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RESEARCH ARTICLE

The interferon –ɤ level associated with human adenovirus detected in urine of Hemorrhagic cystitis patients.

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Abstract

Two hundred bloody urine and 200 blood specimens were collected from200 Patients selected to eliminate other causes of hematuria;(149 male and 51 female, age 4- 46 years) all patients suffering from hemorrhagic cystitis(HC) attending to some of hospitals in Babylon governorate over period between April 2014 to December 2014. Detection of Adenovirus was done by direct chromatography technique by using manual principle of CreTest company as a rapid detection and Direct Immunoflouresecnt Technique according to manual procedure of Vircell company to evaluation of the interferon ɤ among a group of patients infected by Adenovirus. The quantitative determination of human IFN-ɤ concentrations in serum by uses Sandwich-ELISA as the method. This idea refer to the uses the concentration of interferon ɤ as the monitor to the immune status for patients suffering hemorrhagic cystitis. The age range distribution showed that the greatest patients group at age 1- 10 y, 21 (31 %) . The present study as shown the male percentage53(78%) was significantly higher than female 15(22%) at p value (< 0.05) .And show the 9(13%) positive result of Adenovirus by IC, DIF, and IDIF technique. The result showed that higher significant level (IFN-ɤ) at comparison with control samples at P value (< 0.05) of T test. Gender relationship with IFN-ɤ showed the female patients have higher level than male of IFN-ɤ. This study shows detected Adenoviruses in bloody urine by rapid chromatographic test, so this method could be applicable in hospitals laboratories and using of certain immunological factors such as (INF-ɤ) to monitor the effectiveness of such virus on the patients at acute and chronic state .

INTRODUCTION

Hemorrhagic cystitis is distinct by lower urinary tract symptoms that include hematuria and irritative voiding symptoms. It result from damage to the bladder's transitional epithelium and blood vessels by toxins, pathogens, radiation, drugs, or disease(Alkan *et al*., 2006). Infectious causes of hemorrhagic cystitis include bacteria and viruses. Viruses are cause of UTIs in an immunocompetent host; however, viruses are increasingly recognized as the cause of lower UTI, especially hemorrhagic cystitis, amongimmunocompromized patients(Kellogg *et al*.,2010). Noninfectious hemorrhagic cystitis most commonly occurs in patients who have undergone pelvic radiation chemotherapy, or both. Affected patients may develop asymptomatic microscopic hematuria or gross hematuria with clots, leading to urinary retention.(Hatakeyama *et al*., 2006).

