Identification of HCV core regions responsible for false positive reactions

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Abstract

Introduction and purpose: Hepatitis C virus (HCV) is a hepatotropic positive-strand RNA virus from the Flaviviridae family. Core protein of hepatitis C virus is one of the major immunoreactive proteins of the virus. Core antigen, as a recombinant antigen or peptide/s, is used in diagnostic tests for detection of antibodies to HCV. False positive results complicate the diagnosis of hepatitis C. The aim of our work was the detection of core regions responsible for false positive reactions.

Materials and methods: A set of 58 overlapping 15mer peptides spanning HCV core antigen (1 - 129 aa) genotype 1b was synthesized and tested for potential antigenic epitope mapping. 60 well known blood serum samples (33 anti-HCV positive and 27 anti-HCV false positive) were investigated by peptides based ELISA.

Results: At least two strong antigenic epitopes were located in positions 11- 41 and 47 - 71 aa. The most reactive sequence (comprising 17 to 31 aa) reacted with the main part of anti HCV core positive specimens (26 out of 33). Peptide corresponding to 3-17aa was the most reactive with false positive samples (16 out of 27). The majority of false positive reactions (18 out of 27) focused in region 1-23aa with weak reactivity in epitope located in 58-83aa region (6 out of 27).

Conclusions: Two strong antigenic epitopes were identified within the HCV core protein at amino acids positions 11 - 41 and 47 - 71. The majority of false positive reactions were associated with HCV core regions 1-23aa and 53-83aa.

Introduction and purpose

Situation with hepatitis C in the world remains complicated.The World Health Organization estimates that between 130and 150 million people are chronically infected with hepatitis C (HCV) virus worldwide. The accuracy of diagnosis of HCV is of paramount importance because a misdiagnosis can lead to psychological trauma, to the spread of HCV, increase of the diagnosis cost.

Hepatitis C virus (HCV) is a hepatotropic positive-strand RNA virus from the Flaviviridae family. Core protein of hepatitis C virus is one of the major immunoreactive proteins of the virus. Core antigen, as a recombinant antigen or peptide/s, is used in diagnostic tests for detection of antibodies to HCV. There are some problems with false positive reactivity among testing persons. Cross-reacting with antibodies against other viruses and pathogens may be the cause of false positive reactivity. The aim of our work was the detection of core regions responsible for false positive reactions.

Materials and Methods

Core antigen 1-129 aa 1b genotype was used as a model A set of 58 synthetic peptides was synthesized and applied for potential antigenic epitope mapping (Fig.1, Table 1) The peptides were 15 aa long with 2 aa shift. The activity of peptides was assessed by indirect ELISA. Microwells plates were coated with peptides. Detection of anti-HCV core reactivity was carried out with mouse anti-human IgG purified by affinity chromatography and conjugated to horse-radish peroxidase.

60 well known blood serum samples (33 anti - HCV positiveand 27 anti-HCV false positive) were investigated in an experiment.

Hepatitis C virus core polyprotein (subtype 1b)

1 MSTNPKPQRK TKRNTNRRPQ DVKFPGGGQI VGGVYLLPRR GPRLGVRATR KTSERSQPRG

61 RRQPIPKARQ PEGRAWAQPG YPWPLYGNEG MGWAGWLLSP RGSRPNWGPS

111 DPRRRSRNLG KVIDTLTCG

Fig.1

Results

At least two strong antigenic epitopes were located in positions 11-41 and 47-71 aa. 29 HCV positive serum samples were reactive with 11-41aa core region, and 3 samples – with 47-71 aa region. The one HCV positive sample was reactive with 1-23 aa region only. The most reactive sequence (comprising 17 to 31 aa) reacted with the main part of anti HCV core positive specimens (26 out of 33). Peptide corresponding to 3-17aa was the most reactive with false positive samples (16 out of 27). The majority of false positive reactions (18 out of 27) focused in region 1 - 23aa with weak reactivity in epitope located in 58-83aa region (6 out of 27). 15 HCV positive samples were reactive in 1 - 23aa core region with different power, but 14 samples out 15 were also reactive with 11-41aa core region.

Data are represented in Fig. 2 and 3.

Conclusion

Two strong antigenic epitopes were identified within the HCV core protein at amino acids positions 11-41 and 47-71. The majority of false positive reactions were associated with HCV core regions 1-23aa and 53-83aa.

False positive reactivity can be caused by a crosshair between common human pathogens and HCV core sequence, that is confirmed by studies of other researchers. This fact should be considered when choosing the sequence of the protein.

Table 1

# peptide	location aa	# peptide	location aa	# peptide	location aa	# peptide	location aa
1	1 - 15	16	31 - 45	31	61 - 75	46	91 - 105
2	3 - 17	17	33 - 47	32	63 - 77	47	93 - 107
3	5 - 19	18	35 - 49	33	65 - 80	48	95 - 109
4	7 - 21	19	37 - 51	34	67 - 81	49	97 - 111
5	9 - 23	20	39 - 53	35	69 - 83	50	99 - 113
6	11 - 25	21	41 - 55	36	71 - 85	51	101 - 115
7	13 - 27	22	43 - 57	37	73 - 87	52	103 - 117
8	15 - 29	23	45 - 59	38	75 - 89	53	105 - 119
9	17 - 31	24	47 - 61	39	77 - 91	54	107 - 121
10	19 - 33	25	49 - 63	40	79 - 93	55	109 - 123
11	21 - 35	26	51 - 65	41	81 - 95	56	111 - 125
12	23 - 37	27	53 - 67	42	83 - 97	57	113 - 127
13	25 - 39	28	55 - 69	43	85 - 99	58	115 - 129
14	27 - 41	29	57 - 71	44	87 - 101		
15	29 - 43	30	59 - 73	45	89 - 103		

Number of reactive serum samples

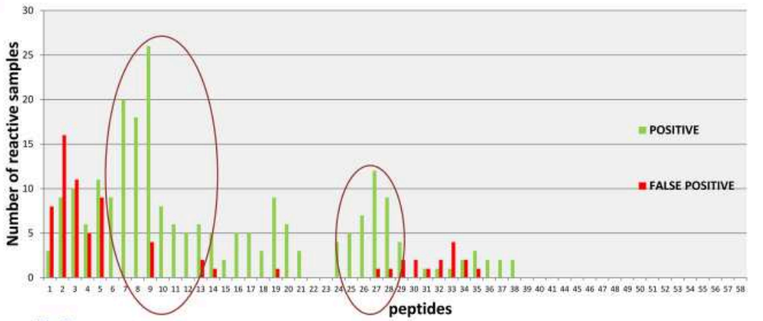

Fig.2

Distribution of average values of optical densities of positive and false positive anti-HCV samples

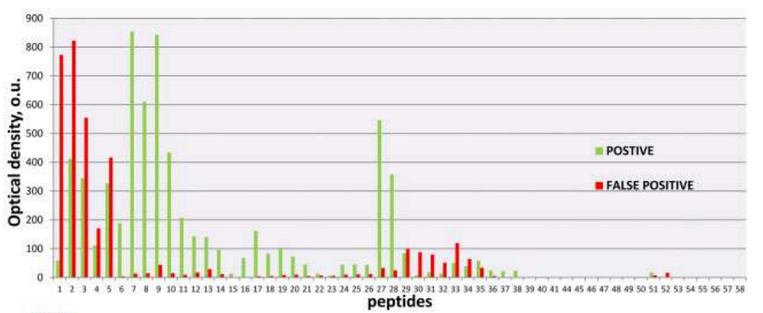

Fig.3