ELISA kits (Elabscience, USA) were used in this study for quantitative determination of IL-2 and IL-8 concentrations respectively, in serum of participant human samples and done according to manufacturing company instruction.

The ELISA results were studied depending on the average of the duplicate readings for each standard and samples optical density. Then create a standard curve by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.

Table 1: Main characteristics features of patients and control group.

Characteristics	Patient *sn* = 70	Control *n* = 70	*P*
Age (years)			
<20, *n* (%)	22 (31.4 %)	23 (32.9)	0.761 ¥NS
20-40, *n* (%)	26 (37.1 %)	25 (35.7)	
> 40, *n* (%)	22 (31.4 %)	22 (31.4)	
Mean ±SD	33.56 ±19.77	33.54 ±19.97	0.995 †NS
Gender			
Male, *n* (%)	42 (60.0)	42 (60.0)	1.000 ¥NS
Female, *n* (%)	28 (40.0)	28 (40.0)	
Governorate			
Babylon, *n* (%)	35 (50.0)	35 (50.0)	1.000 ¥NS
Baghdad, *n* (%)	35 (50.0)	35 (50.0)	
Residency			
Urban, *n* (%)	54 (77.1 %)	50 (71.4 %)	0.439 ¥NS
Rural, *n* (%)	16 (22.9 %)	20 (28.6 %)	
BMI			
Normal (18.5-24.9 kg/m^2), *n* (%)	34 (48.6 %)	47 (67.1 %)	0.024 ¥S
Overweight (25 -29.9 kg/m^2), *n* (%)	19 (27.1 %)	17 (24.3 %)	
Obese ≥ (30 kg/m^2), *n* (%)	17 (24.3 %)	6 (8.6 %)	
Mean ±SD	25.62 ±4.67	24.05 ±3.54	0.026 †S

n: number of cases; SD: standard deviation; ¥: Chi-square test; †: independent samples t-test; NS: not significant at ≤ *P* 0.05.

Results and Discussion

Demographic Characteristics of Patients and Control Group

The demographic characterization of patients and control subjects are demonstrated in table 1. The present study included 70 patients and 70 apparently healthy control subjects. There was no significant difference in mean age between patients and control group (*P* = 0.761); 33.56 ± 19.77 years versus 33.54 ± 19.97 years, respectively. In addition, there was no significant difference in the distribution of patients and control subjects according to age intervals, < 20, 20 - 40 and > 40 years (*P* = 0.761).

With respect to gender, the study included 42 (60.0%) male patients and 28 (40.0) female patients with a male to female ratio of 1.5:1. Based on this male to female ratio, a comparable control gender ratio was intended, thus 42 (60.0%) apparently healthy male and 28 (40.0) apparently healthy female patients were included in the study Table 1.

Half of patients enrolled were from Babylon governorate and the other half was from Baghdad. Therefore, control subjects were selected so that half of them were from Babylon governorate and the other half was from Baghdad Table 1. Moreover, there was no significant difference in the frequency distribution of patients and control subjects with respect to residency, urban versus rural; however, participants from urban areas dominated the sample, 54 (77.1%) versus 16 (22.9%) and 50 (71.4%) versus 20 (28.6%), respectively, as shown in Table 1. Regarding BMI, there was significant difference in mean value between patients and control subjects (*P* = 0.024), 25.62 ± 4.67 kg/m^2 versus 24.05 ± 3.54 kg/m^2, respectively. In addition, the proportions of overweight and obesity were significantly more frequent in patients than in control group (*P* = 0.026), 19 (27.1 %) and 17 (24.3 %) versus 17 (24.3 %) and 6 (8.6 %), respectively Table 1.

