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Genetic Characterization of Wild-Type Measles Viruses Isolated in Peshawar Pakistan.

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ABSTRACT

Background: Measles is a highly contagious disease, caused by the Measles Virus (MV). Knowledge of genotypes prevalent in any region is important in effective measles surveillance; by mapping distribution of prevalent MV genotypes at different points of time, in a region, gives an idea about the success of measles control through vaccination programs, by documenting the interruption of transmission of the endemic viral genotype.
Objectives: To detect genotypes of wild type measles virus isolated from the samples collected of measles patients in Peshawar valley of Khyber Pakhtunkhwa (KPK) province, Pakistan.
Material and Methods: This was a cross-sectional descriptive study. Nasopharyngeal and/or throat swab samples were collected during a measles outbreak, from February to March 2014, from clinically diagnosed measles patients (cases who met the clinical case definition of WHO). Collection and processing were done according to the standard protocols. RNA (Ribo-Nucleic Acid) was extracted directly from samples; cDNA (Complimentary Deoxy-Ribonucleic Acid) was synthesized, amplification and sequencing of 528bp of carboxy-terminal of N gene was carried out. Sequences were analyzed and data were compared with the standard reference strains of measles virus.
Results: A total of nine sequences obtained were of good quality and enough in length to meet WHO criteria for genotype assignment. It was found that all the nine strains of measles virus belonged to clade B, genotype B3 and cluster B3.1. The rest of the 13 sequences which were shorter in length for genotype assignment also showed close resemblance to Genotype B3.
Conclusion: Wild type measles virus strains, isolated from the samples of measles patients, in Peshawar valley of Khyber Pakhtunkhwa province of Pakistan were found to be genotype B3. It shows that genotype B3 may be the predominate genotype prevalent in Peshawar region at the time of this study.
Keywords: B3 genotype, Genotyping of measles, Measles Virus, Molecular epidemiology.

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INTRODUCTION

Measles Virus (MV) belongs to the genus Morbillivirus and family Paramyxoviridae. It is a negative sense single stranded RNA virus. MV is the cause of highly contagious exanthematous infection.[1] An effective vaccine is available for prevention.[2,3] Randers and Peebles were the first to isolate the virus in 1954.[4] Measles still remains one of the leading cause of mortality, mainly in young children and particularly in developing countries of the world, where inadequate vaccination coverage is the primary cause.[5] Large outbreaks still occur all over the world, including countries with high vaccination coverage, where the virus is being imported from regions where it is still endemic[6]. Generally, for effective control and elimination, through vaccination campaigns, one essential step is to investigate the distribution of regional genotype(s) of MV through molecular studies. Currently genotyping of measles virus is based on sequence analysis of WHO recommended 450 bp region coding for the COOH-terminal (150 amino acids of the nucleoprotein, N - 450) which is the minimum amount of sequence data required for assigning a measles genotype.[7] Furthermore, vaccineassociated rash and fever can be distinguished from infection caused by wild type MV only on basis of sequencing.[8,9] Such information are also required to identify the source of importation and pathways of MV transmission.[10]

WHO has placed the MV in 8 clades, designated A- H. The clades contain 24 recognized genotypes, designated A, B1- B3, C1, C2, D1, D2, D3- D11, E, F, G1- G3, H1, and H2. Within certain genotypes (like B3 and H1) some sequences are closely related that is why they are referred to as clusters within that genotype.[11] Sequence data is compared to a set of WHO reference sequences representing each of the recognized genotypes to assign genotype to a newly recovered strain.[12] Genetic characterization of wild-type MV in any region when combined with epidemiological data, is useful in documenting transmission pathways when detected genotypes phylogenetically match with the genotypes detected in another region thus differentiating indigenous from imported viruses and possibly can trace their source of importation. Knowledge of prevalent genotypes can be used to illustrate the progress made

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towards measles elimination, when the indigenous genotypes are no more detected after running a control and elimination program like vaccination (s).[6,7] Previously reported genotypes from Pakistan were D4, D8 and B3 during 2013 to 2015.[13] There is scarcity of information from Pakistan regarding genotypes of the MV prevalent locally. Research at molecular level investigating MV genotypes is required for effective control and surveillance to measure the success of measles vaccination programs by documenting the interruption of transmission of the endemic viral genotype and to be able to detect pathway of transmission and the source, in case, any non endemic genotype is detected. This study was carried out to investigate genotypes of measles virus prevalent locally in Peshawar, Pakistan to add to the data regarding genotypes prevalent in regions of Pakistan.

