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Molecular Detection of Adenovirus type 2 among Conjunctivitis Patients in Khartoum State- Sudan

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Abstract: Background: Adenoviruses are a group of viruses that can infect the membranes (tissue linings) of the respiratory tract, eyes, intestines, and urinary tract. Diagnosis of conjunctivitis and differentiation between bacterial, viral, and noninfectious conjunctivitis are usually clinical. PCR and other rapid, office-based immunodiagnostic tests can be useful especially when the inflammation is severer.
Objective: This study aimed to detect adenovirus among conjunctivitis infected patients to figure out the possibility of using PCR technique to achieve that goal.
Methods: By using PCR for DNA products of 45 conjunctivitis swabs, to detect adenovirus type 2.
The Results: Only one sample gave a positive result out of 45 samples detected (2.2%)
Conclusion: It seems to be no adenovirus spreading enough among patients causing their eye illness, therefore other causative agents for conjunctivitis may be existence, as in Sudan most of the year the climate not suitable for adenovirus survival as it lives in cold and wet environments unlike what above local one. However, if any signs suggest bacterial conjunctivitis (eg, purulent discharge), cultures or other studies may be useful. The prevalence of adenoviral conjunctivitis in the study population was lower than the prevalence in other regions of the world.
Key words: Conjunctivitis, Human adenoviruses (HAdVs), polymerase chain reaction (PCR).

I.   INTRODUCTION

Adenoviruses (AdV) are a major cause of viral conjunctivitis. Human adenoviruses (HAdVs) are classified in the genus Mastadenovirus, which contains seven known HAdV species HAdV-A to HAdV-G[1-2]. It has been discovered in 1953 [3]. Traditionally, the HAdV species were classified by hemagglutination and serum neutralization reactions into different serotypes[1-4].Adenoviruses are non-enveloped double-stranded DNA viruses that can infect a variety of human tissues. They range in size from 65 to 80 nm in diameter. The virion is composed of a protein capsid, made up of 252 capsomeres, and a nucleoprotein core that contains the DNA viral genome (26–46 kbp long, containing 23–46 protein-coding genes) and internal proteins.

The capsid has an icosohedral shape, consisting of 240 hexon components and 12 pentons per virus particle [5-6]. Each penton contains a base plate with fiber. The length of the fibers varies among the different serotypes[7]. DNA homology within the HAdV subgroups ranges from 48% to 99%. HAdV subgroup C serotypes revealed the highest DNA homology (up to 99%) as compared to other HAdV subgroups. However, the DNA homology between HAdV subgroups is less than 20%[8].

Adenovirus is the cause ofepidemic viral conjunctivitis which is the most common infectious and a highly contagious eye disease that occurs worldwide.Cases are more frequent during warmermonths. Epidemiological analysis indicated the regionaland the seasonal distribution dominated in the winter and early spring.

The exact incidence of adenoviralconjunctivitis is still poorly known[9].Conjunctivitis is one of the most frequent ocular disorders observed in clinical practice[10]. Conjunctival infections are caused both in sporadic and epidemic form, due to a variety of microorganisms, including bacteria[11], viruses[12] and parasites[13].

The leading cause of acute viral conjunctivitis is human adenoviruses (HAdVs) [14-15]. About 15–70% of all conjunctivitis cases worldwide are associated with HAdVs 10, where clinical manifestations include epidemic conjunctivitis (EC), pharyngoconjunctival fever and non-specific follicular conjunctivitis[14-16].

Although most adenoviral infections have been described as mild and self-limited, HAdVs have been associated with severe infections in both immunocompromised and healthy individuals [17]. HAdVs cause outbreaks in a wide range of settings, such as military recruits [14-18-19]or hospitals[20].

Polymerase chain reaction (PCR) is a rapid, reliable and sensitive tool for the diagnosis of viral infections of the eye. Conjunctivitis caused by adenoviruses[21].