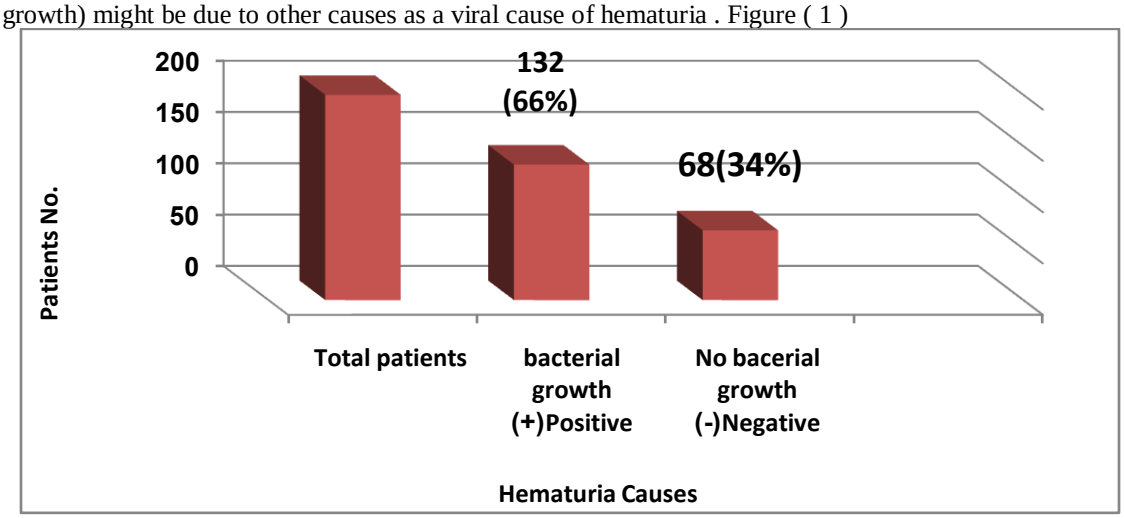
Lower urinary tract infections (UTIs) are common among the general population and are most often caused by bacterial pathogens. Adenovirus, BK virus, and cytomegalovirus are predominant pathogens involved in hemorrhagic cystitis after stem cell and solid organ transplantation, and their early diagnosis and treatment may prevent significant morbidity of hemorrhagic cystitis(Wehrberger *et al*., 2012).. Hemorrhagic cystitis (HC) is characterized by painful hematuria due to hemorrhagic inflammation of the urinary bladder mucosa. It is also recognized as an important complication after hematopoietic stem cell transplantation (HSCT) (Held *et al*., 2010). Adenovirus (particularly serotypes 11 and 21 of subgroup B) is the most common cause of acute viral hemorrhagic cystitis, though it can result from BK virus, CMV virus as well (Hierholzer. 1992). Patients undergoing therapy to suppress the immune system are at risk for hemorrhagic cystitis due to either the direct effects of chemotherapy or activation of dormant viruses in the kidney, ureter, or bladder (Jaffe JC.2001).

Material and method :-

Two hundred bloody urine and 200 blood specimens were collected from200 Patients selected to eliminate other causes of hematuria;(149 male and 51 female, age 4- 46 years) all patients suffering from hemorrhagic cystitis(HC) attending to some of hospitals in Babylon governorate over period between April 2014 to December 2014. The diagnosis of HC is based on the clinical history, physical examination and the exclusion of alternative causes of painful hematuria with excluded samples from female patients suspected of vaginal bleeding and those with possible bacteremia. with included only de novo hematuria that was manifested 4 days after HC. Detection of Adenovirus was done by Direct Chromatography technique by using manual principle of CreTest company as a rapid detection and direct Immunoflouresecnt technique according to manual procedure of Vircell company. The determination of concentration of interferon - ɣ was done by ELISA technique by using manual principle of Elabscience company as the methods.

Results:-

In regarding to patients classification according to causative factor of hematuria two hundred bloody urine specimens were collected from patients and subjected for culturing on blood agar and MacConkey agar. After incubation the samples at 37C for 24-48 hour in ordinary, it was found that 132 specimens (66%) showed positive culture (bacterial isolates) were excluded from the study samples, and 68 samples(34%) showed negative culture (No. growth) might be due to other causes as a viral cause of hematuria . Figure (1)



Figure(1): Patients group according to causative factor of hematuria

The age range distribution showed that the greatest patients group at age 1- 10 y, 21 (31 %) increased in comparison with other age groups as shown in Figure(2) .