MATERIAL AND METHODS

This was a cross sectional descriptive study. Samples were collected by non probability purposive method. Sample size was kept forty (40) depending on available financial resources. Specimens were collected from those patients who met WHO 2007 clinical case definition,[14] which is the criteria generally used for clinical diagnosis of measles infection in local hospitals, i.e. a generalized rash lasting greater than or equal to 3 days, a temperature greater than or equal to 101 °F, any two of cough, coryza, and conjunctivitis. Since the chances of MV RNA retrieval from throat and nasal/nasopharangeal swab decreases after seventh day of rash, measles cases with a rash for more than seven days were excluded. Only those patients who were residing in Peshawar valley for more than three weeks before developing symptoms were included in this study. This duration safely covered the incubation period of infection which is around fourteen days.[15] Afghan nationals residing in Khyber Pakhtunkhwa and patients with a history of measles immunization in less than three weeks were excluded to minimize the chances of collecting imported and vaccine strains of MV. Ethical approval was obtained from the ethical board of the Khyber Medical University, Peshawar No. DIR/KMU-EB/GC/000098 dated 28-1-2014. Consent was taken from guardians of all participating patients as these patients were minors. Nasopharyngeal (NP) and /or Throat Swabs (TS) were collected from each of the patient, transported, stored and processed according to WHO guidelines. General safety precautions as described for measles and rubella were followed as described in WHO lab manual.[14] The collected samples were placed in universal viral transport medium (Universal viral transport combo kit of Bacten, Dickinson and company (BD)USA and stored at - 80°C within two hours of collection.

Samples were thawed at room temperature before extraction of RNA. RNA was extracted from each sample using Pure Link RNA mini kit (Ambion Life Technologies, CA, USA) according to manufacturer's instructions. cDNA was prepared from RNA using RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) and specific primers (Thermo Scientific, Waltham, Massachusetts, USA). Semi-nested PCR was carried out using previously described primers[16] and cycling conditions with minor modifications (Table 01). PCR master mix 2X (ThermoScientific, Waltham Massachusetts, USA) was used. The 528 nucleotides of the N (Nucleoprotein) gene including carboxy terminal 450 nucleotides, recommended minimum requirement for genotyping of measles virus[7] was amplified in the second round of a semi nested PCR. Primers were manufactured by Invitrogen (Waltham, Massachusetts, USA). In the first round of PCR a 644 bp fragment was amplified using MVF1 (nt985±1008, 5'TACCCTCTGCTCTGGAGCTAT GCC 3') as forward and MVB1 (nt162 9±1609, 5'AACAATGATGGAGGGTAGGCG 3') as a reverse primer. A second round semi-nested PCR was used to amplify a 528 bp fragment using primers MVF2 (nt 1101±1121, 5' GATGGTAAGGA GGTCAGCTGG 3') and MVB1 as forward and reverse primers respectively. The PCR cycling program consisted of denaturation for 5 min at 95°C, followed by 30 cycles (1st round) or 20 cycles (second round) of 30 seconds at 95°C, 30 seconds at 60°C and 45 seconds at 72°C, with final extension for 7 min at 72°C[16].All the products were stored at - 40°C till send for sequencing.

Table 01. Primers used to amplify 528 bp containing the C terminus 450 bp of the measles virus N gene required for genotyping.

Gene	Primer	Position	Sequence	Fragment bp
N gene	MVF1	nt 985 ± 1008	5'TACCCTCTGCTCTGGAGCTATGCC 3'	528
	MVB1	nt 1629±1609	5'AACAATGATGGAGGGTAGGCG3'	
	MVF2	nt 1101±1121	5'GATGGTAAGGAGGTCAGCTGG3'	

