CHITOSAN AND SILVER NANOPARTICLES: PROMISING ANTI-TOXOPLASMA AGENTS  
Presenting Author: Maha Reda Gaafar

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EVALUATION OF SERUM MICRO-RNA 122 AND 221 AS BIOMARKERS FOR DIAGNOSIS OF HEPATOCELLLULAR CARCINOMA IN EGYPTIAN PATIENTS WITH CHRONIC HEPATITIS C  
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IMPACT OF DIABETES MELLITUS ON RESPONSE TO HEPATITIS C VIRUS THERAPY  
Presenting Author: El-Sayed Tharwa

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NAFLD FROM BENIGN TO MALIGNANT  
Presenting Author: Ehab Abdelatty

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TRANSLATIONAL RESEARCH, FROM BASIC SCIENCE TO APPLIED THERAPEUTICS. TARGETS AND LEADS IN THE HCV LIFE CYCLE
Presenting Author: Mostafa Yakoot
CHITOSAN AND SILVER NANOPARTICLES: PROMISING ANTI-TOXOPLASMA AGENTS

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Toxoplasmosis is a worldwide infection caused by obligate intracellular protozoan parasite which is Toxoplasma gondii. Chitosan and silver nanoparticles were synthesized to be evaluated singly or combined for their anti-toxoplasma effects as prophylaxis and as treatment in the experimental animals. Results were assessed through studying the parasite density and the ultrastructural parasite changes, and estimation of serum gamma interferon. Weight of tissue silver was assessed in different organs. Results showed that silver nanoparticles used singly or combined with chitosan have promising anti-toxoplasma potentials. The animals that received these compounds showed statistically significant decrease in the mean number of the parasite count in the liver and the spleen, when compared to the corresponding control group. Light microscopic examination of the peritoneal exudates of animals receiving these compounds showed stoppage of movement and deformity in shape of the tachyzoites, whereas, by scanning electron microscope, the organisms were mutilated. Moreover, gamma interferon was increased in the serum of animals receiving these compounds. All values of silver detected in different tissues were within the safe range. Thus, these nanoparticles proved their effectiveness against the experimental Toxoplasma infection.
COMPARISON BETWEEN HUMAN AND FISH SPECIES OF CRYPTOSPORIDIUM AND CYCLOSPORA

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This study was designed to compare Cryptosporidium and Cyclospora species detected in fish with the corresponding species isolated from humans morphologically and genetically. Intestinal contents of 35 Tilapia zillii fish and 50 human stool samples were stained and examined to identify Cryptosporidium and Cyclospora oocysts. Thirty male Swiss albino mice were divided into control (I) and experimental group (II) which was further subdivided into four equal subgroups (IIa, IIb, IIc and IId) that were infected with fish and human Cryptosporidium and Cyclospora species, respectively. Two weeks later, all mice were sacrificed; parts of their small intestines were subjected to histopathological examination, and processed for transmission electron microscopy. DNA was extracted from frozen oocysts present in human stool samples and fish intestinal samples and amplification was performed using specific primers for Cryptosporidium parvum and Cyclospora cayetanensis. Cryptosporidium and Cyclospora of both fish and humans detected in mice intestinal sections were morphologically similar by both light and electron microscope. However, failure of DNA amplification of oocysts of both parasites in fish intestinal samples, following application of the specific primers, indicates that fish species were not identical to human species. As a conclusion species identified in fish are apparently not infectious to humans.
EVALUATION OF SERUM MICRO-RNA 122 AND 221 AS BIOMARKERS FOR DIAGNOSIS OF HEPATOCELLULAR CARCINOMA IN EGYPTIAN PATIENTS WITH CHRONIC HEPATITIS C

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BACKGROUND: MicroRNA (miRNA) is a small noncoding RNA gene product known to post transcriptionally modulate gene expression by negatively regulating the stability of its target mRNAs. Several miRNAs were found to be potential diagnostic, prognostic, or metastatic markers for hepatocellular carcinoma (HCC).

AIM OF THE WORK: this study was designed to explore the potential usefulness of serum miR-122 and miR-221 as novel non invasive markers for diagnosis of HCV related HCC in Egyptian patients.

METHODS: This prospective study was conducted on 90 adult patients of both gender with HCV-related chronic liver disease and chronic hepatitis C related HCC. In addition to 10 healthy control individuals. Patients were stratified into; interferon naïve chronic hepatitis C (CH) (n=30), post-hepatitis C compensated cirrhosis (n=30) and treatment-naïve HCC (n=30). All patients and controls have undergone full clinical assessment and lab investigations in addition to the evaluation of the level of serum miRNA expression by RT-PCR.