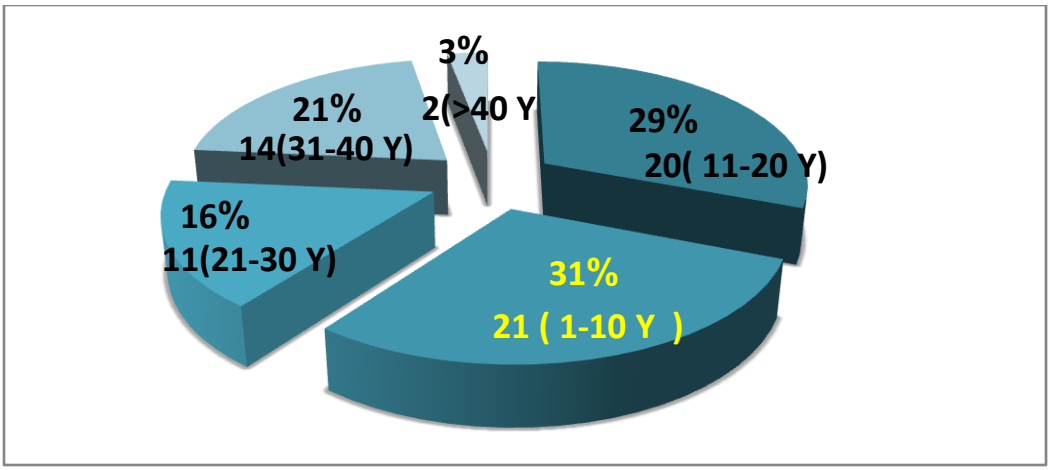


Figure (2): Age groups distribution of Adenovirus patients

Gender group distribution of Adenovirus infection The present study as shown the male percentage53(78%) was significantly higher than female 15(22%) at p value (<0.05) in association with Adenovirus infection. Figure(3).

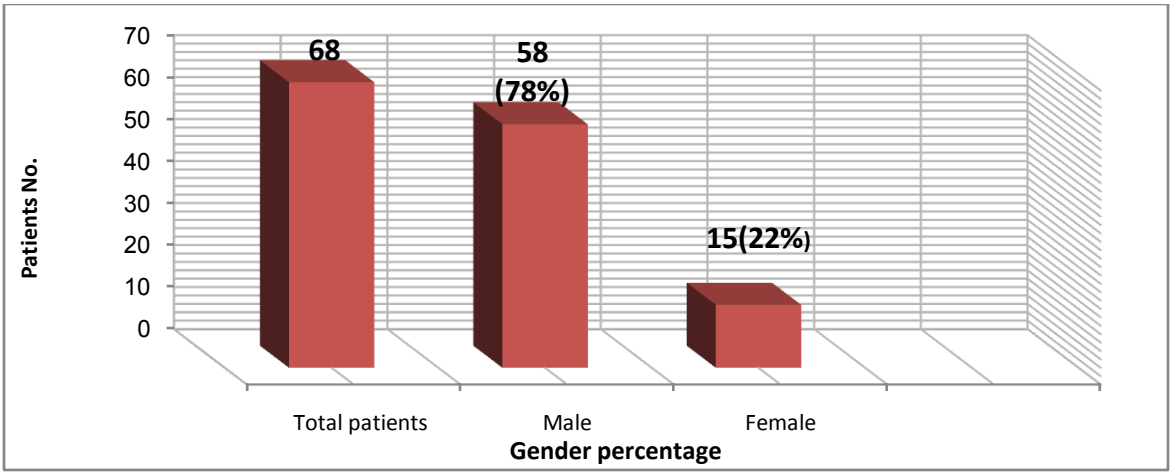


Figure (3) Gender distribution of Adenovirus patients

Detection of human adenoviruses in urine

1. Detection by Immunochromatography(IC)

Based on Immunochromatography (IC) test all negative culture samples 68from patients with viral hemorrahgic cystitisshow that 9(13%) samples were positive for adenovirus samples and 59(87%) were negative , this result might be due to suspected for other causes of hematuria, as shown in Figure (4)

Figure (4): The rapid test (IC) revealed Adenovirus positive(+)

1- (Negative) the green color indicate to the control line.

2- (Positive) blue color indicate to the Adenovirus infection.

2.Detection by direct immunofluorescence

Based on direct immunofluorescence test for the detection and identification of human adenovirus serotypes in urine sample or clinical specimens or cell cultures was done the test utilizes a genus specific monoclonal antibody to detect an epitope of adenovirus hexon proteins which is expressed in all known human adenovirus serotypes. the study showed that 9(13%) samples were positive for adenovirus samples and 59(87%) Figures (5,6 , 7 and 8) for specific DIF pattern of virus infected cells.

