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Serological detection of adenovirus infection by ELISA in Fallujah city

Huda Fawaz Mohamed
Postgraduate student at the College of Education for Pure Sciences, Department of Biology Science, AL-Anbar University
Email: hudafawaz95@gmail.com

Mohammed A. Hamad
Department of Biotechnology, College of Applied Science, University of Fallujah
Email: dr-moh75mnr@uofallujah.edu.iq

***Abstract*---**Adenovirus represent the most common cause of respiratory system disorders and antibody detection can speed up and improve the sensitivity of hAdV diagnosis. Method: This study includes 90 patient who suffering from respiratory symptoms , common colds , or diarrhea with high temperature their ages range from newborn to elderly which collected from Fallujah Maternity and Children's Hospital and teaching hospital in Fallujah in Anbar governorate, for the period from November to May 1-11-2021 to 1-5-2022. Adenovirus was detected by ELISA Test for serum. Results: Out of total 90 serum sample there were 22(24.4%), 68(75.6%) Negative and positive result respectively, from IgG and 87(96.7%) ,3(3.3%) Ne gative and positive result respectively from IgM Result. Conclusion: The presence of positive IgM in ELISA cannot be relied upon as a tool for diagnosing early or acute infections with the virus.

Keywords*---Serological ,detection, Common cold , ELISA, Adenovirus.*

Introduction

Adenoviruses are non-enveloped, double-stranded deoxyribonucleic acid (DNA) viruses that cause a variety of human clinical disorders (Zhang *et al.*, 2020). Adenoviruses can cause diarrhea, mesadenitis, hemorrhagic cystitis, and other pathological disorders (Yamamoto *et al.*, 2017). It represent the most common cause of respiratory system disorders (Xie *et al.*, 2018). More than 100 different human AdV (HAdV) types have been identified, these serotypes are divided into seven groups (Hashimoto *et al.*, 2018). Nasopharyngeal secretions, faeces, conjunctival discharge, sputum, and urine are all sources of pathogens (Desheva, 2018) mainly in individuals with immunosuppression, Pathogens from the upper respiratory tract

are isolated on the 25th day of illness start, Adenoviral infections are spread by the air, where the virus enters the conjunctiva, and possibly through the feces-oral pathway, affecting not just the respiratory tract but also other organs. The incidence of the disease is observed throughout the year, with a peak during the cold season. There have been occasional occurrences as well as pandemic outbreaks. Children aged 6 months to 5 years, as well as military personnel, are the most vulnerable to infection (Khanal *et al.*,2018). It is most prevalent in newly established groups of children and adults (in the first 2–3 months). Antibodies to the virus's most common serotypes can be found in 95% of the adult population in the serum (Kajon *et al.*,2016). Rarely seen is transmission via exposure to cervical canal secretions during birth and in solid organ transplants, especially the liver and kidney. (Bitterman and Kumar, 2021). The virus has demonstrated the capacity to replicate in gastrointestinal epithelial cells, indicating that mucosal lymphocytes serve as a HAdV reservoir. Therefore, in these patients, an increase in the number of copies in stools indicates a higher chance of developing HAdV. Infection that has spread (Hum *et al.*, 2018). Antibodies produced by B cells tend to be relatively type-specific. They have a role in internalizing and neutralizing HAdV, which prevents the un coating necessary for infection, and they can also induce Internalization of opsonized HAdV by cells with activated inflammasomes (Labzin *et al.*, 2019). According to Wu et al., markers for the severity of adenovirus respiratory infection in children include a high serum level of LDH and a low lymphocyte count. (Wu *et al.*, 2020). but only those cases with adenovirus infection had low lymphocyte percentages. Thus, the severity of an adenovirus infection in children may not be accurately predicted by lymphocytes. The level of albumin and clotting function may be affected by the adenovirus's attack on the immune system or hematopoietic system. Children with severe adenovirus pneumonia and low serum albumin may have a bad prognosis (Huang *et al.*, 2017; Zheng *et al.*, 2021). An acute disease is indicated by a high IgG antibody titer, which is seen as a favorable response. (Zhang *et al.*, 2017). Antigen and antibody detection can speed up and improve the sensitivity of hAdV diagnosis. The hAdV IgA, IgM, and IgG antibodies can be found in human serum or plasma by indirect ELISA. Due to their simplicity and speed, (Zhang *et al.*, 2020).

