INTERNATIONAL JOURNAL OF INTEGRATIVE AND MODERN MEDICINE

Clinical Diagnosis of Some Viral Human with Myocarditis Patients by PCR

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Abstract: Three of Human samples from 100 samples of human *Coxsakievirus*, Adenovirus B1. Coronavirus were collected during October, 1st, 2021 to 8 of December 2021. Human *Coxsakievirus*, Adenovirus B1, Coronavirus were including (1month – 95 years). The qPCR method was detected of three samples, for sequence diagnosis the results showed *Coxsakievirus* (98.38%), Adenovirus B1 (95%), Coronavirus (80%) cases. The Population groups studied samples subject groups were distribution into (6) groups including (1month,1-15, 16-31 and 32-47 and 48-63 and 64-79 and 80-95) year, changed age too gender. The samples were isolated from the hospitals including (Al-Sadr, AL- Hakeem, AL- Sajjad). The first study in Iraq to diagnose of human *Coxsakievirus*, Adenovirus B1, Coronavirus with Myocarditis.

Keywords: *Coxsakievirus*, Adenovirus B1, Coronavirus, Myocarditis, qPCR

Introduction

Coxsakievirus B3 was a non-enveloped single-stranded RNA virus belonging to the genus Enterovirus of the picornavirus family. As with all members of the picornaviridae, *Coxsakievirus* B3 was characterized by an icosahedral capsid of approximately 30 nm diameter, which houses the positive-sense (+) RNA genome. The capsid consists of twelve pentamers, each composed of five asymmetric units of the structural proteins VP1–VP4. VP1 to VP3 form the viral shell. VP4 lies at the inner surface of the viral shell making a connection between N-termini of the other capsid proteins and the viral RNA, thereby acting as a stabilizer of the capsid pentamers during virus assembly. The positive-sense (+) RNA genome of *Coxsakievirus* B3 has a length of approximately 7.5 kilobase pairs (kb). It comprises a single large open reading frame (ORF) flanked by a 742 nucleotide (nt) long 50 -untranslated region (50 -UTR) and an about 100 nt long polyadenylated 3‘-UTR. Particularly the long 50 -UTR builds a number of stem-loop structures, among them the cloverleaf (CL) and the internal ribosomal entry site (IRES) which play major roles in viral replication and protein synthesis. The CL interacts with VPg (virus protein genome-linked, also known as 3B), which is covalently attached to the 50 -end of the positive-sense RNA, and with the 30 -UTR and other trans-acting proteins to form the replication complex during RNA synthesis [1]. important pathogen that causes several infectious diseases, ranging from a mild febrile syndrome or respiratory illness to aseptic meningitis, myocarditis, and encephalitis [2]. Enteroviruses spread from person to person by the fecal-oral and by the respiratory routes but indirect transmission may also occur via different routes, including contaminated water, food and fomites [3].

Adenoviruses (AdVs) were non-enveloped icosahedral viruses with a double-stranded DNA genome. Since its discovery in 1953 more than 120 species-specific adenoviral serotypes have been identified in humans, mammals, birds, fish and reptiles. Though human adenoviruses are not generally associated with causing severe disease in immunocompetent humans, they may cause severe infections in immunocompromised people [4]. Apical fibres projecting from capsid surface mediate attachment to host cells. Adenoviruses use either the Coxsackie B virus-Adenovirus receptor (CAR) or CD46 as their primary receptor. Both were expressed in a broad range of cell types, facilitating multiorgan infection and disease. The incubation period ranges from 2 days to 2 weeks depending on the virus type and mode of transmission. Adenoviruses can be spread by droplet inhalation, fomites, the faecal-oral route, and in transplant tissue. Even among nonenveloped viruses, adenoviruses were unusually stable, even in adverse environmental conditions, persisting for a month or more [5].

SARS-CoV-2 was a zoonotic virus belonging to the β-coronavirus genus consisting of crown-shaped peplomers, enveloped as positive-single-strand RNA (+ssRNA) viruses, identifed in the pleomorphic form with a size of 80–160 nm and a genome varying between 27 and 32 kilobases (kb) (Vellingiri et al. 2020; Ji et al. 2020). The virus has four major structural proteins to control the viral structure and function: (1) the protein Envelope (E), (2) the protein Nucleocapsid (N), (3) the protein of the Membrane (M), and (4) the protein Spike (S). The signifcant characteristic of the Coronavirus was because of S-proteins as it forms a crownlike structure on the outermost layer [6]. The possible modes of transmission of SARS-CoV-2 include contact,droplet, airborne, fomite, fecal–oral, Urine, Saliva and animal-to-human transmission. The incubation period for Coronavirus, which was the duration from exposure to the virus (becoming infected) to the onset of symptoms, was on average 5–6 days; however, it can be up to 14 days. The viral load and shedding pattern was different in each patient [7]. was an inflammatory heart disease induced by both infectious (ie, viral, bacterial, fungal) and noninfectious (ie, immune-mediated organ-specific or systemic disease, drugs, toxins) causes. It mainly affects young adults and children, leading to increased cardiac morbidity and mortality.

