Study of Changes in Cholesterol 7 Alpha Hydroxylase in Patients with Gall Stone in Babylon Province

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Abstract

The gallbladder (GB) is an assistant organ of the digestive system, It is located under the liver and attached to the biliary system, And it is responsible for controlling release of bile and the storage. Gallstone disease a major health problem worldwide. In 10–15% of adults contain gallstone disease**.** This study (case control) consists of one hundred sixty (160) patients are included in this study randomly selected and consisted of 130 females and 30 males. All patients are examined 24 hr. Before operation, Intra operatively and 24hr post operatively**.** Decreased gallbladder motility and the decrease of cholesterol 7 –alpha hydroxylase activity, which regulate the novo bile salt synthesis. The rate-limiting step of bile acid synthesis is acting between the liver-specific CYP 7A1 (CYP450). Thus, Alteration in CYP gene control also has an impact on bile acid synthesis and lipid metabolism**.**

Keyword: gallbladder (GB)**,** Gallstone disease (GD), CYP 7A1

الخلاصة

المرارة (GB) هو الجهاز المساعد من الجهاز الهضمي، وهي تقع تحت الكبد وتعلق على نظام الصفراوي، وأنها مسؤولة عن التحكم في إطلاق الصفراء والتخزين .مرض حصوة مشكلة صحية كبيرة في جميع أنحاء العالم .في15–10 ٪ من البالغين احتواء مرض حصوة .هذه الدراسة) مراقبة حالة (ويتكون من (160) 160 وشملت المرضى في هذه الدراسة تم اختيارها عشوائيا، ويتألف من 130من الإناث و 30 من الذكور .ويتم فحص جميع المرضى 24 ساعة .قبل العملية، داخل الجراحة وبعد الجراحة .24hr انخفاض الحركة المرارة وانخفاض الكولسترول alpha– 7 النشاط هيدروكسيلاز، التي تنظم نوفو توليف الصفراء الملح .والحد من معدل خطوة من تركيب الحامض المراري يتصرف بين CYP 7A1 الكبد معين .(CYP450) وهكذا، والتعديلات في السيطرة الجينات CYP أيضا له تأثير على تركيب الحامض المراري والتمثيل الغذائي للدهون.

الكلمات المفتاحية: المرارة(GB) ، مرض الحصوة(GD)، CYP 7A1 .

Introduction

The gallbladder (GB) is an assistant organ of the digestive system, It is located under the liver and attached to the biliary system, And it is responsible for controlling release of bile and the storage (Kennth *et al.*, 2014). Gallstone disease a major health problem worldwide (Abeysuriya *et al.*, 2010). In world 10–15% of adults contain gallstone disease (Ansaloni, 2016)). cholelithiasis is referred to present of stones in the gallbladder (James *et al.*, 2011). Cholelithiasis or Gallstone disease (GD), is one of the general gastrointestinal diseases (Sun *et al.*, 2009). A gallstone is a stone consist of in the gallbladder out of bile components (Stinton *et al.*, 2012). Most people with gallstones (about 80%) don't have symptoms (Lee *et al.*, 2015). Occurs each year in 1–4% of those with gallstones, known as biliary colic, Inflammation of the pancreas, gallbladder and liver consider the complications of gallstones (3). Gallstones may be suspected based on signs (3). Diabetes, contraceptive pill, pregnancy, a family history of gallstones, obesity, or rapid weight loss are consider risk factors for gallstones. It is asymptomatic, even for years. These gallstones do not

require treatment are called "silent stones" (Bethesda *et al.,* 2007; Heuman *et al.,* 2010; https://en.wikipedia.org/wiki/Gallstone_-_cite_note-Acalovschi2003-10). Other complications; gallstones may the gallbladder into adherent abdomen potentially, in cases of chronic inflammation, causing an obstruction termed gallstone ileus (Acalovschi *et al.,* 2003). There may be several reasons of Gallstones, including: weight, genes, Problems with gall bladder or Diet (Derrer *et al.,* 2016). Gallstone is classified as cholesterol, pigment and mixed stones, Based on their chemical composition (Grunhage *et al.,* 2006; Gurusamy *et al.,* 2007; Sikkandar *et al.,* 2011). Deoxycholic acid did not prevent HMG CoA reductase action (Williams *et al.,* 2008).

Aims of the study

1. To investigate the association between serum bile acid and cholesterol 7-alpha hydroxylase and the development of gallstone disease.
2. Evaluate the serum insulin changes and its effect to gallstone formation.
3. The determination of serum superoxide dismutase changes in patients with gallstone.

Patient and Materials

After approval of the study by the ethical committee of college of medicine and consent of patients an explained to them. This prospective study is carried out in Al-Hilla general teaching hospital lasted from November (2015) to May (2016). This study (case control) consists of one hundred sixty (160) patients are included in this study randomly selected and consisted of 130 females and 30 males. All patients are examined 24hr. before operation, Intra operatively and 24hr post operatively. Their ages are between 20-70 years for females and as well as 20-70 years for male patients. Those patients are diagnosed by specialist doctors as uncomplicated, symptomatic gallstones (cholelithiasis), who underwent elective laparoscopic cholecystectomy.

3.2.4.1:Measurement of CYP 7A1 level (Catalog No: E-EL-H208)

Principle:

This ELISA kit uses Sandwich-ELISA as the method. Only those wells that contain CYP7A1, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to CYP7A1.The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. Then a biotinylated detection antibody specific for CYP7A1 and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate successively and incubated. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Free components are washed away. The OD value is proportional to the concentration of CYP7A1. The substrate solution is added to each well. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. You can calculate the concentration of CYP7A1 in the samples by comparing the OD of the samples to the standard curve.

