Journal of Cardiovascular Disease Research

ISSN: 0975-3583, 0976-2833 || VOL 16, ISSUE 03, 2025

COMPARATIVE ANALYSIS OF BILIARY CHOLESTEROL LEVELS IN IRON DEFICIENT AND NON-IRON DEFICIENT PATIENTS OPERATED FOR GALL STONE DISEASE

Dr. Sreethar[1] , Dr. Richard Samuel [2]

1,Professor, Department of GeneralSurgery, Sree Mookambika Institute of Medical Sciences Kanyakumari, Tamil Nadu, India.

2.Junior Resident, Department of General Surgery Sree Mookambika Institute of Medical Sciences College Kanyakumari, Tamil Nadu, India.

Corresponding Author: Dr.Richerd Samuel ,Junior Resident, Department of General Surgery ,Sree Mookambika Institute of Medical Sciences College Kanyakumari, Tamil Nadu, India.

ABSTRACT :

Background: Gall Stone disease is a common clinical entity affecting adult population of both sexes. The old saying, that gall stone sufferer is a fat, fertile, female of fifty is only partly true, as the disease has been observed in women after their first delivery and also in underweight and thin people. Several etiological factors have been studied in the formation of gall stones. There has been recent interest in establishing the role of several nutritional trace elements in the pathogenesis of gall stone disease.Iron Deficiency is a new and particularly interesting parameter which has been studied lately, but with a few studies showing conflicting results. Establishing the role of Serum Iron in the etiology of Gall stones is of special importance in our population group because of the huge prevalence of nutritional deficiencies. It also provides scope for early detection, treatment and risk modification if its role as an etiologic/risk factor is clearly defined.

Methods: In this study was done between December 2022 to September 2023 at the department of General Surgery.50 patients suffering from Cholelithiasis, confirmed by USG, were divided into two groups based on serum iron values. Group A, consists of patients with normal serum iron (non-anaemic) and group B, of patients with less than normal serum iron (anaemic). Serum Iron, Biliary Cholesterol and Serum Cholesterol of all the patients was obtained. The Biliary cholesterol levels and Serum cholesterol levels of both the groups was analysed by using a student t-test.

Results: Out of the 50 Patients, 40 (80%) were female and 10 (20%) were males. The female to male ratio was 4:1. The Biliary Cholesterol values for Group A and B respectively were 754.5 ± 398.3 and 1184.7 ± 405.2 mg/dl. The Biliary Cholesterol levels were significantly higher in the Iron Deficient group (Group B) than compared to the Non-Iron Deficient Group. This result was extremely statistically significant with a p value of <0.0004. Similarly, an independent t-test comparing Serum Cholesterol levels in

ISSN: 0975-3583, 0976-2833 || VOL 16, ISSUE 03, 2025

Non-Iron Deficient (184.8 ± 35 mg/dl) and Iron Deficient subjects (171 ± 49.3 mg/dl) did not find a statistically significant difference. (p=0.2544)

Conclusion: These results suggest that Iron Deficiency has an association with Biliary Cholesterol Levels.

Keywords: Biliary Cholesterol, Iron Deficiency, Gall Stone Disease

INTRODUCTION:

Gall Stone disease is a common clinical entity affecting adult population of both sexes. The old saying, that gall stone sufferer is a fat, fertile, female of fifty is only partly true, as the disease has been observed in women after their first delivery and also in underweight and thin people[1]. Several etiological factors have been studied in the formation of gall stones. There has been recent interest in establishing the role of several nutritional trace elements in the pathogenesis of gall stone disease.[2]
Cholesterol gallstones occur most commonly in multiparous women, but the causes for this phenomenon remain unclear[3]. This same patient population is prone to chronic iron deficiency anemia. With this as the background, Iron Deficiency is a new and particularly interesting parameter which has been studied lately, but with a few studies showing conflicting results. In experimental data from adult prairie dogs,[4] Iron deficiency has been shown to alter the activity of several hepatic enzymes, leading to increased gall bladder bile cholesterol saturation and promotion of cholesterol crystal formation. Iron acts as a coenzyme for nitric oxide synthetase (NOS), and that is important for the maintenance of basal gall bladder tone and normal relaxation[5]. It was found that iron deficiency resulted in altered motility of gall and sphincter of oddi (SO), leading to biliary stasis and thus increased cholesterol crystal formation in the gall bladder bile.[6]

Therefore, we tested the hypotheses that iron deficiency would alter hepatic cholesterol metabolism causing increased biliary cholesterol saturation and hence enhance gallstone formation. Establishing the role of Serum Iron in the etiology of Gall stones is of special importance in our population group because of the huge prevalence of nutritional deficiencies. It also provides scope for early detection, treatment and risk modification if its role as an etiologic/risk factor is clearly defined.
The present study was conducted on the randomly selected individuals of the South Indian Population, suffering from gall stone formation, to study the role of iron deficiency anaemia in gall stone formation.

