Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 09, pp.57935-57938, September, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

A STUDY OF SERUM VITAMIN D3, SERUM CALCIUM & BILIARY CALCIUM IN GALLSTONE DISEASE

***Gurmeet Singh, Athar Parvez, Digamber Chaubey, Sonalini Thakur and Suparna, P. N.**

Vivekananda Polyclinic & Institute of Medical Sciences, India

ARTICLE INFO

Article History:
Received 21st June, 2017
Received in revised form
19th July, 2017
Accepted 02nd August, 2017
Published online 30th September, 2017

Key words:
Vitamin D deficiency,
Significant statistically.

ABSTRACT

The total concentration of calcium in bile is much higher (25 meq/lt) than the plasma concentration due to calcium binding by other components of bile, especially bile salts[2].Vitamin D plays an important role in regulation of bile salts and megalin thus preventing gallstones. Since an increase in Biliary Calcium was found during gallstone formation in various studies. In present study an attempt was done to see if Vitamin D deficiency causes a rise in Biliary Calcium and precipitating gallstones.In present study a negative poor correlation was also observed between Biliary Calcium and Serum Calcium (r= -0.02) i.e. if Serum Calcium levels fall the Biliary Calcium level may increase but such finding was not significant statistically (p=0.83). In all patients calcium in bile was higher than serum suggesting the ability of gallbladder to concentrate calcium in gallbladder bile that may lead to stone formation.In the present study the mean Serum Vitamin D3 level was 13.16±6.76 ng/ml which shows deficiency of vitamin D3 in patients of gallstones.

Copyright©2017, Gurmeet Singh et al. *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

Citation: Gurmeet Singh, Athar Parvez, Digamber Chaubey, Sonalini Thakur and Suparna, P. N. 2017. "A study of serum vitamin d3, serum calcium & biliary calcium in gallstone disease", *International Journal of Current Research*, 9, (09), 57935-57938.

INTRODUCTION

There is a steep rise in incidence of cholelithiasis in Indian population. There is no definitive theory is there to explain this rise in incidence of gallstone disease. The most accepted hypothesis is the changing dietary habits. Other reason of rise in incidence may be the advent of ultrasonography leading to early and easy diagnosis of asymptomatic gallstones patients. Although no gene has been identified in human beings but LITH gene has been identified in mice having role in formation of cholesterol gallstone (Helen, 2010). This study is done to assess the role of Calcium in pathogenesis of Gallstones and Correlation of Serum Vitamin D3 and Biliary Calcium levels in Gallstone Disease. The total concentration of calcium in bile is much higher (25 meq/lt) than the plasma concentration due to calcium binding by other components of bile, especially bile salts (Henry, 2007). The binding-affinity of bile salts for Calcium ions is particularly strong and accounts for the majority of bound Calcium in bile. Thus total calcium in bile may be separated into two fractions bound and free calcium (free ionized calcium). All calcium in bile is in the ionized state and free ionized calcium is synonymous with unbound calcium (Williamson, 1979; Williamson, 1980; Rajagopalan, 1982; Moore, 1982; Shiffman, 1992 and Gleeson, 1994).

****Corresponding author: Gurmeet Singh,***
Vivekananda Polyclinic & Institute of Medical Sciences, India

The calcium buffering action of bile salts prevents precipitation of calcium and thus formation of stones. If this buffering action of bile salts is absent or there is high level of calcium in bile then gallstones may get precipitated. Fisher *et al* (2009) found Vitamin D3 deficiency in 45.6 % of patients with biliary and pancreatic disorders (Fisher, 2009). So, the present study was designed to see the levels of Serum Calcium, Serum Vitamin D3 and Biliary Calcium in gallstone patients and to see if there exists any correlation in levels of Serum Vitamin D3, Serum Calcium and Biliary Calcium.

