Original Article

D4 is the measles virus which caused the measles outbreak in 2004 in Iraq

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Summary:

Background: no previous study is done in Iraq about the isolation and the identification of measles virus although the outbreaks were continuous in the previous years.

Aim of the study: To identify our local strain of measles virus, which had caused measles outbreak in the year 2004.

Patient and methods: About (55) Urine samples and (80) throat swabs were collected from 88 measles suspected patients all over the country during measles outbreak of the year 2004. Serological (ELISA) and virological test were used for this purpose.

Results: Measles virus was isolated successfully in 16 patients who had symptoms of measles infection from mid and south of Iraq. These isolates were obtained on B95a and Vero Slam cell line in 2004. Measles isolates was identified in WHO RRL as D4 genotype.

Conclusion: The study shows that Vero Slam is batter than B95a for measles isolation and this is the first attempt to get measles isolates on B95a cell line in comparison with using Vero Slam cell line. In addition to that it was the first study had identified the endemic strain of measles virus (D4) in Iraq.

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Introduction:

Measles is a highly contagious disease characterized by fever, coryza, cough, and conjunctivitis, followed a generalized maculopapular rash[1]. Measles is successfully controlled in many parts of the world through the use of a live, attenuated vaccine. However, measles still causes nearly 30 million infections and results in more than 875,000 deaths each year[2]. Measles virus is considered monotypic, but sequence analysis of wild type strains has shown that a number of lineages exist and co-circulate[3].

The WHO has recommended genotyping of wild isolates of the measles virus. Thus far, eight clades from A to H and a total of 20 genotypes are considered to be associated with geographical location and chronological isolation; it has been considered that a genotype can reveal its own origin and transmission pathway[3].

The present study includes the first successful attempt to identify our local strain of measles virus which was endemic in Iraq and causing measles outbreak for 2004.

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Material and methods:

Collection and processing of Specimens:

About (55) Urine samples , (80) throat swabs and 88 blood samples were collected from 88 measles patients during measles outbreak of the year 2004. All samples collected within the first 5 days of rash onset and sent to the national measles laboratory of Iraq within 3 days of collection. Specimens were processed directly at the time of collection and store in refrigerator (4 C^0) until cultured as soon as possible on Vero Slam and B95a cell lines. Measles isolates is identified and confirmed by IF test (Microimmune company). Specific measles IgM antibodies were determined as described in the leaflet of commercial kit (Enzygnost; Dade Behring). Measles isolates were confirmed in WHO regional reference laboratory in Tunis. Z test was used for detection of statistical significant difference [4].

Results

The mean level of MDA in the healthy subjects was (0.67±0.23µmol/L). However the level showed a definite significant stepwise increase in the different forms of thyroid disorders. In patients with hypothyroidism the mean level was (1.12±0.69µmol/L). in patients with hyperthyroidism the level was (1.28±0.59 µmol/L). While the highest level of MDA was recorded in patient with thyroid carcinoma as it was (1.59±0.73µmol/L), as shown in figure (1)

The results shown in the figure indicate a highly significant difference between each type of the thyroid disorders compared to that of the control ($P<0.05$).

Table (1): Positive isolates of Measles suspected cases for the year 2004

Lab. code	Provinces	District	TYPE OF SPCIMEN	CPE/Vero Slam & B95a	ELISA/Measles IgM	
					Result	OD values
113 S	Baghdad	Resafa	supernatant	Positive	Positive	0.4
129 U	Baghdad	Kerkh	urine	Positive	Positive	0.6
115 U	Baghdad	Resafa	urine	Positive	Positive	0.5
124 D	Baghdad	Alsader	deposit	Positive	Positive	0.4
142 U	Dyala	Mukdadya	urine	Positive	Positive	0.5
150 U	Baghdad	Alsader	urine	Positive	Positive	0.7
175 D	Baghdad	Alsader	deposit	Positive	Positive	0.5
180 D	Baghdad	Alsader	deposit	Positive	Positive	1.178
158 D	Baghdad	Alsader	deposit	Positive	Positive	0.4
162 S	Baghdad	Alsader	supernatant	Positive	Positive	0.5
105 S	Baghdad	Alsader	supernatant	Positive	Negative	0.0
131 S	Wasit	Alsueara	supernatant	Positive	Positive	0.7
70 U	Theqar	Alshatra	urine	Positive	Positive	0.4
69 U	Theqar	Alshatra	urine	Positive	Positive	0.5
143 U	Dyala	Mukdadya	urine	Positive	Positive	0.7
28 D	Dyala	Mukdadya	deposit	Positive	Positive	0.4

Table (2) shows that the positive results for Anti measles IgM is 66 out of 88 measles suspected cases and just 16 of these cases gave positive result for measles isolation

Table (2): comparison between serological and virological test for detection of Measles infection among measles suspected cases in Iraq.

