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ANALYSIS OF LEVEL OF BILE OBSTRUCTION AND ITS ASSOCIATION WITH INCREASED CYP7A1 EXPRESSION IN BILE ACID

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Abstract:

Introduction: *Bile acids are synthesized from cholesterol exclusively in the liver. The rate of bile acid synthesis is mainly controlled by transcriptional regulation of cholesterol 7α-hydroxylase (CYP7A1), which encodes the rate-limiting enzyme in the classic bile acid synthesis pathway.*

Objectives of the study: *The main objective of the study s to analyze the level of Bile Obstruction and its association with increased CYP7A1 and MIR33a expression in bile acid.*

Materials and Methods: *This cross-sectional study was conducted in Health department Punjab during 2019. The data comprises of 100 patients of both genders. Blood sample was drawn for the analysis of PCR. Expression levels of CYP7A1 and three bile acid hepatic transporters (Abcg5, Abcg8 and Ldlr) was carried out by quantitative real time PCR using RNA extracted from the rat livers. Using Trizol reagent (Invitrogen, Carlsbad, CA) total RNA was extracted from the livers of killed rats, according to manufacturer's protocol with slight modifications.*

Results: *The data was collected from 100 patients. An analysis of 7α-hydroxylase mRNA levels in the rat livers revealed marked increase in the expression of CYP7A1 in bile ligated rats while the non-ligated rats showed a lower expression level. It was also observed that the rats on high fat diet had (WD) had higher levels of CYP7A1 mRNA in livers than the ones on regular laboratory chow, both in ligated and non-ligated groups.*

Conclusion: *It is concluded that in such times of impaired release of bile acids to intestine, secondary pathway may be activated. Ligated rats showed increased CYP7A1 levels while 27-hydroxylase in the serum of such rats was high as compared to non-ligated group.*

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INTRODUCTION:

Bile acids are synthesized from cholesterol exclusively in the liver. The rate of bile acid synthesis is mainly controlled by transcriptional regulation of cholesterol 7α-hydroxylase (*CYP7A1*), which encodes the rate-limiting enzyme in the classic bile acid synthesis pathway. When bile acid levels increase, bile acids repress their own synthesis and stimulate biliary lipid secretion. These mechanisms allow the liver to efficiently maintain lipid homeostasis [1]. Bile acids activate farnesoid X receptor (FXR) and the G protein-coupled receptor TGR5, and also several cell signaling pathways to regulate bile acid synthesis and lipid metabolism. Pharmacological activation of either FXR or TGR5 receptor has been shown to improve lipid, glucose and energy homeostasis, glucose tolerance and insulin sensitivity [2]. Paradoxically, loss of FXR in obese and diabetic mice reduced body weight and improved peripheral insulin sensitivity, and decreasing bile acid pool size with the specific FXR agonist GW4064 caused increased susceptibility to diet-induced obesity, fatty liver and hypertriglyceridemia. It is likely that activation of different bile acid signaling in different mouse models might have different effects on hepatic metabolism, diabetes and obesity [3]. Conversion of dietary cholesterol intake as lipoprotein particles into bile acids is the main metabolic pathway for clearance of these lipoproteins from a person's blood [4]. This process is carried out in liver and is the major pathway for eradication of cholesterol from the body. Rate of synthesis of bile acids is directly related with many factors like increased synthesis of cholesterol in liver, increased uptake of lipoproteins mediated by cell surface receptors [5]. These data suggest that changes in availability of hepatic cholesterol result in compensatory alterations in bile acid synthesis pathways and hence controlling the cholesterol homeostasis [6].

Almost 50% of the daily turnover of cholesterol is eliminated by conversion into bile acids in a chain of enzymatic reactions. This series of reactions converts the cholesterol into amphipatic compounds [7].

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OBJECTIVES OF THE STUDY:

The main objective of the study s to analyze the level of Bile Obstruction and its association with increased CYP7A1 and MIR33a expression in bile acid.

