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Original Research Paper

General Surgery

EVALUATION OF SERUM FERRITIN LEVEL AND IRON REGULATORY PROTEIN-1 GENE EXPRESSION IN PATIENTS WITH CHOLELITHIASIS

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ABSTRACT

BACKGROUND: Cholelithiasis is a major problem affecting females, which can be attributed to iron deficiency anaemia.

AIM: To evaluate serum ferritin level and iron regulatory protein-1 (IRP-1) gene expression in patients with cholelithiasis and compare mRNA expression of IRP-1 gene in patients of cholelithiasis with their first degree relatives.

METHODS: 55 patients of cholelithiasis and 55 controls were included, and a fasting blood sample was taken for evaluation of S.ferritin levels and IRP-1 gene expression. The patients were divided into anaemics and non-anaemics. S.ferritin level of these groups were compared and IRP-1 gene expression of patients was compared with their respective controls.

RESULT: 92.7% (51/55) patients were females. 61.8% of the patients (34/55) were anaemic. Out of these 34 anaemic patients, 33 (97.1%) were females. The p-value of ferritin (0.043) was less than 0.05 indicating a significant change in ferritin values in the two groups. 1.22 fold change upregulation was observed when all patients were considered as a test group and all relatives as controls.

CONCLUSION: Significant decrease in S.ferritin was found in anaemics with cholelithiasis. IRP-1 gene showed upregulation in all anaemics with cholelithiasis as compared to controls.

KEYWORDS : Cholelithiasis, Ferritin, Iron Deficiency Anaemia, Irp

INTRODUCTION

Cholelithiasis is one of the most common problems associated with the biliary system, affecting millions of people worldwide.(Einarsson, Nilsell, Leijd, & Angelin, 1985; Shaffer, 2006). The highest prevalence of cholelithiasis is seen in the Native American Pima Indians where 73% of the Pima women more than 30 years of age suffer from gallstones(Sampliner, Bennett, & Comess, 1970). The exact cause of higher incidence in females, particularly in multiparous women, still remains unknown. Iron deficiency anaemia is also seen in these multipara women with cholelithiasis suggesting some association between an iron deficiency state and gallstone formation.

The role of iron in the pathogenesis of gallstone disease has not been well established so far. Iron deficiency has been shown to alter the activity of several hepatic enzymes, leading to increased cholesterol saturation of bile within the gall bladder and hence promoting cholesterol crystallization[4] . The serum iron concentration is found to be least in patients harbouring pigment type of gallstones, hence enforcing the role that iron may play in gall stone formation[5].

Levels of serum iron, total iron binding capacity and transferrin saturation are not good indicators of iron status. In an infection free situation, **serum ferritin** estimation is an ideal indicator for diagnosis of iron deficiency and to assess the response to iron therapy[6]. If the prevalence of iron deficiency in a population must be described with a single parameter, serum ferritin should be used; which can be complemented with haemoglobin in all programme evaluations.

Since, iron could have a significant role in gallstone pathogenesis, its regulation through ferritin requires to be studied; as ferritin is the most specific marker for iron levels in the body. Correspondingly, at genetic level the expression of genes controlling ferritin levels viz **Iron regulatory protein (IRP-1)** might be considered to play a significant role in pathogenesis of cholelithiasis.

If we can predict which factors contribute to the development of cholelithiasis then their prevention could be affected by modifying these factors, which can have large scale implications to reduce the burden, both economic and health status, in the given population.

AIMS & OBJECTIVES

- To evaluate serum ferritin level and iron regulatory protein-1 (IRP-1) gene expression in patients with cholelithiasis.
- To compare mRNA expression of IRP-1 gene in patients of gall stone disease with their first degree blood relatives.

MATERIALS AND METHODS

The study was conducted in Department of Surgery in collaboration with Department of Biochemistry, UCMS & GTB Hospital, Delhi.

STUDY DESIGN:

- To determine the level of serum ferritin in patients of gall stone disease- Cross sectional study
- To compare mRNA expression of IRP-1 gene in patients of gall stone disease with their first degree blood relatives - Case control study

55 patients and 55 controls (first-degree relatives) were enrolled in the study.

