Establishment of a Sandwich ELISA Method for Detection of Vascular Endothelial Growth Factor in Serum Samples of Hepatocellular Carcinoma Patients[1]

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Objective To establish a sandwich ELISA method for detecting vascular endothelial growth factor (VEGF) in sera of population and the patients with hepatocellular carcinoma (HCC). **Methods** Full length and two truncated human VEGF cDNA sequences were amplified from a commercial plasmid pBLAST49-hVEGF by PCR and inserted into the prokaryotic-expression plasmid pET-32a or pGEX-2T. Various VEGF proteins were expressed and purified from *E. coli* in His-Trx or GST fusion forms. The specific VEGF antibodies were elicited in experimental rabbits and mice by immunization of the full length VEGF fusion protein His-Trx-VEGF1-165. After purification of antibodies with chromatograph of Protein G, a sandwich ELISA technique was established. Serum VEGF levels were evaluated in 229 adults and 291 HCC patients. **Results** SDS-PAGE displayed that the molecular weights of the expressed full length (His-Trx-VEGF1-165), N-terminal (His-Trx-VEGF1-100) and C-terminal (GST-VEGF100-165) human VEGF fusion proteins were about 38KD, 31KD, and 33KD, respectively. Western blots confirmed that the prepared antisera were able to recognize both prokaryoticly and eukaryoticly expressed recombinant VEGF proteins. Assays of serially diluted His-Trx-VEGF1-100 by the established sandwich ELISA method showed that the linear range of the standard curve was 0.625-320 ng/mL, with the squared correlation coefficient R^2=0.991. Screening of a serum panel containing 291 serum samples of HCC patients and 229 health adults revealed that the average VEGF level in HCC patients was higher than that in healthy controls, with a statically significant difference. **Conclusion** The established sandwich ELISA reflects the level of serum VEGF and provide scientific basis for screening metastasis and recurrence of HCC using serum VEGF as an index.

Key words: Hepatocellular carcinoma; Vascular endothelial growth factor; Enzyme-linked immunosorbent assay

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