In the present study the mean age of patients with keratoconjunctivitis was 33.56 ± 19.77 years and the age range was very wide from 1 to 68 years. This indicates that no age is immune and human beings are risk to acquire the disease at any age. In addition, a proportion of patients admitted to eye hospital have previous similar attacks. In an Indian study including 468 patients with keratoconjunctivitis, the mean age was 12.00 ± 6.63 years (Saboo *et al.,* 2013), which is far less than that seen in

the current study. This Indian study was also a hospital-based study. Actually, the disease is known to be a disease of childhood however, several previous studies, in accordance with the findings of the current study, have reported the occurrence of the disease in adult population. It was found in one study that 12% of patients were above 20 years of age, of these 3.5% patients had an adult onset of disease and others had childhood disease which had persisted beyond the age of 20 years (Saboo *et al.*, 2013). Leonardi *et al*., found 4% of patients above the age of 20 years and Shafiq *et al*., reported 6% of patients with keratoconjunctivitis above the age of 20 years in a hospital-based study in Pakistan (Leonardi *et al.*, 2006; Shafiq and Shaikh, 2009).

Certainly, we had larger number of patients beyond 20 years of age in our population. On the other hand, a small proportion of patients have chronic illness particularly diabetes and these disorders may render the immune system less effective in fighting viral infections. Moreover, it is well known according to the theory of aging of immune system that elderly individuals will have less potent immune system and thereby more liable for development of viral illnesses such as skin infections, pneumonia and eye infections (Montecino-Rodriguez *et al*., 2013; Castelo-Branco and Soveral, 2014; Pinti *et al.*, 2016; Weyand and Goronzy, 2016; Sadighi Akha, 2018).

In another study, which was a community based one and included 574 children; the mean age of participants was 9.74 ± 4.0 (Alemayehu *et al.*, 2019). One of the inclusion criteria in the later study was school age children; therefore, the mean age was far less than that of the present study. However, in another hospital-based study, the mean age of patients with Adenovirus type 54 keratoconjunctivitis was 60.9 ± 10.0 years and all participants were adults (Matsuura *et al.*, 2019), in line with the finding of the current study that the disease can be recognized in adults as well as in children.

On the other hand, the studies previously mentioned with young mean of age included mostly cases of allergic keratoconjunctivitis, whereas the current study included cases mostly due to Adenovirus infection. For instance, in an American study dealing solely with Adenoviral keratoconjunctivitis the median patient age was 45 years (range = 9 months–90 years) (Killerby *et al.*, 2017), which almost in line with the findings of the current study.

In this study, most of the patients were males and the male to female ratio was 1.5:1. In one study the male: female was 6.4:1 (Saboo *et al.*, 2013). Leonardi and co-workers in two separate observations including a multicentric study from Italy found a male: female ratio between 3.3 and 3.5 (Leonardi *et al.*, 2006; Lambiase *et al.*, 2009). Generally, all other series have reported M: F ratio between 4:1 and 2:1 (Tabarra *et al.*, 1999; Akinsola *et al.*, 2008). Ukponmwan, reported a female preponderance (M:F ratio of 1:1.3) from Nigeria (Tabarra *et al.*, 1999; Ukponmwan, 2003); however, another report from that region suggested M:F ratio of 1.27:1 (Akinsola *et al.*, 2008). The male: female ratio in current study is in line with previous reports from other parts of the world and confirms the global pattern of male preponderance of keratoconjunctivitis with exception of Tabarra, *et al.*, report.

The predilection of male patients in cases with keratoconjunctivitis is explained by more frequent exposure of male patients to hot dry climate (Singhal *et al.*, 2019), but why in our study male patients are more commonly affected despite the etiology in almost all cases was viral rather than being allergic, we don't exactly know. Reviewing available published articles also did not provide us with an acceptable reason for the higher incidence rate of Adenoviral keratoconjunctivitis in male patients, but probably males in our community are more liable to acquire the disease at work since it is a communicable disease that can be transferred from patient to patient by direct contact and especially considering that most of our cases comes from urban areas in which cultural habits make men more liable to get the infection at workplaces, whereas, women are often housewives.

