A Voyage on the hormones and biomarkers related to Cholelithiasis associated Non Alcoholic Fatty Liver.

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Abstract

Gall stone disease (Cholelithiasis) is the most prevalent and chronic hepatobiliary disease affecting humans, especially fertile women. They represent a significant burden throughout the world and is the most common disorder leading to emergency. The prevalence of this disease varies by age, gender, region, lifestyle and dietary habits. The pathogenesis involves many factors and may arise from the complex interactions between genetic and environmental factors. The present review is focused on overall information about the etiology, risk factors, clinical manifestations, pathogenesis, role of diet, sedentary lifestyle, hormones and biomarkers in the development of stones in the gall bladder and the relation between cholelithiasis and Non Alcoholic Fatty Liver.

Keywords: Cholelithiasis, Gall bladder, Non alcoholic fatty liver, Pathogenesis, Hormones, Biomarkers.

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Introduction

Gallbladder is a thin walled pear shaped sac located just below the liver which concentrates and stores the bile. The formation of stones in the gallbladder is known as Cholelithiasis. This is the most prevalent gastro-intestinal disease, occurs as a result of impaired metabolism of cholesterol, bile acids, bilirubin in common bile duct or gallbladder [1]. These form when substances in bile reach their limits of solubility. As age increases, the risk of formation of stones also increases. Gallstones have various compositions and etiologies [2]. The Gallstones may further develop complications like cholangitis, cholecystitis, pancreatitis and cholangiocarcinoma [3].

Etiology

Cholelithiasis is a multifactorial disease. Major factors that benefits the formation of gallstones are:

- Cholesterol super saturation of bile
- Crystallization core formation
- Impairment in absorption, contraction, secretion of gallbladder
- Dysfunctioning of bile acids in enterohepatic circulation

Some causes of cholelithiasis include high fat diet, hormonal replacement therapy, heredity, medications like estrogen, progesterone, clofibrate, and somatostatin. The risk factors include female gender, pregnancy, obesity, genes, metabolic syndromes, quick weight loss, extended fasting, and Crohn's disease [4].

Signs and Symptoms: Many of the cholelithiatic patients don't show any symptoms, only 10% will exhibit symptoms like pain on upper right side of abdomen especially following fatty meals, loss of appetite, nausea , vomitings, jaundice, low grade fever, increased number of WBC, tea coloured urine and light c].

Gallstones: The bile containing high level of cholesterol gets concentrated, becomes hardened, crystalline and doesn't move into the intestine. This hardened deposits of digestive fluid in the gallbladder are called gallstones, made of cholesterol or bilirubin. Gallstones can vary in size and number and may or may not produce symptoms. Gallstones block bile duct and cause abdominal pain. The size ranges from a sand grain to a golf ball. The types include:

- Cholesterol stones
- Pigment stones
- Mixed stones

Cholesterol Stones: 50-90% of gallstones are of cholesterol type, [6] composed of pure cholesterol or a mixture of cholesterol and substance like mucin. The causes for cholesterol stones: Hyper secretion of cholesterol into bile: obesity, high calorie food, polyunsaturated fatty diet, contraceptives or pregnancy, DM, inherited hypercholesterolemia. Hypo secretion of bile acids: dysfunction in the synthesis of bile and extreme loss of bile salts. Impaired gallbladder with irregular stasis: in late pregnancy and by oral contraceptives, associated with neuro-endocrine dysfunction.

Pigment Stones: Accounts for 10%. Composed of bile pigments with minimum cholesterol, calcium [7] and Bilirubin as major constituent. Some are associated with bacterial infections which release glucuronidases; deconjugates bilirubin leading to precipitation of calcium salts and also due to deficiency of Cholecystokinin. These are of two types:

Black pigment stones: Most common type, formed in gallbladder, multiple, hard, made of calcium, bilirubin, phosphates and bicarbonates. Common in haemolytic disorders, cirrhosis.

Brown pigment stones: Less common, infrequently formed in gallbladder, developed in bile duct, associated with bile movement and infection due to E.coli.

