Expression of Hepatitis C Virus Core and E2 antigenic recombinant proteins and their use for development of diagnostic assays

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A B S T R A C T

Background: Early diagnosis of HCV infection is based on detection of antibodies against HCV proteins using recombinant viral antigens. The present study was designed to select, clone and express the antigenic regions of Core and E2 genes from local HCV-3a genotype and to utilize the antigenic recombinant proteins (Core & E2) to develop highly sensitive, specific and economical diagnostic assays for detection of HCV infection.

Methods: The antigenic sites were determined within Core and E2 genes and were then cloned in PET-28a expression vector. The right orientation of the desired inserted fragments of Core and E2 were confirmed via sequencing prior to expression and were then transformed in BL21 (DE3) plys5 strains of E. coli and induced with 0.5 mM Isopropyl-b-D-thiogalactopyranoside (IPTG) for the production of antigenic recombinant proteins. The produced truncated antigens were then purified by Nickel affinity chromatography and were confirmed by western blotting, immunoblotting and enzyme-linked immunosorbent assay (ELISA).

Results: The expressed Core and E2 recombinant antigens were used to develop immunoblotting assay for the detection of anti-HCV antibodies in sera. With immunoblotting, a total of 93-HCV infected sera and 35-HCV negative individuals were tested for the presence of anti-HCV antibodies to the Core and E2 antigens. Recombinant antigen showed 100% reactivity against HCV infected sera, with no cross reactivity against HCV-negative sera. The immunoblot assay mixture of recombinant antigens (Core+E2) showed a strong reaction intensity in the test area (TA) as compared to the individual truncated Core and E2 recombinant antigens. In the in-house ELISA assay, mixed Core and E2 recombinant antigens showed 100% reactivity against a standardized panel of 150-HCV-positive sera and non-reactivity against a standardized panel of 150 HCV-negative sera while also being non reactive to sera positive for other viral infections. The antigenic recombinant antigens also were tested for the 30 sera of known genotypes. The antigens did not detect antibodies to genotype-3a, but detected antibodies to all genotypes and did not discriminate them genotype wise. A panel of 175 of HCV-suspected serum samples was subjected to comparative analysis with our in-house ELISA assay and with commercial HCV screening assays. After subjecting the results to the formulas for determining the quality parameters, immunoblot assay had 100% sensitivity and specificity, while the ELISA assay had 100% sensitivity and 98.8% specificity as compared to commercially available assays.

Conclusion: This study indicates that a mixture of Core and E2 antigens are potentially valuable antigens and there is the possibility of developing serological assays for monitoring HCV infection.

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1. Introduction

Hepatitis C virus (HCV) may be called a silent killer virus because most HCV-infected people are unaware of the HCV presence in their bodies, even decade after infection. Globally more than 170 million subjects are infected with HCV, often via contact with contaminated blood. HCV is small single stranded RNA (+) virus classified in the genus *Hepacivirus* within the *Flaviviridae* family. Amongst HCV proteins, Core antigen, besides its significance for diagnostic application, is an important target in the efforts to develop preventive and therapeutic strategies against this pathogen. HCV Core antigen is highly antigenic, induces cellular and humoral specific responses and most probably plays a key role in the pathogenesis of HCV viral infection. Several studies confirm that one of the main components of routine kits for recent detection of HCV infection is Core antigen.

The first identified conformational protein was the envelope E2 antigen, which might have played an important role in immuno-reactivity in HCV-infected patients. E2 antigen is considered a prime candidate for anti-HCV vaccine. In one study anti-E2 antibodies were seen in >90% of HCV RNA positive individuals using E2 antigen, and the E2 protein might be useful for monitoring of HCV infection.

Several efforts have been made to develop efficient serological tests for the screening of HCV infection. The ELISA assay or immunoblot assays uses HCV recombinant viral antigens corresponding to multiple polypeptides from various viral fragments, including structural proteins and non-structural proteins. These serological screening assays combine improved sensitivity and specificity, however still fail to diagnose HCV infection more accurately.

Countrywide, no serological screening tests are available for HCV, and only imported kits are used for the diagnosis of HCV. However these imported kits are unable to screen the local population for HCV infection accurately. Moreover, Pakistan is a developing country and there is a financial burden associated with importing the HCV screening kits. Therefore, the purpose of our study was to develop highly sensitive, inexpensive and simple serological assays for the screening of HCV infection utilizing a mixture of recombinant (Core & E2) antigens to detect the presence of HCV antibodies in serum specimens. To the best of our knowledge this is the first report from Pakistan in which the mixture of Core and E2 antigens of HCV-3a was used for the in-house screening of HCV infection.