II.   MATERIAL AND METHOD

This cross sectional study enrolled among 45 well diagnosed conjunctivitis subjects, they were attended to ophthalmology hospitals in Khartoum state, samples taken to detect Adenovirus were swabs from their conjectivita of the eyes by swabbing, which later preserved in sterile added normal saline containers, then frozen at -20 for later work, which was DNA extraction which conducted via DNA extraction kit (Analytika Jena, Germany), provide fast and easy silica-based DNA purification in convenient spin-column and 50-well-plate formats. Most samples were directly lysed with proteinase K, eliminating the need for mechanical disruption and reducing hands-on time. Optimized protocols for specific sample types provide reproducible purification of high-quality DNA. Purification of DNA using the DNeasy Blood & Tissue Kit can be automated on the QIAcube (QIAGEN trade mark, Germany). Then the DNA products used for polymerase chain reaction PCR technique, the adenovirus primers (P1 - 5' GCCGCAGTGGTCTTACATGCACATC 3', P2 - 5' CAGCACGCCGCGGATGTCAAGT 3') were designed according to the DNA sequence of the hexon region of adenoviruses types 2 and 5. This pair of primers amplifies a fragment of 300 bp from the hexon gene of many serotypes [22]. The PCR reaction was carried out as described elsewhere [23]. The PCR cycle was 1 cycle at 94ºC/5min, 35 cycles at 94º C/30s, 55º C/30s, 72oC/1min and 1 cycle at 72ºC/15min., The PCR products wereelectrophoresed on 2% agarose gel containingEthidium bromide. The bands were visualizedusing an ultraviolet Trans illuminator.

III.   RESULT

Forty five conjunctival swabs were collected from patients referred for acute conjunctivitis to ophthalmology hospital. They were 42% females and 58% males as in figure 1, their age mean+SD as 33.2+20.6years. They were having pink or red color of the eye, swelling of the eyelid, watery discharge during the day and crusting noted in the morning, itchy, burning of eyes and eye discharge, sometimes yellow and others white. They were between 1 to 2 weeks of getting the eye infection.
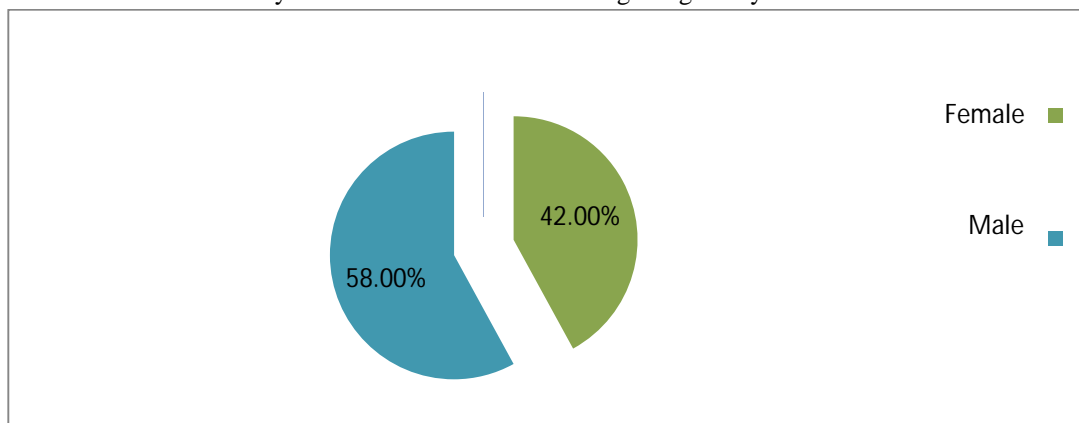

Figure 1: gender distribution among patients with conjunctivitis, 42% females and 58% males.

