Expression Analysis of Liver-Specific Circulating MicroRNAs in HCV-Induced Hepatocellular Carcinoma in Egyptian Patients

Lobna Mourad[1*], *Eman El-Ahwany[2], Mona Zoheiry[2], Hoda Abu-Taleb[3], Marwa Hassan[2], Ali Abdel Rehim[4], Moataz Hassan[4], Suher Zada[1]*

*[1]Biology Department, American University in Cairo, Cairo, [2]Immunology Department, [3]Environmental Department, [4]Hepato-Gastroenterology Department, Theodor Bilharz Research Institute, Egypt. *Lobna.mourad@gmail.com*

Introduction: In Egypt, hepatitis C virus (HCV) infection is the leading cause for the high hepatocellular carcinoma (HCC) incidence, which is diagnosed at late stages. Due to lack of biomarkers for early detection of HCC, circulating microRNAs have recently emerged as great candidates. MicroRNAs are small non-coding RNA molecules responsible for regulating gene expression. Therefore, the aim is to investigate using circulating microRNAs as a non-invasive diagnostic tool for early detection of HCV-induced HCC.

Methods: Seven miRNAs (miR-125a, miR-139, miR-34a, miR-221, miR-16, miR-145 and miR-199a) were selected due to their involvement in hepato-carcinogenesis. A total of 165 patients were enrolled, from which serum samples were collected and categorized into four patient groups: 42 chronic hepatitis C (CHC) without cirrhosis, 45 CHC with cirrhosis (LC), 38 HCC with HCV patients, and 40 healthy controls. The expression profile of the miRNAs was analyzed using real-time RT-PCR. Additionally, the HCC conventional markers, α-fetoprotein (AFP) and des-γ-carboxyprothrombin (DCP) were measured using commercial kits.

Results: Serum levels of miR-125a, miR-139, miR-145 and miR-199a were significantly decreased ($P< 0.01$) in HCC than in the CHC and LC groups (Table 1). MiR-16 and miR-34a were significantly increased ($P< 0.01$) in HCC patients compared to the normal group. No significant difference was shown in the expression of miR-16, miR-34a, and miR-221 between the CHC, LC, and HCC groups. MiR-34a showed the highest sensitivity and specificity among all miRNAs, followed by miR-221, miR-125a, miR-139, miR-145, and miR-199a.

Conclusions: These results indicate that measuring the expression of liver circulating microRNAs can be a reliable diagnostic tool for HCC. Our results demonstrated that the up-regulation of miR-16, miR-34a, and miR-221 differentiates between normal individuals and liver disease patients. Meanwhile, the down-regulation of miR-125a, miR-139, miR-145 and miR-199a in HCC patients indicates that these microRNAs can differentiate HCC from CHC and LC.

Table 1: Expression levels of serum microRNAs of the patient groups

MicroRNAs	Normal	CHC	LC	HCC
miRNA-16	14.26±0.69	24.09±0.44**	23.29±0.46**	22.35±0.54**,a
miRNA-34a	27.32±0.19	32.69±0.34**	30.01±0.54**,b	32.50±0.94**
miRNA-221	22.82±0.38	27.17±1.44**	28.22±0.41**	28.51±0.46**
miRNA-125	20.57±0.54	96.01±4.36**	100.54±0.81**	29.96±0.57**,d
miRNA-139	29.96±0.57	94.63±0.38**	86.02±0.40**,e	30.03±0.43
miRNA-145	20.65±0.52	85.31±0.53**	80.74±0.59**	20.64±0.57
miRNA-199	80.23±0.72	330.38±0.74**	311.98±0.72**	66.16±0.44c,d

***p<0.01 significant increase than control; [a]p<0.01 significant decrease than CHC; [b]p<0.01 significant decrease than CHC and HCC; [c]p<0.01 significant decrease than control; [d]p<0.01 significant decrease than CHC and LC; [e]p<0.01 significant decrease than CHC*

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Lobna Mourad is a PhD student in the Biotechnology program at the American University in Cairo (AUC). She received her Bachelor degree from AUC in 2007, graduated with Cum Laude, majoring in Biology with a concentration in molecular genetics and minoring in Clinical Chemistry. By late 2010 she completed her Master's Degree at AUC in collaboration with NAMRU-3 in Molecular Microbiology where she studied antibiotic resistant bacteria in hospitalized patients in Oman. Her PhD at AUC is in collaboration with TBRI, specializing in HCV-induced liver cancer diagnostics. She aims to contribute to scientific research in the world and in Egypt in particular, since Egypt has the highest rates of Hepatitis C infection in the world leading to very high incidence of liver cancer