RESULTS: There was significant fold changes in the expression levels of miRNA genes in patients’ era of different HCV associated liver disease when compared to the control group; Chronic hepatitis C group showed a significant fold increasing in the expression level of miR-122 and miR-221, Cirrhosis group showed significant fold decreasing in expression level of miR-122 and significant fold increasing in expression level of miR-221 in addition, HCC group displayed significant fold elevation in miR-122 and significant fold decrease in miR-221.(p=0.01)

Comparing fold changes in miRNAs in HCC group versus non HCC group (CH and Cirrhosis), there was significant fold decreasing in miR-221 and non-significant fold elevation in miR-122 in HCC versus non HCC (p=0.03).

Comparing HCC group with individual groups within non HCC group showed, An increasing tendency towards statistical significant fold elevation in expression of miR-122 in serum of HCC patients in comparison to liver cirrhosis (p=0.83) and a statistical
significant fold decreasing in expression level of miR-221 in serum of HCC patients in comparison to liver cirrhosis with \( P=0.05 \) as well as in comparison to CH with \( P=0.06 \).

CONCLUSION: Serum miR-221 could represent potential non invasive diagnostic marker for early detection of HCV related HCC in Egyptian patients.

KEY WORDS: Hepatocellular carcinoma, serum miR expression level and non invasive

**HCC RISK FACTORS SYNERGISM AND HOW TO PREVENT ?**

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Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. The burden of HCC has been increasing in Egypt with a doubling in the incidence rate in the past 10 years. There is a geographic correlation between the incidence of HCC and the prevalence of chronic hepatitis B and C, suggesting that these two viral infections are the most important risk factors of HCC worldwide. Several other risk factors for the development of HCC have been reported such as aging, gender, alcohol intake and NASH. Visceral fat accumulation reportedly increases the risk of HCC development in patients with chronic liver disease. Co-infection with HBV and HCV is associated with a higher risk for developing HCC than either infection alone. The more risk factors the patients have, the higher occurrence of HCC was shown. A synergistic interaction between cigarette smoking and HCV and between cigarette smoking and alcohol consumption was observed for men and women, respectively.

Proper prophylaxis, early detection and treatment of HCV and HBV will reduce cases of HCC. Public awareness and health education of controllable risk factors such as smoking and DM will also reduce cases of HCC.
IMPACT OF DIABETES MELLITUS ON RESPONSE TO HEPATITIS C VIRUS THERAPY

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Background: The HCV epidemic in Egypt is unique in the world and well documented in the international medical scientific literature. Up to one third of patients with chronic HCV develop type 2 diabetes mellitus (DM). This prevalence is much higher than that observed in the general population. Further, HCV seropositivity in patients with DM appears to be higher than in the general population. Aim: The aim was to study the virological and biochemical responses to anti-viral therapy (pegylated INF & ribavirin) in diabetic versus non-diabetic patients with HCV infection. Subjects and methods: The study included 100 patients, 50 of them were diabetic and the other 50 were non-diabetic. All patients were subjected to history taking and complete clinical examination. HCV RNA (with commercial PCR-based assays) and HbA1c were done before, 3 months, 6 months and at the end of treatment. Follow up of patients who responded to treatment was done after 6 months by HCV RNA to assess sustained virological response (SVR). Results: End of treatment response (ETR) was higher in non-diabetic group (33 patients, 66%) than diabetic group (25 patients, 50%) with insignificant statistical difference (p-value > 0.05). Sustained virological response (SVR) was higher in non-diabetic group (26 patients, 52%) than diabetic group (18 patients, 36%) with insignificant statistical difference (p-value > 0.05). HbA1c in those who achieved SVR in the diabetic group decreased at the end of treatment with a mean of 6.39±0.80 g/dL. Conclusion: Response to HCV therapy is affected by the presence of DM type 2; it was found to be higher in non-diabetic patients compared to diabetic patients. Good control of diabetes should be achieved before starting treatment for HCV.
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Hepatocyte steatosis, defined as accumulation of fat droplets in hepatocytes, is a histological feature of a group of liver diseases including not only metabolic or alcoholic disorders but also HCV and drug-induced liver disease. Steatosis is a very common lesion in chronic HCV, seen in more than half of patients.

