



## IL28B GENETIC VARIATION ASSOCIATION WITH SPONTANEOUS CLEARANCE OF HEPATITIS C VIRUS IN WOMEN AND CHILDREN

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### INTRODUCTION

Despite the rapid development of theoretical and clinical medicine in the twenty-first century, nevertheless, a number of unresolved and problematic diseases remain. One of the most acute and global problem for modern medicine is hepatitis C. Despite significant changes in the pathogenic landscape of the viral society of the earth, in terms of incidence, hospitalization and disability, hepatitis C is still the leader among other diseases of public health importance [6]. There are currently more than 170 million people infected with Hepatitis C in the World, which accounts for more than 3% of the total civilian population [4]. Globally, an estimated 71 million people have chronic hepatitis C virus infection. Antiviral medicines can cure more than 95% of persons with hepatitis C infection, thereby reducing the risk of death from cirrhosis and liver cancer, but access to diagnosis and treatment is low. [14]

Georgia belongs to high prevalence countries of HCV. According to the latest epidemiological research conducted in the country, 7.7% of the country's population is positive for hepatitis C antibodies. [8]

In industrialized countries, in children, HCV is the most common cause of chronic liver disease. The HCV is transmitted from mother to infant in 1 from 6 cases. Infections can occur through vertical transmission both during vaginal delivery and cesarean section. Most infected newborns, 8-9 cases from 10, has persistent form of virus, which is accompanied by an entire set of health problems. The number of new cases of HCV infection in Georgian among children and women of reproductive age increases annually. More than 200 cases of HCV have been reported in children annually, according to the data by the National Center for Diseases Control and Public Health of Georgia (NCDC).

The majority (70%–80%) of HCV infections persist and about 30% of HCV infected patients can clear the virus spontaneously after acute infection and avoid the progression to chronic hepatitis C, which can develop into cirrhosis and hepatocellular carcinoma [10, 11]. Several epidemiological, viral and host factors have been associated with the differences in HCV clearance or persistence and studies have demonstrated that a strong host immune response against HCV favors viral clearance [2].

Host genetics is assumed to influence the outcomes of HCV infection, in both the abilities to achieve sustained viral response (SVR) after antiviral treatment and spontaneous clearance. SVR was more frequently observed in patients of European ancestry than those of African ancestry [3]. This ethnic difference was also found in spontaneous clearance of HCV [13], even across individuals infected by the same strain of HCV [5].

Accumulating studies have assured that two single nucleotide polymorphisms (SNPs) around interleukin-28B (IL28B) locus, rs12979860C/T and rs8099917T/G, have the strongest association with the spontaneous clearance of HCV infection. [12] Analysis performed in Caucasian populations indicated that rs12979860CC and rs8099917TT contributed to HCV spontaneous clearance. Patients

who were homozygous for the C allele at rs12979860 locus were nearly four times more likely to clear HCV spontaneously. This effect was negatively correlated with male proportion and age, female gender was a protective factor for HCV viral control. Young age (<40 years) was reported to be one of the protective factors of HCV viral control [7]. The mechanism explaining the association of IL28B gene and spontaneous HCV clearance remains unclear.

The aim of this study was to assess the immunogenetic factors which influence the spontaneous clearance or Chronic of HCV among infected youngsters and pregnant population.

### MATERIAL AND METHODS:

The Analysis of anti-HCV antibodies and HCV RNA have been performed to determine which form of HCV infection we were dealing with - spontaneously cured form or chronic infection. A special questionnaire has been developed to evaluate co-variables corresponding to the well-defined risk-factors.

Study subjects were recruited from the retrospective case-control study of HCV infection in pregnant and pediatric populations and the risk factors affecting the clinical course of HCV infection. The study population consisted of women from Tbilisi maternity hospitals who met the following criteria: spoke Georgian or Russian, and agreed to participate in the study after discussing informed consent and answering questions.

The study involved children from different pediatric clinics and from different regions of Georgia. The blood samples were collected at clinical settings or alternatively at their household places. The screening data on hepatitis C antibodies status were available from the HCV elimination state program databases of Georgia.

