Introduction: Aflatoxin-B1 (AFB1) and hepatitis-B virus (HBV) are the most common etiological factors associated with the high incidence of hepatocellular carcinoma (HCC) in Asia [1]. Epidemiological studies have also suggested a strong correlation between AFB1 exposure and a signature mutation on the tumor suppressor gene, p53. The latter is often mutated at codon 249 in HCCs that arise from areas of high risk factors, resulting in an amino acid change from arginine (AGG) to serine (AGT), which inactivates its function [2]. Thus we have investigated the HCC model that closely mimics human HCC, induced by expression of the Hepatitis surface antigen as a transgene from the albumin promoter (HBsAg) after treatment with AFB1. In the present study we used magnetic resonance imaging (MRI) and spectroscopy (MRS) and in vitro MRS to expose the biochemical changes that are associated with a high risk of HCC.

Methods: All the in-vivo and ex-vivo experiments were done in compliance with and approved by the local Biological Resources Center (BRC). In this study we employed nine mice over three categories. The first group involved HBsAg mice treated with Aflatoxin B1 (AFB1) acronymed as HBsAg + AFB1 (n = 3). The second group (n = 3) consisted of HBsAg mice treated with corn oil (no AFB1 treatment). Third group included non-transgenic wild type mice (n = 3) injected with only corn oil, to serve as a control. AFB1 and corn oil treatment was performed on the 7th day after birth. The HBsAg mice develop HCCs around 9 months after AFB1 injection, which resemble the human HCC pathologically. All animals were subjected to localized MRS and diffusion tensor imaging (DTI). Prior to in vivo experiments, animals were anaesthetized with 1-2 % v/v isofluorane mixed with medical air and medical oxygen. All MR experiments were performed with a 7 T Clinscan MRI/MRS scanner (Bruker, Karlsruhe, Germany) equipped with a 72-mm volume resonator for RF transmission, in combination with a 20-mm surface receive-only coil. All experiments were respiratory gated to obviate the effects of liver motion. MRI experiments included gradient echo, spin echo; and multi-directional DTI (b = 0, 1000 s mm \(^{-2}\)) was employed with 20 gradient directions over the entire liver. A volume-localized PRESS sequence was employed on normal live [21:201 –216. (3) Susumo Mori et al., DTI, studio version 3.2.6. (4) Lindstedt G. et. al., J. Biol. Chem.1965;240:516-321.]

Results and Discussion: Figure 1 shows the in vivo \(^1\)H NMR spectrum obtained from the HCC tumor of HBsAg + AFB1 mouse at the age of 54 weeks. Prominent resonances in the region of 0.78 ppm to 2.8 ppm were assigned to lipids. The resonance from the -N(CH\(_3\))\(_3\) of choline was detected at 3.20 ppm. In addition to the choline signal we also observed the carnitine peak at 3.38 ppm in tumors of HBsAg group treated with AFB1. The HRMAS spectrum (Figure 2) of intact tumor tissue, included multidiirectional DTI, confirming the assignment of carnitine. The average choline-to-water ratio (Figure 3) in the HBsAg + AFB1 group of mice was 0.0039 ± 0.0002, compared to the control group at 0.0019 ± 0.0002 (p = 0.01). The carnitine resonance was absent from the control group of animals. Bioisynthesis of carnitine involves the hydroxylaion of trimethyllysine to 3-hydroxy-6-N-trimethyllysine. Then the hydroxytrimethyllysine is cleaved to yield butyrolytae aldehyde that is then oxidized to butyrobetaine in turn is finally hydroxyolated to yield carnitine [4]. Carnitine plays a major role in fatty acid transport into the mitochondria, prior to \(\beta\)-oxidation and hence energy transduction. The average ADC value of the lipid in the tumors of HBsAg + AFB1 mice was 7.26 \(\times\) 10\(^{-4}\) mm\(^2\) s\(^{-1}\) compared to normal liver tissue where it was 9.67 \(\times\) 10\(^{-4}\) mm\(^2\) s\(^{-1}\) (p = 0.00001); this is consistent with the marked changes in cellularity.

Conclusions: We investigated HCC in vivo in HBsAg transgenic mice model that simulated the human condition. We detected carnitine for the first time, to our knowledge, in HCC tumors of HBsAg + AFB1 mice model. Therefore relatively high concentrations of carnitine may be a biomarker for the early detection of HCC.