2. Methodology

2.1. Amplification and sequencing of Core and E2 genes of HCV-3a

Chromically HCV infected serum samples with genotype-3a were kindly provided by the Division of Molecular Virology and Molecular Diagnostics, National Centre of Excellence in Molecular Biology (CEMB), Lahore, Pakistan. The HCV RNA was isolated from genotype-3a using a RNA extraction kit (Genta, life technologies, USA) according to kit protocol. The viral RNA was reverse transcribed to cDNA using 100 U of M-MLV RTEs at temperature of 37 °C for 50 min. cDNA was utilized as a template for the amplification of full length core and E2 genes using gene specific primers (Table 1). Primers were designed using Primer3 software by Gu2944841.1 as a reference sequence. Amplified products of Core and E2 genes were sequenced using an automated gene sequencer (Applied Biosystems), and sequences of the Core (accession number KC796013) and E2 (accession number KC796015) genes were submitted to the gene bank.

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence</th>
<th>Amplified gene product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core-F</td>
<td>5’-ATGACACACTCTTCAAAACCTCA-3’</td>
<td>573-bp</td>
</tr>
<tr>
<td>Core-R</td>
<td>5’-TGGCTCT CCTGGATGTAATGCC-3’</td>
<td>573-bp</td>
</tr>
<tr>
<td>E2-F</td>
<td>5’-CACACATACACCCGTG-3’</td>
<td>1056-bp</td>
</tr>
<tr>
<td>E2-R</td>
<td>5’-CTGCGACAGGAGGAGGA-3’</td>
<td>1056-bp</td>
</tr>
</tbody>
</table>

2.2. Selection and amplification of antigenic sites of Core and E2 genes

Antigenic sites (truncated regions) were selected within the Core and E2 genes of HCV-3a using software (http://www.bioinformatics.org/jaMBW/3[1]/7/). The antigenic nucleotide sequences of Core (nt-336) and E2 (nt-342) genes were amplified using forward primers containing HindIII restriction sites, while the reverse primers contained XhoI restriction site (Table 2).

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequences</th>
<th>Restriction site</th>
<th>Amplified product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core-F</td>
<td>5’-AAGCTGCCACCATGACACACTCTTCAAAACCTCA-3’</td>
<td>HindIII</td>
<td>336-bp</td>
</tr>
<tr>
<td>Core-R</td>
<td>5’-CTGCTCTGGATGTAATGCC-3’</td>
<td>XhoI</td>
<td>342-bp</td>
</tr>
<tr>
<td>E2-F</td>
<td>5’-AAGCTGCCACCATGACACACTCTTCAAAACCTCA-3’</td>
<td>HindIII</td>
<td>336-bp</td>
</tr>
<tr>
<td>E2-R</td>
<td>5’-CTGCGACAGGAGGAGGA-3’</td>
<td>XhoI</td>
<td>342-bp</td>
</tr>
</tbody>
</table>

2.3. Construction of expression plasmids and bacterial transformation

The amplified products of truncated Core and truncated E2 were treated with HindIII and XhoI restriction enzymes and pET-28a vector as well. The digested Core and E2 products were cloned into digested pET-28a vector to construct the expression plasmids. The pET-28a vectors harbouring the truncated Core insert and truncated E2 insert were digested with restriction enzymes (HindIII & XhoI). The right orientation of the desired fragments was confirmed by DNA sequencing. Transformation was carried out using competent BL21 DE3 pLysS), bacterial cells and 1 μl of isolated plasmid of pET-28a harbouring antigenic Core and E2, and then heat shocking for 90 second at 42 °C. LB medium of 500 μl free of antibiotics was added and incubated for 1 h at 37 °C. Subsequently about 150 μl transformants of truncated Core and E2 was spread on LB-agar plates containing kanamycin (25 mg/ml) and chloramphenicol (25 mg/ml) and incubated overnight (O/N).