In this study the majority of patients were from urban areas. The most likely explanation for the predominance of viral keratoconjunctivitis in urban areas is that the disease is transmitted by direct contact from person to person as well as from contaminated objects, instruments and tools and because of overcrowding of population in urban settings; therefore, one can expect the disease to be more frequent in urban areas than rural areas (Neiderud, 2015).

In the present study there was significant difference in mean body mass index (BMI) between patients and control subjects (P = 0.024), 25.62 ± 4.67 kg/m^2 versus 24.05 ± 3.54 kg/m^2, respectively. In addition, the proportions of overweight and obesity were significantly more frequent in patients than in control group (P = 0.026), 19 (27.1 %) and 17 (24.3 %) versus 17 (24.3 %) and 6 (8.6 %), respectively. These findings indicated an association between overweight and obesity with Adenoviral keratoconjunctivitis.

Indeed, the association between Adenovirus and obesity has been supported by several other authors

whose found direct correlation between obesity and different pathogens infection (Almgren *et al.*, 2012; Ponterio and Gnessi, 2015). In general, obese patients are more likely to develop infections (Jubber, 2004; Campitelli *et al.*, 2014). Obesity has been associated with increased risk of complications due to surgical site infections (Choban and Flancbaum, 1997; Calle *et al.*, 1999; Mullen *et al.*, 2009). Obese individuals have increased risk of *Helicobacter pylori* (Arslan *et al.*, 2009) infection and overweight children show a three times greater risk of *Neisseria meningitides* (Uberos *et al.*, 2010). Obesity is also a risk factor of severe infection and death caused by the pandemic influenza strain H1N1 (Morgan *et al.*, 2010).

Overall, these observations indicate that excessive adipose tissue expansion predisposes individuals to various infections. On the other hand, new data have been generated in the last few years suggesting infectious agents being the cause of obesity in addition to being more easily hosted in an obese individual. Among the multitude of infectious agents, Adenoviruses are the human pathogens that more than others are causatively and correlatively linked with animal and human obesity, respectively, and seem to directly influence the adipose tissue (Hegde and Dhurandhar, 2013; Huttunen and Syrjanen, 2013).

In animals, Adenovirus serotype AdV31 and AdV9 correlate with obesity and are adipogenic in animal cells culture (Verlaeten *et al.*, 2001; Pasarica and Dhurandhar, 2007). An Avian Adenovirus in India and the Human Adenovirus type 36 (AdV36) have been associated with obesity and there are reported suggesting significant role of Adenoviruses in the development of human obesity (Mitra and Clarke, 2010). To the best of our knowledge, the current study is one of the first studies that linked obesity to keratoconjunctivitis caused by Adenovirus infection; therefore more research work may be needed to validated the findings of the current study and explore the mechanisms by which obesity renders individuals more liable to Adenovirus keratoconjunctivitis more than normal weight people.

Clinical Appearance of Patients with Keratocon-junctivitis

The clinical features, signs, symptoms and other associated clinical features of patients enrolled in this study are shown in Table 2. Patients were generally suffering the following symptoms: Pain, itching and tearing were seen in 70 subjects (100.0%), whereas burning sensation, blurry vision and photophobia was seen in 66 subjects (94.3%), while foreign body sensation was seen in 58 patients only (82.9%).

The following signs were identified up on examination of patients with keratoconjunctivitis: the condition was bilateral in 35 (50.0%), Red Eye was seen in 70 (100.0%), conjunctivitis was seen in 70 (100.0%), discharge was seen in 17 (24.3%) and chemosis was seen in a single patient (1.4%); however, conjunctival haemorrhage, Pseudomembranes, conjunctival membrane and subepithelial infiltrates were not recognized in any enrolled patient Table 2.

Regarding other associated manifestation, Rhinorrhea was seen in 4 (5.7%), whereas, Pharyngitis was seen in a single patient (1.4 %) Table 2.

