Chemiluminescence Immunoassay of Alpha-Fetoprotein

Petrit Gecaj[a], Fatime Kullolli[a], Qazim Lataj[b], Mirela Lika[b]

[a]Klinika Orthodhokse "Ungjillëzimi", Rruga "Dibrës", (Nr. 159) ND. 110 H7, Tirana, Albania

[b]Tirana University, Faculty of Natural Sciences, Department of Biology, Bulevardi "Zog I", Tirana, Albania

Abstract

Early diagnosis and treatment of cancer are the keys to improve patient survival rate. As the levels of tumor markers in serum are associated with the stages of tumors, reliable and sensitive determination of tumor markers plays a significant role in early cancer screening and evaluation. Protein detection methods**,** owing to the limited specificity of single markers in cancer diagnosis, multiplex immunoassays for analyzing a panel of tumor markers in complex serum samples to improve the diagnostic accuracy have attracted considerable attention.

A sensitive chemiluminescence (CL) imaging immunoassay method for detection of multiple tumor markers with high throughput, easy operation, and low cost was developed. The immunosensor was applied to detect α-fetoprotein, carcinoma antigen 125, and carcinoembryonic antigen and to screen patients with liver cancer. The high throughput and acceptable stability, reproducibility, and accuracy showed good applicability of the proposed multiplex CL imaging immunoassay in clinical diagnosis.

KEYWORDS: chemiluminescence, immunosensors, α-fetoprotein, cancer, liver.

Introduction

The liver is a vital organ of vertebrates and some other animals. In the humans is located in the upper right quadrant of the abdomen, below the diaphragm. The liver has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of biochemical necessary for digestion (1,3). There is currently no way to compensate for the absence of liver function in the long term, although liver dialysis techniques can be used in the short term (2,5,10).

The liver is a gland and plays a major role in metabolism with numerous functions in the human body, including regulation of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It is an accessory digestive gland and produces bile, an alkaline compound which aids in digestion via the emulsification of lipids . The liver's highly specialized tissue consisting of mostly hepatocytes regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (4,13).

A tumor marker is a biomarker found in the blood, urine, or body tissues that can be elevated in cancer, among other tissue types (7,11,14). There are many different tumor markers, each indicative of a particular disease process, and they are used in oncology to

help detect the presence of cancer. An elevated level of a tumor marker can indicate cancer; however, there can also be other causes of the elevation.

Tumor markers can be produced directly by the tumor or by non-tumor cells as a response to the presence of a tumor. Most tumor markers are tumor antigens, but not all tumor antigens can be used as tumor markers (8**,**12).

Although mammography, ultra sono-graphy, computed tomography, magnetic resonance imaging scans, and tumor marker assays help in the staging and treatment of the cancer, they are usually not definitive diagnostic tests. The diagnosis is confirmed by biopsy.

Uses of tumor markers can broadly be classified as follows:

Diagnosis of specific tumor types, particularly in certain brain tumors and other instances where biopsy is not feasible.

As stated in the BMJ 2009, tumor markers should not generally be used for the purpose of diagnosis of cancers, as opposed to monitoring purposes in certain cancers, or in certain cases, screening purposes (9,13). The use of these tests without understanding their utility has resulted in inappropriate use of tumor marker blood tests, which has also resulted in further inappropriate over-investigation for cancers.

MATERIALS AND METHODS

Materials and Reagents.

The capture antibodies (Ab1) and Ab2 of α-fetoprotein (AFP) (mouse monoclonal antibodies, clones bsm-1021 and bsm-1022) were purchased from Beijing Biosynthesis Biotechnology Co. Ltd. (Beijing, China).

The corresponding electrochemiluminescent (ECL) immunoassay reagent kits for reference detection were provided by Roche Diagnostics GmbH (Germany).

Clinical serum samples were from the ambulatory and hospital person.

RESULTS AND DISCUSSION

The collected CL intensity was proportional to the logarithm of analytic concentration over the ranges of (5.0×10^{-5})−10 ng/mL or – 5,8 Unit/mL, for AFP. Positive detection rates in 102 serum samples by the CL imaging immunoassay are shown in Table 1.

