Cholesterol and its esters as serum biomarkers in malignant Obstructive Jaundice: A single step 1H NMR metabonomic approach

ABHINAV ARUN ARUN SONKAR[1], SHATAKSHI SRIVASTAV[2], SANTOSH KUMAR SINGH[1], HARI OM GUPTA[1], DEVENDRA SINGH[1], JITENDRA KUSHWAHA[1], ABHIJIT CHANDRA[3], and RAJA ROY[2]

[1]SURGERY, KG MEDICAL UNIVERSITY, Lucknow, UTTAR PRADESH, India, [2]CBMR, SGPGI, Lucknow, UTTAR PRADESH, India, [3]GASTROSURGERY, KG MEDICAL UNIVERSITY, Lucknow, UTTAR PRADESH, India

INTRODUCTION: In obstructive jaundice (OBJ), composition of bile and cholesterol metabolism changes drastically with growing severity towards malignancy. The timely diagnosis of underlying malignancy with OBJ is complex, as malignant tumors are often asymptomatic in their earlier course and thus preclude its curative resection. The complex regional anatomy of hepatopancreatobiliary system confounds the recognition of potentially resectable lesion and hinders the pre-operative histological confirmation of malignancy. Therefore, objectives of the present study include: (i) identification of the variations in low molecular weight metabolites of serum under the pathological state of benign OBJ and hepatopancreatobiliary malignancy induced obstructive jaundice from normal conditions, (ii) to explore the role of serum cholesterol (Chol) and cholesterol esters (CE) and their relative ratio estimation in sera of benign and malignant obstructive jaundice with the help of ^{1}H NMR spectroscopy. In turn, these biomarkers will identify the alterations in associated metabolic processes which play a crucial role in cancer cell biology and, (iii) the evaluation of status of bile acids, cholesterol and choline containing compounds in bile and their contribution towards resolving the diagnostic dilemma for malignant and benign OBJ, for assessing their clinico-pathological status.

MATERIAL AND METHODS: Serum and bile specimens from benign OBJ patients (n=28), malignant OBJ patients (n=36) and serum of healthy controls (n=57) were analysed by ^{1}H NMR spectroscopy and through standard biochemical liver function tests. The serum samples (10µL) were dissolved in 490µL of DMSO-d_6 and standardized for single step cholesterol and cholesterol esters estimation. Relative- and semi-quantitation of serum metabolites viz. isobutyrate, lactate, alanine, acetone, glutamine, creatine, threonine and 1-methylhistidine, total cholesterol (tCho), cholesterol (Chol) and cholesterol ester (CE) were performed. In bile, total bile acids (BA), cholesterol, phosphatidylcholine (PC) and glycerophosphatidylcholine (GPC) were quantified. The effect of benign and malignant OBJ on small metabolites and lipids was analysed by non-parametric Mann-Whitney U test.

RESULTS: Six serum metabolites were significantly lower in both classes OBJ patients and glutamine levels were further lowered under malignant conditions. The serum Chol and CE were significantly altered in all the three groups. The bile acids and lipids decreased significantly in case of malignant OBJ patients when compared with benign ones (Figure 1, 2 and 3).

DISCUSSION: The alterations in amino-acid metabolism, biosynthesis and degradation along with ketone body metabolism, which has been represented by lower levels of alanine, glutamine, threonine, 1-methylhistidine and acetone in serum, are suggestive of impaired liver function. This is also suggested by increased levels of ALP, SGOT and SGPT concentrations in serum. Since ALP is located in hepatic sinusoidal and biliary canalicular membranes, its synthesis gets increased under the condition of OBJ.

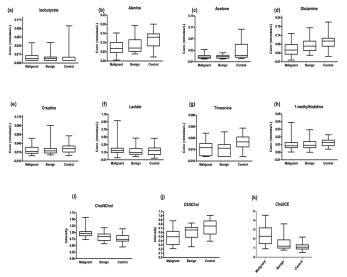

Figure 1: The Box and Whiskers plot for comparisons and depiction of median and range of concentrations (mmoles/L) of all the serum metabolites in three different groups.

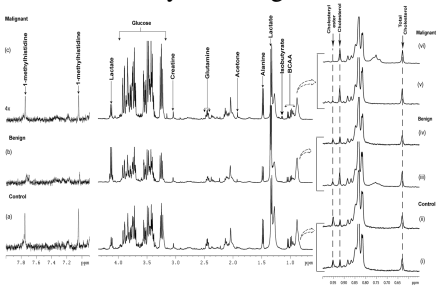

Figure 2: The representative serum spectra of all three different groups .i.e. healthy control, malignant and benign patients, where (a-c) depict small metabolites and (i-iv) represent relevant portions of 1H-NMR (10µL in DMSO-d_6) spectra of representative serum specimen of patients, indicating variation in tCho, cholesterol and CE signals as affected by the presence and absence of OBJ. All spectra were plotted on the same vertical scale and hence the different peak intensities shown truly relate to relative concentration changes among groups.

The obstruction of biliary tracts results in hypercholesterolemia due to the inefficiency of liver to remove excess cholesterol from blood and liver cell damage during severe obstruction. This alters the level of Chol and modifies its nature from predominant esterified Chol to its free form and thus ratio of Chol: tChol increases and CE: tChol decreases. Since the entry of DMSO through cell membranes is directed and not random, therefore, a semi-quantitative ratio analysis of serum cholesterol and its esters also seems to provide biochemical acuity in obstructive jaundice. Thus, ratio of Chol and CE to tChol and Chol/CE presents visualization for malignant pathology which goes in hand with previous biochemical reports. The phospholipid metabolism has earlier been correlated with malignant transformations and disruption in cell integrity. It has been suggested that multidrug-resistant protein 3 (MDR3) activity plays a vital role in maintaining complex balance between bile salts and phospholipids by modulating the PL export into bile.

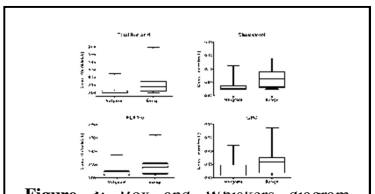

Figure 3: Box and Whiskers diagram indicating median indices of Total BA, Chol, PtdCho and GPC (mmol/L) in two groups of patients: benign OBJ and malignant OBJ.

CONCLUSIONS: The single step estimation of alterations in serum Chol and CE may have potential for early and differential diagnosis of malignant and benign OBJ. This may augment the novel insights in local and systemic effects of OBJ patients.

REFERENCES: Geier A, Wagner M, Dietrich CG, Trauner M. Biochimica et biophysica acta 2007;1773:283-308.