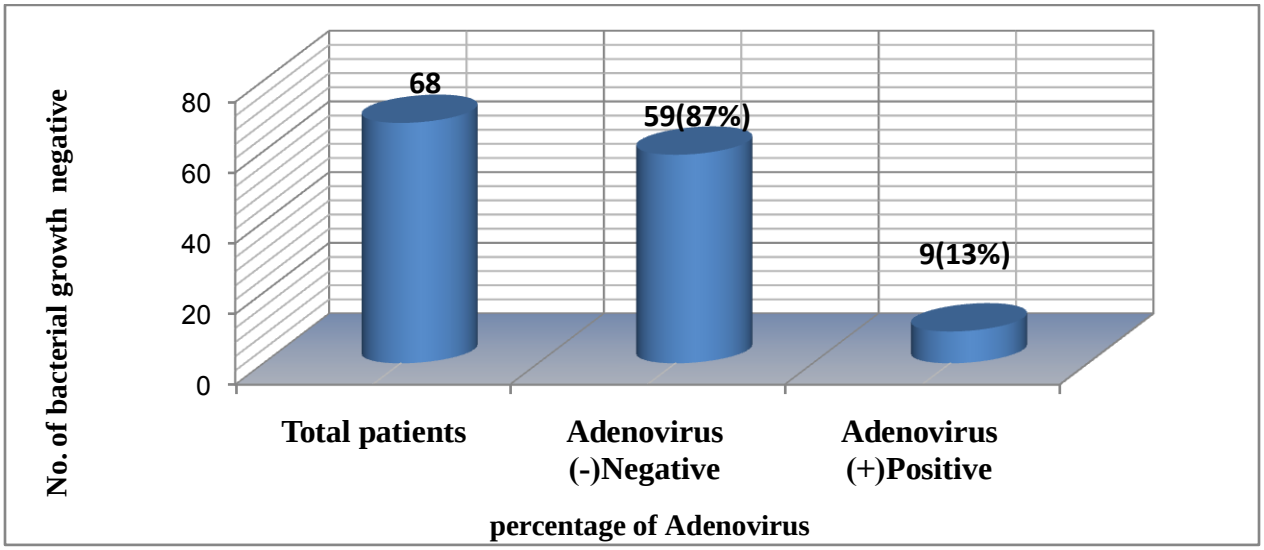
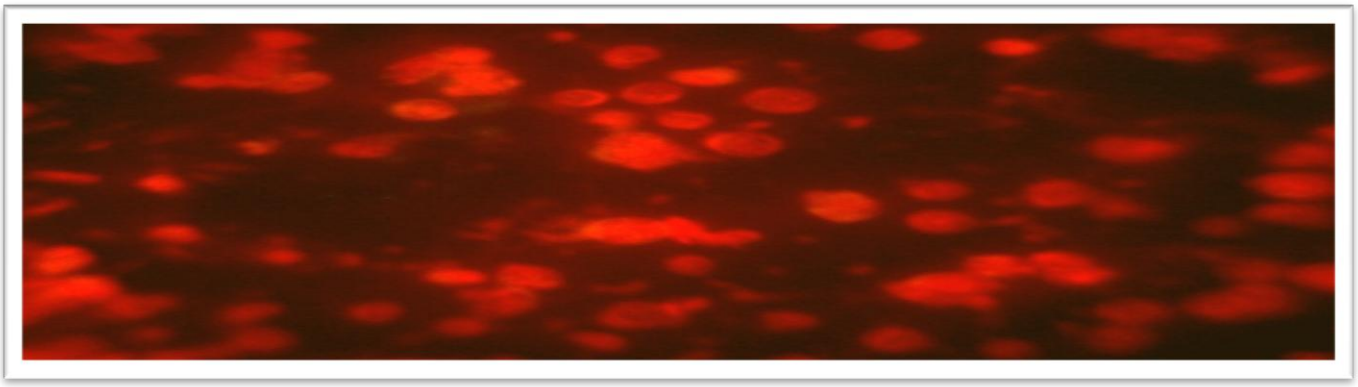


