بسم الله الرحمن الرحيم

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A study of the individualized immune response of expressed hepatitis C virus antigens in peripheral blood mononuclear cells from healthy blood donors

A thesis submitted for the degree of Doctor of Philosophy in Science in Microbiology

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بسم الله الرحمن الرحيم

Dedication

To Allah;

My savior, sustainer and the All Merciful

Papy;

Who left a part of himself alive in every word I speak and every gesture I make,

who paved a road of kindness to keep us safe wherever we go.

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ABSTRACT

Background and objectives. Hepatitis C virus (HCV) infection is a universal health threat, and Egypt is among the countries with a high prevalence of infection. the importance of vaccine development was emphasized due to the reported risks associated with direct-acting antivirals, the viral drug resistance, challenges of reinfection or relapses, and the drug cost burden, especially in developing countries. In the present study, we focus on developing an HCV envelope protein-based DNA vaccine tailored to mimic the spontaneous clearance event responses. The vaccine candidate was designed to elicit cellular and humoral responses. Also focuses on its *ex vivo* comparative evaluation in human cell populations from different individuals and *in vivo* evaluation in individual mice.

Materials and Methods. HCV E1/E2 DNA construct (EC) was designed based on the most immunogenic epitopes of E1 position 313-327 and an E2 stretch including the E2 neutralizing face of positions 412-538. Our study workflow followed four consecutive levels; *in silico, in vitro, ex vivo,* and *in vivo*. The *in silico* studies were conducted to confirm the broadness of the antibodies' neutralization effect among different HCV genotypes (Gt). The study was established on the E1-epitope and the E2 sequence stretch using 1095 and 1003 sequences, respectively representing the 7 HCV Gts known till today. Also, the expected cellular response in different population ethnicities to the designed vaccine candidate was analyzed. The *ex vivo* studies included testing the E1/E2 epitopes expression level was tested in PBMCs of five HCV-uninfected healthy donors transfected with the EC via real-time qPCR. Serum samples from twenty HCV antibody-positive patients were used to detect each individual PBMCs expressed antigens via ELISA. The *in vivo* studies included the immunization of two groups, five Swiss albino mice each, with either the EC or a control construct. The absolute numbers of lymph nodes' $CD4^+$ and $CD8^+$ T-lymphocytes were assessed.

Abstract

Results. The selected E1-epitope conservation level exceeded 90% of the residues except for the Gt7 with 85% conservancy. This reflects high conservation and a higher degree of broad neutralization with Gt1 through Gt6. The E2-stretch showed a range of conservancy between 81.39-86.11% with the other genotypes except for Gt4 where it reached 90.25%. The worldwide individuals predicted to respond to the epitopes used as a vaccine candidate reached 91.83% of the individual presented by the used database. While the coverage in Afrocentric ethnicity ranged from 76.99-to 90.16% of the individuals in East, West, Central, and North Africa. While South Africa showed a lower coverage of 34.16% only. Donors' PBMCs showed different levels of EC expression, using D5 as a ground value of 1; expression levels ranged between 0.83-2.61-fold in four donors, while donor-3 showed 34.53-fold expression. The antigens expressed in PBMCs were significantly reactive to the twenty HCV antibody repertoire (all p=.0001). All showed slightly different reactivity except for donor-3 who showed the lowest reactivity level. There was an increase in the $CD4^+$ T-cell % in four of the five EC immunized mice compared to the control group (p=.03). No significant difference in CD8+ T-cells % was observed (p=.89).

Conclusion. We can conclude from this study that the described HCV vaccine candidate might result in a natural humoral response that is developed by natural infection. Also, it can elicit an early $CD4^+$ T-cell response, which is the desired response to mimic the spontaneous clearance events that will prime $CD8^+$ T-cell development. On the other side, we conclude that immune response differences might be related to individual differences in antigen processing and presentation. Also, the individual antigen expression and presentation might be independent of the antigenic reactivity levels. This stresses the importance of extending the individual-wise research in vaccine approach evaluation.

Keywords. HCV, PBMCs, DNA vaccine, envelope 1, envelope 2, $CD4^+$ T-cell, $CD8^+$ T-cell, Individualized response, T-lymphocyte.

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