This study describes the prevalence and distribution of Hepatitis C virus (HCV) genotypes in Myanmar. A total of 154 anti-HCV antibody-positive serum samples were collected from four different border cities of Myanmar. The overall prevalence of HCV infection in general was 11.6% (154/1333) and regionally, it was 13.5% (47/349), 12.8% (64/501), 4.2% (16/380) and 26.2% (27/103) in northeastern, northwestern, southern and western city, respectively. HCV was genotyped in 145/154 (94.2%) samples by reverse transcriptase polymerase chain reaction, direct DNA sequencing and phylogenetic analysis on the partial core genome. Genotype 6 was the most prevalent genotype in this study [71/145 (49%)], followed by genotype 3 [57/145 (39.3%)], genotype 1 [16/145 (11%)], and genotype 2 [1/145 (0.7%)]. Genotype 6 was mostly found in the northern cities and genotype 3 in the southern and western cities of Myanmar. We successfully characterized multiple HCV genotypes/subtypes as 1a, 1b, 2a, 3a, 3b, 6m, 6n, and a new 6 subtype. Among them, subtype 6n was the most predominant subtype (38.6%), followed by subtype 3b (29.7%), 3a (9.6%), 6m (9%), 1b (6.9%), 1a (4.1%), new 6 subtype (1.4%) and 2a (0.7%). Subtype 6n was more widely distributed in the northern cities whereas subtype 3b in the western city. The newly discovered genotype 6 subtype was from the northern cities. Our study revealed the regional differences of HCV genotype distribution in Myanmar and the distinct geographic variation with other Southeast Asian countries in terms of the existence of the new genotype 6 subtype.