PCR products were sent to Macrogen, Korea (Macrogen, Beotkkot-ro Geumcheon-gu, Seoul Republic of Korea) for purification and sequencing. Sequencing was done by dideoxy method. Sequencing primers were MVF2 and MVB1 as described above. The obtained electropherograms were viewed and analyzed with the help of Chromasprogram (Chromas Lite version 2.1 (2012), Technelysium Pty Ltd, Queensland, Australia) as well as edited manually when required, after proofreading. The isolated virus strains were characterized according to the WHO recommendations.[17] Reference sequences of MV representative strains were downloaded from GenBank by accession numbers.[17] Nucleotide sequences were aligned with the Clustal W function of MEGA-6 software.[18] Phylogenetic tree was constructed using the nucleotide Kimura-2 parameter and the neighbor-joining method for the sequences of reference and test strains.[19] Bootstrap analyses were performed through 1,000 re-sampling of the data sets. The neighbor-joining method was implemented by using MEGA-6.[18] The obtained sequences had been submitted to GenBank, and the corresponding accession numbers are given in (Table 2).Sequences shorter than 450 bp were also analyzed with Chromas and edited manually and compared with GenBank sequences (www.ncbi.nlm.nih.gov)[20] using BLAST (Basic Local Alignment Search Tool) (www.ncbi.nlm.gov/BLAST). These sequences also show similarity to genotype B3 (Table 03).

RESULTS

Out of forty collected samples from clinically diagnosed measles patients admitted into Lady Reading Hospital, Peshawar, Khyber Teaching Hospital, Peshawar, Hayatabad Medical Complex, Peshawar and Siffat Ghayoor Memorial Hospital Peshawar. Samples from seven patients did not show visible bands of corresponding length after PCR amplification and gel electrophoresis confirmation, so were excluded from analysis. In the remaining 33 cases, sequences were successfully obtained for 22 patients. These 22 sequences were included in further analysis. Out of 22 patients in whom N gene sequences were obtained, both NP and TS were taken from 16 patients, while only TS were collected from 06 patients. Age of patients ranged between 01-11years. Four patients had history of measles vaccination; 11 were not vaccinated before while history of immunization was not available/confirmed in 07cases.

Amplification of the desired 528 bp fragment was confirmed by gel electrophoresis (Fig 01). Although visible bands of desired length were obtained for all 22 samples, good quality sequences were obtained only for 09 samples which were of adequate length for measles genotype assignment, including at least 450 bp encoding measles N gene carboxy terminal proteins as per WHO criteria Table 2. For other 13 samples, different lengths of N gene sequences (ranging from 299 to 466) were obtained Table 3. The 09 strains for which sequence length was adequate for genotype assignment according to WHO criteria analysis of sequences showed that the isolates were in one cluster and members of WHO clade B and closest to WHO reference strain measles genotype B3.1, Abadan Group Fig. 2. Bootstrap analysis of test strains N-gene sequences and the WHO reference sequences showed 95% confidence in the group containing these test virus strains and reference strain and 99 % confidence in group containing only test MV strains. Strains of MV investigated in the current study should be considered as members of WHO genotype B3.1. These wild type MV strains from Khyber Pakhtunkhwa province of Pakistan were named according to WHO guidelines[17] and GenBank accession numbers were obtained (Table 02). The remaining sequences shorter in length for genotype assignment also showed close similarity to genotype B3 strains on analysis using BLAST (Table 03).

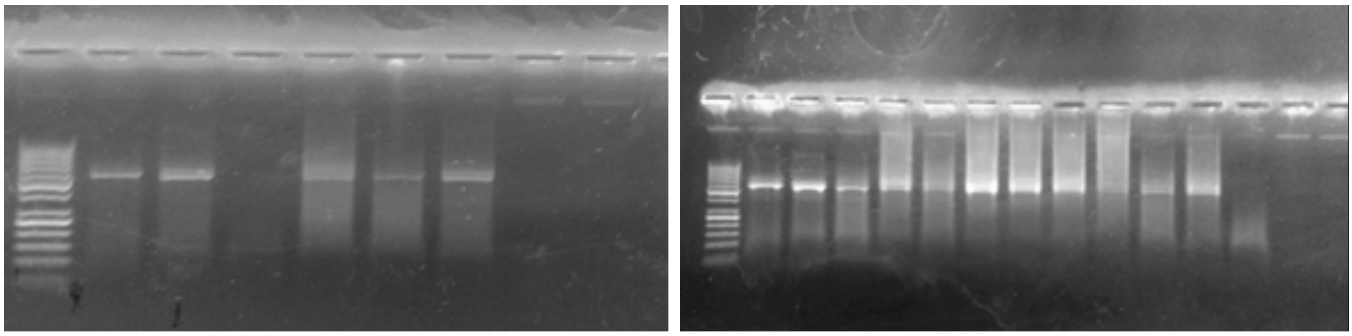


Fig 01. Two photographs of RT-PCR amplifcation products separated by electrophoresis on 1.5% agarose gel using 1000 kb ladder as size marker. Showing the characteristic bands, which lie between 500 and 600bp of size marker for N gene.