In the present study, pain, itching, burning sensation, tearing, foreign body sensation, blurry vision and photophobia were the main clinical features experienced by majority of patients with Adenovirus keratocon-junctivitis; whereas, physical examination revealed the following clinical signs: red eye, conjunctivitis, discharge and chemosis. This is in accordance with most of literatures dealing with clinical features of Adenoviral keratoconjunctivitis (Pihos, 2013; Chigbu and Labib, 2018; Uemura *et al.*, 2018; Matsuura *et al.*, 2019).

Molecular Identification of Adenovirus DNA in Patients with Keratoconjunctivitis

Real-time PCR was carried out on samples obtained from each enrolled patient for the purpose of identification

Table 2: Clinical features of patients with keratoconjunctivitis.

Clinical features	*n*	%
Symptoms		
Pain	70	100.0
Itching	70	100.0
Burning	66	94.3
Tearing	70	100.0
Foreign body sensation	58	82.9
Blurry Vision	66	94.3
Photophobia	66	94.3
Signs		
Bilateral	35	50.0
Red Eye	70	100.0
Conjunctivitis	70	100.0
Discharge	17	24.3
Chemosis	1	1.4
Conjunctival hemorrhage	0	0.0
Pseudomembranes	0	0.0
Conjunctival membrane	0	0.0
Subepithelial infiltrates	0	0.0
Other associated clinical features		
Rhinorrhea	4	5.7
Pharyngitis	1	1.4

Table 3: PCR detection of Human Adenovirus in patients infected with epidemic keratoconjunctivitis.

Characteristic	Value
Positive Cases, No. (%)	64 (91.4 %)
Negative Cases, No. (%)	6 (8.6 %)
Viral Load Median (IQR), Copy /ml	1.14×10^7 (3.59×10^{15})
Range	$1.32 \times 10^3 – 6.62 \times 10^{23}$

IQR: inter-quartile range

Table 4: Correlation of viral load to demographic characteristics and chronic illnesses.

Characteristic	*R*	*P*
Age	0.076	0.531NS
Gender	0.061	0.618NS
Governorate	0.081	0.503NS
Residency	0.064	0.599NS
BMI	0.019	0.874NS
Chronic Disease	-0.003	0.983NS
Pre-infection	-0.184	0.126NS

r: Correlation coefficient according to spearman correlation; NS: not significant at $P \leq 0.05$.

Adenovirus DNA and quantification of viral load and the results were shown in Table 3. Real time PCR provided evidence of Adenovirus infection in 64 out of 70 accounting for (91.4%), whereas, negative results were seen in 6 cases (8.6%).

Viral load was assessed also by real time PCR and was expressed in terms of copy / ml and the results were as following: median and inter-quartile range of 1.14×10^7 (3.59×10^{15}) and a range of $1.32 \times 10^3 – 6.62 \times 10^{23}$ copy /ml Table 1.

Correlation of Viral Load to Demographic Characteristics and Chronic Illnesses

Correlations of viral load to demographic characteristics and chronic illnesses are demonstrated in Table 3. These correlations were assessed according to Spearman correlation test. Viral load was not significantly correlated to any of the demographic characteristic of patients enrolled or their possession of chronic medical illness ($P > 0.05$).

In present study, majority of cases with clinical suspicion of Adenoviral infection were proved to have molecular evidence of Adenovirus DNA based on real time PCR results. In the current study, we were able to quantitatively assess viral load and expressing it in terms of copy number per ml. The range was extremely wide and non-parametric statistical methods were used to describe viral load and its correlation to demographic and clinical characteristics.

Another important finding of the present study is that there was no apparent difference in clinical severity between the higher virus DNA copy number group and lower virus DNA copy number group obtained by quantitative PCR method. These findings are in accordance with Uemura and his colleagues. These results seem contradictory. There are several possible explanations for this discrepancy. Neutralizing antibody, that acts to suppress viral proliferation during the clinical course, is reported to rise around 10 days after the onset in Human Adenoviral conjunctivitis (Uemura *et al.*, 2018).