AIM AND OBJECTIVES OF THE STUDY:

The study is aimed at:

ISSN: 0975-3583, 0976-2833 || VOL 16, ISSUE 03, 2025

- Determining the association of iron deficiency in the super saturation of bile with respect to cholesterol.
- Determining the association of iron deficiency with serum cholesterol levels.
- Sex and Parity specific distributions in the serum iron levels and their correlation with biliary cholesterol levels

MATERIALS AND METHODS:

50 patients suffering from Cholelithiasis, confirmed by USG, were divided into two groups based on serum iron values. Group A, consists of patients with normal serum iron (non-anaemic) and group B, of patients with less than normal serum iron (anaemic). Gall bladder Bile cholesterol and serum cholesterol of both the groups are compared.The study was conducted over a period of 10 months at department general surgery, from December 2022- September 2023. The study protocol was approved by the ethical committee of our institute. It was a retrospective analysis. The patients were selected, based only on the USG confirmation of their gall stones, irrespective of their age, sex, physique, parity, etc. Only those patients were included, whose serum as well as bile could be procured for analysis. Patients with empyema and mucocele of gall bladder were excluded.

All the patients, who were included in the study were given a serial number 1 to 50, in the order of their admission to the surgery department for Cholecystectomy. Thus their bile and serum samples were also labeled 1 to 50 accordingly. The numbered samples were sent to the Biochemistry department for analysis. All the numbered samples with less than normal serum iron (n=23) were put in the anaemic group, B and all the samples with normal serum iron (n=27) were put in the non anaemic group, Group A.Serum iron was estimated by Ferrozine kit method for determination of iron. The normal reference values supplied with the kit, for males (60-160 μg/dl) and for females (35-145 g/dl), were used to label the patients as anaemic and non- anaemic i.e. males with serum iron < 60 g/dl and females with serum iron <35 μg/dl were labeled as anaemic. During the operation for open cholecystectomy, bile was aspirated with an aspiration needle mounted on a sterilized syringe. The aspiration needle was passed obliquely into the fundus of gall bladder and as much of bile as possible, was withdrawn from the gall bladder. Similarly during laparoscopic cholecystectomy, bile was aspirated under vision through a long venflon needle or a veress needle just before delivering out the gallbladder at the port site.

Bile was kept in a sterile labeled container and sent for analysis. Serum cholesterol and gall bladder bile cholesterol of all the patients were estimated. Bile was first subjected to the Folch method to extract lipids and then the cholesterol contents were estimated as for serum cholesterol. In the Folch method, lipids from bile were extracted by using water, Methanol and Chloroform mixture in the ratio of 3:4:8 v/v and from the extracted lipids, cholesterol was estimated by Enzopak kit, based on the cholesterol oxidase/peroxidase

ISSN: 0975-3583, 0976-2833 || VOL 16, ISSUE 03, 2025

method. The enzymes used only the cholesterol as substrate and hence Bilirubin is automatically eliminated, from the procedure of cholesterol estimation.

Statistical analysis was done using the statistical package for social sciences (SPSS).Different statistical methods were used as appropriate. Mean ± SD was determined for quantitative data and frequency for categorical variables. The independent t- test was performed on all continuous variables. The normal distribution data was checked before any t-test. The Chi-Square test was used to analyze group difference for categorical variables. In logistic regression models, age was adjusted for estimation of each or all the independent effects of hypertension, ischemic heart disease and diabetes mellitus . A p- value < 0.05 was considered significant.

RESULTS:

During the 10 month period from December 2022- September 2023, a comparative study of Biliary Cholesterol levels was done between 2 groups, divided based on Serum Iron levels among 50 consecutive patients admitted to the Department of Surgery, at sree mookambika medical college hospital with a diagnosis of Symptomatic Gallstone Disease who underwent Cholecystectomy. The results are as follows:
Out of the 50 Patients, 40 (80%) were female and 10 (20%) were males.