- The study aimed to diagnosis of infection with adenovirus (Advs) that causes common colds and enteric disease in human and detection of the virus using serological methods (ELISA), as well study the presence of IgM and IgG antibodies in correlation with adenovirus infection.

Method

Serum samples were collected from patient their ages range from children to elderly aged , suffering with acute gastroenteritis from respiratory symptoms , common colds , or diarrhea with high temperature which collected from Fallujah Maternity and Children's Hospital and teaching hospital in Fallujah in Anbar governorate, admitted with to hospitals or outpatient wards, a total of (90) serum samples obtained From 1st November 2021 to 1st May 2022.

Samples obtained from the patients were

Sample collection

The blood was drawn from the patients and placed in a gel tubs then separated using the centrifuge to obtain serum for 5 minutes (3000 x), the serum was transferred to a plain tube for preservation and the samples were kept in the freezer (-20^{o}c) until the time of the experiment.

Detection of anti-AdenoVirus IgM and IgG by ELISA

Serum samples were used for detection of IgM and IgG antibodies to identify infection presence according to the instructions of anti-Adenovirus IgM ELISA kit and anti- Adenovirus IgG ELISA Kit supplied by Demeditec Diagnostics GmbH, Germany. The results of the reaction were mesured using microplate reader device (LABOMED IN , USA) at 450/620 nm within 30 min after addition of the stop solution.

The cut-off results were calculated, which is the average absorbance value of the cut-off control decisions.

If the cut-off >11U,the result is positive, which antibodies against the pathogen are present

If the cut-off is 9 = 11U, the result is equivocal, meaning that antibodies against the pathogen cannot be clearly detected.

If the cut-off is <9U,the result is negative, meaning the sample does not contain antibodies against the pathogen.

Results and Discussion:

Human adenovirus (HAdV) is one of the common cause of respiratory diseases ranging from minor to acute infections and conjunctivitis (Crenshaw *et al.*,2019) in addition to acute gastroenteritis to severe disseminated disease (Hiwarkar *et al.*,2018; Kuo,2019; Souza *et al.*, 2021). It represent one of the main pathogens that cause diarrhea significantly (Kumthip *et al.*,2019 ; Zaraket *et al.*, 2020). HAdV epidemiological studies conducted in a many countries including Iraq, which have shown that the virus primarily infected young infants under the age of two (Kumthip *et al.*, 2019).

This study included 90 patient who suffering from respiratory symptoms ,common colds , and /or enteric symptoms including diarrhea with fever most of those patients were males (57.7%) flowed by females (42.3%), their ages range from newborn (37.7% was less than 6 months 22% between 6 month end 1 year)to elderly which collected from Fallujah Maternity and Children's Hospital and Fallujah Teaching hospital in Anbar governorate, during the period from 1st November 2021 to 1st May 2022.

The results of our study demonstrated that patients with suspected common cold caused by adenovirus suffered from Fever(88%), Fatigue (98%) , Diarrhoea (64%)

, Bronchitis (77%) , Sorethroat (91%) Flue (98%) and conjunctiva (3.3%) as showed in Figure (4.1).