Mixed Stones: Composed of cholesterol and carbonates, phosphates, palpitates of calcium

Pathophysiology

Gallstones are formed due to weakened relation between bile components, cholesterol, phospholipids and bile acids [8]. Saturation, crystallization and growth are the most important steps involved in the pathogenesis. Supersaturation [9] of bile with cholesterol is a mandatory step that contributes to GS formation. Pro-nucleating and anti-nucleating factors, functional status of gallbladder also plays a vital role.

Pathway in the pathogenesis of Cholelithiasis

Super saturation of Cholesterol: Generally, bile dissolves the cholesterol that is excreted by the liver. But, if more quantity of cholesterol is produced, excess will be precipitated forming crystals that get trapped in the mucus forming sludge. As time increases, the crystals may increase in size to form stones, blocks the ducts resulting Cholelithiasis [10].

Hyperbilirubinemia: Some hematological conditions cause the liver to produce excess bilirubin, which doesn't excrete, get blocked causing gallstones.

Hypo motility of Gallbladder: Impaired gallbladder contraction or motility concentrates the bile leading to gallstones.

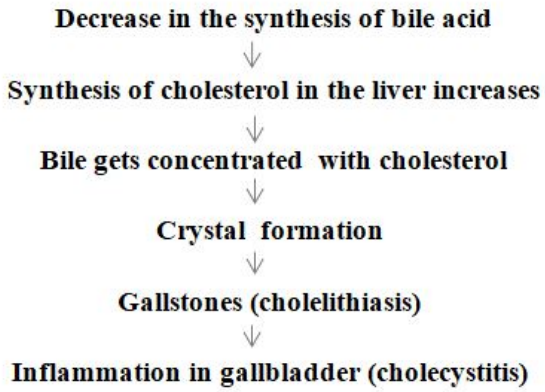

Figure 1. *Representing the pathophysiology of gallstone formation.*

Relation between NAFLD and Cholelithiasis

Non Alcoholic Fatty Liver is a chronic metabolic liver disorder [11] characterized by accumulation of triglycerides intracellularly without alcohol intake and ranges from Non Alcoholic Steato Hepatitis (NASH) to Cirrhosis. The association includes sharing of same risk factors like Insulin Resistance, obesity, Type 2 DM, dyslipidemia etc. Koller et al demonstrated that NAFLD is a predictor of Cholelithiasis [12]. The severity of the liver disease plays an important role in enhancing the prevalence and incidence of Cholelithiasis. The development of gallstones in NAFLD is more in females [13]. The prevalence increases progressively with severity of fibrosis, necrosis and inflammation of liver. Some of the biomarkers [14] like Interleukins, Tumour Necrosis Factor Alpha, Adiponectin and free fatty acids can relate both.

The pathogenesis of Gallstone Disorder is multifactorial intervening both the environmental as well as the genetic factors leading to the super saturation of bile, hypo motility of GB, precipitation of cholesterol microcrystals [15]Obesity is commonly associated with an increase in the biliary cholesterol secretion and changes in the hypo motility of GB. The presence of more concentration of triglycerides affects both composition of bile and GB emptying and diabetes affects the motor functions of GB. Moreover, the prevalence of bacterial growth in small intestine is more in NASH assessed by higher levels of TNF-α [16] Reduced fiber intake by NAFLD patients reduces the intestinal transit time and possibly increases biliary deoxycholic acid that is associated by supersaturation of bile and the formation of gallstones, a mechanism for NAFLD-GD association [17].

Insulin resistance is the strong contributor of developing gallstones in patients with NAFLD [18]. The aggregation of visceral fat has major role in the formation of stones in Gallbladder because the visceral adiposity enhances IR [19] and hyperinsuinemia [20] Cholecystokinin is the hormone needed for the normal and effective contraction of the gallbladder. During hyperinsuinemia, the response of gall bladder to cholecystokinin decreases. In fatty liver, the gall bladder dismotility can also leads to the development of gallstones [21]. The activation of the enzyme Hydroxy Methyl Glutaryl CoA Reductase will be promoted by IR, increasing cholesterol secretion [22].