- The Male to Female Ratio was 1:4.
- In the present study the minimum age was 17 yrs and the maximum age was 68 yrs.
- The number of patients was highest in the age group of 40-50 yrs having 14 patients (28%) followed by 30-40 yrs having 13 patients (26%). The least was in the age group 60-70 and 0-20 yrs having 2 patients each (4%).
- Mean age was 40.6 yrs. Standard Deviation 12.1 yrs
- Median Age was 39.5 yrs.
- The Majority of Patients Presented with all the 3 symptoms of cholelithiasis—31(62%) of the 50.

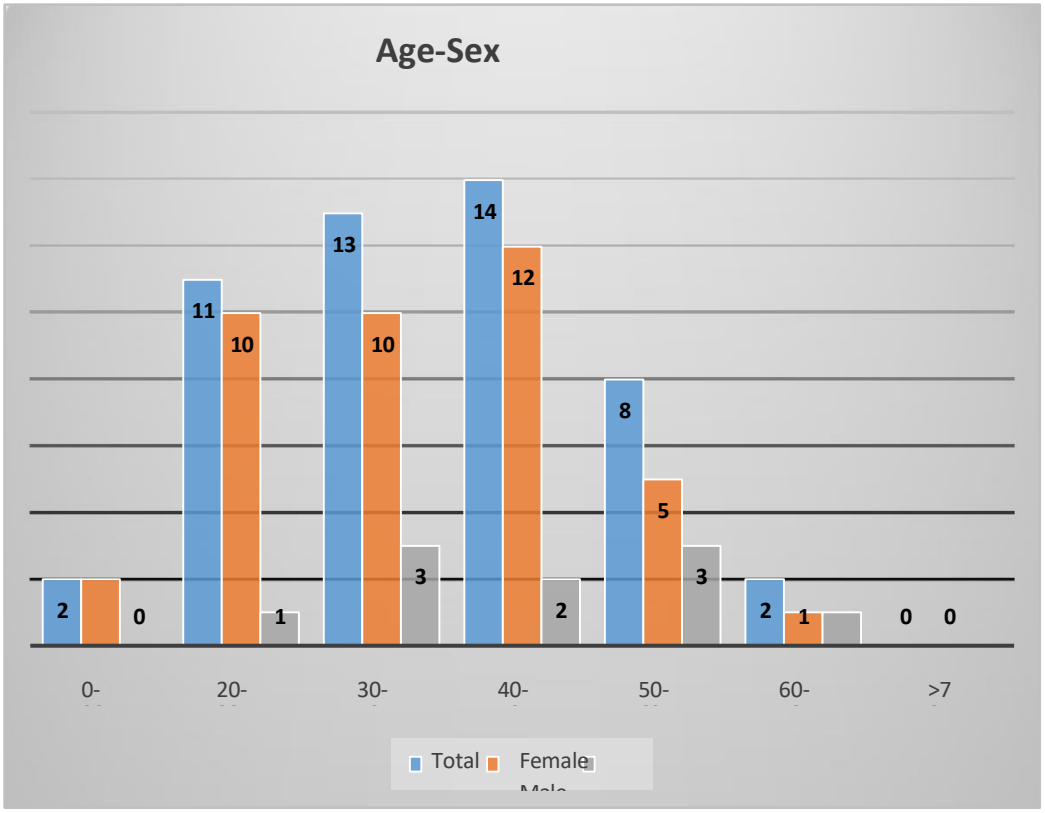
Combined Age and Sex distributions:

Age Range	Total	Females	Males
<20 yrs	2	2	0
20-30 yrs	11	10	1

ISSN: 0975-3583, 0976-2833 || VOL 16, ISSUE 03, 2025

30-40 yrs	13	10	3
40-50 yrs	14	12	2
50-60 yrs	8	5	3
60-70 yrs	2	1	1
>70 yrs	0	0	0

Combined Age and Sex Distributions:

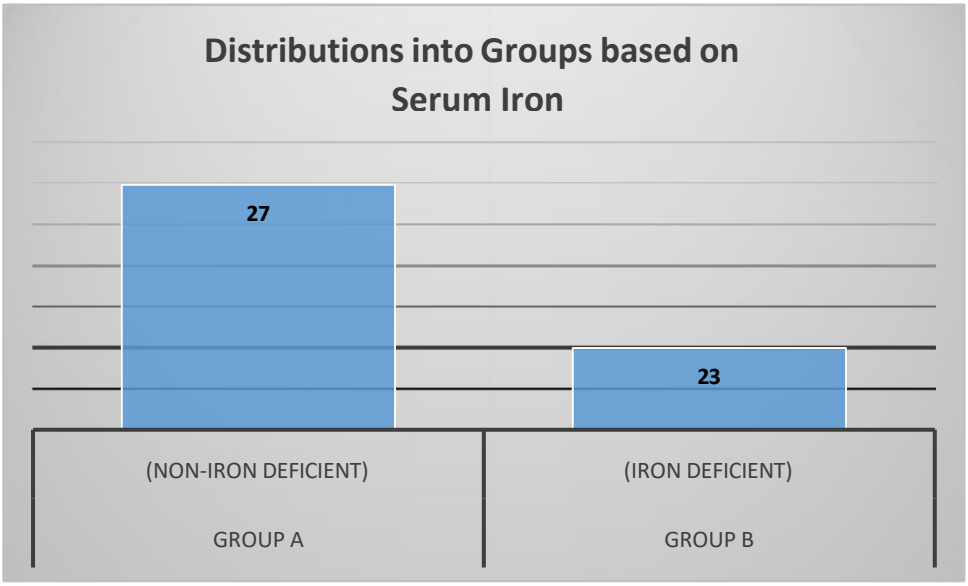


Distribution of patients into Group A and Group B:

ISSN: 0975-3583, 0976-2833 || VOL 16, ISSUE 03, 2025

Group A (Non-Iron Deficient)	Group B (Iron Deficient)
27	23

Distribution of patients into Group A and Group B:



Serum Iron Contents in Group A & Group B Patients

Group	No. of Patients	Serum Iron Range µg/dl	Serum Iron Mean ±S.D
A (Non-Iron Deficient)	27	36-163	93.2 ± 34.6
B (Iron deficient)	23	7-40	26.8 ± 8.6
P-Value			<0.0001

ISSN: 0975-3583, 0976-2833 || VOL 16, ISSUE 03, 2025

Serum Cholesterol Levels in Group A & Group B Patients

Group	No. of Patients	Serum Cholesterol Range mg/dl	Serum Cholesterol Mean ±S.D
A (Non-Iron Deficient)	27	93-254	184.8 ± 35
B (Iron deficient)	23	79-270	171 ± 49.3
P-Value			0.2544

Serum Iron, Biliary Cholesterol and Serum Cholesterol Levels with P- Values after T- Test Analysis

Groups	Serum Iron (µg/dl)	Biliary Cholesterol (mg/dl)	Serum Cholesterol (mg/dl)
A=27 (Non- Iron Deficient)	93.2 ± 34.6	754.5 ± 398.3	184.8 ± 35
B=23 (Iron Deficient)	26.8 ± 8.6	1184.7 ± 405.2	171 ± 49.3
P-Value	<0.0001 ***Extremely Significant***	<0.0004 ***Extremely Significant***	0.2544 ***Not Significant***

BILIARY CHOLESTEROL LEVELS:

Group	Group A (Non- Iron Deficient)	Group B (Iron Deficient)

ISSN: 0975-3583, 0976-2833 || VOL 16, ISSUE 03, 2025

Mean Biliary Cholesterol	754.500	1184.700
SD	398.300	405.200
SEM	76.653	84.490
N	27	23

SERUM CHOLESTEROL LEVELS:

Group	**Group A (Non- Iron Deficient)**	**Group Two (Iron Deficient)**
Mean Serum Cholesterol	184.800	171.000
SD	35.000	49.300
SEM	6.736	10.280
N	27	23

DISCUSSION:

Cholelithiasis is a very common condition encountered in surgical practice. Though most cases may be asymptomatic, a definitive percentage of these patients may develop symptoms of Gall Stone disease. A few will develop complications that may even be life threatening.

Over the past century the mortality and morbidity related to Gall Stone Disease has decreased due to the early recognition and treatment of symptomatic cholelithiasis. By and large, surgical management has been the treatment of choice, and with the advent of laparoscopy, Laparoscopic cholecystectomy has replaced Open Cholecystectomy as the Gold Standard in the surgical management of uncomplicated gall stone disease.

There have been numerous studies done to assess the risk factors for Cholelithiasis. These have been discussed earlier in this study. Also its of paramount importance to identify easily modifiable risk factors as these can help in risk stratification and also prevention.