The positive detection rates of AFP−CEA panel for 20 cases of liver cancer and CEA for 15 cases. As controls, no positive results were obtained in seven normal control samples. These results demonstrated the improved positive detection rates and diagnostic value for cancer screening, showing potential application in clinic diagnosis.

Table 1. Positive Detection of Clinical Sera

Cancer	**Samples**	**Tumor marker**	**Positive cases**
Liver cancer	120	AFP	20

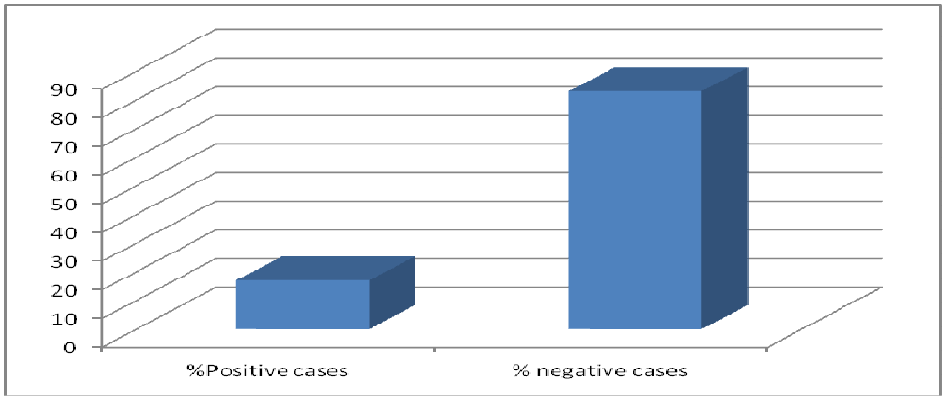

Figure 1: Percentage of the cases with AFP

If we observed the figure 1, we can say that the most of the people who are tested for AFP resulted negative and only 16,6% of the total cases are positive by chemiluminiscence.

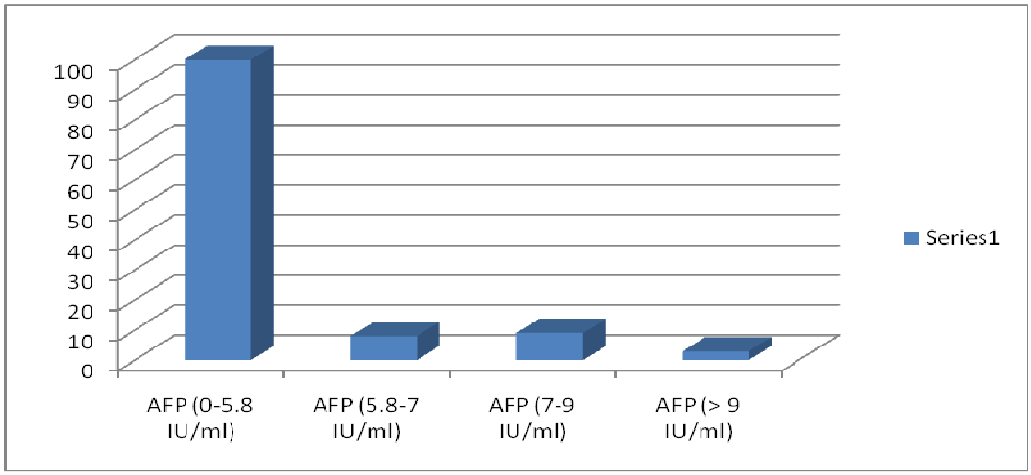

Figure 2: The cases with AFP

Basing on the AFP values we observed that the most of the positive cases are in values 7-9 IU/ml, and all the patients suffered from liver cancer.

CONCLUSIONS

From our results we can say that a chemiluminescence immunoassay of multiple tumor markers is low cost, small consumption, simple operation, and high sensitivity for combination diagnosis of certain tumors. The availability of this system to measure simultaneously the levels of multimarkers in serum samples with sensitivity is a suitable to carry out large-scale screening of cancers in the early stage.

The level of relatively specific marker to certain tumor, for example, AFP to liver cancer, has the biggest difference from its cutoff value.

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