Figure (5): Percentage of Adenovirus by direct immunofluorecent in urine

Figure (6): The appearance of control positive(+) result of Adenovirus in urine by using immunofluorescence microscope that characteristic bright apple-green fluorescence is seen within the cytoplasm and/or nucleus of infected cells, contrasting with the red background staining of uninfected cells.(power 40X)**.**

Figure (7): The appearance of positive(+) result Adenovirus in urine by using immunofluorescence microscope that characteristic bright apple-green fluorescence is seen within the cytoplasm and/or nucleus of infected cells, contrasting with the red background staining of uninfected cells.(power 40X)

Figure (8): The appearance of control negative of Adenovirus in urine by using immunofluorescence microscope.



No green color after incubation with patient urine and conjugate that mean no reaction and negative result (power 40X).

Interferon level in patient with H.C.

The present study deals with interferon (IFN-ɤ) to evaluate the immune function in association with such viral infection and to maintain the disease status. The result showed that higher significant level at comparison with control samples at P value (< 0.05) of T test (Table 1) .

Table(1): Interferon- ɤ level in comparison with control

Parameters	Variable Group	N	Mean ±SD	P value of T- test
INF - ɤ	Test	68	26.28 ± 4.29	
	Control	20	19.74 ± 2.39	*H.S 0.010

Age relationship with IFN-ɤ

The patients at age > 40 years revealed higher level of IFN-ɤ than other result of age group distribution in M ± SD(45.42±5.54) with a significant difference after comparison with control samples (19.74 ± 2.39)at LSD value 2.27. With a significant level of result in comparison between each individual age groups. Table(2).

Table(2) :Age relationship of IFN-ɤ among patient groups

Age groups	N	IFN-ɤ
		Mean ± SD
1 -10 sraey	11	37.96 ± 5.96
11-20 years	20	22.97 ± 3.21
21-30 years	21	25.06 ± 3.23
31-40 years	14	20.93± 3.54
>40 years	2	45.42± 5.54
Control	20	19.74 ± 2.39
LSD		2.27

Gender relationship with IFN-ɤ

The female patients have higher level than male of IFN-ɤ at M±SD (31.57 ±5.44) in a significant difference after comparison with control 19.74 ±2.39,at LSD value (3.94).

Table (3): Gender distribution of IFN-ɤ among patient groups

Gender	NO.	IFN-ɤ
		Mean ± SD
Male	53	24.78 ±3.71
Female	15	31.57±5.44
Control	20	19.74±2.39
Total	88	24.79±4.07
LSD		3.94

Dissuasion:-

Due to the serious morbidity of viral infections, we planned to conduct a study to detect HAdVs infection in hemorrhagic cystitis and consequently, determine the frequency rate of the infection in the viral hemorrhagic cystitis cases in patients attending to some of hospitals in Babylon governorate. Apart from which, we would also like to identify the clinical features pertaining to HAdVs hemorrhagic cystitis which might help us in differentiating it from other causes of viral hemorrhagic cystitis and hence, assist us in diagnosis and managing these cases. In this study, the study was aimed to use of direct chromatography technique as a rapid detection and Direct Immunoflouresecnt Technique for direct detection HAdVs from urine specimens of hemorrhagic cystic patients. To

evaluation of patients infected of Adenovirus the present study was detected by rapid chromatography test in urine specimens of 68 patients were negative bacterial culture and revealed that 9 (13%) of samples were positive for adenovirus in comparison with 59 (87%) were negative for adenovirus infection. The percentage of negative result might be due to suspected for other viral causes of hematuria.