However, it is also reported that a rise in antibody titer is observed in 75% of patients with Adenoviral keratoconjunctivitis, meaning that some patients are non-responders to neutralizing antibody. Combined with this information, the inhibitory effect of neutralizing antibody is very complicated and variable in Adenoviral conjunctivitis. Another aspect is that the host immune status affected clinical findings regardless of Adenovirus DNA copies. This was presumed from the atypical clinical appearance in specific immunological situations such as immunocompromised cases (Sarbay *et al.*, 2016).

The wide range of date of sample collection for real time PCR method might also have affected our results. Further study will be needed for more detailed evaluation of the relation between the number of viral DNA copies and the clinical severity of Adenoviral keratoconjunctivitis, using the same period of infection in an experimental animal model. Since specific anti-adenoviral agents have not been introduced locally or systemically at present, it can be considered that the Adenovirus DNA copies in our study were not affected by therapeutic measures. However, variation in number of viral copies might be due to different stage of virus infection and different replication cycle level.

Interleukin-2 (IL-2) and Interleukin-8 (IL-8) Serum Levels in Patients and Control Patients

The serum levels of IL-2 and IL-8 are shown in Fig. 1 and 2. Following performance of Kolmogorov-Smirnov test, IL-2 and IL-8 appeared to be not normally distributed, therefore, they were expressed as median and inter-quartile range rather than mean and standard deviation in addition comparison of their levels between patients and control groups was carried out using Mann Whitney U test rather than independent samples t-test.

The serum level of IL-2 was significantly higher in patients' group in comparison with control group, 17.83 (16.86) versus 10.87 (4.04), respectively, ($P < 0.001$) (Fig. 1).

Moreover, the serum level of IL-8 was significantly higher in patients' group in comparison with control group,

113.75 (82.72) versus 18.85 (26.52), respectively, (P < 0.001) (Fig. 2).

Correlations of serum IL-2 and IL-8 to demographic characteristics of patients with keratoconjunctivitis are shown in table (5). IL-2 was not significantly correlated to gender, also there was non-significant correlation to age, but its level was significantly higher in patients from Baghdad in comparison to those from Babylon governorate (P < 0.001), in addition, there was non-significant correlation to residency as urban versus rural and was not significantly correlated to BMI.

Interleukin-8 was significant high in female gender than in male (P = 0.024), it was not significantly correlated to age, governorate, residency as urban versus rural and was not significantly correlated to BMI Table 5.

Although, there was marked increase in both IL-2 and IL-8 levels between HAdVs infected patients and non-infected individuals yet there were non-significant differences between them.

Neither IL-2 nor IL-8 showed significant correlation to presence of chronic illness, diabetes and pre-infection (P > 0.05), with the exception that serum IL-8 was significant in the presence of chronic illness (P = 0.014) Table 5. In addition, there was non-significant difference in the level of IL-2 and IL-8 with respect to Real-time PCR evidence of presence or absence of Adenovirus infection Table 7.

Table 5: Correlations of IL-2 and IL-8 serum levels to demographic characteristics of patients with keratoconjunctivitis.

Characteristic		IL-2	IL-8
Gender	Male	16.37 (12.19)	99.23 (79.57)
	Female	23.24 (23.29)	160.48 (80.82)
	P †	0.115NS	0.024S
Age	<20,	18.51 (19.15)	109.96 (126.29)
	20-40	17.09 (18.48)	107.43 (78.93)
	> 40,	14.31 (16.35)	127.63 (75.46)
	P €	0.825NS	0.435NS
Governorate	Babylon	12.67 (11.38)	125.11 (108.61)
	Baghdad	25.82 (15.79)	109.96 (75.77)
	P †	< 0.001HS	0.421NS
Residency	Urban	15.38 (15.89)	130.80 (79.88)
	Rural	17.52 (18.19)	110.59 (89.66)
	P †	0.695NS	0.727NS
BMI	Normal	17.94 (18.58)	113.75 (90.92)
	Overweight	13.31 (17.24)	103.49 (93.51)
	Obese	11.68 (16.43)	99.86 (97.25)
	P †	0.332NS	0.938NS

BMI: body mass index; Data were expressed as median (inter-quartile range); †: Mann Whitney U test; €: Kruskal Wallis test; NS: not significant at $P \leq 0.05$; HS: highly significant at $P \leq 0.01$.

