Paper #:
V-009

Urine Proteomic Pattern Analysis for Early Hepatitis and Hepatitis-Related Liver Cancer Detection

M. Abdalla[1], and Y. Haj-Ahmad[1,2]. [1]Brock University, St. Catharines, ON, CANADA, [2]Norgen Biotek Corp., Thorold, ON, CANADA

Contact Information:
3430 Schmon Parkway
Thorold, ON, CANADA
Ph: (905) 227-8848
Fax: (905) 227-1061

Abstract

- The early detection of Hepatitis and Hepatitis-related liver cancer is critical for its influential control and prevention.
- Although there are a few biomarkers for the early detection for Hepatitis and Hepatitis-related liver cancer (i.e. alpha-fetoprotein, des-g-carboxy prothrombin and isozyme of alkaline phosphatase specific for liver cancer), they are of low specificity especially against a background of chronic hepatitis.
- As a specific filtrate of the blood, the protein components of urine are qualitatively similar to those of blood. Identification of reliable, reproducible, and noninvasive markers for Hepatocellular Carcinoma (HCC) has a significant impact on public health, especially in the light of the high burden of hepatitis C virus infection which is considered one of the most the most causative agents in the development of HCC.
- In this study, urine samples were collected from patients with Hepatocellular carcinoma post Hepatitis C virus infection, patients with Hepatitis C and Hepatitis B virus at different stages of the diseases as well as normal individuals. The proteins were isolated from 1 mL of those samples using the Proteospin™ Urine Protein Concentration Micro Kit (Norgen Biotek), and the protein profiles from all the samples were analyzed qualitatively and quantitatively using 1D SDS-PAGE and 2D electrophoresis.
- The results revealed the appearance or the disappearance of some bands in both HCC-post HCV samples or the HCV samples when compared with the normal control group. Those proteins might be of a great significance and of a great value for the early detection of HCC or HCV/HBV infection using only 1 ml of a urine sample.

Introduction

- A major goal in the field of clinical proteomics is to identify disease biomarkers in the biological fluids that can be measured relatively inexpensively for early diagnosis of disease.
- An important challenge in this process is to develop a rational means of reducing the complexity of the proteome of body fluid sample to enhance the detectability of relatively low-abundance proteins that may have a special patho-physiological significance (1).
- Urine represents a bodily fluid which can be used as an abundant, non-invasive source of biomarkers that can aid in the early diagnosis or monitoring of certain diseases.
- As a specific filtrate of blood, the protein components of urine are qualitatively similar to that of blood which make of a great importance as an easily accessible body fluid.
- A catalog of these urinary proteins cannot only improve our knowledge of the kidney's physiology, but can also allow the identification of novel proteins associated with pathological states (2).
- Most recent biomarkers research has been focusing on various forms of cancer due to the assumption that a growing cancer will affect the physiology of the organism to such an extent that measurable changes will be of a significant difference (3), thus allowing the detection of a proteomic pattern in biological fluids.
- HCC is one of the most well-known malignancies worldwide. The number of cases of HCC is rising, and this is likely due to increased HCV and HBV infections (4).
- HBV is the most common and best investigated etiological agent of HCC, but the mechanism is poorly understood (5). HBV, HCV and HCC post HCV are known to alter the normal physiological function of liver cells.
- We hypothesize that analyzing the urinary proteomic profiles of patients infected with HBV, HCV and HCC post HCV should yield valuable information which will help in understanding both the mechanism of infection and the progression of theses infections to the cancerous state, and may lead to early detection.

Methods

- Urine samples were collected from the Alexandria University General Hospital and the institute of Liver Diseases at El Monofya University (Egypt) upon the approval of the Brock University Research Ethics Board.
- 122 different urine samples were analyzed:
  - 16 from HBV infected patients.
  - 74 from HCV infected patients.
  - 32 from HCC post HCV patients.
- 10 urine samples from healthy Egyptian individuals were also used as a control for the protein profiling.
- Total proteins were isolated from 1 mL urine samples using the Proteospin™ Urine Protein Concentration Micro Kit (Norgen Biotek).
- Proteins samples were quantified using the Bio-Rad Protein Assay.
- Protein samples (20 µL out of each 100 µL elution) were run on SDS-PAGE gels for qualitative analysis.
- Proteins samples (30 µg) from 4 highly concentrated samples were separated on IPG strips (4-7) for 2D SDS-PAGE analysis.

Results

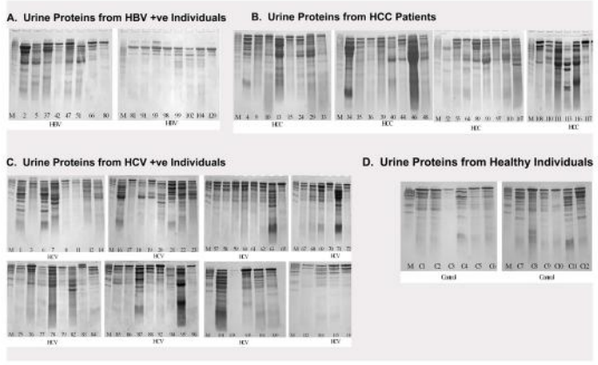

Figure 1. SDS-PAGE gels of total proteins isolated from 1 mL urine samples (ProteoSpin™ Urine Protein Concentration Micro Kit, Norgen Biotek) of individuals with HBV (Panel A), HCC post HCV (Panel B), HCV (Panel C) and healthy individuals (Panel D). A total of 20 µL out of each 100 µL elution was loaded onto a 15% SDS-PAGE gel and run at 200 V for 75 minutes. M is the ProteoLadder 150 Protein Ladder (Norgen Biotek). Pictures were taken using an AlphaImager™ 2200 from AlphaInnotech.