Myocarditis was usually considered an uncommon disease, though its real incidence seems to be largely underestimated. Postmortem studies on sudden cardiac death (SCD) in young people revealed active myocarditis in 2% to 42% cases.2,3 Moreover, in the years 1990 to 2015, increased morbidity and mortality from myocarditis were recorded. In approximately 50% of patients, acute myocarditis resolves itself spontaneously, while in the remaining cases, it evolves into serious complications and/or to dilated cardiomyopathy (DCM). Finally, due to the variability of clinical presentation and its unpredictable prognosis, myocarditis still poses many diagnostic and therapeutic challenges [8]. The spectrum of clinical symptoms was rather wide and unspecific, ranging from mild discomfort due to palpitations, non-specific chest pain, or fatigue to more severe clinical manifestations such as acute coronary syndrome-like presentations, acute (with or without cardiogenic shock) or chronic heart failure, brady- and tachyarrhythmias, as well as conduction abnormalities. Infectious prodrome with fever, myalgia, and respiratory or gastrointestinal symptoms can be present in cases of infectious myocarditis, whereas in other cases, symptoms associated with systemic diseases can be of relevance. Owing to the unspecific nature of clinical presentation, many cases of myocarditis may go undetected, are accidentally discovered during autopsy, or were discovered too late when the patient already developed end-stage heart disease [9].

Material and Methods

Collect affected specimens of Human *Coxsakievirus*, Adenovirus B1,Coronavirus:

Samples were collected of *Coxsakievirus*, Adenovirus B1, Coronavirus through a start interval 1 October 2021 up to 8 December 2021. Sixty eight for *Coxsakievirus* including (32) female, (36) male, sixty three for Adenovirus B1 including (31) female (32) male twenty five for Coronavirus including (13) female, (10) male positive caseswith infected human patients of age ranged one month up to ninety five years of specimens.

q PCR Technique

This method was used to diagnose human *Coxsakievirus*, Adenovirus B1,Coronavirus, via (this primer was designed based on the NCBI, GoTaq® Green Mater Mix Kit (Cat,Lot.0234854645001,,abm,Canda). Viral DNA was extracted by using Viral Nucleic Acid Extraction Kit (gSYNC TM DNA extraction kit) (Geneaid, Lot No. FA30411-GS, USA) ;(TRIpure Total RNA Extraction Reagent, Lot No. T: 86-27-59760950, ELK Biotechnology, UK and European. All qPCR products were electrophoresed on agarose gel with red assay bromide and visualized under UV light. The technique was performed in the Faculty of Science graduate laboratory in the department of laboratory investigation by using (MULTI GENE OPTIMAX) device.

Table 1. Primer design of human *Coxsakievirus*, Adenovirus B1, Coronavirus depended to NCBI.

Oligo\Name	Sequence	Bases	PCR product size
Coxsakievirus-F	5-TAATGCAGTGGCTGCTTGTC -3	20	234
Coxsakievirus-R	5-TCGCACCTGAAGGCTTAACT-3	20	
Adenovirus B1-F	5-TGCAACATCAGGTAGGGTCA-3	20	215
Adenovirus B1-R	5-TCGCACCTGAAGGCTTAACT-3	20	
S-spike *Coronavirus* - F	5-GAGGCGGAGGTACAAATTGA-3	20	138
S-spike *Coronavirus* -R	5-GTGGTAGCCCTTTCCACAAA-3	20	

Results

Genetic analytic method for diagnosis of *Coxsakievirus*,Adenovirus B1,Coronavirus through q-PCR

Three cases from positive (68) *Coxsakievirus*, (63) Adenovirus, (25) Coronavirus samples of collected serum of different hospitals were diagnosis by qPCR in figure 1.