2.4. Expression and purification of Core and E2 antigens

BL21 (DE3) pLysS transformed colonies were picked using a sterile loop, immersed into a tube containing kanamycin (25 mg/ml) and chloramphenicol (25 mg/ml) antibiotic LB media, and then incubated O/N at 37 °C for 200 rpm. The O/N culture was further diluted with 1:50 dilution in sterile LB medium containing antibiotics, and the inoculums were allowed to grow in autoclaved 1L flasks, incubated for 250 rpm at 37 °C to attain the optical
density (OD600 = 0.6). 0.5 mM IPTG was added to the culture for 3 h at 37°C for the maximum production of truncated Core and E2 antigens. The IPTG induced culture was then centrifuged (Beckman USA) at 600 rpm for 15 min at 40°C. The pellet was re-suspended in 1X PBS, sonicated (Mixonson USA) four times in an ice bath for 30 seconds with 4 pulses, and centrifuged at 13,000 rpm for 15 min at 4°C, and then the supernatant was shifted into a fresh tube. Recombinant proteins were then purified with Ni-NiTA column containing Ni2+-nitrioloacetate agarose resin (Invetrogen, USA). Protein supernatants was poured into the column and allowed to run. After the sample was completely poured into column, the column was washed with washing buffer (1X PBS and 40 mM imidazole, pH 8). The conjugated His-Core and His-E2 truncated proteins were then eluted from the column by adding elution buffer (1X PBS and 250 mM imidazole, pH 8). The purified protein was quantified using Bradford assay (Bio-rad, USA).

2.5. SDS-PAGE and western blot analysis of recombinant antigens

The recombinant truncated Core and E2 proteins were electrophoretically separated on 12% SDS-PAGE and dyed with Coomassie blue. For western blotting analysis the protein was transferred from gel and then shifted to nitrocellulose membrane using a semi-dry blotting apparatus (Biorad, USA) for 90 min at 16 V. The membranes were blocked with 5% blocking buffer for 1:30 h at room temperature (RT) and washed with PBST and then incubated with mouse anti-His6 monoclonal antibodies at 4°C O/N. The membranes were washed and then incubated with Alkaline Phosphatase (AP) conjugated anti-mouse secondary antibody IgG diluted for 1 h at RT followed by washes with PBST, and then the membrane was treated with NBT/BCIP substrate for 15 min at 37°C. The membrane was dried and then photographed.

2.6. Detection of anti-HCV antibodies in human sera by immunoblotting

The nitrocellulose membrane was cut into strips, and we applied 1.6 µl of truncated Core and E2 recombinant antigens at concentrations of 24 µg/µl and 28 µg/µl in the test area (TA), respectively. Subsequently, 1.6 µl of the truncated Core & E2 antigens and a mixture with same concentrations was applied in the TA and 1.5 µl of extracellular recombinant protein A (Sigma USA) at the concentration of 6 µg/µl was applied in the control area (CA) and allowed to dry for 1 h at RT and then was blocked for 1 h at RT using blocking buffer. The strips were washed two times with PBST and then incubated for 2 h with HCV infected sera diluted 1:50 in PBST at 4°C. Strips were washed and then incubated for 1 h with horseradish peroxidase (HRP) conjugated rabbit anti-human IgG antibodies diluted 1/1000 in PBST and washed with PBST again. For the recognition of the bound anti-human IgG-HRP, 4-chloro-1-naphthol and H2O2 solution was utilized as a substrate. The strips were then dried and photographed. A similar procedure was followed for the HCV negative sera.

2.7. Detection of antibodies to HCV in serum samples by ELISA

The immunoreactivity of the mixture of Core and E2 recombinant proteins with antibodies circulating in the HCV positive sera was observed with microtiter immune plates coated with 100 µl of the mixed (Core & E2) antigens, at 0.3 µg/ml concentration per well. The mixture of (Core+E2) antigens was adsorbed on to the micro ELISA plates for O/N at 4°C. Wells were blocked following washing with PBST. We then added 100 µl (diluted 1/50) of serum samples positive & negative for HCV and for other viral diseases, i.e. HBV, HEV and dengue virus, to each well of the plate and then incubated the plate at 37°C for 1:30 h. In brief 100 µl of the mixture (Core+E2) of recombinant antigens was added to each well of the plate and incubated O/N at 4°C. The wells were blocked with blocking buffer and then washed with PBST. After this, 100 µl of each serum sample of HCV known genotypes (1a, 1b, 2a, 3a & 3b) was added per well of the plate and incubated at 37°C for 1:30 h. The plates were washed and then incubated with 100 µl rabbit anti-human IgG conjugated to HRP at 37°C for 30 min. The ultimate immune complexes were seen by adding 100 µl TM substrate solution, the wells were blocked, and then the absorbance at 450 nm of each well was read using an ELISA reader (Bitec, USA). To distinguish between positive and negative HCV sera the cutoff value was calculated as the mean value of the OD of HCV 150 negative samples plus 2 Standard Deviations (SD) equal to 0.09. According on this calculated cut-off value the findings of anti-HCV screening assay were calculated as described earlier.16

2.8. Data analysis

The calculations of sensitivity, specificity, positive predictive, and negative predictive values were carried out by applying the formulas.17 Receiver operating curves (ROC) were generated, area under the curve (AUC) and its standard errors & 95% confidence intervals were calculated using SPSS-17 version.