INCLUSION CRITERIA-

All consenting patients with cholelithiasis within age group of 18-50 years

EXCLUSION CRITERIA-

Patients taking haematinics for past two months, with previous history of biliary tract surgery, or with comorbid conditions

METHODOLOGY

All patients with cholelithiasis (as per inclusion criteria) and their first degree relatives underwent a detailed history, clinical, radiological and biochemical investigation. A fasting blood sample of 5 ml was withdrawn for basic haematological evaluation, Serum Ferritin level and IRP-1 gene expression. In first degree relatives, only IRP-1 gene expression was done and compared with corresponding patients. Serum ferritin

level was estimated by ELISA method as per the protocol of the commercially available kit used i.e. Calbiotech®. Estimation of IRP-1 Gene Expression comprised of 3 steps: Extraction of RNA, Synthesis of complementary DNA (cDNA) and quantification of IRP-1 gene expression by quantitative Real Time PCR. All the molecular biological studies were performed in a Laminar Air Cabinet® (Toshiba, India).

RESULTS

Majority of the patients(32/55) were in the age group of 40 to 50 years (Figure 1). Amongst the studied population, 51 (92.7%) patients were females while only 4 (7.3%) were male patients.

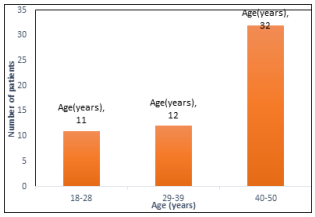

Figure 1- Age distribution

The patients of cholelithiasis were divided into 2 groups: Anaemics (34 patients) & Non anaemics (21 patients).

Table 1- Gender distribution of mean Hb in anaemic and non-anaemic patients

Total no. of patients (55)*	Anaemic (34)		Non-Anaemic (21)	
	Male(1)	Female(33)	Male(3)	Female(18)
Mean Hb - g%**	9.9	10.7	14	12.7
	Mean - 10.7		Mean - 12.91	

*(Number of patients), **(Hb g%)

The mean Hb of all 55 patients was 11.5 g%. Out of the 34 anaemic patients, 33 (97.1%) were females and 1 (2.9%) was male. Out of the total non-anaemic patients 18 (85.7%) were females and 3 (14.3%) were males (Table 1). The mean Hb of anaemic groups was 10.7 g% and that of non-anaemics was 12.91 g%.

Mean corpuscular haemoglobin (MCH) was reduced in anaemic patients with cholelithiasis. Anaemic patients had mean MCH value of 26.9 pg/rbc as compared to 28.2 pg/rbc in non-anaemics. The normal range of MCH is 28-33 pg/rbc.

SERUM FERRITIN

The mean of serum ferritin of the 55 patients was 25.65 g/l. The lowest value was 2.82 g/l and highest was 78.53 g/l. 60% of patients (33/55) had serum ferritin value in 1-20 g/l group (Figure 2).

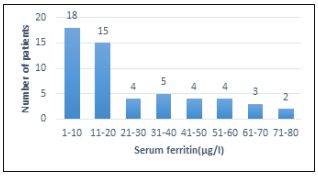

Figure 2- Distribution of serum ferritin values in 55 patients

Mean serum ferritin level in anaemic group (34/55) was **21.67 g/l** and that in non-anaemic (21/55) was **32.11 g/l**. This difference in ferritin values gave the p-value of **0.043**, which is less than 0.05 indicating that there is a **significant change in the ferritin values in the two groups viz anaemic and non-anaemic** (Figure 3)**.**

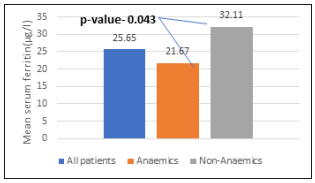

Figure 3- Mean serum ferritin in various group

The p-value of **MCH (0.043)** showed statistical significance with serum ferritin suggesting that MCH values decrease as ferritin decrease and vice versa.

IRP-1 GENE EXPRESSION

We could include only 53 patient-relative gene expression results owing to impurity in RNA samples due to human error in 2 of the samples. IRP-1 gene expression (up/downr egulation) was observed when each relative was considered as control for the respective related patient (Figure 4). 31/53 patients showed IRP-1 gene upregulation while the rest 22/53 patients showed downregulation when compared to their respective relative, showing that there is a IRP-1 gene upregulation trend in patients with cholelithiasis. However, no statistical significance **(p-value= 0.411)** could be observed with this assessment when each relative was considered a control for the respective patient.