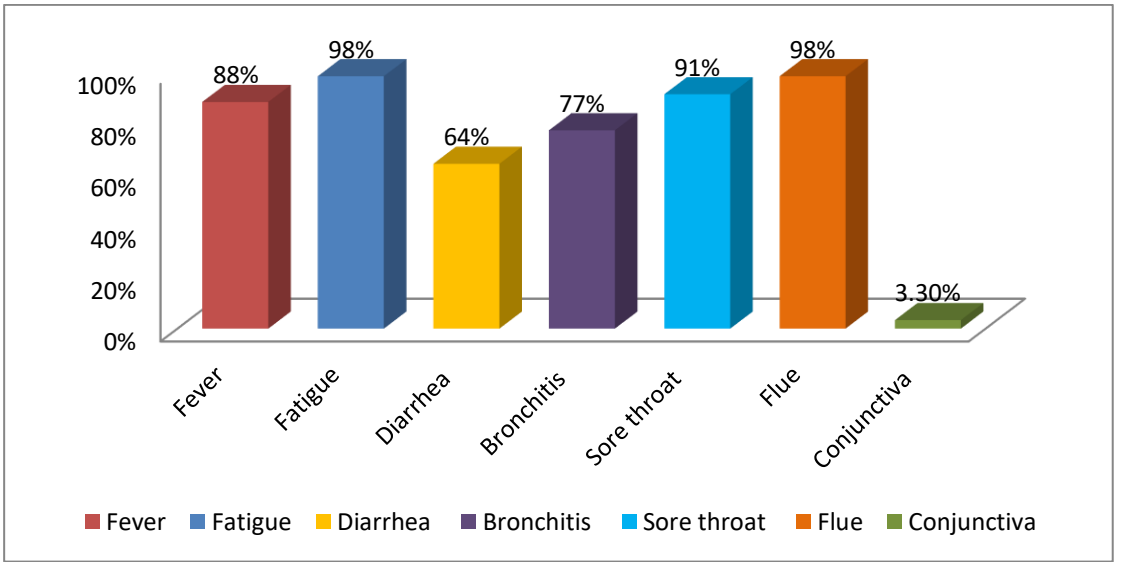

Figure 1: the percentage of symptoms that appear in study patients

Adenoviruses can produce a variety of cold-like symptoms, such as fever, sore throat, cough, pharyngitis, and rhinorrhea. Bronchitis, bronchiolitis, and pneumonia are examples of lower respiratory diseases that can be severe and even fatal which would be more complex in patients with weakened immune systems (Shieh, 2021). Children typically experience prodromes like fever and myalgia with worsening when they have lower respiratory tract infections (LRTIs), such as bronchiolitis and pneumonia. They present with a cough and dyspnea and do not improve with antibiotic treatment (Khanal *et al.*,2018; Shi *et al.*,2020). Where Viral gastroenteritis symptoms include watery diarrhea, stomach pain, nausea or vomiting, and possibly fever (Demers-Mathieu *et al*, 2018). The results of Thewainy and Hasony (2019) study in Iraq, Basra presented that Dehydration, vomiting, and fever were the three main clinical signs of viral gastroenteritis (80%, 60%, and 60% respectively) and Adenovirus-caused gastroenteritis is less likely to cause vomiting and dehydration. Since our study conducted on two types of symptomes: respiratory and enteric, most of study patients suffer from diarrhoea that would related colsely with both types of infcetions which agree with Kumthip *et al* . (2019) study, who suggested that adenoviruses, both enteric and non-enteric, are linked to diarrhea.

hospitalized with illnesses, particularly respiratory ones, is hospital infection. The maximal excretion of adenovirus in patients with gastroenteritis occurs 3 to 13 days after the symptoms have arisen, which means that many cases in older individuals may not be identified or missed out due to virus shedding during a symptomatic stage (Jaff *et al.*, 2015).

Antigen and antibody detection can speed up and improve the sensitivity of hAdV diagnosis. The hAdV IgG, and IgM antibodies can be found in human serum or plasma by indirect ELISA. Only 3 (3.3%) of our study samples showed positive result for IgM while the other (87) 96.7 % of the samples showed negative results for both enteric and non-enteric hAdVs. Indicating low presence of IgM antibody significantly (P≤0.01) as presented in Table (3).

Table (3) : Showed the results of ELISA (IgM)

Type of diagnostic test	Positive		Negative		Total
	No	Percentage %	No	Percentage %	
Elisa IgM	3	3.3	87	96.7	90
Chi-Square - x^2 (P-value)	78.40 ** (0.0001)				--