The exact mechanism of Gall Stone formation is a complex process that is not solely

ISSN: 0975-3583, 0976-2833 || VOL 16, ISSUE 03, 2025

dependent on any single factor. Thus Gall stone disease has a multi factorial etiology. Newer risk factors/ etiological agents have been studied time and again, with some showing to have a significant impact on the disease process.

As stated already, animal studies had suggested that Iron may have a role to play in the pathophysiology of gall stone disease. More importantly the finding that iron deficient mammals were more prone to develop gall stones, has sparked interest in its probable role in humans. Also the classical teaching is that a females, in her forties, obese and multiparous is more prone for gall stone disease. When this is viewed, keeping the recent findings in perspective, it may suggest that apart from the other risk factors in this group, these are also the patients who are most iron deficient. Hence, it makes perfect sense, more so in the Indian population, to define the role of Iron or its deficiency in the pathophysiology of gall stone disease.

As data from other studies suggest, Iron deficiency changes the activity of many liver enzymes. This may be a factor in promoting cholesterol supersaturation and increased cholesterol crystal nucleation. Also Iron acts as a coenzyme for nitric oxide synthase. Nitric oxide helps in the normal gall bladder tone and relaxation. An alteration in this fine balance may promote gallbladder stasis which results in cholesterol stone formation.

The current study was done in a randomly selected South Indian Population who presented to our hospital with symptomatic gall stone disease.

Of the 50 patients with gall stone disease, 40 were females (80%) and 10 were males(20%). This is consistent with the known sex prevalence, that gall stone disease is more common in females than males,The main sufferers of gall stone disease in our study were females as compared to males. The female to male ratio was 4:1. This was similar to that observed in his study by Zuhair R, Ganey et al.[7] and Major Alok Sharma et al, series . In contrast, this was higher than that observed by Frazee et al[8],U.Berggren et al[9] and the Battacharyaseries.This is higher than that reported by most western studies indicate the female to male ratio as between 3:1 to 2:1,The reason for high incidence in females could be that pregnancy and child birth have a direct influence on biliary tract disease, acting by bile stasis , weight gain and consequently hypercholesteremia,The maximum number of patients-14 (28%) of the 50- were in the age group of 40-50 yrs followed by 30-40 yrs having 13 patients (26%). The Mean age was 40.6 yrs SD-12.1 yrs,Similar incidence is seen in the studies of Herman et al and Hanif series showed peek incidence in 5th decade,In western studies the peak incidence is in the 5th and 6th decades,Similar findings are noted in the studies of Ganey et al[7] and Moreaux et al[10].

The non-Iron deficient group (Group A) had an above average value of93.2 ± 34.6 micro gm/dl as compared to the Iron deficient group (Group B) which had a value of 26.8 ± 8.6 micro gm/dl.

The Biliary Cholesterol values for Group A and B respectively were754.5 ± 398.3 and 1184.7 ± 405.2 mg/dl.The Serum Cholesterol values for Group A and B respectively were

ISSN: 0975-3583, 0976-2833 || VOL 16, ISSUE 03, 2025

184.8± 35 and 171 ± 49.3 mg/dl.

In our study the Biliary Cholesterol levels were significantly higher in the Iron Deficient group (Group B) than compared to the Non-Iron Deficient Group. This result was extremely statistically significant with a p value of
<0.0004.Also, there was no significant difference between the values of serum cholesterol levels in the Iron Deficient (Group B) and Non-Iron Deficient Group. (Group A). P value of 0.2544.
The current study suggests that deficiency in serum iron could play a role in the increased saturation of biliary cholesterol. Biliary cholesterol supersaturation is an independent factor in the formation of cholesterol gall stones. As mentioned previously, the probable explanation for this is the defective cholesterol metabolism and gall bladder stasis promoted a deficiency in serum iron.

CONCLUSION:

To conclude, there was a **significant difference** in the Biliary Cholesterol Levels for Non-Iron Deficient (M=754.5 mg/dl , SD=398.3) and Iron Deficient Subjects(M=1184.7 mg/dl, SD=405.2) t=3.77, p =<0.0004.

Similarly, an independent t-test comparing Serum Cholesterol levels in Non-Iron Deficient (184.8 ± 35 mg/dl) and Iron Deficient subjects (171 ± 49.3 mg/dl) did not find a statistically significant difference. (p=0.2544)These results suggest that Iron Deficiency has an association with Biliary Cholesterol Values.