The Immunochromatography(IC) test has proven to be a useful method for diagnosis of HAdVs in patients with hemorrhagic cystitis (HC) because that HAdVs infection is difficult to diagnose the main causes of HC by the presence of symptoms alone. The (IC) test is a rapid detectable test of adenovirus in bloody urine specimens of patients with(HC)as mentioned by (Teramura *et al.*,2004) they said that IC test are more rapid, and they rely on the presence of threshold amount of reactive adenoviral antigen within the specimens.

This may vary considerably with bloody urine, based on the time and course of infection at which the specimen is taken and the amount of adenovirus infected epithelial cells have a major influence on the antigen content of bloody urine . In the present study was observed that IC test was useful, easier to be conducted , with advantage of providing faster result and for early diagnosis of adenoviral hemorrhagic cystitis(Kimura *et al* ., 2009) . IC is a useful method for diagnosing HAdV diseases at the bedside because it has high sensitivity and specificity (IC) can be completed within 15-30 minutes without any special equipment and can therefore be performed in a doctor's office, in an outpatient clinic or a hospital ward. The diagnostic sensitivity and reliability of such rapid tests remains to be a topic of discussion.(Ryan-Poirier *et al.,* 1992).The present study examined urine and serum samples from hemorrhagic cystitis patients and identified a subset that presented a positive reaction in the direct(DIF)and indirect(IDIF)fluorescent-antibody test designed for the demonstration of antigen by (DIF) and anti-adenovirus antibodies by (IDIF).This method allows for the specific identification of proteins with high sensitivity and within a short time, it was soon adapted for the rapid detection of common viruses in clinical samples obtained from patients with upper and lower urinary tract infection (Aldous,*et al.,* 2005). In the present study the *Adenovirus* was detected byDIF and IDIF test in urine specimens of 68 patients were negative bacterial culture and revealed that 9 (13%) of specimens were positive for adenovirus in comparison with 59 (87%) were negative for adenovirus infection. The percentage of negative result might be due to suspected for other viral causes of hematuria. And revealed that equal percentage result between positive result 9(13%) in urine specimens by detection of IgG specific against Adenovirus in serum compared with (IC) , this result might be due to different characteristics will depend on the virus detected, duration of disease , age of the patient, time of sample collection after onset of symptoms, study groups that used during this study . The result of present study was agreement with the study done by(Booth, et al.,2006) they stated that rapid IF methods for viral antigen detection generally showed excellent specificity and very good sensitivity of DIF and IDIF test .Finding specific antibodies at any titer by indirect immunofluorescence supports the clinical diagnosis of hemorrhagic cystitis caused by HAdVs. The result of present study relatively consistence with result of other studies such as (Percivalle,*et al*.,2003) which reported that specific antibodies to AdenovirusIgM and IgG antibodies can be measured in serum and confirmed by R-T PCR technique . The decreased level of interferon -ɤ among younger patients was accompanied by significantly lower proliferative responses, IFN-γ secretion, due to viral infection. (Robin *et al*., 2007).Because frequencies and responses toward polyclonal stimuli were unaffected, this decline in immune responses was not a sign of a generalized immunosenescence in older individuals .(Watcharananan *et al*., 2010).The present study showed that increased IFN -ɤ in older age group of patients more than 40 years at M±SD(45.42±5.54) have higher level than other at a significant difference as well as after comparison with control samples at LSD value (2.27) these result is correlated with the result obtained by Biron, (1998) who found out that the IFN-ɤ is play an important role in the bladder surface inflammatory processes, it is a highly sensitive indicator of various types of cystitis disease .The female is more susceptible for infection with HAdVs rather than other male, this might be due to hyper gamma-globulinemia , and ethnic group (Ghez D *et al*, 2001) . In older study done by(Ebner K*et al*., 2005) , they stated that HAdVs is a diverse condition that can affect both sexes and spares no age group. Overall, the heightened cellular and humoral responses characteristic of female suggest that female sex results in a heightened initiation response and a reduced immunoregulatory response to viral antigens in HAdVs (Berk AJ., 2007) .

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