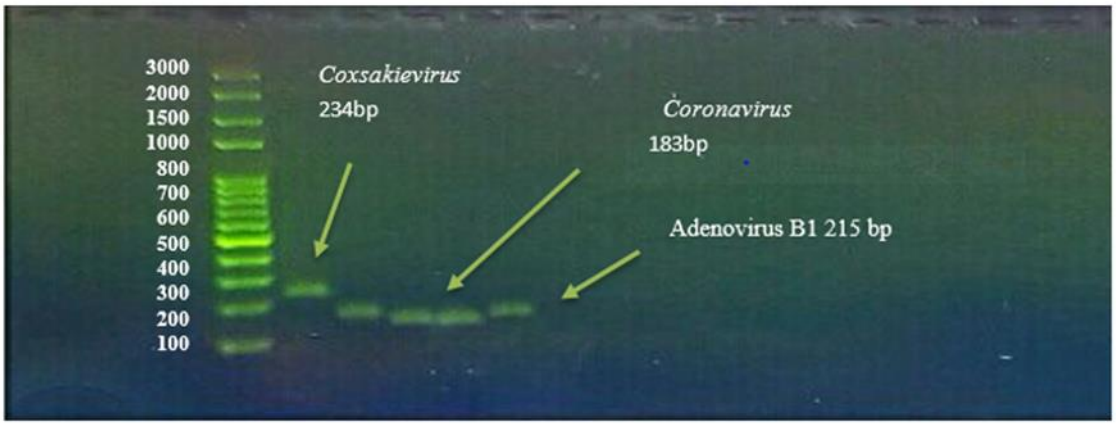

Figure 1. Diagnosis of Human *Coxsakievirus* by Quantitative Polymerase chain reaction (qPCR) product size 234bp,Adenovirus product size 215bp and Coronavirus product size 138bp, 5 µl PCR Ladder was loaded on a 1.5 % agarose in 1x TBE and stained with red assay. dsDNA ladder with bands from 100 bp to 3.000 bp,

DNA Sequencing Method

Three samples were selected out of *Coxsakievirus* (68) case, Adenovirus B1 (63) case, S-sipke Coronavirus (25) case positive for genetic Sequencing. Conventional PCR products of positive were sent to Macrogen Company in Korea by (Alamin center for advanced research and biotechnology) for performing the DNA sequencing by (AB DNA sequencing system). The DNA, RNA sequencing analysis for *Coxsakievirus*,Adenovirus B1, Coronavirus genotyping The multiple alignment analysis was based on Clustal W alignment analysis, and NCBI-BLAST for the homology sequence identity. The NCBI-BLAST Homology Sequence identity (98.38-80%) between local human *Coxsakievirus*, Adenovirus B1and Coronavirus isolate and NCBI-BLAST submitted human *Coxsakievirus*, Adenovirus B1and Coronavirus isolate in table 2.

Table 2. The NCBI-BLAST Homology Sequence identity (98.38-80%) between local human *Coxsakievirus*, Adenovirus B1and Coronavirus isolate and NCBI-BLAST submitted human *Coxsakievirus*, Adenovirus B1and Coronavirus isolate.

Local isolate No.	NCBI-BLAST Homology Sequence identity (%)		
	NCBI-BLAST identical Genotypes	Genbank Accession number	Identity (%)
Human *Coxsakievirus* in Myocarditis isolate No.1	Homo sapiens chromosome 21 *Coxsackievirus* and *Adenovirus* receptor (CXADR) gene.	AH009718.2	98.38%
Human *Adenovirus* B1 in Myocarditis isolate No.2	Homo sapiens *Coxsackievirus* and *Adenovirus* receptor (CXADR) gene and ANA gene.	AF200465.1	95 %
Human s-spike *Coronavirus* in Myocarditis isolate No.3	spike protein Human *Coronavirus* NL63 .	HCNV63gp2	80%

Discussion

Diagnosis of human myocarditis the study is considered in Najaf Governorate and at the level of Iraq as well by designing primers depending on the Location "NCBI" by Quantitative Polymerase chain reaction (qPCR) technicality which resembled with [10] diagnosis of *Coxsakievirus* by Quantitative Polymerase chain reaction (qPCR) that corresponds to our study. [11] diagnosis of Adenovirus by Quantitative Polymerase chain reaction (qPCR)that also corresponds to our study. Evgeniya et al.,2022 diagnosis of Coronavirus by Quantitative Polymerase chain reaction (qPCR) that corresponds to our study. sanger sequencing of our study show identity (98.38-80%) between local human *Coxsakievirus*, Adenovirus B1and Coronavirus isolate and NCBI-BLAST submitted human *Coxsakievirus*, Adenovirus B1and Coronavirus isolate. 98.38% for *Coxsakievirus*,95% for Adenovirus,80% for Coronavirus. [12] they were sequenced in forward and reverse directions with the respective PCR primers. The obtained enterovirus sequences were compared with the corresponding ones available in the GenBank using Basic Local Alignment Search Tool (BLAST) in order to identify the enterovirus type. [13] were diagnosis of Adenovirus by sequencing. [14] diagnosis of Coronavirus by whole genome sequencing will us diagnosis by sanger sequencing.

According to his study [15] most of the infections with the Coronavirus are common with respiratory viruses in different proportions, in addition to the fact that the age group (51-61) year is more infected, and (110) case of Influenza shares with the Coronavirus more infection and the results of Sanger sequences between (99.92 - 78 %) in

The samples of Adenovirus had been gathered of eye excretion of infected groups, this results complied by way of [6], [16] Most ages 16–30 years, further exposed to infection during the study showed to be reliance of age. A study showed (63.76%) of males were more influenced than females (36.23%) of [17].

Conclusion

According to our study, the ratio of the analysis of the genome bank was from (99.92 - 78 %) to the study viruses diagnosed by patients with myocarditis through an examination PCR and only three

samples were sent for different viruses and they were chosen depending on the concentration of the virus through our study in a previous research Alameedy., et al, where the virus was diagnosed through (RT-qPCR). The concentration of the virus was known in addition to gender and age, and we noticed the similarity ratios through genetic analysis on the (NCBI) where new primers were designed for the purpose of diagnosis.

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