BIBLIOGRAPHY

1. Angwafo FF, Takongmo S, Griffith D. Determination of chemical composition of gall bladder stones: basis for treatment strategies in patients from Yaounde, Cameroon. World J Gastroenterol 2004;10(2):303–5.
2. Cuevas A, Miquel JF, Reyes MS, Zanlungo S, Nervi F. Diet as a risk factor for cholesterol gallstone disease. J Am Coll Nutr 2004;23(3):187–96.
3. Novacek G. Gender and gallstone disease. Wien Med Wochenschr 2006;156(19-20):527–33.
4. Johnston SM, Murray KP, Martin SA, Fox-Talbot K, Lipsett PA, Lillemoe KD, et al. Iron deficiency enhances cholesterol gallstone formation. Surgery 1997;122(2):354–62.
5. Salomons H, Keaveny AP, Henihan R, Offner G, Sengupta A, Lamorte WW, et al. Nitric oxide and gallbladder motility in prairie dogs. Am J Physiol 1997;272(4 Pt 1):G770–8.
6. Goldblatt MI, Swartz-Basile DA, Choi S-H, Rafiee P, Nakeeb A, Sarna SK, et al. Iron Deficiency Transiently Suppresses Biliary Neuronal Nitric Oxide Synthase.

ISSN: 0975-3583, 0976-2833 || VOL 16, ISSUE 03, 2025

Journal of Surgical Research 2001;98(2):123–8.
7. Festi D, Reggiani MLB, Attili AF, Loria P, Pazzi P, Scaioli E, et al. Natural history of gallstone disease: Expectant management or active treatment? Results from a population-based cohort study. J Gastroenterol Hepatol 2010;25(4):719–24.
8. Mattson MW, Sterchi JM, Myers RT. Accuracy of ultrasonography and oral cholecystography in the diagnosis of cholelithiasis. Am Surg 1981;47(2):80–1.
9. Maglinte DD, Torres WE, Laufer I. Oral cholecystography in contemporary gallstone imaging: a review. Radiology 1991;
10. Szpakowicz J, Kulig J, Popiela T. [Value of ultrasonography for the diagnosis of cholelithiasis in light of surgical verification]. Prz Lek 1990;47(2):283–6.
11. Barakos JA, Ralls PW, Lapin SA, Johnson MB, Radin DR, Colletti PM, et al. Cholelithiasis: evaluation with CT. Radiology 1987;162(2):415–8.
12. Mandelia A, Gupta AK, Verma DK, Sharma S. The Value of Magnetic Resonance Cholangio-Pancreatography (MRCP) in the Detection of Choledocholithiasis. J Clin Diagn Res 2013;7(9):1941–5.
13. Byrne MF. Gallstone pancreatitis--who really needs an ERCP? Can J Gastroenterol 2006;20(1):15–7.
14. Dykes EH, Wilson N, Gray HW, McArdle CS. The role of 99mTc HIDA cholescintigraphy in the diagnosis of acute gallbladder disease: comparison with oral cholecystography and ultrasonography. Scott Med J 1986;31(3):170–3.
15. Ibáñez Aguirre J, Santoyo Santoyo J, Rico Selas P, Bercedo Martínez J, Gómez Sanz R, Mansilla Molina D, et al. [Intraoperative cholangiography: its value in simple cholelithiasis]. Rev Esp Enferm Apar Dig 1989;75(3):252–5.
16. Strasberg SM, Clavien PA. Overview of therapeutic modalities for the treatment of gallstone diseases. Am J Surg 1993;165(4):420–6.
17. Ganey JB, Johnson PA, Prillaman PE, McSwain GR. Cholecystectomy: clinical experience with a large series. Am J Surg 1986;151(3):352–7.
18. Frazee RC, Roberts JW, Symmonds R, Snyder SK, Hendricks J, Smith R, et al. What are the contraindications for laparoscopic cholecystectomy? Am J Surg 1992;164(5):491–4–discussion494–5.
19. Berggren U, Gordh T, Grama D, Haglund U, Rastad J, Arvidsson D. Laparoscopic versus open cholecystectomy: hospitalization, sick leave, analgesia and trauma responses. Br J Surg 1994;81(9):1362–5.
20. Moreaux J. Prospective study of open cholecystectomy for calculous biliary disease. Br J Surg 1994;81(1):116–9.