Results	Anti Measles IgM	%	Isolation of measles Virus	%
Positive results	66	75	16	18
Negative results	22	25	72	82
Total	88	100	88	100

Discussion:

This result show that measles virus replicated on Vero Slam cells more efficiently than in B95a cells and it need higher concentration of measles virus to grow in B95a. The stability of cell lines may explain the reason behind that. A stability of cell lines which is used for measles isolation is differing from each other and the stability of Vero Slam is better than B95a cells. The later cell line need changing medium every 3 days while Vero Slam don't need that, it remain stable in the culture flask for 10 days, because of that it may keep the titer of this virus in good concentration. In B95a, the concentration of measles virus may dilute during changing the medium. This may lead to delay the CPE in B95a cells and huge syncytia consisting of several hundred cells developed within 1 to 2 days after infection and expanded to almost the entire monolayer, subsequently detaching from dishes and it may be lost during changing medium continuously. Therefore, titers for MV viruses in Vero Slam cells reached their peaks at day 3 while its need more time to develope in B95a cells. These results is compatible with the results which was obtained by Kaoru Takeuchi, et .al.[5]

Table (1) shows that all measles isolates appeared positive results for both virological test and serological test but laboratory surveillance must depend upon the serological test for confirmation of measles cases (Table 2) especially that the statistic analysis shows highly significant difference ($p<0.01$) between positive results obtained by ELISA test (Anti Measles IgM) and positive results of measles isolation test. Isolation of measles virus indicates active virus replication but the sensitivity of this methods is low, negative results do not excluded measles virus infection, Virus isolation can take several weeks to complete and successful virus isolation need careful timing of specimen and testing of more than one specimen

In conclusion, the most convenient specimens to collect are urine samples and/or throat swabs and it is acceptable to collect both a urine and respiratory sample. Those samples are excellent sources of measles virus. Specimens for virus isolation should be obtained as soon as possible after the onset of rash and its better to collect these samples within 5 days of rash onset. Specimens for virus isolation should be collected in addition to a serum sample, but urine or respiratory samples should never be substituted for serum samples. Vero Slam is the best choice for Measles isolation although all positive results of measles isolates give CPE on both B95a and Vero Slam cells. Virological test (isolation of measles virus) is an important component of measles surveillance because these studies enhance our ability to identify the source and transmission pathways of the virus. Molecular

surveillance is most beneficial when it is possible to observe the change in virus genotypes over time in a particular region. Such information can help to document the interruption of transmission of measles virus and thus provide an important method for assessing the effectiveness of vaccination programs. It is recommended that virus surveillance be conducted during all phases of measles control and be expanded to give an accurate description of the global distribution of measles genotypes.

The virus of the observed transmission chain in all provinces in which this virus were isolated and the type is D4 viruses. Viruses from genotype D4 were isolated from eight chains of transmission during 1997–2001; in seven of these chains, a foreign source of infection was identified (India, Kenya, Ethiopia, Pakistan, Japan, unknown), Importations from Kenya were associated with a 15-case outbreak in Virginia in 1999 and a sporadic case in Minnesota in 2001. At this time, no information is available about the genotypes of wild-type measles viruses circulating in Kenya, but a genotype D4 virus was imported into the United States from Kenya in 1996 [3] . A genotype D4 virus imported from Ethiopia was responsible for a six-case outbreak in Vermont during 2000. In 2001, a genotype D4 virus was isolated from a small outbreak in Massachusetts, which was traced to a student from Pakistan. During 1999, two genotype D4 viruses were isolated from unlinked, imported cases from India. Genotype D4 is known to be circulating in Pakistan, Southern Africa, India, and Ethiopia [6,7, 8, 9]. A genotype D4 virus (CA1-00) was isolated from a single imported case in California that was traced to Japan. This finding was unusual because genotype D4 viruses have never been detected in Japan despite extensive virological surveillance.

Monitoring the pattern of measles genotypes in an area can help document the effectiveness of control measures which underscore the need to improve the mechanism for obtaining appropriate specimens for viral isolation from all suspected cases, especially outbreak-associated cases when we be in the elimination phase of measles control, obtaining specimens for viral isolation at first contact with all suspected measles cases is important.

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