3. Results

3.1. PCR amplification and restriction digestion of full and truncated Core and E2 genes

Full length and antigenic (truncated) Core and E2 genes of HCV-3a were amplified using each gene specific primer as described in methods and materials. The amplified gene products of full and truncated genes were confirmed by restriction digestion and sequencing.

3.2. Expression, western blotting and purification of Core & E2 antigens

Total cell lysates induced and uninduced from BI21 (DE3) pLyS3 were separated on a 12% SDS-PAGE and the expected sizes of truncated Core (12.3-kDa) and truncated E2 (12.5-kDa) antigens were seen. The purified proteins were checked using SDS polyacrylamide stained with silver staining to demonstrate the purity of purified antigens. The quantity of Core antigen was 1.5 mg and of E2 antigen was 2.2 mg respectively after purification. The antigenic recombinant proteins were further confirmed by western blotting for the identification of antigenic (Core & E2) recombinant products.

3.3. Immunoblot analysis of Core and E2 recombinant antigens

The immunoblotting assay was carried out for 128 serum samples, out of which 93 serum samples were confirmed positive for anti-HCV and RNA PCR and 35 samples were HCV negative. The 1.6 µl of truncated recombinant Core at a concentration of 24 µg/µl and E2 at a concentration of 28 µg/µl and a mixture of the truncated recombinant proteins (Core+E2) of the same concentrations were applied in test area (TA). The 1.5 µl of recombinant A at a concentration of 6 µg/µl was applied in control area (CA). There was no reaction in the CA area, only a mark in the control area was seen while treating with negative serum samples. The results of developed immunoblot tests and applying the results to formulas for determining the sensitivity, specificity, positive and negative predictive values were 100%. Immunoblotting tests can be seen in Fig. 1.
3.4. ELISA assay for the detection of anti-HCV antibodies against mixture of Core & E2 antigens

We evaluated the immunoreactivity of the mixture of Core and E2 antigenic recombinant proteins in the ELISA assay. For the validation of the ELISA assay a standardized panel was established by the Division of virology CEMB, and 150-HCV positive sera and 150-HCV sera were screened in the ELISA using the mixed recombinant antigens. All of the 150-HCV positive sera presented values above the cut-off value (0.09) and all of the 150 HCV negative sera presented values below the cut-off value. The 30 sera of known genotypes (5 with genotype-1a, 4 with genotype-1b, 4 with genotype-2a, 11 with genotype-3a, & 6 with genotype-3b) were received from CEMB diagnostic lab to confirm the antigen reactivity against infected sera of selected genotypes. All of the selected genotype sera presented values above the cut off value and showed reactivity regardless of genotype specificity. Furthermore patient sera positive for other viral infections including 7 sera positive for HBV, 7 sera positive for HIV and 7 sera positive for dengue virus were also tested, and presented values below the cut-off value (Fig. 2).

3.5. Validation of developed in house ELISA assay via HCV suspected sera

For the validation of ELISA analysis, 175 sera from patients suspected of HCV were randomly selected from almost all regions of Pakistan. Comparative analysis of suspected sera by the present study using four assays is summarized in Table 3. Out of a total 175 sera, 85 sera were positive by our diagnostic ELISA assays and PCR assays, and were used as a reference standard while one serum out of 85 was observed negative by the commercial anti-HCV Ag assay and two sera out of 85 positive sera were found negative by another commercial HCV CORE Ag assay, respectively. The remaining 90 sera were found negative for HCV by PCR assay, while in 90 of these negative samples, our assay confirmed 89 as negative and gave one false positive result, whereas commercial anti-HCV Ag and Core Ag assays gave 2 and 4 false positive results. It is confirmed that the in-house anti-HCV ELISA diagnostic assay is high sensitivity (100%) and specificity (98.8%) and is valuable for the diagnosis of anti-HCV antibodies in infected-sera, while the sensitivities and specificities of commercial CORE Ag and anti-HCV Ag assays were 97.6%, 98.8%, 95.5% & 97.7%, respectively in comparative analyses. The use of the mixture (Core+E2) of antigenic recombinant proteins is a novel method in the ELISA diagnostic method; countrywide the mixture of antigenic peptides (core+E2) were used for the first time for the screening of HCV infection and have shown high sensitivity and specificity as compared to commercial assays. The sensitivities, specificities, positive predictive values (PPV) and negative predictive values (NPV) of the in-house ELISA assay and commercial assays are shown in Table 4. The receiving operating curves (ROC) demonstrate the association between the true positive ratio (sensitivity) and false positive ratio (1-specificity). In the present study we used ROC to distinguish sensitivity and specificity for the in-house ELISA assay and commercial assays (Fig. 3) and to compare the areas