Role of food habits and lifestyle in inducing NAFLD

NAFLD is associated with obesity. Energy intake during a meal is usually larger with outside food as it contains more fats [23]. The patients with NAFLD consume high calorie diets. The increased consumption of carbohydrates in the form of beverages increase the total energy intake. Soft drinks [24] are the leading source of added sugar and have been linked to NAFLD. Enlarged meal volume increases the energy intake, which results in obesity and NAFLD.

Fast foods, restaurant foods and fried foods [25] are the representatives of high energy dense diet. As per study, the fast food of 5753 K.cal for 4 weeks showed an increase in body weight and serum ALT levels resulting in obesity and NAFLD [26]. Inappropriate meal timings and eating manners like missing breakfast, eating too rapidly, eating too much at night etc. are often observed in patients with NAFLD and obesity [27]. Night workers and shift workers are recently shown to be at high risk of obesity, metabolic syndrome and fatty liver disorder [28]. Multiple pregnancies in women is also the main reason for Cholelithiasis.

Over ingestion of carbohydrates, lipids, [29] cholesterol and deficiency of polyunsaturated fatty acids, vitamins D and E can

also result in Insulin resistance, metabolic syndrome, obesity, NAFLD. Rats fed a Western Diet (high-fat/high fructose corn syrup) [30] with vitamin D depletion had significantly poor liver fat, lobular inflammation, NAFLD activity. The excess carbohydrates is also associated with steatosis and obesity, maybe due to the activation of sterol regulatory element binding protein-1c which enhances the enzymatic expression in the synthesis of fatty acids [31].

High fat, refined sugar and low fiber in the diet increases the risk of formation of gallstones. Sedentary habits, [32] stress, physical inactivity, high saturated fats and high waist hip ratio emerged as significant predictors in cholelithiasis. Lack of physical activity leads to an increase in weight gain which ultimately increase the cholesterol levels in bile.

Role of hormones

Thyroid hormones

Gender, dietary factors, obesity, pregnancy, Crohn's disease, gastric surgery, hereditary spherocytosis, sickle cell diseases and thalassemia may be considered to be the risk factors in developing gallstones. The pathophysiology involves a network of systems affecting both bile flow and content. In hypothyroidism, different agents may guide the formation of gallstones [33]. In hypothyroidism, decrease or absence of thyroxine:

- Decreases the rate of cholesterol metabolism [34] in liver leading to supersaturation of bile with cholesterol, weaken the gallbladder motility, contractility and filling, causes collection of cholesterol crystals, nucleation and growth of gallstones.
- Causes ruination in the clearance of precipitate due to decrease in bile secretion from hepatocytes [35].
- Relaxation of Sphincter of Oddi reduces leading to delayed bile flow, resulting in gallstones and accumulates in the ducts [36].

The association between the malfunctioning of thyroid gland and disruption in the metabolism of lipids [37] successively cause a change in the composition of the bile [38]. The sphincter of Oddi has thyroid hormone receptors and thyroxine has a direct prorelaxing. Impaired function of Sphincter of Oddi and low bile flow are considered to be the important functional mechanisms which attenuate the formation of gallstones [39]. In hypothyroid patients, decline in the secretion of bile, weakened cholesterol metabolism in liver and lowered sphincter of Oddi relaxation may contribute to the formation and/or accumulation of gallstones.

Effect of hypothyroidism: Hypothyroidism increases the risk for both gallbladder-originated and de novo Common Bile Duct stones. Due to the lack of thyroxine:

- Cholesterol metabolism [40] in the liver decreases resulting in the supersaturation of bile with cholesterol, which lessen motility, contractility and filling of the gallbladder, contributing to the preservation of cholesterol crystals, nucleation and growth of gallstones
- Abolishes discharge of bile from hepatocytes [41] resulting in diminished clearing of precipitates from bile ducts
- Diminishes Super Oxide relaxation [42] leading to delay in the bile flow and hence contributes to the genesis of stones.

In vertebrate tissues, Thyroid Hormones control different activities, explained by their interaction with the nuclear receptors, present in a tissue and developmental stage specific fashions [43]. The Sphincter of Oddi of expresses thyroid receptors TR β_1 and β_2. THs involve a transcriptional and also non genomic mechanism that involves extra nuclear sites [44]. Generally, thyroid hormones exert intracellular action that needs the transport across the plasma membrane with the help of thyroid hormone receptors. These are very active and specific in nature. Some of them are monocarboxylate transporter 8, MCT8, MCT10 and organic anion transporting polypeptide [45,46].