Assay procedure Centrifuge the sample again after thawing before the assay. Bring all reagents and samples to room temperature before use. **Avoid foaming.** It's recommended that all samples and standards be assayed in duplicate. **All the reagents should be mixed thoroughly by gently swirling before pipette.**

1. **Add Sample:** Add 100µL of Standard, Blank, or Sample per. The blank well is added with Reference Standard & Sample diluent. Solutions are added to the

bottom of the micro ELISA plate well. Cover the plate with sealer we provided. Incubate for 90 minutes at 37℃. A void inside wall touching and foaming as possible. Mix it gently

2. **Biotinylated Detection Ab:** don't wash, Remove the liquid of each. Immediately add 100μL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37°C.
3. **Wash:** Wash by filling each with Wash Buffer (approximately 350μL) (a squirt bottle, Complete removal of liquid at each step is essential. After the last wash, manifold dispenser, multi-channel pipette, or automated washer are needed). Aspirate each well and wash, repeating the process three times.
4. **HRP Conjugate:** Incubate for 30 minutes at 37°C., Add 100μL of HRP Conjugate working solution to each well.
5. **Wash:** Repeat the wash process about five times as conducted in step 3.
6. **Substrate:** Add 90μL of Substrate Solution to each well. Incubate for about 15 minutes at 37°C. Protect the plate from light. When apparent gradient appeared in standard wells, Cover with a new Plate sealer. The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes.
7. **Stop**: The order to add stop solution should be the same as the substrate solution. Add 50μLof Stop Solution to each well. Then, the color turns to yellow immediately.
8. **OD Measurement**: Determine the optical density (OD value) of each preheat the instrument, well at once and set the testing parameters. Using a micro-plate reader at 450 nm.
9. After experimenting, until their expiry. Put all the unused reagents back into the refrigerator according to the specified storage temperature respectively.

Important note: 1. **ELISA Plate**: The just opened ELISA Plate may appear water-like substance.

2. **Add Sample**: The interval of sample adding between the first well and the last well should not be too long, which will significantly affect the experiment's accuracy and repeatability, otherwise will cause different pre-incubation time.
3. **Incubation**: proper adhesion of plate sealers during incubation steps is necessary, To prevent evaporation and ensure accurate results. Do not let the strips dry at any time during the assay.
4. **Washing**: Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against the absorbent paper in the washing process. The wash procedure is critical.
5. **Reagent Preparation**: As the volume of Concentrated Biotinylated Detection Ab and Concentrated HRP Conjugate is very small, To ensure that pipette are calibrated is minimize imprecision caused by pipette. Liquid may adhere to the tube wall or the tube cap when being transported, It is recommended to suck more than 10μL for once pipette. Please pipette the solution for 4-5 times before pipette.. Do not reuse the standard solution, working solution of Detection Ab and HRP Conjugate, which have been diluted, Please carefully reconstitute Standards, working solutions of Detection Ab and HRP Conjugate according to the instructions.
6. **Reaction Time Control:** Please mange reaction time strictly following this product description.
7. **Substrate:** Please protect it from light, Substrate Solution is easily contaminated.

8. **Stop Solution:** As it is an acid solution, please pay attention to the protection of your face, eyes, hands, and clothes when using this solution.
9. **Mixing:** You'd better use micro-oscillator at the lowest frequency, you can knock the ELISA plate frame gently with your finger before reaction., as sufficient and gentle mixing is particularly important to reaction result.
10. **Security:** please perform, Please wear lab coats and latex gloves.
11. Washing buffer and stop solution can be an exception) (Do not use components from different batches of kit).
12. To avoid cross-contamination, between reagent adding and sample adding, change pipette tips, and between adding of each standard level. Also, use separate reservoirs for each reagent.

Result

4.3.1. Cholesterol 7-alpha hydroxylase (CYP 450)

Table (4-8) The cytochrome p450 (CYP 450) level in both patients and control groups. It is highly significant lower in patient groups when compared with control groups (P<0.01).

Table (4-8): The cytochrome p450 (CYP 450) level in both patients and control group.

Parameter	Group	mean± SD.	P. Value
Cholesterol 7-alpha hydroxylase (µM)	Patients	15.68±2.69	**0.001****
	Control	21.87±1.63	

**** highly significant differences at P<0.01**

Discussion

Cholesterol 7-alpha hydroxylase (CYP 450)

Table (4-8) shows the cytochrome p450 (CYP 450) level in both patients and control groups. It is highly significant lower in patient groups when compared with control groups (P<0.01). And these results are agreeing with another study done by (G.Schulthess et al., 2000).

Decreased gallbladder motility and the decrease of cholesterol 7 –alpha hydroxylase activity, which regulate de novo bile salt synthesis (Einarsson *et al.,* 1985). Additionally to this central role of hepatic CYPs in the handling of xenobiotics, the rate-limiting step of bile acid synthesis is acting between the liver-specific CYP 7A1 (CYP450). Thus, Alteration in CYP gene control also has an impact on lipid metabolism and bile acid synthesis (Aleksunes *et al.,* 2012). CYP7A1 and HMGR expression levels were high, resulting in increase synthesis of hepatic cholesterol and bile acids, Also HDL metabolism was impaired (Akiyama *et al.,* 2000; Shih *et al.,* 2001). In contrast, given of fibrates to humans does inhibit CYP7A1 expression, resulting in an elevated risk for gallstone formation (Stahlberg *et al.,* 1995; Caroli –Bosc *et al.,* 2001).

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