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HCV NS3 inhibitors resistance mutations in the telaprevir started Turkish patients with chronic HCV

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Background: Now with the new triple therapie options sustained viral response rates increased very much in genotype 1 patients. This therapie is very suitable for Turkey because of dominance of genotype1 inTurkey (∼95%).

Methods & Materials: HCV RNA isolated with magnetic particle technique and detected with Bosphore HCV Kit v2 on the real-time PCR platform (Anatolia Biotechnology, Istanbul, Turkey). HCV NS3 region (30 - 181 aa) has been analysed for telaprevir and boceprevir resistance mutation after population based sequencing. NS3 inhibitors resistance mutation determination by geno2pheno drug resistance tool (coreceptor.bioinf.mpi-inf.mpg.de). The fold change of NS3 inhibitors resistance mutation is based on the IC50 values of the drugs for the different mutations and the wild type and fold change cutoff was 1.2. Consensus sequence from strain HPCPLYPRE, HPCCGAA, HPCJCG, HPCHUMR, HPCCGS and AY051292 for HCV genotype 1b and H77 for type 1a sequences has been used in the resistance mutation analysis.

Results: We analysed 36 patients. HCV RNA from 6 different proviencesof Turkey before beginning telaprevir treatment and found in 3 (8,3%) patient telaprevir resistance and in 1 (2,8%) patient boseprevir resistance.

Only telaprevir is now available in Turkey. All this 36 patients are relapsed after first pegylated interferon and ribavirin treatment or treatment naive cirrhotic patients.

Table 1; Demographic features and results of HCV NS3 inhibitors resistance mutation analysis in the telaprevir started treatment naive Turkish patients with chronic hepatitis C.

Characteristics	Study group
Gender, M/F, n(%)	12 (33%)/24 (66%)
Age, median (range)	56.6 (25 - 76)
HCV RNA load, median (range), IU/ml	4.2 + E7 (33 + E2 - 10 + E9)
HCV genotype, n(%)	1b; 35 (97.2)
	1a; 1 (2.8)
ALT, median (range), U/L	67 (16 - 202)
AST, median (range), U/L	49.7 (8 - 116)
NS3 inhibitors resistance, n(%)	Telaprevir; 3 (8.3)
	Boceprevir; 1 (2.8)
NS3 inhibitors resistance mutation	Telaprevir
	T54S
	L155I
	I132V
	Boceprevir
	R109K
NS3 inhibitors resistance mutation	T54S; 1.9
fold change (fold change cutoff: 1.2)	L155I; 4.3 - 16.4
	I132V; 1.8
	R109K; 1.2

Conclusion: Despite this resistance profile all the patients HCV RNA at the first month of therapy were under 1000 IU/mL. We will see clinical aspects of this resistance pattern at the end of the therapy.

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Molecular characterization of rotavirus in diarrhoeic children 0-5 Years of age in Kano, Nigeria

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Background: Rotavirus (RV) is the most common cause of severe diarrhoeal illness in infants and young children 0-5 years of age in both developing and developed countries. However, limited data exist on rotavirus (RV) infection in Kano, North-Western Nigeria. This Study was aimed at determining the prevalence and genotyping of rotavirus among these children using Enzyme Linked ImmunoSorbent Assay (ELISA) and Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

Methods & Materials: A total of 285 stool samples were collected from infants and children 0-5 years of age, who reported with diarrhea in six different hospitals in Kano, Nigeria between November 2009 and July 2010. The diarrhoeic Stools were analyzed for RV antigen (ELISA), and the RV positive stools were further subjected to VP7 and VP4 genotyping using gene specific primers (RT-PCR).

Results: Rotavirus was detected in 36.5% (104/285) of the diarrhoeic children. The infection occurred throughout the study period with higher peaks in the drier month of April 77.6% (38/49) and lowest in July 12.2% (5/41) (Pearson Chi Square analysis: $X^2 = 27.720$, $P < 0.05$, $df = 1$). The highest prevalence of RV infection was in children 41-50 months 50% (3/6) The RV was detected more in male 37.2% (61/164) than female 35.4% (43/121) children and no statistically significant difference was observed ($P > 0.05$). Three different rotavirus P-genotypes (P[8], P[4], and P[6]) were detected in this study and P[6] (48.5%) was the most commonly detected. Mixed infection were detected and consisted only of P[8] + P[6]. Six different G-genotypes were detected. The predominant genotype was G2 35.0% (36/103). The most common G and P combination was

found to be G2P [6] with 19.4% (20/103) frequency of occurrence. A single GNTP [8+6] mixed combination of rotavirus strains was also detected during the study. Strains such as G6, G9 and G12 were also detected at very low levels.