DNA from the entire specimen was extracted andsubjected to PCR assay using adenovirus specific primer. From the total of 45 specimens tested one (2.2%) sample was positive by PCR test and the rest 44 (97.8%) were negative for adenovirus, as in figure 2. And the appearance of the PCR as in figure 3.
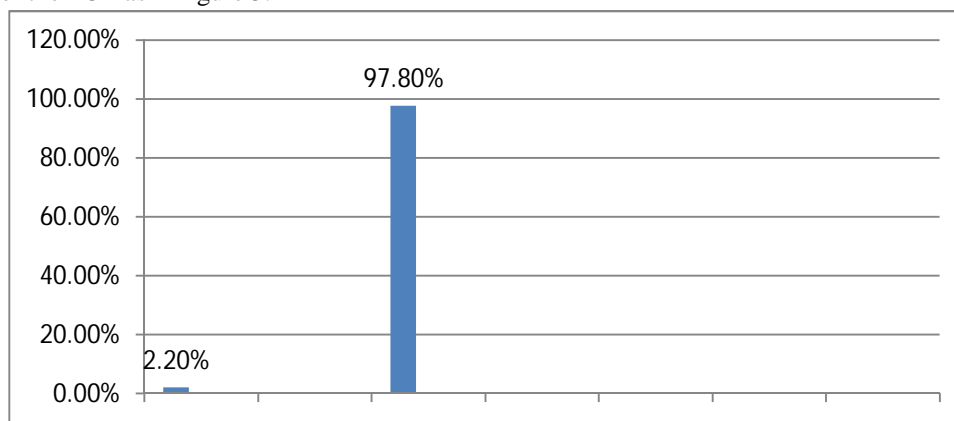

Figure 2: frequency of adenovirus among study group. Shows positive result via PCR 2.2% and negative result 97.8%.
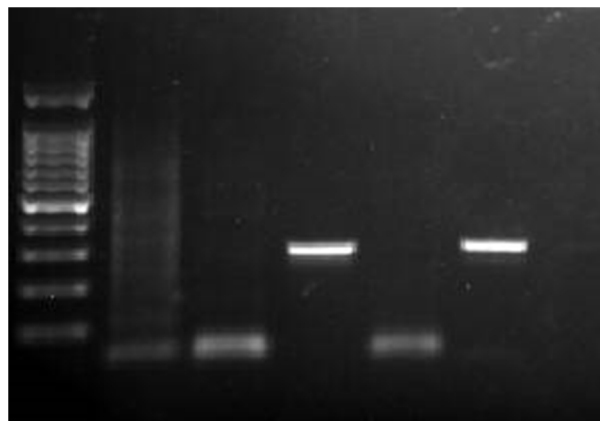
Figure 3: PCR result for positive result

IV. DISCUSSION

Conjunctivitis is the most frequent ocular disorder that is observed in ophthalmic clinics. Several viruses are associated with conjunctivitis and most important group is adenoviruses, Adenoviruses are the leading cause of acute conjunctivitis[24,25]. We found that the prevalence of adenoviral conjunctivitis was 2.2% (1 out of 45) in all patients presenting with a clinical diagnosis of infectious conjunctivitis, this result is the lowest prevalence report until now, isn't in agreement with the findings of similar previous studies. Possible reasons for negative PCR results include the presence of viral conjunctivitis caused by non-adenoviral species, allergic conjunctivitis, Chlamydia and inclusion conjunctivitis. Less common causes include herpetic viruses, picornaviruses, Epstein-Barr virus, influenza viruses, paramyxovirus and poxviruses. Results from studies conducted in Brazil showed that adenoviruses were involved in 59% of all viral cases of conjunctivitis in that country[26], whereas worldwide, adenoviruses have been found to be involved in 15% to 70% of all cases of infectious conjunctivitis[27–30]. It is often difficult to clinically distinguish a disease caused by an adenovirus from other etiologies of conjunctivitis and comparison of laboratory studies of acute conjunctivitis shows that the accuracy of clinical diagnosis ranges from 40% to 75%[31,32]. Other Brazilian researchers studied 75 eye swabs and reported that 60% of the patients had positive PCR results for an adenovirus[33]. A laboratory confirmation of an adenovirus-related etiology may aid the physician in making an accurate diagnosis. The correct identification of patients with adenoviral conjunctivitis may reduce the spread of the disease and limit its toxicity in addition to allergic reactions and antibiotic resistance associated with unnecessary empirical treatments. No laboratory tests are routinely performed at the clinical level in all hospital in Sudan. A rapid, inexpensive and accurate method for diagnosing adenoviral ocular infections is needed not only to limit the transmission of the virus within the community but also to avoid the expensive, unnecessary, and ineffective use of antibiotic therapies.

V. CONCLUSION

Using PCR technique to diagnosis adenovirus conjunctivitis did bring a promising data can make it a tool of diagnosis.

A. Recommendation

Adenovirus infection detection method should be expanded to easy techniques to ensure right results can be obtained.

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