MATERIAL AND METHODS

The prospective study design was used. The study was conductedon a group of 115 patients who were admitted for Cholecystectomy (Laproscopic) during period of 1 year 20 days in Department of General Surgery in multispeciality hospital, Lucknow, India. The study was approved by the ethical committee and scientific committee of the institute and the consent was taken from each patient. Patients suffering from cholelithiasis confirmed by ultrasonography admitted in the surgical ward for laproscopic cholecystectomy were included in the study irrespective of their age, sex and parity. Patients suffering from empyema and mucocele of the gall bladder were excluded from the study. Blood sample was taken for evaluation of Serum Calcium and Serum Vitamin D3 one day before surgey. For evaluation of Biliary Calcium, bile sample was taken from gallbladder at the time of laproscopic

cholecystectomy. Serum Calcium was estimated using calcium reagent by a timed endpoint method. In this reaction calcium combines with Arsenazo III to form a bluish purple coloured product. The SYNCHRON CX System automatically proportions the appropriate sample and reagents volumes into the cuvette. The ratio used was one part sample to 100 parts reagent. The system monitors the change in absorbance at 650 nm. The change in absorbance is directly proportional to the concentration of calcium in the sample and was used by the system to calculate and express calcium concentration. Serum Vitamin D3 was estimated using enhanced Chemiluminescence method. For the estimation of Biliary Calcium, bile was centrifuged at 3000 rpm for 3 minutes followed by estimation of calcium using calcium reagent similar to that of estimation of Serum Calcium.

DATA ANALYSIS AND RESULTS

The following results were found on analysis of data obtained from gallstone patients.

Age and Sex distribution of Gallstone patients

Table 1. Age and Sex distribution of the Gallstone patients

Age in years	Male		Female		Total	
	No.	%	No.	%	No.	%
<30	3	15.0	17	85.0	20	17.4
30-40	7	21.2	26	78.8	33	28.7
41-50	6	22.2	21	77.8	27	23.5
51-60	3	13.0	20	87.0	23	20.0
>60	3	25.0	9	75.0	12	10.4
Total	22	19.1	93	80.9	115	100.0

Chi-square=1.29, p=0.86 (Age vs. gender)

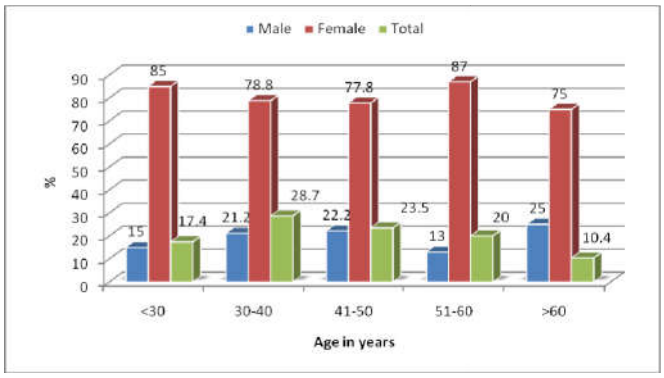

Fig. 1. Age and sex distribution of the patients

Table no. 1 and Figure no. 1 depicts the age and sex distribution of the patients of gallstone disease. The average age of the patients was 42.45 (±14.05) years. 52.2% of patients were from age group 30-50 years of which most were females (30-40 yr: 78.8%) (41-50 yr: 77.8%). 20% of total patients were of age group 51-60 yrs, 17.4% were less than 30 yrs and 10.4% of patients were more than 60 year old. 89.9% of total patients were females while only 19.1% were males. There was no statistically significant difference between age and gender (p>0.05) of gallstone patients.

Biochemical parameters among gallstone patients

Table no.2 shows the mean value of different biochemical parameters among the gallstone patients included in study. The mean Serum Vitamin D3 level was 13.16±6.76 ng/ml showing the deficiency of Vitamin D3 in study group. The mean Serum Calcium level was 9.01±0.64 mg/dl which is within normal range whereas mean Biliary Calcium was 14.02±2.76 mg/dl showing less than normal levels of calcium in bile but mean calcium in bile is higher than mean Serum Calcium showing calcium getting concentrated in gallbladder bile.

Table 2. Mean value of biochemical parameters in gallstone patients in study

Biochemical parameters	Normal range	Mean Value (n=115)
Serum Vitamin D3	<20 ng/ml (Deficiency) 20-30 ng/ml(insufficiency) 30-100 ng/ml (Sufficiency) >100 ng / ml (Toxicity)	13.16±6.76 ng/ml
Serum Calcium	8.5-10.2 mg/dl	9.01±0.64 mg/dl
Biliary Calcium[7]	50 mg/dl	14.02±2.76 mg/dl

Correlation between Serum Vitamin D3, Serum Calcium and Biliary Calcium

Table 3 below depicts the correlation among the Vitamin D3, Serum Calcium and Biliary Calcium.