Overall, NAFLD patients have a higher risk of all-cause death than the general population, mainly due to CVD or liver-related causes. NASH has been proposed as a probable cause of cryptogenic cirrhosis. NASH is characterized by insulin resistance, and the resistance is thought to be involved in hepatocarcinogenesis. Emerging evidence has established multiple independent risk factors for the development of HCC including obesity, diabetes, and iron deposition. Moreover, hepatic steatosis increases the risk for the development of HCC in patients with chronic HCV.

The majority of patients diagnosed with NAFLD are asymptomatic. The elevations in ALT and AST are typically mild. GGT in the serum is frequently elevated in patients with NAFLD. Ultrasonography (US) is currently the most common method for screening asymptomatic patients with elevated liver enzymes and suspected NAFLD. US findings of fatty liver include hepatomegaly, diffuse increases in the echogenicity of the liver parenchyma.
RELATION OF INSULIN RESISTANCE AND HEPATOCELLULAR CARCINOMA IN NON-OBESE NON-DIABETIC HCV POSITIVE PATIENTS

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Background: The higher incidence of insulin resistance (IR) in patients with HCV infection is becoming an increasing problem. IR has emerged as a risk factor for a wide variety of cancers. The aim of the present study is to assess the relationship between insulin resistance and HCC in non-obese non-diabetic HCV positive Egyptian patients.

Patients & methods: The study included 30 HCV+ve patients with HCC (group I), 30 HCV+ve patients without HCC (group II) and ten healthy individuals (control group). Full history taking, clinical examination and abdominal ultrasonography were done for all patients and controls. A blood sample was withdrawn for CBC, AST, ALT, albumin, bilirubin, PT%, insulin, FBG, PP, urea, creatinine, HBsAg, HCV Ab, quantitative PCR for HCV-RNA and AFP. HOMA-IR was calculated. Diagnosis of HCC is further confirmed by triphasic CT of the liver.

Results: Serum insulin level of group I were significantly higher than group II and controls (P=0.01). HOMA-IR level of group I were significantly higher than group II and controls (P=0.001). Serum insulin and HOMA-IR levels of group II were significantly higher than controls (P=0.03).

Multivariate logistic regression analysis showed that HOMA-IR and insulin level were independent predictors for the risk of HCC development (P=0.04 and 0.03 respectively).

Conclusion, This study showed a significantly higher degree of insulin resistance in patients with HCV infection and HCC compared with HCV infection alone or healthy controls. We hypothesize that the presence of a vicious cycle triggered by HCV infection leads to increased insulin resistance with subsequent increased risk of HCC.
RISK FACTORS FOR POST ERCP PANCREATITIS: A PROSPECTIVE MULTICENTER STUDY IN UPPER EGYPT

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Background and Study Aims: Endoscopic retrograde cholangiopancreatography (ERCP) has become widely available for diagnosis and treatment of pancreatic and biliary diseases. Pancreatitis is the most common and serious complication to occur after ERCP resulting in substantial morbidity and occasional mortality. The aim of this study is to evaluate the potential patient and procedure-related risk factors for post-ERCP pancreatitis (PEP) in a prospective multicenter study.

Patients and Methods: Consecutive ERCP procedures were prospectively studied at 5 centers (2 universities, 3 private). Data were collected on patient characteristics and endoscopic techniques prior to the procedure, at the time of procedure and 24-72 h after discharge. Post-ERCP pancreatitis was diagnosed and its severity graded according to consensus criteria.

Results: Pancreatitis occurred after 104 (8.9%) of 1162 consecutive ERCP procedures and was graded mild in 66 (63.5%), moderate in 30 (28.8%), and severe in 8 (7.7%) cases. By univariate analysis, 11 of 18 evaluated variables were found to be significantly associated with PEP. By multivariate analysis, significant risk factors with adjusted odds ratios (OR) were: difficult cannulation (OR: 10.2), previous PEP (OR: 8.1), previous pancreatitis (OR: 7.9), ≥2 pancreatic duct injection (OR: 3.1), pancreatic duct cannulation (OR: 2.7), difficult stone extraction (OR: 2.2), and precut sphincterotomy (OR: 1.2).