** (P≤0.01).

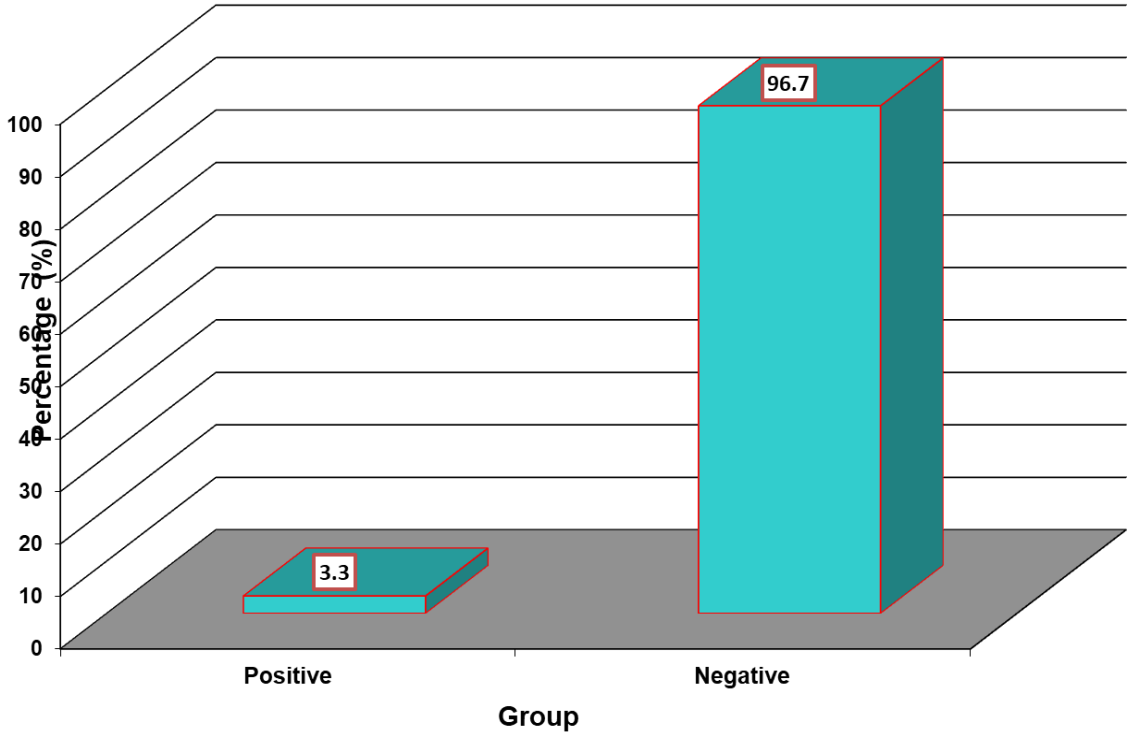

Figure 3. Showed the results of ELISA (IgM)

Despite the fact that IgM was thought to be an immediate detection marker for the infection, the IgM antibody response was variable and had no clear relationship to either the children's age or the severity of their clinical symptoms.our results was significantly lower than the results obtained by Huang *et al*, (2020) 48% of the youngsters had antibody responses, indicating that they were present. On the other hand, our results differ from results obtained by AL-Sadawi *et al*. (2017), where in 77% of the cases, antibody enzyme immunoassay was capable of detecting the IgM which is higher than our percentage that may be attributed to the difference in infection stage where IgM formed after specific period of infection and decline early before symptoms resolution. Where The antigen-binding regions of natural IgM antibodies are polyreactive which interact weakly with many different antigens with only low affinity that makes natural IgM an important component of the innate immune system for recognizing microbes and allows IgM to function as the first line of defense against invading pathogens before antigen-specific IgG come into play. (Allen and Byrnes, 2019),

In comparison with IgM results, the patients illustrated higher positively presence of IgG in the study sample 68 (75.6%) which is significantly higher than negative results 22 (24.4 %) significantly (P≤0.01) as shown in Table (2). those divergent results between IgM and IgG results indicated different stages of disease and different immunological response between those patients.

Table (2) : Showed the results of ELISA (IgG)

Type of diagnostic test	Positive		Negative		Total
	No	Percentage %	No	Percentage %	
Elisa IgG	68	75.6	22	24.4	90
Chi-Square - x^2 (P-value)	23.51 (0.0001)			**	--
** (P≤0.01).					