Table 3. Correlation between the Serum Vitamin D3, Serum Calcium and Biliary Calcium

		Serum Vitamin D3	Serum Calcium	Biliary Calcium
Serum Vitamin D3	Pearson Correlation coefficient (r) p-value	1		
Serum Calcium	Pearson Correlation coefficient (r) p-value	-0.06 0.50	1	
Biliary Calcium	Pearson Correlation coefficient (r) p-value	-0.18 0.06	-0.02 0.83	1

There was negative poor correlation between levels of Serum Vitamin D3 and Serum Calcium (r= -0.06) in the studythat was not statistically significant (p=0.50). The correlation between Serum Vitamin D3 and Biliary Calcium (r= -0.18) was also negative poor correlation showing a rise in Biliary Calcium with deficiency of Vitamin D3 although this correlation was not statistically significant (p=0.06). A negative poor correlation was also observed between Biliary Calcium and Serum Calcium (r= -0.02) showing a rise in bile calcium with deficiency of Serum Calcium but this was also not found to be significant statistically (p=0.83).

DISCUSSION

The present study was designed to see levels of Serum Calcium, Biliary Calcium and Serum VitaminD3 in patients of cholelithiasis, to see correlation in levels of Serum Calcium, Biliary Calcium and Serum Vitamin D3 in patients of cholelithiasis and to see if there exists any role of Vitamin D3 and abnormal calcium metabolism in these patients. In the present study the average age of the patients was 42.45 (±14.05) years of which most were from age group 30-50 yrs. About one fourth of the patients were in the age groups of 30-40 (28.7%) and 41-50 (23.5%) years. However, 20% of the patients were in the age group of 51-60 years and 17.4% were below 30 years. Only 10.4% of the patients were above 60 years. Majority of the patients were females (80.9%). Sayeed*et*

al., (2011) studied the prevalence of gallbladder diseases in Northern India (Uttar Pradesh and Bihar)[10]. The ultrasonography revealed a prevalence of gallstone disease 4.15% of which 5.59% were females. A significantly increased risk was seen in females >50 years, multi-para and a genetic history. In the present study, the mean Serum Calcium was 9.01±0.64 mg/dl that was within normal range. Verma*et al* (2002) had also reported higher serum level in gallstone formers[11]. Shareef *et al* (2009) in his study on the correlation between chemical components of gallstones and metal contents of sera of gallstone formers found that there was moderate (positive and negative) correlation between the calcium content of the serum and the cholesterol, pigment and mixed gallstones (r=0.202, -0.213 and -0.210 respectively). In a study, the mean serum concentration for calcium in gallstone formers (13.2±4.6mg/dl) compared to control subjects (9.96 ±4.5mg/dl) was higher[12]. This finding was in line with other investigators who reported that high levels of cholesterol and calcium in serum leads to increase in their level in bile followed by their co-precipitation as calcium salt of cholesterol (Cholesterol + calcium carbonate) to form cholesterol gallstones[13].