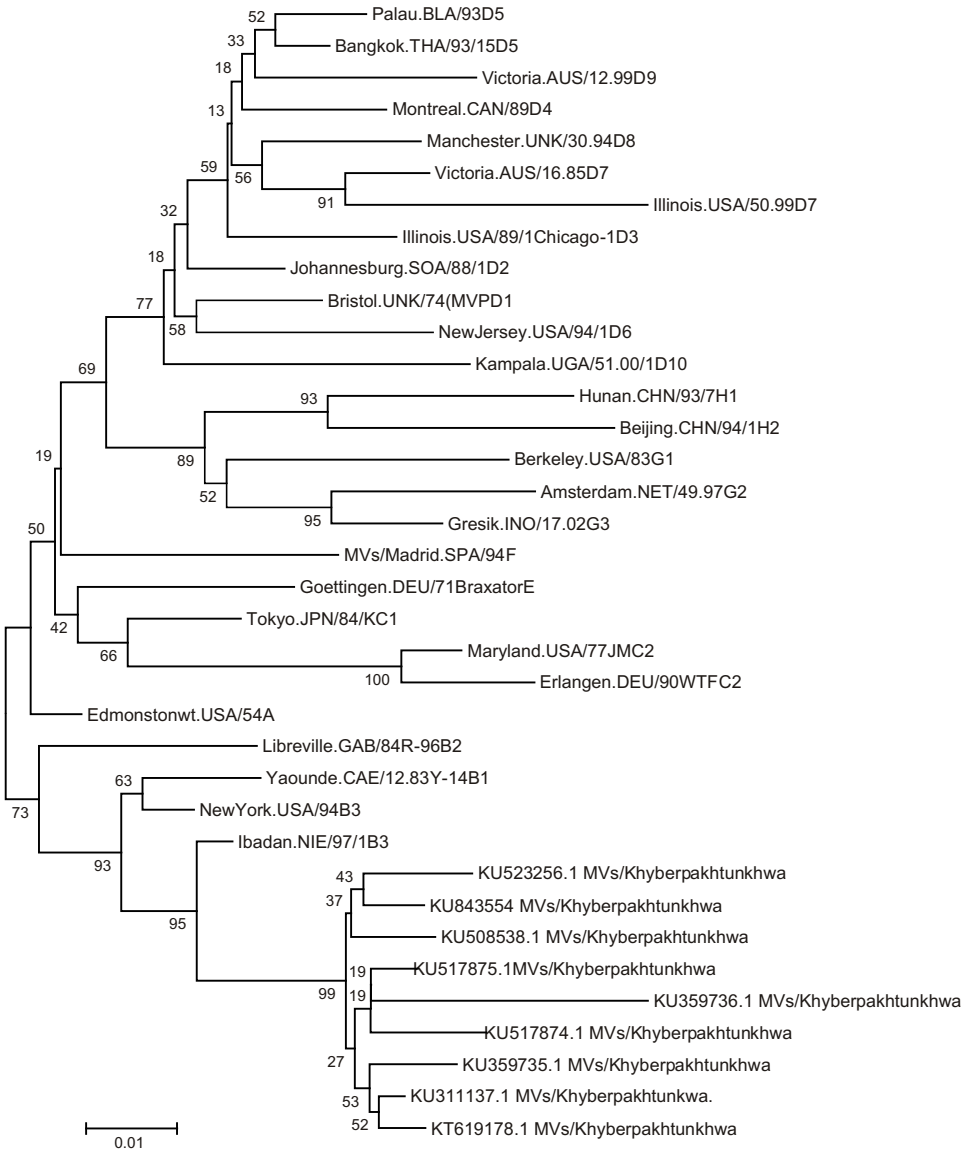


Fig 02. Phylogenetic analysis of MVs genotype reference strains and the nine measles strains from Peshawar Valley of Khyber Pakhtunkhwa province of Pakistan in 2014. The dendrogram (phylogenetic tree) was constructed using MEGA 6 software 18. The analysis involved WHO reference strains and Measles Virus strains isolated in Peshawar valley, given with their accession numbers.17

Table 02. Information of nine patients infected by the measles in Peshawar Valley of KPK for whom measles virus genotype was determined.