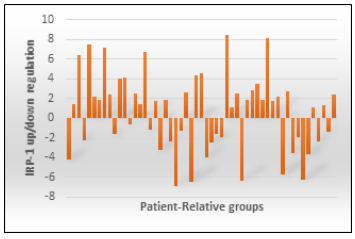

Figure 4- IRP-1 gene expression in 53 patient-relative groups (each relative as a control for respective patient)

When all patients were considered as a test group and all relatives were considered as a control group, then **1.22 fold change upregulation** of IRP-1 gene was observed (Figure 5).

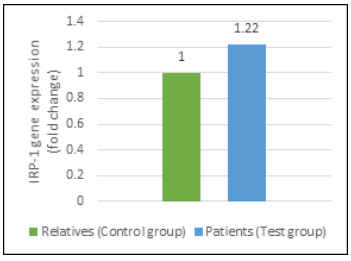

Figure 5- IRP-1 gene expression in patients (test group) vs relatives (control group)

Amongst anaemics (two of the patients not assessed were of anaemic group), 22/32 (68.7%) showed IRP-1 gene upregulation and 10/32 (31.3%) showed its downregulation. Amongst non-anaemics, 9/21 (42.9%) showed IRP-1 gene upregulation and 12/21 (57.1%) showed its downregulation. This showed that IRP-1 gene upregulation is a trend seen in majority of anaemic patients (64.7%) (Figure 6).

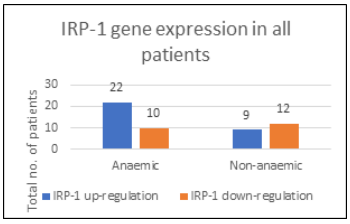

Figure 6- IRP-1 gene expression in anaemics vs non-anaemics

The Mean serum ferritin was 21.88 µg/l in those with IRP-1 upregulation and 29.05 µg/l in those with downregulation suggesting that the value of **serum ferritin decreases as IRP-1**

upregulation occurs.

DISCUSSION

The old dictum that cholelithiasis is common in fat, fertile, flatulent females of fifty is not always true[6]. It is also seen in young and underweight females suffering from iron deficiency anaemia. Since cholelithiasis is a multifactorial disease, we intend to fathom the factors causing gall stones at molecular level. In our study an increasing trend of incidence of gall stones from 3rd to 5th decade was observed.

There was female preponderance which is a well-established fact in literature. This gender related difference could be linked to pregnancy and iron deficiency, as multiparous women and women with longer length of fertility appear to have higher likelihood of developing gallstones than those who remain nulliparous. During pregnancy, biliary sludge formation is seen in upto 30% and gallstones develop in 1-3%[8,9] Hence, it is postulated that iron deficiency anaemia may influence the biliary lipid profile to initiate stone formation.

As evident from stages of iron deficiency[10], a person is said to be anaemic when he/she reaches the 3rd stage wherein the Hb level falls. However, the vicious cycle of iron deficiency may have already been manifesting inside the body for years before the eventual fall in Hb. Thus, decrease in serum iron (Stage 2) and more importantly serum ferritin (Stage 1) are important predisposing factors for developing iron deficiency anaemia (Figure 7).

This in turn explains as to why some people may have decreased level of serum ferritin and serum iron but may still have normal Hb. Such people are at high risk of developing iron deficiency anaemia and its related consequences if corrective therapy is not undertaken[10].

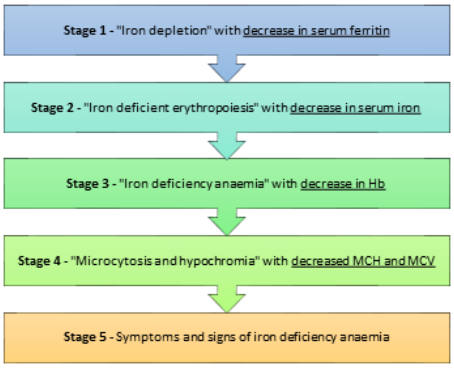

Figure 7 - 5 Stages of iron deficiency(Ganesan, 2016)

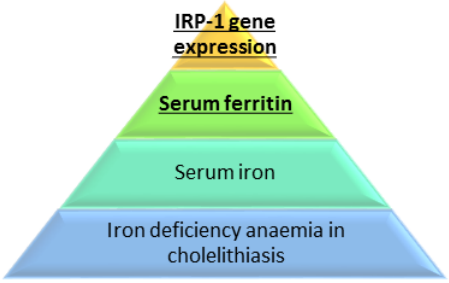

Figure 8- As we go towards the molecular level, the number of studies done on cholelithiasis vs iron deficiency anaemia decreases