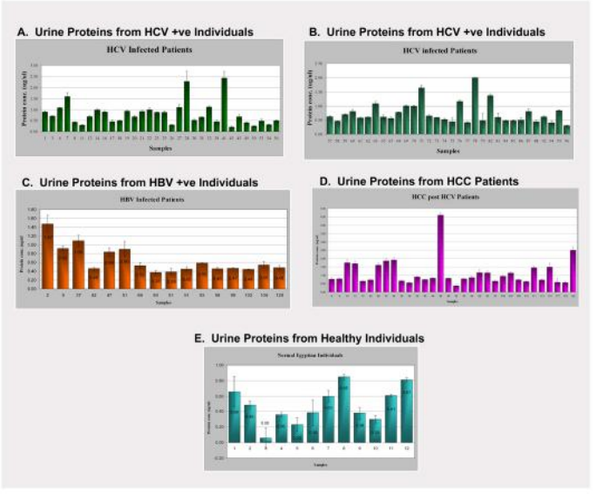

Figure 2. Graphs showing the concentrations of total protein isolated from 1 mL urine samples (ProteoSpin™ Urine Concentration Micro Kit, Norgen Biotek) of individuals with HCV (Graphs A and B), HBV (Graph C),HCC post HCV (Graph D) and healthy individuals (Graph E). Total proteins were quantified using the BioRad Protein Assay.

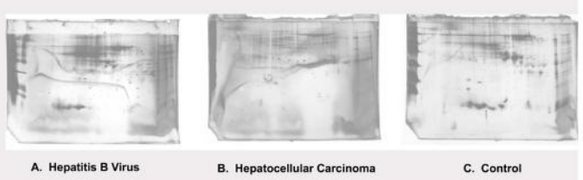

Figure 3. 2D SDS-PAGE gels of total proteins isolated from 1mL of urine samples (ProteoSpin™ Urine Protein Concentration Micro Kit, Norgen Biotek) for selected samples (47 of HBV, 34 of HCC and 11 from the control). A total of 30 µg protein was loaded to the ReadyStrip™ IPG strip pH 4-7 and focused on PROTEAN IEF Cell at 250V for 20 min, 4000 V for 1 hour and then to 20000 V. The strips were then transferred to the second dimension were it was loaded onto a 12% SDS-PAGE and run at 200 V for 75 minutes. The gels were stained using the Silver Stain Plus Kit from Bio-Rad. Pictures were taken using an AlphaImager™ 2200 from AlphaInnotech.

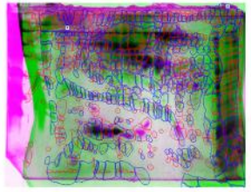

Figure 4. Analysis of the control sample against the HBV samples using Progenesis SameSpots software from Nonlinear Dynamics. The two gels were wrapped and overlaid to show the common spots as well as the difference between the two gels. The blue spots belong to the control sample whereas the red spots belong to the proteins detected on the HBV sample. The spots from the 2 gels are matched together with the pink lines. The pink spots are for proteins only found in the control samples whereas the green spots are unique to that of the HBV sample.

Discussion

- 1D SDS-PAGE showed that some bands are consistently present across the board.
- Comparing the protein pattern within each group and between the different groups and the control result in the presence of a specific pattern which is unique to each group of patients as well as showing a possible relation between different groups as all the 3 investigated cases are common in their site of infection, leading to the same outcome which is HCC.
- 2D SDS-PAGE for specific sample from each group compared with a normal sample showed some proteins of interest which were either over-expressed or disappeared.
- The spots of interest are now under further investigation to be related with the case of the disease and will be then tested for the rest of the samples for consistency purposes and for validation
- Unique protein(s) populations appeared or disappeared consistently within each group which could be a consequence of the viral infection or due to the tumor.

Conclusions

1. Valuable information can be obtained from analyzing the proteomic profiling of urine in patients infected with HBV, HCV and HCC post HCV.
2. Yield of urinary proteins varied within each disease group and between groups.
3. Some protein bands were consistent in all samples, which may be of a great significance and could be related to the state of the disease.
4. Preliminary analysis of urinary proteomics has revealed a wealth of information which can be of a great importance in the field of either the early detection of HBV and HCV infections or in the field of HCC biomarker discovery.

References

1. PNAS, Sep 2004; 101: 13368-13373.
2. Thongboonkerd V, Klein JB, Pierce WM, et al: Sodium loading changes urinary protein extraction: A proteomic analysis. Am J Physiol Renal Physiol 284: F1155-F1163, 2003.
3. Michener C.M., Ardekani A.M., Petricoin E.F., Liotta L.A., Kohan E.C. Genomics and proteomics: Application of novel technology to early detection ad prevention of cancer. Cancer Detect Prev 2002;26:249-255.
4. Ince N, Wands JR. The increasing incidence of hepatocellular carcinoma. Semin Liver Dis 1999;19:263-269.
5. Tabor E. Viral hepatitis and liver cancer. In Goldin RD, Thomas HC, Gerber MA eds. Pathology of viral hepatitis. London. Arnold; 161-177.

Acknowledgements

The authors would like to thank The Egyptian Government and the Egyptian Cultural & Educational Bureau in Canada for their financial support of this project, as well as Dr. Ahmad Raafat Bassiouny for his helping in the collection of the urine samples. The authors would also like to thank the staff of Norgen Biotek for their technical assistance, and Pam Roberts for her help in preparing this poster.

Brock University

NORGEN BIOTEK CORPORATION