Data entry, verification, management and statistical analysis of the data obtained through the questionnaire were carried out by statistical package SPSS version 20. The  $\chi^2$  (Chi-square) test or the Fisher's exact test was used to determine the statistical significance of the associations between qualitative factors.

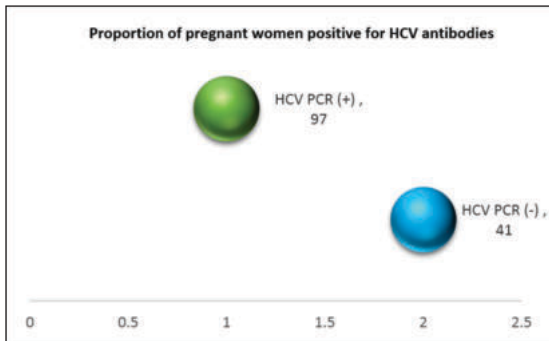
Prior to the study, the informed consent forms approved by the Institutional Review Board of the relevant institutions were obtained from the study subjects or from the parent / guardian.

We studied the polymorphism of the interleukin 28 (IL28) gene (namely rs12979860 gene locus) by real-time polymerase chain reaction (SACACE Biotechnologies IL28B rs17 / rs0 Real-TM PCR Kit, Como, Italy). This real-time PCR test allowed us to distinguish the following genetic variants: homozygous CC, TT, and heterozygous CT. At the data analysis stage, two groups of CC and non-CC were formed to group the functional variants, since the variant of protection against infection is predominantly the non-CC variant and it was important to compare it together with all other variants.

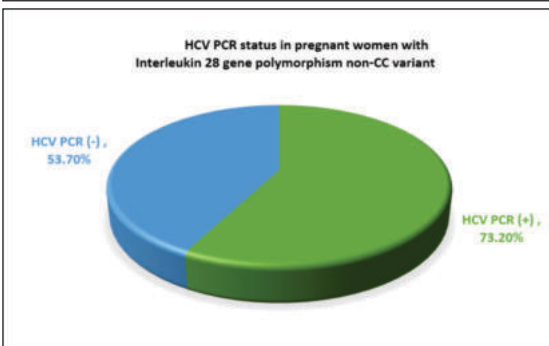
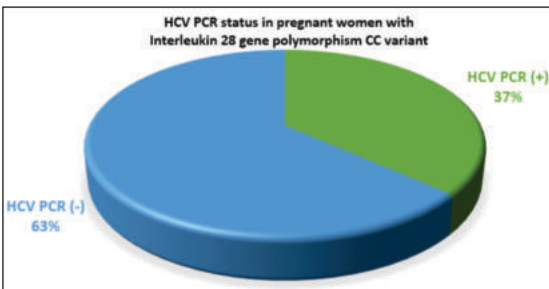
### Results and their discussion.

In our study the total number of pregnant women positive for HCV

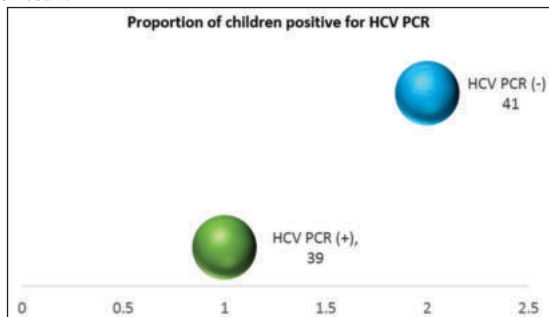
antibodies were 138. The study has shown that 97 (70%) of the 138 pregnant women were positive for hepatitis C virus and 41 pregnant women (30%) were HCV PCR negative. Of the HCV PCR positive pregnant women (total 97), 26 (26.80%) were diagnosed with the CC variant of the IL 28 gene; Of the HCV PCR-negative pregnant women (total number 41), the CC variant of the IL 28 gene was detected in almost half - 19 pregnant women (46.30%). The non-CC variant of the IL28 gene was detected in 73.20% of HCV PCR pregnancies (71 pregnant women) and 53.70% of pregnancies (22 pregnant women) with this variant were HCV PCR negative. There was a statistically significant difference between the occurrence of the IL 28 gene CC variant in Anti-HCV positive vs. negative pregnant women at  $p = 0.03$ , the odds ratio  $OR = 2.34$  and the 95% confidence interval (95% CI) was [1.08; 5.06].