Estrogen and Progesterone

In women, pregnancy and parity are considered to be the major risk factors in the genesis of cholesterol gallstones. The increase in the levels of female sex hormones [47] during pregnancy may cause some changes in the metabolism of hepatobiliary system, favouring bile to get concentrated with cholesterol and attenuate cholelithogenesis [48] Estrogen promotes the cholesterol lithogenesis [49] by promoting the activities of estrogen receptors present in liver and gallbladder. Estrogen receptors like ERα and ERβ are present in liver cells, ERα [50] is a vital steroidal receptor generating the biological outcome of estrogen. 17 β-estradiol significantly increases the expression of ERα.

The mechanism of action of estrogen mediated biliary cholesterol involves a rate limiting step in the biosynthesis of cholesterol i.e., 3-hydroxyl-3-methylglutaryl coenzyme reductase [51] accompanied by an increase in the secretion of cholesterol into bile from hepatic de novo synthesis. The cholesterol from the diet also initiates the super saturation of bile, can be increased by estrogen.

In vertebrates and mice, the lipid homeostasis is maintained by a group of membrane bound transcription factors attributed to sterol regulatory element-binding proteins (SREBPs) [52]. The genomic sequence in mammals encodes for three different SREBP isoforms, SREBP-1a, 1c, 2. During normal levels of gene expression, SREBP-2 preferentially attenuates the biosynthesis of hepatic cholesterol that regulates release of cholesterol into bile containing in micelles and vesicles. Most of the SREBP-2-responsive genes in cholesterol biosynthesis pathway encodes for the enzymes: HMG-CoA reductase, HMG-CoA synthase, Farsenyl diphosphate synthase and lathosterol synthase [53]. The studies from the cultured fibroblasts and hepatocytes depict the mechanism of action of SREBP-2 genes. When these are in need of cholesterol, initiates a two-step proteolytic process which releases NH2-terminal domains of SREBP-2 creating an easy entry into the nucleus [54]. Then, it interacts with sterol regulatory elements in the promoter regions encoding for the enzymes. This binding causes transcription, resulting increase in the synthesis

of cholesterol and the supersaturation of bile that prone to precipitation of solid cholesterol crystals and leads to the genesis of gallstones.

Progesterone is a strong inhibitor of hepatic hepatic acyl-coenzyme A: cholesterol acyl transferases (ACAT) which decreases the synthesis of cholesteryl esters in liver, thereby allowing large quantity of free cholesterol into the intrahepatic cavity for bile secretion [55]. Gallbladder motility is also influenced by estrogen and progesterone [56]. Progestin's when administered without estrogen exhibit minute or no effect on kinetics or composition of bile acids. Progestin's inhibit gallbladder contraction, encourage bile stasis and decrease gall bladder's response to Cholecystokinin [57]. During pregnancy, the hormonal changes are responsible for the formation of gallstones. Gradually, the levels of cholesterol in bile increases from first to third trimesters accompanied by a continuous rise in the occurrence of biliary sludge (pre-cursor for gallstones) and finally form stones.

Insulin

After a meal or a hormone release like glucagon or catecholamine's, the pancreas will release insulin in response to the changes in glucose concentration [58]. Insulin strictly maintains plasma concentrations and glucose metabolism

- By increasing the uptake of glucose in skeletal muscle and liver (for glycogen storage), in adipose tissue (synthesis of triglycerides).
- By inhibiting glucose production in liver. On lipid metabolism, insulin favours the re-esterification of fatty acids into triglycerides in adipose tissue and liver, and inhibits peripheral lipolysis in adipose tissue.