Conclusion: Technique-related risk factors are probably more numerous and potent than patient-related ones in determining high-risk predictors for post-ERCP pancreatitis.
SERUM AND ASCITIC FLUID HEPcidIN IN HCV POSITIVE LIVER CIRRHOSIS WITH AND WITHOUT HCC

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Aim: Is to investigate the role of hepcidin in HCV+ve liver cirrhosis patients in relation to disease progression and HCC development. Patients & Methods: The study population consists of: 20 HCV+ve patients without HCC (HCV patients), 20 HCV+ve patients with HCC (HCV-HCC patients) and 10 controls. They were subjected to laboratory check-up for serum ferritin, AFP and hepcidin. Results: There was a significant difference among HCV and HCV-HCC patients and controls with regard serum ferritin and hepcidin (P=0.0001). Serum hepcidin of HCV and HCV-HCC patients were significantly lower than controls (P=0.0001). Serum and ascitic fluid hepcidin of HCV-HCC patients was significantly lower than HCV patients (P=0.01). Serum ferritin was significantly higher in HCV and HCV-HCC patients than control (P=0.001). Serum ferritin of HCV-HCC patients was significantly higher than HCV patients (P=0.02). Ascitic fluid hepcidin was negatively correlated with Child-Pugh score in HCV (r=-0.55, P=0.01) and HCV-HCC patients (r=-0.53, P=0.02). Ascitic fluid hepcidin was negatively correlated with bilirubin in HCV (r=-0.43, P=0.04) and HCV-HCC patient (r=-0.47, P=0.04). Ascitic fluid hepcidin was positively correlated with serum albumin in HCV (r=+0.44, P=0.04).

Conclusion: Low levels of hepcidin may be involved in the pathophysiological mechanism of iron overload in patients with chronic HCV with and without HCC. Moreover, there is a positive relationship between hepcidin levels and synthetic liver function suggesting that uniform suppression of hepcidin may be linked to disease progression and HCC development. Further analysis is still required to evaluate its usefulness as a marker for early detection of HCC by serial measurement of hepcidin in blood and ascitic fluid.
THERAPEUTIC ENHANCEMENT OF NEWLY DERIVED BACTERIOCINS AGAINST GIARDIA LAMBLIA

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Trials for identifying efficient anti-giardial agents are still ongoing. Nowadays, bacteriocins have attracted the attention as potential antimicrobial compounds. For the first time, the current study evaluated the therapeutic efficacy of bacteriocins newly derived from newly isolated Egyptian strains of probiotics Lactobacilli; L acidophilus (P106) and L. plantarum (P164) against Giardia lamblia. Bacteriocins’ efficacy was evaluated both in vitro; by growth inhibition and adherence assays, and in vivo; through estimation of parasite density, intestinal histopathological examination and ultrastructural analysis of Giardia trophozoites. In vivo bacteriocins' clinical safety was assessed. In vitro results proved that 50 µg of L acidophilus bacteriocin induced reduction of the mean Giardia lamblia trophozoites by 58.3±4.04%, while at lower concentrations of 10 µg and 20 µg of both L. acidophilus and L. plantarum, non significant reduction of the mean parasite density was achieved. In vitro trophozoites adherence was susceptible to the tested bacteriocins at all concentrations studied with variable degrees, while the highest adherence reduction was demonstrated by 50 µg of L acidophilus bacteriocin. In vivo, oral inoculation of 50 µg/mouse L. acidophilus bacteriocin for five successive days resulted in a noteworthy decline of the intestinal parasite density, along with amelioration of intestinal pathology of infected mice. Ultrastructural examination proved that five doses of L. acidophilus bacteriocin showed marked changes in cellular architecture of the trophozoites with evident disorganization of the cell membrane, adhesive disc and cytoplasmic components. This is the first reported study of the safe anti-giardial efficacy of L. acidophilus P106 derived bacteriocin, hence highlighting its great promise as a potential therapeutic safe alternative to existing commercial drugs.
TRANSLATIONAL RESEARCH, FROM BASIC SCIENCE TO APPLIED THERAPEUTICS.
TARGETS AND LEADS IN THE HCV LIFE CYCLE

Authors: Mostafa Yakoot
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The optimal therapy for chronic hepatitis C virus infection (HCV), analogous to HIV (HAART), is now believed to require a combination of > 3 antivirals targeting multiple aspects of the viral life cycle. Three HCV "NS3-4A protease" and HCV "NS5B polymerase inhibitors were recently approved by FDA; while many HCV "NS5B", " NS5A assembly" inhibitors and virus-liver cell entry blockers are currently under phases of development. Here, we present the life cycle of the HCV and the state of the art in basic and translational research to find solutions and lead substances to target the key processes and steps in the viral life cycle from the virus entry to assembly.