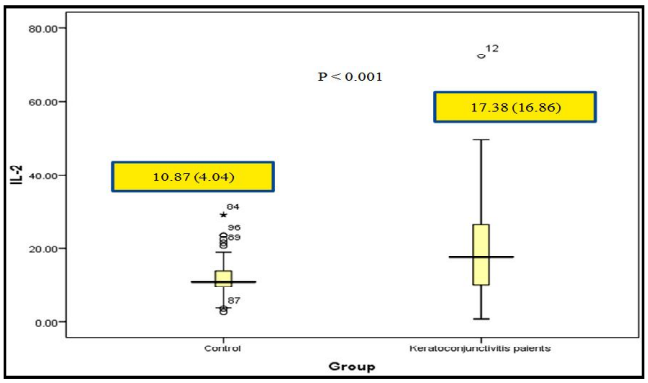

Fig. 1: Comparison of median serum IL-2 between control and patients' group.

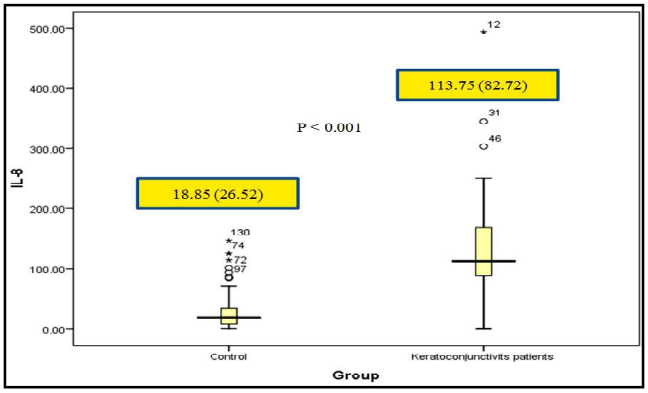

Fig. 2: Comparison of median serum IL-8 between control and patients' group.

Log IL-2 was significantly and positively correlated to log viral load (r = 0.268; P = 0.032), whereas, log IL-8 was not significantly correlated to log viral load (r = 0.076; P = 0.550), as shown in Fig. 3 and 4, respectively.

In the current study, the serum levels of IL-2 and IL-8 were both significantly higher in patients' group in comparison with control group. In Fig. 1 and 2.

Moreover, there was highly significant positive correlation between log IL-2 and log IL-8 (r = 0.500; P < 0.001) Fig. 5.

Interleukin-8 is a member of the chemokine family. The chemokines are specialized cytokines produced and secreted by a variety of normal and neoplastic human cell types, which have been defined by their ability to cause directed migration of leukocytes (Shahzad *et al.*, 2010). They are generally secreted in response to growth factors, inflammatory cytokines, and pathophysiologic conditions. Among the first chemokines discovered, IL-8 was identified as a chemotactic factor secreted by activated monocytes and macrophages that promotes the directional migration of neutrophils, basophils, and T-lymphocytes (Brat *et al.*, 2005; Shahzad *et al.*, 2010). It

was later found to play an important role in autoimmune, inflammatory, and infectious diseases. Because of its potent pro-inflammatory properties, IL-8 is tightly regulated, and its expression is low or undetectable in normal tissues (Brat *et al.*, 2005).