The Biliary Calcium concentration plays a part in bilirubin precipitation and gallstone calcification. Many patients with gallstones have increased Biliary Calcium, with supersaturation of calcium carbonate. Calcium intake seems to be inversely associated with gallstone prevalence. Dietary calcium decreases cholesterol saturation of gallbladder bile by preventing the reabsorption of secondary bile acids in the colon [14.] Mean Biliary Calcium level was 14.02±2.76 mg/dl which was well above the Serum Calcium levels reflecting concentration of calcium in bile within the gallbladder. In study bile samples from 62 patients with cholesterol gallstones, 12 with pigment gallstones, and 10 with normal gallbladders undergoing operation wereexamined, both total and ionized calcium were linearly related to total bile salt concentration. When groups of patients were compared, no differences in calcium concentrations were found between control patients and patients with either type of gallstone. It was suggested for such a difference may be as the operation for symptomatic gallstones occurs years after gallstone formation**.** Calcium elevations might be transient and these data could not exclude calcium abnormalities during active formation of gallstones[15].In canines elevated Biliary Calcium was found to play a role in gallstone formation[16]. Study in patients undergoing Gastric bypass found that increased calcium in bile was present during formation of gallstones but not later after gallstone stone formation or at time of surgery [17-18]. In present study a negative poor correlation was also observed between Biliary Calcium and Serum Calcium (r= -0.02) i.e. if Serum Calcium levels fall the Biliary Calcium level may increase but such finding was not significant statistically (p=0.83). In all patients calcium in bile was higher than serum suggesting the ability of gallbladder to concentrate calcium in gallbladder bile that may lead to stone formation. In another study Prasheeda*et al* (2005) [19] observed a significant positive correlation between Calcium in Cholesterol stone and Bile while an insignificant correlation between calcium in serum and bile. In mixed stones negative insignificant correlation was seen between calcium of stone and bile while there was insignificant positive relation between calcium in serum and bile. In pigment stones an insignificant negative correlation was seen in calcium of serum and bile.

In the present study the mean Serum Vitamin D3 level was 13.16±6.76 ng/ml which shows deficiency of vitamin D3 in patients of gallstones. In 1979, Kobayashi *et al* measured Serum 25-hydroxy-vitamin D (25-OHD) concentrations in 49 patients with hepatobiliary disease in infancy. Low mean values were found in groups of patients with biliary atresia, neonatal hepatitis, choledochal cyst, and chronic intrahepatic cholestatic syndrome[20]. Fisher *et al* (2009) [9] determined vitamin D status in patients with biliary and pancreatic disorders. In 90 consecutive patients (mean±SD age, 65.5±17.7 years; 45 females) undergoing endoscopic retrograde cholangiopancreatography (68 with choledocholithiasis, 14 with other benign condition, and 8 with cholangiopancreatic cancers) fasting concentrations of carboxylated (cOC), the 25-hydroxyvitamin D was measured. Vitamin D deficiency (25-hydroxyvitamin D <50 nmol/L) was found in 45.6% of patients. In the present study, there was poor negative correlation between Serum VitaminD3 and Serum Calcium (r= -0.06) which was statistically not significant p=0.50). Vitamin D plays an important role in regulation of bile salts and megalin thus preventing gallstones. Since an increase in Biliary Calcium was found during gallstone formation in various studies. In present study an attempt was done to see if Vitamin D deficiency causes a rise in Biliary Calcium and precipitating gallstones. In present study, Serum VitaminD3 and Biliary Calcium (r= -0.18) showed a poor negative correlation reflecting a rise in calcium levels in bile if Vitamin D3 was deficient but this correlation was poor and statistically not significant (p=0.06).

Conclusion

On the basis of the data analyzed in the study group, the following conclusions were drawn about the Gallstone Patients involved in study:

- The mean Serum Vitamin D3 level was 13.16±6.76 ng/ml suggesting a deficiency of vitamin D3 in the study group. Mean level of Serum Vitamin D3 was found to be lower among males (12.40±4.00ng/ml) as compared to females (13.34±7.26 ng/ml) in the study. Serum Vitamin D3 was least among 41-50 yr age group (12.36±5.24 ng/ml) and 51-60 yr age group (12.83±4.40 ng/ml).
- The mean Serum Calcium level was 9.01±0.64 mg/dl and mean Biliary Calcium was 14.02±2.76 mg/dl showing much higher levels of calcium in bile of gallstone disease patients indicating concentrating ability of gallbladder for calcium.
- There was negative poor correlation between Serum Vitamin D3 and Biliary Calcium (r= -0.18). Thus deficiency in Serum Vitamin D3 may cause increase in Biliary Calcium levels. Although this correlation was not found to be statistically significant as p=0.06.
- A negative poor correlation was also observed between Serum Calcium and Biliary Calcium (r= -0.02). Thus a decrease in Serum Calcium may lead to increase in Biliary Calcium levels but this correlation was not statistically significant (p=0.83).

REFERENCES

Chandran, P., Kuchhal, N.K., Garg, P., *et al.* 2007. An extended chemical analysis of gallstone. *Ind J ClinBiochem.,* 22(2): 145-150.