In the presence of IR, [59] pancreas become energized to increase the secretion of insulin to control the deficiency in the absorption of glucose peripherally and to decrease production of glucose in the liver. Insulin contributes a crucial role in regulating the metabolism of cholesterol. IR decreases the absorption of cholesterol by intestines and promotes the synthesis of cholesterol [60] and VLDL [61]. Insulin inhibits CYP7 A1, which regulates the most important step in the synthesis of bile acid. Insulin Resistance is also responsible for the atypical movement of the gallbladder, then making prone to the formation of cholesterol gallstones [62]. The present approved pathogenic relationship between Insulin Resistance and cholesterol supersaturated bile and the formation of cholesterol stones is the increase in synthesis of cholesterol in the body and increased secretion of cholesterol in the bile, as found in obesity.

Mice with hepatic insulin resistance developed gall stone because of disruption of Insulin Receptors (LIRKO), thereby the output of biliary cholesterol and saturation are increased, as stated by [63]. All these animals developed gall stones whenever exposed to hyperlipidic diet compared to controls [64]. In this type of mouse models, an increase in ABCG5 and ABCG8 gene expression are responsible for inducing cholesterol gall stones. ABCG5 and ABCG8 are the sterol export pumps in canaliculi that work like obligate heterodimers favouring secretion of biliary cholesterol and deciding the discharge of cholesterol from hepatocytes into the bile [65].

Biomarkers of Cholelithiasis

Serum Adiponectin

The main cause of cholesterol gallstones is the abnormal metabolism of cholesterol [66] and pigment gallstones is due to the bacterial degradation and also by mechanical obstruction in the biliary tract. Changes in the adiponectin levels are correlated with metabolic deviations and low levels are linked to different types of diseases like cancers, obesity. Wang et al., investigated the relation between adipokines and gallstones. The blood samples collected from controls and from cholelithiatic patients before cholecystectomy were radio immune assayed to measure serum adiponectin levels. The adiponectin [67] levels were noticeably high in the patients of pigment gallstones and remarkably low in cholesterol gall patients. Based on multiple investigations, age and high levels of adiponectin; female sex hormones and low levels of adiponectin were considered as predictors of pigment and cholesterol gallstones respectively. The gallstone formation is favoured by adiponectin and hence regarded as a biomarker for both types of gallstones.

Markers of inflammation and Cytokines

TNF-alpha, Interleukins, chemokine CC-chemokine ligand-2 and high sensitivity C reactive protein (hs-CRP) [68] are included under chemokine's and inflammatory cytokines. The down regulation of hepatocellular insulin receptors is possible by suppression of cytokine signalling-3, encouraging the acquired hepatic insulin resistance [69].

Interleukin-1 (α, β), IL-4, IL-6 and Tumour Necrosis Factor α (TNF-α) are essentially produced from the monocytes and macrophages, exhibit a great diversity of cytokines during immune reaction and inflammation, act as pro-inflammatory proteins. TNF-α acts by energizing expression of different inflammatory mediators along with IL-6 that influences the functions like absorption, secretion and contraction of smooth muscle in gallbladder. IL-1α [70] present intracellularly, functions like both cytokine and transcription factor. IL-4 deficiency also causes gallstones by increasing the serum concentrations of bilirubin [71]. The trans-signalling activity of IL-6 is of utmost important in maintaining disease condition by encouraging transition from acute to chronic inflammation. Lipopolysaccharides provoke the secretion of IL-1 which causes inflammation in gallbladder. IL-1α, TNF-α were found to affect the functions of gall bladder epithelial cells like absorption and secretion that favours the development of gallstones.

High calorie diet was found to increase the activity of myeloperoxidase, IL-1 and thickness of mucosa. In a mouse model of cholelithiasis, along with obesity, [72] more intake of purified carbohydrates was observed to induce infiltration of fats in the walls of gall bladder and amplify the production of

IL-1 and TNF-α. Obesity alone can increases the free fatty acids and IL-6 [73] in gallbladder. The serum collected from cholelithiatic patients has high concentration of IL-1, IL-6, TNF-α [74] that reflects inflammatory phase. Inflammation in gallbladder is an initial sign of gallstone formation. Inflammatory cytokines released in the gallbladder also initiate induction of NO Synthase, increased production of Nitric Oxide and induce damage in DNA in normal epithelial cells.