S. No	Age Years	Gender	Site of Sample	Vaccination History	Nomenclature	NCBI Accession No.	Genotype
1	3.2	Male	TS	Positive	MVs/Khyberpakhtunkhwa. PAK/15.14[B3]	KT619178	B3
2	1.8	Male	NP	Not Available	MVs/Khyberpakhtunkhwa. PAK/15.14[B3]	KU311137	B3
3	3.6	Female	TS & NP	Positive	MVs/Khyberpakhtunkhwa. PAK/15.14/3[B3]	KU359735	B3
4	2.2	Female	TS & NP	Positive	MVs/Khyberpakhtunkhwa. PAK/16.14/3[B3]	KU517874	B3
5	0.9	Female	TS & NP	Negative	MVs/Khyberpakhtunkhwa. PAK/16.14/5[B3]	KU508538	B3
6	1.6	Male	NP	Negative	MVs/Khyberpakhtunkhwa. PAK/16.14/5[B3]	KU523256	B3
7	5	Female	TS	Negative	MVs/Khyberpakhtunkhwa. PAK/16.14/5[B3]	KU359735	B3
8	3	Female	TS & NP	Positive	MVs/Khyberpakhtunkhwa. PAK/16.14/5[B3]	KU517875	B3
9	1	Male	NP	Negative	MVs/Khyberpakhtunkhwa. PAK/17.14[B3]	KU843554	B3

Named according to WHO nomenclature following the scheme: measles virus (MV), clinical specimen (s)/place. Country/epidemiologicweek. Year/sample identity [genotype]NP: Nasopharyngeal TS: Throat swab.

Table 03. Epidemiological data of patients (n=13). The N gene sequences obtained closely resembled measles genotype B3 when compared with measles N gene sequences in GenBank by nucleotide BLAST but either length of sequences was short for genotype assignment according to WHO criteria or only one sequence read, either forward or reverse was obtained.

S No.	Age Year	Gender	Month-Year of sample collection	Length of sequence matched to MV B3	Name &GenBank accession # of strain to which close similarity was found on BLAST to our sequences on similarity search.
1	2.6	Female	April-2014	466 bp	MVs/London.GBR/3.14/2/[B3] KT732220.1
2	1	Female	April-2014	372 bp	MVi/Manchester.GBR/31.13/[B3] KT732215.1
3	4	Male	April2014	452 bp	MVs/London.GBR/4.14/2/[B3] KT732223.1
4	7.2	Male	April-2014	299 bp	MVs/London.GBR/4.14/2/[B3] KT732223.1
5	9	Male	April-2014	363 bp	MVs/London.GBR/4.14/2/[B3] KT732223.1
6	3	Male	April-2014	412 bp	MVs/London.GBR/4.14/2/[B3] KT732223.1
7	3	Female	April-2014	393 bp	MVs/London.GBR/4.14/2/[B3] KT732223.1&
8	4	Female	April-2014	302 bp	MVs/London.GBR/4.14/2/[B3] KT732223.1
9	5.8	Male	May-2014	467 bp	MVs/London.GBR/4.14/2/[B3] KT732223.1
10	4.4	Female	May-2014	434 bp	Mvs/Aichi.JPN/52.13AB914699.1
11	1	Male	May-2014	401 bp	MVs/London.GBR/3.14/2/[B3] KT732220.1
12	1	Male	May-2014	445 bp	Mvs/Aichi.JPN/52.13 AB914699.1
13	2	Male	May-2014	401 bp	MVs/California.USA/8.14/3[B3] & KJ638891. MVs/London.GBR/3.14/2/[B3] KT732220.1