Channa, N.A., Khand, F. and Soomro, A.M. 2012. Comparison of serum calcium, copper and iron levels in serum samples from gallstone patients and control subjects. *Pak J Med Sci.* 28(4): 769-772.

Darracq, S., Bernhard, B. C., Bourrie, B., *et al.* 2004. Heavy metals transfer from soil to rapeseed oil. Proceedings of conference on Waste Contaminants: Lifecycle and Entry into *Food Chain*. 61-64

Dawes L. G, Nahrwold D.L. and Rege R.V. 1988. Increased Total and Free Ionized Calcium in a Canine Model of Pigment Gallstones. *Archives of Surgery.* 104: 86-90.

Dawes L. G. and Rege R. V. 1990. Calcium Binding in Human Gallstone Disease. *Archives of Surg.* 125: 1606-9.

Fisher, L., Byrnes, E. and Fisher, A.A. 2009. Prevalence of vitamin K and vitamin D deficiency in patients with hepatobiliary and pancreatic disorders. *Nutr Res.*, 29(9): 676-83.

Gleeson, D., Murphy, G. M. and Dowling, R.H. 1994. Changes in Serum Calcium Levels Influence Biliary Calcium Levels in Humans. *Gastroenterology.* 107(6), 1812-18.

Grant W B. The Health Benefits of Solar Irradiance and Vitamin D and the Consequences of Their Deprivation. Holick MF ed. Vitamin D Physiology,Molecular biology and Clinical Applications. Humana Press. USA. 2010: 753.

Helen H W, Piero P, Wang Q H, *et al.*Lith Gene and Genetic Analysis of Cholestrol Gallstone Formation. *Gastroentrology Clinics of North America*. 2010; 39(2): 185-207.

Henry A P and Gadacz T R. Anatomy, Embryology, Anomalies and Physiology. In: Yeo Charles J. Shackelford's Surgery of the Alimentary Tract 6/e. Elsevier Book Aid International. China. 2007: 1455

Moore E.W, Celic L. and Ostrow J. D. Interactions Between Ionized Calcium and Sodium Taurocholate: Bile Salts Are Important Buffers for Prevention of Calcium-Containing Gallstones. Gastroenterology.1982; 83, 1079-89.

Prasheeda C, Garg P. and Pundir C.S. Correlation between chemical components of billary calculi and bile & sera and bile of gallstone patients. *Indian Journal of Clinical Biochemistry.* 2005; 20 (2): 81-85

Rajagopalan N. and Lindenbaum S. The Binding of Ca2+ to Taurine- and Glycine-Conjugated Bile Salts Micelles. Biochem Biophys Acta. 1982; 711, 66-74.

Shiffman M. L, Sugerman H. J, Kellum J.M, *et al.* Calcium in Human Gallbladder Bile. *Journal of Laboratory and Clinical Medicine.* 1992; 120(6), 875-84.

Shiffman M. L, Sugerman H.J, Kellum J.M, *et al.* Calcium in Human Gallbladder Bile. *Journal of Laboratory and Clinical Medicine.* 1992; 120(6): 875-84.

Shiffman M. L, Sugerman H.J, Kellum J.M, et al. Gallstones in Patients with Severe Obesity. Relation to Body Weight, Weight Loss, and Gallbladder Bile Cholesterol Solubility. *International Journal of Obesity & Related Metabolic Disorders.* 1993; 17(3): 153-8.

Unisa, S., Jagannath, P., Dhir, V., *et al.* 2011. Population-based study to estimate prevalence and determine risk factors of gallbladder diseases in the rural Gangetic basin of North India. HPB (Oxford). 13(2): 117–125

Verma, G.R., Pandey, A.K., Bose, S.M., *et al.* 2002. Study of serum calcium and trace elements in chronic cholelithiasis. *ANZ J Surg.* 72(8): 596-599.

Williamson, B. W. A. and Percy-Robb, I. W. 1979. The Interactions of Calcium Ions with Glycocholate Micelles in Aqueous Solutions. *Biochem. J.* 181: 61.

Williamson, B. W. A. and Percy-Robb, I. W. 1980. Contribution of Biliary Lipids to Calcium Binding in Bile. Gastroenterology. 78: 696-702.

****