Figure 1. Detection of anti-Core, and anti-E2 antibodies in human sera by immunoblot analysis using the Core, E2 and mixture (Core & E2) of antigenic recombinant proteins at concentration of 24 µg/µl, and 28 µg/µl respectively. Nitrocellulose sheets, cut into strips and reacted with human sera. Strips 1-93 HCV-positive sera; row 1: Core antigen in test area (TA), 2: E2 antigen in TA, 3: mixture of Core & E2 antigens, 4: extracellular recombinant protein A (Sigma) in control area (CA) at a concentration of 6 µg/µl. Strips 94-128 HCV negative control serum only a mark was seen in CA.
under the curves (AUC) for the in-house ELISA assay and commercial assays. Areas under the curve and its Standard errors (SEs) & 95% confidence intervals are summarized in Table 5.

4. Discussion

The serological diagnosis of hepatitis C virus infection has been based on the identification of anti-HCV specific antibodies since 1989, when HCV was first cloned,\(^1\)\(^2\)\(^3\) and many immunoassays for the identification of anti-HCV antibodies in infected sera have been used.\(^1\)\(^2\) In this report we mapped the antigenic sites in Core and E2 genes of HCV-3a and cloned in pET-28a expression vector for the production of truncated Core and E2 recombinant antigens, which were then studied for their potential assessment as diagnostic antigens for the identification of anti-HCV antibodies in HCV infected sera. The production of truncated recombinant Core and E2 antigens was confirmed by western blotting, and their serological reactivity was examined by immunoblotting and ELISA.

Our truncated Core and E2 recombinant antigens were reactive against the HCV-positive sera, and non-reactive against HCV negative sera.

Several studies have indicated that a truncated Core protein is a major antigenic component in diagnostic ELISA kits and shows better reactivity,\(^1\)\(^8\) while other studies demonstrated that the full-length Core protein has better reactivity than truncated Core protein.\(^1\)\(^9\) However our expressed truncated Core antigen showed strong reactivity in 100% of PCR-positive samples and can act as a potential diagnosis tool for HCV infection. These results are markedly similar with the previous study in which the expressed HCV Core antigen showed 100% reactivity of PCR positive sera.\(^1\)\(^5\)

In one study the percentage of sera of HCV infected patients that reacted with E2 antigen was about 70%,\(^1\)\(^1\) which is lower than another previously published report that 90% of chronically HCV infected patients reacted with E2 protein.\(^1\)\(^0\) Our antigenic E2

<table>
<thead>
<tr>
<th>Patient sera</th>
<th>Mixed antigens(^a)</th>
<th>Anti-HCV Ag(^b)</th>
<th>HCV CORE Ag(^c)</th>
<th>PCR reference standard(^d)</th>
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</thead>
<tbody>
<tr>
<td>HCV true positive</td>
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<td>84</td>
<td>83</td>
<td>85</td>
</tr>
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<td>88</td>
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<tr>
<td>HCV false-negative</td>
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<tr>
<td>Total</td>
<td>175</td>
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</table>

\(^a\) Mixed antigens (C+E2) of HCV-3a were used in our in-house ELISA assay  
\(^b\) The commercial anti-HCV kit was used for comparative analysis  
\(^c\) The commercial HCV CORE antigen kit was used for comparative analysis  
\(^d\) The HCV RNA PCR was used as a reference standard in the comparison

<table>
<thead>
<tr>
<th>Diagnostic Assays</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive values</th>
<th>Negative Predictive values</th>
</tr>
</thead>
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<tr>
<td>In-house ELISA assay</td>
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<td>98.8%</td>
<td>98.8%</td>
<td>100%</td>
</tr>
<tr>
<td>Anti-HCV Ag</td>
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<td>98.8%</td>
</tr>
<tr>
<td>HCV CORE Ag</td>
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<td>95.5%</td>
<td>97.64%</td>
<td>97.72%</td>
</tr>
</tbody>
</table>