Cholecystokinin

Cholecystokinin (CCK) [75] takes utmost role in favouring the process of digestion in small intestine. Cholecystokinin is produced by the epithelial cells in the mucosa of small intestine and facilitates the discharge of the digestive enzymes from pancreas and bile from the gall bladder into the small intestine. The rise in the concentration of CCK has two important roles which favours digestion [76].

Secretion of enzymes from the pancreas into duodenum that encourage the digestion. (cholecystokinin also called pancreozymin). Contracts gallbladder, moving bile into duodenum (cholecystokinin – to move the gallbladder)

Once absorption completes, the cholecystokinin secretion ceases. Cholecystokinin makeup most of the hormonal stimulus for emptying of gallbladder after meals. CCK exhibits the contractile effect mainly by interacting with receptors present on the smooth muscle cells of gallbladder directly and by interacting with cholinergic nerves. CCK can increase the nicotinic ganglionic transmission in the serosal layer by releasing Acetylcholine.

CCK interacts with CCKA [77] receptors and contracts the muscular strips of the gallbladder with a potency of 1000 times more than Gastrin. The human CCKA [78] receptor has N-linked complex carbohydrates and also a protein core but don't have sialic acids. The receptor binding region might be present in the protein core. CCK and CCKA gall bladder receptor are the main governors of post prandial gall bladder emptying.

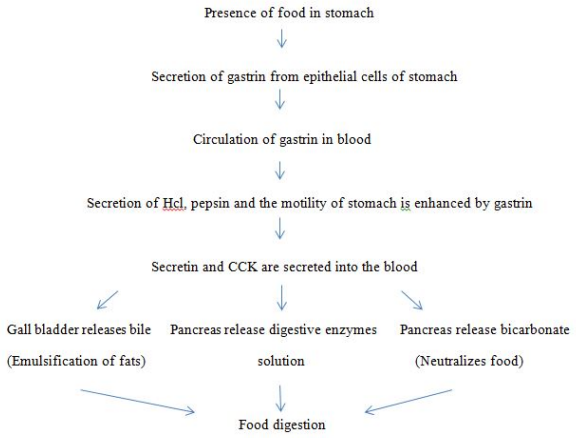

Figure 2. *Representing the actions of Cholecystokinin which is released along with Secretin into the blood. Cholecystokinin enters both the pancreas and gall bladder to facilitate the process of digestion.*

Transforming Growth Factor-β

TGF-β controls growth and differentiation in different cells and tissues, has an important role in diseases. They may exhibit a dual role in the tumour suppression and cancer metastasis [79]. These will function through TGF-β receptors. Epigenetic or genetic modification, leads to change in TFG-β signaling axis [80,81]. The rise in expression of TGF-β receptors I and II in the gallbladder was correlated with chronic inflammatory stages of gallbladder disease.

TGF-β is a versatile cytokine. $TGF\beta_1$ located in biliary epithelial cells, capable of reducing alloantigen-specific immune reactions [82]. $TGF\beta_1$ can also induces the maturation of epithelial cells [83] and controls the transitional events like epithelial-mesenchymal transition and is up-regulated in the patients with cholelithiasis compared to cholecystectomised patients without gallstones [84-86]. Upon exposure to Lipopolysaccharide bacteria, [87] the production of TGF- β from Gallbladder Epithelial Cells increases. The rise in the levels of TGF- β comes up with fibrosis in gallbladder and advancement of disease.

Discussion

Gallstones form due to supersaturation of cholesterol with bile [88] Cholelithiasis is considered to be the most common gastrointestinal disease worldwide [89]. The pathogenesis reveals that GD is a multifactorial disease. The symptoms remain unclear but some of the manifestations were included. Fertile women are at more risk when compared to non-fertile women and men [90]. The association existed between NAFLD and Cholelithiasis shares the common risk factors and also some of the biomarkers. The role of diet, life style and sedentary habits play an important role in inducing the NAFLD. The endocrine hormones like thyroid hormone, insulin, estrogen and progesterone influences the formation of gallstones. There is lack of information regarding the biomarkers involving in the formation of gallstones, however, a series of biomarkers including Serum Adiponectin, Cytokines and inflammatory mediators like Interleukins [91], TNF-α, TGF-β mark the risk of producing gall stones have been detailed.

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