Conclusion: Rotavirus was found to be an important cause of diarrhoea in children 0-5 years of age in Kano, North-Western Nigeria. Further characterization of RV strains circulating in the study area is also needed to provide information needed for implementation of RV vaccination and vaccine effectiveness studies.Rotavirus vaccination should be considered as part of routine immunization in Nigeria.

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Divergence of chikungunya virus in India: Tale of two cities

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Background: Chikungunya (CHIK) is a serious health issue in India and exists as single or co-infections along with Dengue (DENV). Initially restricted to the Southern parts of the country, CHIK has spread to most of the country with 18 states/Union Territories of the 26 states/UTs affected currently. The present study was conducted to understand the divergence of CHIKV from two parts of the country - Mumbai, where the virus was in circulation since a long time, and from Delhi, where the virus was witnessed since 2009. Clinical and genetic information of patients were compared to comprehend the divergence and evolution of the virus within the country.

Methods & Materials: Blood samples were collected over a period of three years (2010-2012) from patients after obtaining their consent and clinical information. Clinical correlation was performed within the two states. Dengue status was analysed with respect to clinical manifestation of symptoms. RNA was extracted from sera and partial gene of E1 envelope protein was amplified. The PCR products were purified, sequenced and phylogenetic analyses performed on the sequences.

Results: A total of 459 samples were collected over the period of three years from the two states. Serosurveillance showed 68% of patients to be positive for CHIKV IgM. Patients showed distinct differences in restriction of joint movements in case of CHIK only and CHIK/DENV co-infections. Platelet was another important feature to distinguish between CHIK and CHIK/DENV co-infection. Genetic characterisation of the samples revealed that all Indian samples were of ECSA genotype and showing K211E mutation as reported earlier showing the expansion and establishment of this sub-clade since 2010. Further, several mutations were seen in selected samples which resulted in amino acid changes in the protein.

Conclusion: This study has thrown insights to the trend of Chikungunya spread in India. Whole genome sequencing of the isolates from the two states will provide important information with respect to the virulence of the virus over the years.

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Identification of surface glycoprotein as novel attachment factor for EV71 early infection by glycoproteomic approaches

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Background: *Enterovirus* 71 (EV71) is a positive-stranded RNA virus, belong to genus *Enterovirus* within the family *Picornaviridae*. It is one of the common causative agent of hand-foot-and-mouth disease (HFMD) and causes severe neurologic diseases such as central never system (CNS) injury in infants. Although two EV71 receptors SCARB2 and PSGL-1 and several cell surface molecules have been indicated facilitate the process of virus attachment during the past few years, the mechanisms of EV71 early infection remain unclear. Therefore, to discover the new factors which mediated the recognition or entry of EV71 should be an important issue for evaluating the mechanisms of EV71 infection. Our previous report demonstrated that sialylated surface proteins influence the binding of EV71 to host cells. In this study, we applied the glycoproteomic approaches to identify EV71-associated glycoproteins that may involve in the early phase of EV71 infection. We also evaluated and verified the functions of candidate protein in EV71 virus attachment.

Methods & Materials: Sialylated glycoproteins were purified by Glycan chromatography from the cell membrane extraction of RD cells. The isolated glycoproteins were precipitated with EV71 viral particles, and identification by LC MS/MS analysis. The functions of candidate protein were evaluated by virus binding assay and infection assay.

Results: According to our results, we suggest that the candidate protein participates in EV71 virus attachment. EV71 binding and virus entry markedly reduced after si-RNA silencing. According to our results, we suggest this candidate protein was a potential receptor for EV71 infection.

Conclusion: We successfully apply new glycoproteomic methodology to identify the EV71-associated glycoproteins and indicated the candidate glycoprotein which facilitate the early phase of virus infection. Our finding also contribute to understanding the mechanisms of EV71 early infection and the therapeutic drug development.

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