Statistical analysis of the results obtained by studying the prevalence of IL 28 gene non-CC variant in HCV PCR positive and negative pregnant women revealed statistically significant difference at  $p = 0.03$ , the confidence interval was 95% CI [1.08; 5.06]; A study of the odds ratio even showed that  $OR = 2.34$



In our study the total number of children positive for HCV antibodies were 86. It was found that 39 of them (45.30%) had positive HCV PCR test result, while the remaining 41 (54.70%) had negative HCV PCR test result.



Of the HCV PCR (+) children, the CC variant of the IL 28 gene was identified in 19 (48.70%); Of the HCV PCR (-) children, the CC variant of the interleukin 28 gene was identified in 13 (27.70%). Bivariate analysis showed that  $p = 0.04$ , the 95% confidence interval was [1,01; 6,17] and the mean odds ratio (OR) was 2.46.

**CONCLUSION**

Based on the study, for the first time in Georgia, the immunogenetic factor of clearance and chronicity of hepatitis C infection in children and pregnant population were studied and the importance of interleukin IL28B polymorphism in the clinical course and outcome of HCV infection was documented.

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**REFERENCES**

- Balistreri, J. E. (2017). Hepatitis C virus infection in children and adolescents. *Hepatology Communications*.
- Cooper S, et al. Analysis of a successful immune response against hepatitis C virus. *Immunity*. 1999;10(4):439-449 [6] Rehermann B, Nascimbene M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol*. 2005;5 (3):215-229.
- Conjeevaram, H.S., et al., 2006. Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. *Gastroenterology* 131,470-477.
- Davila, J. A., Morgan, R. O., Shaib, Y., McGlynn, K. A., & El-Serag, H. B. (2004). Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. *Gastroenterology*, 127(5), 1372-1380.
- Kenny-Walsh, E., 1999. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. *Irish Hepatology Research Group*. *N. Engl. J. Med.* 340, 1228-1233.
- Lee, M. H. (2014). Epidemiology and natural history of hepatitis C virus infection. *World J Gastroenterol*, 20(28).
- Ming-Hua Zheng, Yu Li, Dong-Dong Xiao, Ke-Qing Shi, Yu-Chen Fan, Li-Li Chen, Wen-Yue Liu, Ying-Wan Luo, Yong-Ping Chen 2012. Interleukin-28B rs12979860C/T and rs8099917T/G contribute to spontaneous clearance of hepatitis C virus in Caucasians: a meta-analysis. *Gene* 518, 479-482.
- NCDC official information; <http://www.ncdc.ge/Handlers/GetFile.ashx?ID=f7a28a1e-0489-49a0-b183-eb8674244541>.
- Pratima Dibba, Rosann Cholankeril, Andrew A Li, Meera Patel, Mariam Fayek, Christy Dibble Nnenna Okpara, Autumn Hines, Aijaz Ahmed. 2018 Apr 27 Hepatitis C in pregnancy.
- Shepard, C.W., Finelli, L., Alter, M.J., 2005. Global epidemiology of hepatitis C virus infection. *Lancet Infect. Dis.* 5, 558-567.
- Seeff LB. Natural history of chronic hepatitis C. *Hepatology*. 2002;36(5 Suppl 1):S35-46.
- Shi, K.Q., Liu, W.Y., Lin, X.F., Fan, Y.C., Chen, Y.P., Zheng, M.H., 2012. Interleukin-28B polymorphisms on the SVR in the treatment of naive chronic hepatitis C with pegylated interferon-alpha plus ribavirin: a meta-analysis. *Gene* 507,27-35.
- Thomas, D.L., et al., 2009. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 461, 798-801.
- WHO, Epidemiology of Hepatitis C, 27 July 2020.