Much data supports an essential role for IL-2 in immune tolerance. This idea is much different from the early paradigm in which IL-2 is central for protective immune responses. This change in thinking occurred when a T regulatory cell defect was shown to be responsible for the lethal autoimmunity associated with IL-2/IL-2R deficiency. This realization allowed investigators to explore immune responses in IL-2-nonresponsive mice rendered autoimmune-free. Such studies established that IL-2 sometimes contributes to optimal primary immune responses, but it is not mandatory. Emerging findings, however, suggest an essential role for IL-2 in immune memory (Malek, 2008).

In view of these evidences we can explain our observation that both IL-2 and IL-8 have risen in that they synergistically interact to initiate and maintain a proinflammatory response to neutralize and eliminate Adenoviral infection, this of course through a T helper response but on the same time IL-2 interact with its specific receptors to provide T regulatory response to prevent excessive immune response from damaging host on tissues with subsequent catastrophic outcome.

Indeed, our observation that IL-8 and IL-2 levels increases in response to Adenoviral infection is supported by the observations of other authors (Chodosh *et al.*, 2000; Chigbu and Labib, 2018). Indeed, our hypothesis can explain the observation that "There was highly significant positive correlation between log IL-2 and log IL-8".

Table 6: Correlations of IL-2 and IL-8 serum levels to chronic illness, diabetes and pre-infection in patients with keratoconjunctivitis.

Characteristic		IL-2	IL-8
Chronic Disease	Yes	26.47 (10.80)	178.79 (57.78)
	No	16.59 (16.25)	105.54 (87.45)
	P †	0.113NS	0.014S
Diabetes	Yes	25.25 (21.18)	164.90 (92.19)
	No	17.02 (16.39)	108.70 (89.34)
	P †	0.373NS	0.111NS
Pre-infection	Yes	18.10 (19.25)	94.80 (93.14)
	No	17.38 (18.44)	115.01(86.51)
	P †	0.585NS	0.637NS

Data were expressed as median (inter-quartile range); †: Mann Whitney U test; NS: not significant at *P* d" 0.05; S: significant at *P* d" 0.05; HS: highly significant at *P* d" 0.01.

Table 7: Comparison of median values of serum IL-2 and IL-8 in Adenovirus positive and negative cases.

IL	Adenovirus positive		Adenovirus negative		*P*†
	Median	IQR	Median	IQR	
IL-2	22.03	17.76	17.02	17.72	0.495NS
IL-8	137.74	94.40	113.75	85.56	0.706NS

IQR: inter-quartile range; †: Mann Whitney U test; NS: not significant at $P \leq 0.05$.

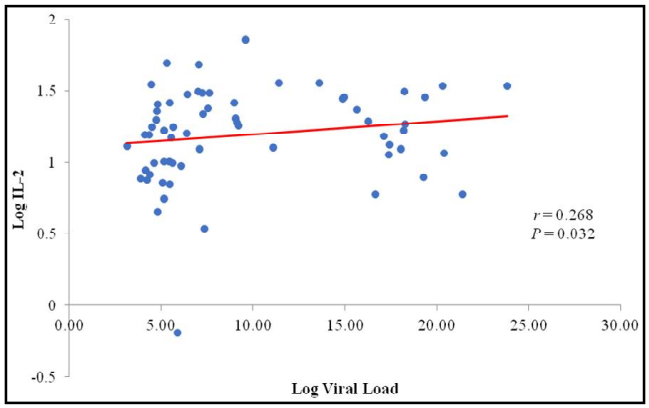

Fig. 3: Scatter plot showing the correlation between log viral load and log IL-2 in patients with keratoconjunctivitis.