DISCUSSION

Sequencing based genotyping, to identify MV strains, circulating in a region is one of the preliminary steps, to achieve goal of control and elimination of measles infection through vaccination.[21] Knowledge about genotypes prevalent in any region is required in efforts going on all over the world for this purpose. At present in some parts of the world, measles is eliminated while in other parts including Pakistan efforts are underway to reduce morbidity and mortality and to achieve the targets of control and elimination through vaccination programs.[22,23] Pakistan has been facing difficulties in surveillance and control due to multiple and diverse reasons. Outbreaks of measles infections still occur besides endemic cases in the country and attempts to achieve control are going on by participating in supplementary vaccination campaigns in addition to routine vaccination programs[24]. Around 14,984 cases were reported in the outbreaks during 2012 and more than 25,000 cases reported in 2013, with an incidence rate of = 10 and < 50/1,000,000 population.[25] In Pakistan, at present, there is scarcity of data regarding molecular, epidemiological and other aspects of measles 'the disease' and 'the virus'. Knowledge regarding the genotypes of wild type me asles viruses prevalent in different parts of Pakistan is yet to be explored to get a complete picture of the genotypes prevalent all over the country. There is no proper surveillance structure for genotype detection on the country level. To participate in the struggle against measles control and elimination, awareness regarding genotypes prevalent in region is need of time.

The genotypes of wild type measles virus reported previously from Pakistan, were members of clade D and genotype was either D4[7-13] or D8[26] whether the virus was collected from patient within Pakistan[7] or was obtained from patients who were suspected to have imported the virus from Pakistan to other countries where genotyping was done[4] genotype B3 was also detected from some parts of Pakistan in later years.[27]

Pakistan is a member of the 23 countries of WHO Eastern Mediterranean region. Detection of prevalent measles genotypes is being carried out in countries of the region from time to time. Genotypes of wild type measles virus reported from countries of Eastern Mediterranean region reported prior to 2009 included, B3 from Djibouti, Kuwait, Lebanon, Libya, and Sudan while D4 from Afghanistan, Bahrain, Egypt, Iran, Iraq, Jordan, Qatar, Sudan and Syria and D8 from Morocco.[7] The genotypes mostly reported after 2009 are B3 from Oman, Bahrain, Iran, Saudi Arabia, UAE, Libya and Jordan, D8 from Syria, Lebanon, Jordan, Bahrain, Oman, Iraq and Kuwait, D4 from Egypt and UAE, while C2 was reported from Morocco. Most of these reports are of viruses from epidemics during 2013 and 14. There is ample evidence that after an outbreak in Philippine (Harare) in 2012, B3 genotype which was previously considered to be the main genotype prevalent in African region, is being widely spread throughout the word in 2013 and 2014.[25] Main genotypes of the wild type measles virus reported from neighboring countries of Pakistan among which China and India are not members of EMR include, H1 from China[28] D4 from Iran[29] D4, D7, D8 earlier and B3 in 2012 from India[30] and D4 from Afghanistan. B3 genotype was earlier considered to be main genotype prevalent only in African region[31] but in 2013 and 14 it was detected in countries all over the world.[25] In our study we found that the genotypes of wild type MV circulating in Peshawar valley at the time of study was B3.In this scenario detection of B3 genotype from Peshawar region not only gives a snapshot of genotypes which are prevalent in this region, but it might also be helpful in contributing to the whole picture of genotype distribution in the world. When there is a change in prevalent genotypes from a region in subsequent genotyping studies, it helps in assessing effectiveness of vaccination programs and the route of transportation and source can also be detected when a different genotype is found by comparing phylogenetically with the genotypes prevalent in neighboring regions. Moreover, detection of B3 genotype also adds to the evidence that this genotype has been spread widely during previous few years[25] including to Pakistan. This was a small scale study done with limited resources, but information obtained at that time regarding genotypes of MV prevalent in Peshawar valley need to be made part of country wide records of measles genotype distribution. The weakness of the study was that only clinical diagnostic criteria were used to include patients, and antibody for acute infection confirmation was not done. Many samples were wasted due to frequent electricity outages. Strength of the study is that it is the only MV genotype data available from Peshawar at that time. Samples were taken from different major

hospitals of Peshawar thus representing almost entire Peshawar region. This limited baseline data might contribute to country wide picture of MV genotype distribution may pave the way for larger, integrated, country wide studies exploring measles molecular epidemiology, to fully characterize viral strains prevalent in the country and add to global knowledge of MV genotype distribution.

CONCLUSION

After molecular characterization of Wild type MV, strains isolated from samples of measles patients in Peshawar valley of Khyber Pakhtunkhwa, they were found to be of genotype B3 and were phylogenetically closest to Ibadan. NIE/97/1B3. This study showed that B3 was the predominant genotype of wild type MV circulating in Peshawar at the time of study. It also shows that the rapid world wide spread of B3 genotype of MV, is also involving Peshawar region of Pakistan.