MATERIALS AND METHODS:

This cross-sectional study was conducted in Health department Punjab during 2018 to 2019. The data comprises of 100 patients of both genders. Blood sample was drawn for the analysis of PCR. Expression levels of CYP7A1 and three bile acid hepatic transporters (Abcg5, Abcg8 and Ldlr) was carried out by quantitative real time PCR using RNA extracted from the rat livers. Using Trizol reagent (Invitrogen, Carlsbad, CA) total RNA was extracted from the livers of killed rats, according to manufacturer's protocol with slight modifications. Briefly, 50 mg tissue samples were homogenized in 1 ml Trizol reagent and left on room temperature for 5 hours. RNA was extracted using 0.2 ml of chloroform and precipitated using 0.5 ml 100% isopropanol. The pellets were washed with 75% ethanol and after air drying samples were suspended in 30ul of water (RNAse free). M-MuLV reverse transcriptase (Thermo Scientific, Waltham, MA) was used to synthesize first strand cDNA, as per manufacturer's protocol. 2ul of the cDNA was used as a template in real time PCR using SYBR Green dye.

STATISTICAL ANALYSIS:

All the statistical analysis was carried out using SPSS version 21.0. Linear regression analysis of expression levels was carried out plotting CYP7A1 on X-axis and comparing the expression with individual hepatic bile acid transporters plotted on Y-axis.

RESULTS:

The data was collected from 100 patients. An analysis of 7α-hydroxylase mRNA levels in the rat livers revealed marked increase in the expression of CYP7A1 in bile ligated rats while the non ligated rats showed a lower expression level. It was also observed that the rats on high fat diet had (WD) had higher levels of CYP7A1 mRNA in livers than the ones on regular laboratory chow, both in ligated and non-ligated groups

Table 1: Primer Sequences for Real Time Quantitative PCR

Gene	Forward Primer	Reverse Primer	Ref
Abcg5	5'-TTGCGATACACAGCGATGCT-3'	5-TGACTGCCTCTACCTTCTTGTTGT–3'	(Song, et al., 2010)
Abcg8	5'-CCGTCGTCAGATTTCCAATGA-3'	5'-GGCTTCCGACCCATGAATG-3'	-do-
Ldlr	5'-GCTCCATAGGCTATCTGCTCTTCA-3'	5'-CTGCGGTCCAGGGTCATC-3'	-do-
CYP7A1	5'-CCATGATGCAAAACCTCCAAT-3'	5'-ACCCAGACAGCGCTCTTTGA-3'	-do-
MIR33a (Probe sequence)		5′- GUGCAUUGUAGUUGCAUUG-3′	(Li, et al., 2013)

Table 2: Serum concentration of cholesterol derivatives

	Serum 7α-hydroxycholesterol levels (nmol/ml)	
Diet	**Ligated**	**Un-Ligated**
Regular Chow	0.8 ± 0.1	1.4 ± 0.3
Western Diet	1.2 ± 0.5	2.4 ± 0.7

DISCUSSION:

Homeostasis of dietary cholesterol in mammalian body is maintained by converting it into bile acids in liver. These bile acids are transported to intestine via hepatic portal system and perform key functions in the digestion of lipids and fats. It has long been known that the bile acids are involved in the formation of micelles within the intestine where they perform digestion of dietary fatty acids [8]. The first step in conversion of dietary cholesterol to bile acids is the addition of a hydroxyl group at the 7^{th} position. This 7-hydroxylation is mediated by cytochrome p-450 class enzyme 7α-hydroxylase (CYP7A1) and represents the major pathway for bile acid synthesis [9]. However, in case of any impairment in this pathway, a secondary pathway can also be activated which employs 27-hydroxylase and the hydroxyl group is added to the position 27 of side chain [10]. The CYP7A1 mediated bile synthesis pathway is under tight negative and positive feedback control. It has been observed that increased accumulation of bile acids inside liver as a consequence of any kind of bile duct obstruction, result in suppression in the synthesis of bile acids. Bertolotti, et al reported that in cholestasis patients, bile acid synthesis is markedly suppressed [11].

Our results indicate that in spite of the expected decreased production as well as increased accumulation of acids in the liver as a result of bile duct ligation expression of CYP7A1 increased significantly. Our results are consistent with the observation made by Bertolotti, et al in human cholestasis patients [12] in which it was observed that CYP7A1 expression increased in cholestatic patients in comparison with normal controls.

CONCLUSION:

It is concluded that in such times of impaired release of bile acids to intestine, secondary pathway may be activated. Ligated rats showed increased CYP7A1 levels while 27-hydroxylase in the serum of such rats was high as compared to non-ligated group.

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