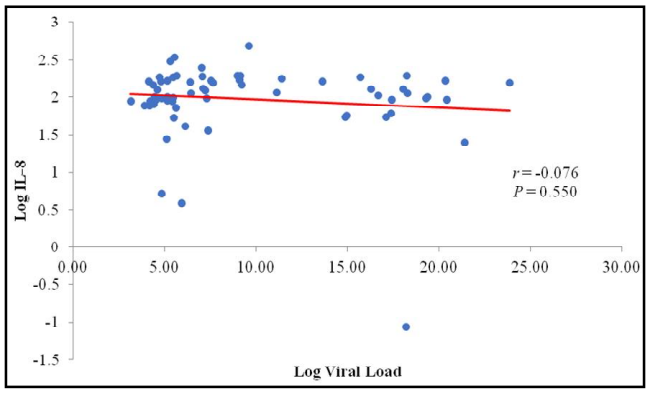

Fig. 4: Scatter plot showing the correlation between log viral load and log IL-8 in patients with keratoconjunctivitis.

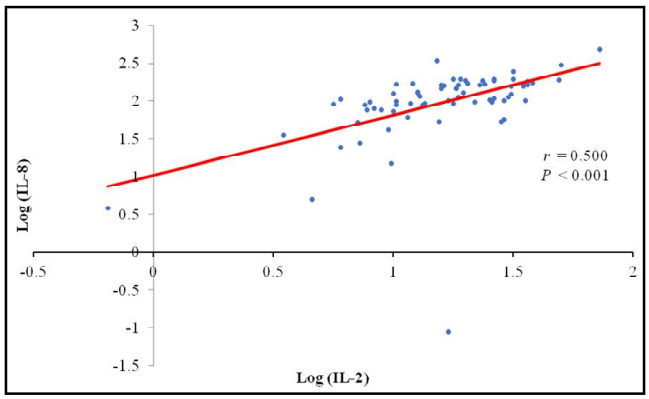

Fig. 5: Scatter plot showing the correlation between log IL-2 and Log IL-8 in patients with keratoconjunctivitis.

In the present study, serum IL-8 was significantly higher in female gender and chronic illness. Indeed, females are more liable to autoimmune diseases and the high level of IL-2 may be partly explained by the ability of female to produce more potent immune response that may even cause damage to host tissue with subsequent autoimmunity. This observation is supported by many researches (Sayad *et al.,* 2013).

The high level of IL-2 in association with chronic illnesses may indicate its role in the pathogenesis of these illnesses, a finding that need much research work to be validated.

Conclusions

All age groups are susceptible for infection with Adenovirus keratoconjunctivitis, with higher rate incidence in age group of 20 – 40 years. As well as higher of infection in male rather than in female. In addition, the disease was more frequent in urban than in rural areas. However, patients suffering from diabetes, chronic diseases, obesity or pre-infected are susceptible to HAdV infection in comparison with normal patients.

Serum level of both Interleukin-2 and Interleukin-8 in patients' group were significantly higher than that of control heathy group. Besides, there was highly significant positive correlation between log IL-2 and log IL-8 (r = 0.500; $P \leq 0.001$).

The serum level of IL-2 was significantly higher in patients from Baghdad than those from Babylon province ($P \leq 0.001$). In addition, serum level of IL-8 was significantly higher in female gender than male and in patients with chronic illnesses (P = 0.014, P = 0.024) respectively.

Log IL-2 was significantly and positively correlated to viral load (r = 0.268; P = 0.032). whereas, log IL-8 was not significantly correlated to log viral load (r = 0.076; P = 0.550).

Recommendations

We recommended that large scale study must be carried to include all Iraqi governorates population to detect more prevalent human Adenovirus circulated in Iraq, so that to improve database for future studies and planning programs to include type of vaccine for human Adenovirus effective vaccine after isolation and identification of causative virus genotype to prevent and control of this disease.

We recommended also an Immunological study to investigate other cytokines and its role in pathogenesis of the disease.

We recommended that the Ministry of Heath prepare intensive media programs targeting kindergartens through attractive cartoon programs and other targeting for adult to raise the cultural level of people and increase their knowledge about this virus and its transmission ways as well as medical signs and symptoms. In addition, how to avoid their exposure to it.

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