Recommendations

Vaccinations programs for measles control and elimination has been running in country and Peshawar region since this data was collected, a further genotyping study might show that the vaccination programs have been effective if a different genotype distribution is found prevalent, and its phylogenetic analysis may show link to MV of the region from where it is possibly imported. Country wide periodic genotype characterization in future might help to provide a map of measles genotype distribution to asses measles control and elimination programs. Further research is also recommended on different aspects B3 genotype of MV, to investigate reason for its rapid and wider spread.

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Ethical Consideration

This study was approved by the ethical board of Khyber Medical University, Peshawar, Pakistan.

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Conflicts of interest

Authors declare no conflicts of interest.

REFERENCES

1. Plattet P, Alves L, Herren M, Aguilar HC. Measles Virus Fusion Protein: Structure, Function and Inhibition. Viruses. 2016;8(4):112-22. https://doi.org/10.3390/v8040112
2. Tahara M, Ito Y, Brindley MA, Ma X, He J, Xu S, et al. Functional and structural characterization of neutralizing epitopes of measles virus hemagglutinin protein. Virol J. 2013;87(1):666-75. DOI: https://doi.org/10.1128/jvi.02033-12
3. Griffin DE. Measles Vaccine. Viral Immunol. 2018;31(2):86-95 https://doi.org/10.1089/vim.2017.0143.
4. Torner N, Anton A, Barrabeig I, Lafuente S, Parron I, Arias C, et al. Epidemiology of two large measles virus outbreaks in Catalonia: what a difference the month of administration of the first dose of vaccine makes. Hum Vaccin Immunother. 2013;9(3):675-80. https://doi.org/10.4161/hv.23265
5. Necula G, Lazar M, Stanescu A, Pistol A, Santibanez S, Mankertz A, et al. Transmission and molecular characterisation of wild measles virus in Romania, 2008 to 2012. Euro Surveill. 2013;18(50):pii=2065 https://doi.org/10.2807/1560-7917.ES2013.18.50.206588.
6. Magurano F, Fortuna C, Marchi A, Benedetti E, Bucci P, Baggieri M, et al. Molecular epidemiology of measles virus in Italy, 2002-2007. Virol J. 2012;9:284-92. DOI:https://doi.org/10.1186/1743-422X-9-284
7. Rota PA, Brown K, Mankertz A, Santibanez S, Shulga S, Muller CP, et al. Global distribution of measles genotypes and measles molecular epidemiology. J Infect Dis. 2011;204 Suppl 1:S514-23. , https://doi.org/10.1093/infdis/jir118
8. Rota PA, Bankamp B. Whole-Genome Sequencing During Measles Outbreaks. J Infect Dis. 2015;212(10):1529-30. https://doi.org/10.1093/infdis/jiv272
9. Roy F, Mendoza L, Hiebert J, McNall RJ, Bankamp B, Connolly S, et al. Rapid Identification of Measles Virus Vaccine Genotype by Real-Time PCR. Journal of Clinical Microbiology. 2017;55(3):735-43. DOI:https://doi.org/10.1128/jcm.01879-16
10. Li S, Qian X, Yuan Z, Sun X, Li C, Tang X, et al. Molecular epidemiology of measles virus infection in Shanghai in 20002012: the first appearance of genotype D8. Braz J Infect Dis. 2014;18(6):581-90. https://doi.org/10.1016/j.bjid.2014.05.018
11. Dia N, Fall A, Ka R, Fall A, Kiori DE, Goudiaby DG, et al. Epidemiology and genetic characterization of measles strains in Senegal, 2004-2013. PLoS One. 2015;10(5):e0121704-e. https://doi.org/10.1371/journal
12. Magurano F, Fortuna C, Baggieri M, Filia A, Benedetti E, Bucci P, et al. Molecular epidemiology of measles virus in Italy during 2008. Ann Ist Super Sanita. 2013;49(1):50-5. DOI: 10.4415/ANN_13_01_09