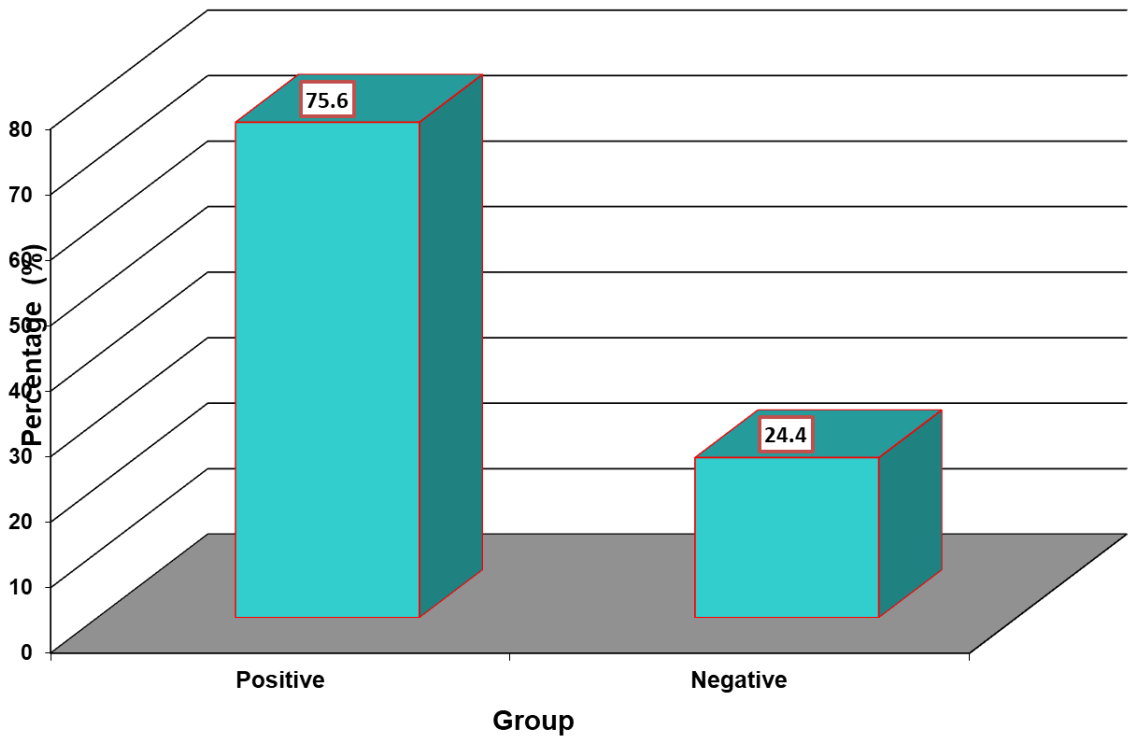

Figure 2. Showed the results of ELISA (IgG)

Despite the fact is good detection results obtained using ELISA, but a different immunological responses formed in patients with variable rates and forming periods, that could be enhanced and produced differentially, moreover, IgG could be considered late response that produced in late onset of the disease, and could not considered an early diagnostic tool. In the weeks following infection with AdV or exposure to AdV, the adaptive immune system produces high-affinity anti-AdV IgG antibodies. (Allen and Byrnes, 2019), which makes suggestion of longevity of infection period in our study patients reaching to late onset of the disease compared with the low rate of IgM that considered early marker of disease.

The positive detection of the IgG with opposite to negative IgM, may suggest late onset of disease because IgM considered early marker that decline after period of time as genus-specific antibodies to Ad usually rise from 9 to 12 days after the onset of infection and type-specific antibodies rise from 10 to 14 days after the onset of infection (El-Sayed and Abd-El Fatah, 2007) while IgG considered late onset marker of the infection, where By the end of the second week (about days 12–14) following the onset of symptoms, adenovirus-specific IgG antibodies started to become detectable during the course of primary infection (Stephan *et al.*, 2003). On the other hand, the antigen of Ad in infected cells persists for 1 to 3 months in 22%–30% of patients even after therapy or by fact that not all patients produce IgM during infection. (El-Sayed and Abd-El Fatah, 2007).

The low rate of infection detection using IgM- ELISA in comparison with IgG may accompany with other studies which found convergent finding. In the study of El-Sayed and Abd-El Fatah (2007) on the serological response to Adenovirus, IgM was negative in two cases with a positive culture that could be attributed to the dynamic of appearance of antibodies. Furthermore, only 3 infants with proven primary infection and low IgM levels close to the cutoff value were able to show virus-specific IgM antibodies in Stephan *et al*(2003) .'s investigation; detection of such antibodies cannot be recommended as a reliable diagnostic tool.

Another assumption of this difference, is that majority of patients enrolled in our study was children mainly less than 6 months followed by patients less than 1 year, where their immunological system were not fully developed, which may reflect the decreased production of antibodies. But It was established that IgG antibodies to adenoviral antigens disappear from circulation after about 10 years after the disappearance of the infection but infants were considered in less incidence of reinfection on recent infection effect (Trojnar *et al.,* 2014).

Conclusions

It is possible to get infected with adenovirus, and the presence of IgM is not detected .The presence of positive IgM in ELISA cannot be relied upon as a tool for diagnosing early or acute infections with the virus .The positive IgG in ELISA more reliable in detection of Adenovirus infection especially in the advanced stage of infection .

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