Figure 2. Diagrammatic representations of the OD 450 nm values obtained from the mixture of (Core+E2) antigenic recombinant proteins. ELISA-based test for the detection of the anti-Core, and anti-E2 antibodies in human sera samples, 1-7: HBV-positive sera, 8-14: HEV-positive sera, 15-21: Dengue virus-positive sera, 22-42 HCV infected sera of known genotypes (1a, 1b, 2a 3a & 3b), 42-271: HCV-positive sera and 272-341 HCV-negative sera.

Table 3
Comparative analyses of in-house ELISA assay, commercially available ELISA assays and HCV RNA PCR as reference standard

Table 4
Parameters for the in-house ELISA diagnostic method and commercial ELISA assays

Figure 3. Receiver operation curve (ROC) distinguish sensitivity and specificity of in-house ELISA and commercial ELISA assays.

<table>
<thead>
<tr>
<th>Diagnostic Assays</th>
<th>AUC</th>
<th>Std. Errors</th>
<th>95% confidence intervals</th>
</tr>
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<tbody>
<tr>
<td>In-house ELISA</td>
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<td>0.006</td>
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<tr>
<td>Anti-HCV Ag</td>
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<td>0.011</td>
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</tr>
<tr>
<td>HCV CORE Ag</td>
<td>0.966</td>
<td>0.16</td>
<td>0.935-0.997</td>
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</table>
recombinant protein showed strong reactivity (>99%) of PCR-positive samples and might have a potential for the screening of HCV infection.

Considering the above potential and the diagnostic value of Core and E2 antigens, we used a mixture of both recombinant antigens (Core & E2) in immunoblot and ELISA diagnostic assays to achieve the maximum sensitivity and specificity for monitoring HCV infection. To accomplish the highest possible sensitivity and specificity for the serological screening of HCV, we used the mixture (Core+E2) of recombinant antigens in immunoblot assay. In the immunoblotting there was the highest reaction intensity in the test area (TA) when the mixture of Core and E2 antigens were used collectively, as compared with the individual Core and E2 proteins. Notably, these results are in agreement with recently developed immunoblot assay.17

In the CA of the immunoblotting assay we used the extracellular recombinant protein A, which has been used for some time to develop immunochromatographic tests, together with particle capture conjugated to colloidal gold as the line capturing test for antibodies in sera.20 The sensitivity and specificity of the immunoblot assay were 100%, so these results verified that there is the possibility to construct an economical clinical in-house immunochromatographic test for the detection of anti-HCV antibodies using mixture of recombinant Core and E2 antigens. The ELISA assays was designed with the results achieved in the assessment of the immunoblot assay, so we used only the mixed antigens (Core+E2) in ELISA analysis. In an ELISA assay the mixed (Core+E2) antigens showed 100% reactivity against HCV infected sera, they did not show any cross-reactivity in the presence of HCV-negative sera with exception of one patient who showed reactivity for an unknown reason, and further did not show any reactivity against serum samples positive for other viral infections (HBV, HEV & Dengue virus). Mixed antigens also showed 100% reactivity against the sera of HCV known genotypes regardless of genotype specificity, and thus ruled out the use of these antigenic peptides for establishing an immunoassay for HCV specific genotypes. However, several published reports also rule out the genotype-specific nature of expressed recombinant antigens.21 The sensitivity and specificity of our developed in-house ELISA assay was 100% and 98.8% respectively as compared to the commercials assays, which specificity is higher than the specificity (87.5%) of a recently developed ELISA assay using truncated Core and E2 recombinant antigens.17 Thus there is also a possibility to develop a local ELISA assay for the screening of HCV. Our developed serological methods for HCV screening are more suitable for circumstances in Pakistan, to reduce the import of HCV diagnostic kits and to be optimized more accurately for the screening of local HCV strains

5. Conclusion

Based on our results the mixture of antigenic recombinant proteins (Core+E2) used in immunoblotting and ELISA assays showed maximum sensitivity and specificity. Thus the expressed proteins were potentially valuable antigens for developing a clinical immunochromatographic test and ELISA diagnostic method for in-house screening of HCV-infection.

Conflict of Interest: There is no conflict of interest

Ethical approval: This study was approved by ethical committee of the University

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References