SERUM FERRITIN

In our study, the value of serum ferritin ranged from 2.82-78.53 µg/l with majority of the patients (33/55) having value under 20 µg/l. Thus, the mean serum ferritin in anaemics was 21.67 µg/l and that in non anaemics was 32.11 µg/l which was on a lower side of the range of our study group. Since even the non-anaemic patients of our study had low serum ferritin values, we may postulate that these patients might be in stage-1 or 2 of iron deficiency (Figure 7) wherein the fall in Hb has not occurred yet. Nevertheless, the iron deficient status of these non-anaemic individuals might have also predisposed them to gallstone formation. Moreover, these patients may become overtly anaemic in future. Moreover, on evaluating the correlation of serum ferritin levels with parameters such as age and Hb indices, **a statistically significant correlation was found only between mean corpuscular haemoglobin (MCH) and serum ferritin (p-value 0.043)**. This is in tune with the fact that MCH decreases in stage 4 of iron deficiency[10]. Thus, MCH too is a reliable indicator of anaemia(Cook, Lipschitz, Miles, & Finch, 1974; Ganesan, 2016).

IRP-1 GENE EXPRESSION

IRP controls the translation of ferritin in bidirectional manner as described below-

- When intracellular concentrations of iron are low IRPs binding to IRE cis-elements (UPREGULATION) ferritin translation repressed[12,13].
- When intracellular concentrations of iron rise IRPs are unable to bind to IRE cis-elements (DOWNREGULATION) ferritin mRNA is efficiently translated(Santamaria et al., 2006; Torti & Torti, 2016)

In our study, **1.22 fold change upregulation** was observed when all patients were considered as a test group and all relatives were considered as a control group, reinforcing the fact that IRP-1 gene may have a role in the pathogenesis of cholelithiasis.

A trend of IRP-1 gene upregulation in anaemics concurs with our previous results where anaemia plays a part in predisposition for development of gallstones. So, at genetic level too, the gene controlling iron metabolism i.e. IRP-1 through its upregulation does tend to establish that it has a role in cholelithiasis in patients with anaemia. We further went forward to compare the serum ferritin levels in those with IRP-1 gene upregulation vs downregulation. The mean serum ferritin was 21.88 g/l in those with IRP-1 upregulation (31/53) and 29.05 g/l in those with downregulation (22/53) suggesting that the value of serum ferritin decreases with occurrence of IRP-1 upregulation.

We could not find any study done on IRP-1 gene expression in patients with cholelithiasis. We could only postulate our results as described below (Figure 9).

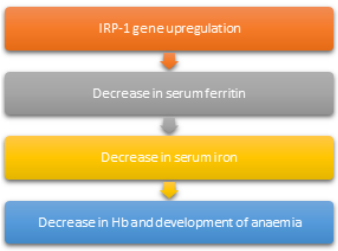

Figure 9- Steps leading to development of anaemia and cholelithiasis

Increase in IRP-1 levels by up-regulation of IRP-1 gene leads to decrease in ferritin, which is the storage form of iron, thus causing decrease in serum iron and increased chances of developing iron deficiency anaemia. More patients of cholelithiasis occurred in anaemic group vs non-anaemic group, thus substantiating that cholelithiasis is predisposed to iron deficiency anaemia.

This upregulation of IRP-1 gene reinforces that the decrease in serum ferritin could be brought about by this gene's

expression, denoting the molecular basis of development of iron deficiency which would predispose to cholelithiasis[14,15]. Findings in the present study suggest that low levels of serum ferritin and IRP-1 gene upregulation may have a role in causation of gallstones. Since serum ferritin and IRP-1 gene are integral to iron metabolism, it is concluded that iron deficiency anaemia does play a role in etiopathogenesis of cholelithiasis. **The decrease in serum ferritin via IRP-1 upregulation in patients with cholelithiasis is visibly apparent from our study.**

CONCLUSION:

In conclusion, we found that there is a significant decrease in Serum ferritin in anaemic patients with cholelithiasis as compared to non-anaemic patients. A significant correlation is also found between Serum ferritin and Mean corpuscular hemoglobin (MCH). At a molecular level, IRP-1 gene showed upregulation in all anaemic patients with gall stones as compared to the control group.

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