13. Nishimura T, Komase K, Terao K, Agboatwalla M, Shahid M, Akbani Y, et al. Sequence analysis of the H, F and N genes of wild measles virus strains isolated in Karachi, Pakistan. Nihon University Journal Of Medicine. 1997;39(6):323-36.
14. WHO. Manual for the laboratory diagnosis of measles and rubella virus infection. 2007. www.who.int/vaccines-documents/
15. Vainio K, Steen T, Arnesen T, Rønning K, Ånestad G, Dudman S. Measles virus genotyping an important tool in measles outbreak investigation in Norway, 2011. Europe. 2011;12(13). https://doi.org/10.2807/ese.17.50.20340-en
16. Chibo D, Birch CJ, Rota PA, Catton MG. Molecular characterization of measles viruses isolated in Victoria, Australia, between 1973 and 1998. J Gen Virol. 2000;81(10):2511-8. https://doi.org/10.1099/0022-1317-81-10-2511
17. Measles virus nomenclature update: 2012. Wkly Epidemiol Rec. 2012;87(9):73-81.
18. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30:2725-9. https://doi.org/10.1093/molbev/mst197
19. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4(4):406-25. https://doi.org/10.1093/oxfordjournals.molbev.a040454
20. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, et al. GenBank. Nucl Acids Res. 2011:gks1195.
21. Namaki S, Gouya MM, Zahraei SM, Khalili N, Sobhani H, Akbari ME. The elimination of measles in Iran. The Lancet Global Health. 2020;8(2):e173-e4. DOI:https://doi.org/10.1016/S2214-109X(20)30002-4
22. Filia A, Bella A, Rota M, Tavilla A, Magurano F, Baggieri M, et al. Analysis of national measles surveillance data in Italy from October 2010 to December 2011 and priorities for reaching the 2015 measles elimination goal. Euro Surveill. 2013;18(20):Pii=20480. https://doi.org/10.2807/ese.18.20.20480-en
23. Eom H, Park Y, Kim J, Yang J-S, Kang H, Kim K, et al. Occurrence of measles in a country with elimination status: Amplifying measles infection in hospitalized children due to imported virus. PLoS One. 2018;13(2):e0188957-e. https://doi.org/10.1371/journal.pone.0188957
24. Niazi AK, Sadaf R. Measles Epidemic in Pakistan: In Search of Solutions. Ann Med Health Sci Res. 2014;4(1):1-2.
25. Featherstone D. Report on 12th WHO global Measles and RubellaLaboratory Istambul; 2014.
26. Ramsay ME, Jin L, White J, Litton P, Cohen B, Brown D. The elimination of indigenous measles transmission in England and Wales. J Infect Dis. 2003;187(Supplement 1):S198-S207. https://doi.org/10.1086/368024
27. Zaidi SSZ, Hameed A, Suleman Rana M, Alam MM, Umair M, Aamir UB, et al. Identification of measles virus genotype B3 associated with outbreaks in Islamabad, Pakistan, 20132015. Journal of Infection and Public Health. 2018;11(4):540-5. https://doi.org/10.1016/j.jiph.2017.10.011
28. Wei C, Shi J, Liu B, Shi Y, Zheng J, Xu G, et al. Molecular characterization of the measles virus genotypes in JiLin Province, China. PLoS One. 2012;7(10):e46011. https://doi.org/10.1371/journal.pone.0046011
29. Naseri M, Salimi V, Mokhtari-Azad T, Esteghamati A, Gooya M, Nadji S, et al. Molecular Epidemiology of Measles Virus before and after the 2003 Mass Vaccination Campaign for Measles/Rubella in Iran. Iran J Public Health. 2011;40(1):41-9.
30. Kuttiatt VS, Kalpathodi S, Gangadharan ST, Kailas L, Sreekumar E, Sukumaran SM, et al. Detection of measles virus genotype B3, India. Emerging Infect Dis. 2014;20(10):1764-71. doi:10.3201/eid2010.130742
31. Kopel E, Amitai Z, Savion M, Aboudy Y, Mendelson E, Sheffer R. Ongoing African measles virus genotype outbreak in Tel Aviv district since April, Israel, 2012. Euro Surveill. 2012;17(37):pii=20272.

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AUTHOR'S CONTRIBUTION
The following authors full fill authorship criteria as per ICMJE guidelines;

Irshad Z: Analysis and interpretation of data, critical revision, substantial contribution to conception and design, drafting the manuscript, final approval, agreed to be accountable for all the work.

Khan ZI: Analysis and interpretation of data, data acquisition, drafting the manuscript, final approval, agreed to be accountable for all the work.

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