CORRELATION BETWEEN CHEMICAL COMPONENTS OF BILIARY CALCULI AND BILE & SERA AND BILE OF GALLSTONE PATIENTS

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ABSTRACT

Total cholesterol, total bilirubin, calcium, oxalate, inorganic phosphate, magnesium, iron, copper, sodium and potassium were analyzed quantitatively in gallstones, bile of gall bladder and sera of 200 patients of cholelithiasis (52 cholesterol, 76 mixed and 72 pigment stone patients) and their contents were correlated between calculi and bile and sera and bile in these three type of stone patients. A significant positive correlation was observed between total cholesterol, total bilirubin of calculi and bile, copper of bile and sera of cholesterol stone patients, copper of calculi and bile, total bilirubin, oxalate, magnesium, potassium of sera and bile of pigment stone patients and oxalate and iron of stone and bile, total bilirubin, oxalate, sodium of sera and bile of mixed stone patients. A significant negative correlation was found between magnesium of serum and bile of cholesterol stone patients, oxalate of calculi and bile of pigment stone patients and magnesium of serum and bile of mixed stone patients.

KEY WORDS

Bile, Biliary calculi, Chemical Composition Trace metals, Cholelithiasis, Gallstone, Serum.

INTRODUCTION

Gallstones have been classified into three classes based upon their physico-chemical characteristics: cholesterol stones, pigment stones and mixed stones. Cholesterol stones are formed in gallbladder, while pigment stones are mostly formed in bile canaliculi of liver and mixed stones in entire biliary tract and ultimately grow and settle down in gallbladder (1). Chemical analysis of these gallstones has revealed that cholesterol stones contain cholesterol as a chief constituent, while pigment stones have calcium bilirubinate as principle constituent and mixed stones consist of salts of cholesterol and bilirubin as major constituents. The other substances found in gallstones are calcium salts of phosphates, carbonate, fatty acid, phospholipids and some trace elements like sodium, potassium, copper, magnesium, manganese and iron (2). All these substances are normally found in the sera and bile of stone patients. However the correlation of these components between calculi, sera and bile has received scant attention. We have

reported a positive correlation between the content of inorganic phosphate, calcium and iron of calculi and sera of cholesterol stone patients, total cholesterol, total bilirubin and iron content of calculi and sera of pigment stone patients and iron content of calculi and sera of mixed stone patients (3). Earlier a significantly higher bile/serum ratio of calcium, copper and zinc in patients of chronic cholelithiasis was also observed (4). The present report describes the correlation between the various chemical components of gallstones and bile and sera and bile of these stone patients.

MATERIALS AND METHODS

Gallstones (52 cholesterol, 76 mixed and 72 pigment calculi) blood sample (5 ml) and bile from 200 patients of cholelithiasis were collected after cholecystectomy at local hospital (Pt. B. D. Sharma PGIMS, Rohtak hospital). The stone was powdered in a pestle and mortar. To determine total cholesterol and total bilirubin, 30 mg stone powder was dissolved in 3 ml chloroform in a test tube. The tube was kept in boiling water bath for 2 min. The stone solution thus obtained was used for determination of total cholesterol and total bilirubin. The stone solution was stored at 2-8°C when not in use. To determine calcium, oxalate, phosphate, magnesium, iron, copper, sodium and potassium, 30 mg stone powder was dissolved in 3 ml 1N HCl in graduated 10 ml tube and its final volume was made up to 10 ml with distilled water. The tube

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was kept in boiling water bath for 1 hr. The solution thus obtained was stored at 2-8°C until use. The blood samples of gallstone patients were centrifuged at 2000 rpm for 20 min. and the supernatant i.e. serum was collected and stored at 4°C until use. The bile samples from gallstone patients were also collected during cholecystectomy in sterilized vials and stored immediately at -20°C until use. Total cholesterol by commercial enzokit method of Bayer Diagnostic India Ltd. based on enzymic colorimetric estimation employing cholesterol esterase, cholesterol oxidase and peroxidase (5), total bilirubin by commercial kit method of Accurex Biomedical Pvt. Ltd. based on biochromatic methods (6), oxalate by the method of Satyapal and Pundir (7) calcium by commercial OCPC kit method of Miles India Ltd (8), inorganic phosphate by method of Fiske and Subba Row (9), magnesium by method of Neill and Neely (10), iron and copper by Atomic Absorption Spectrophotometer (Make Hitachi, Japan) and sodium and potassium by Flame photometer (Systronics 'Mediflame' 127, Hyderabad) were measured in stone solution ,bile and sera and their contents were correlated using regression equation.

RESULTS

The content of total cholesterol, total bilirubin, calcium, oxalate, inorganic phosphate, magnesium, iron, copper, sodium and potassium in biliary calculi, bile and sera of all three type of gallstone patients is given in Table 1. The correlation coefficient (r) of contents of different chemical constituents between stone and bile and serum and bile of cholesterol stone, mixed stone and pigment stone patients is summarized in Table 2. There was a significantly positive correlation between total cholesterol ($r=0.552$) and calcium ($r=0.421$) of gallstone and bile of cholesterol stone patients, which was insignificant in mixed ($r=-0.139$, $r=-0.087$ respectively) and pigment ($r=0.014$, $r=-0.024$ respectively) stone patients. A significantly positive correlation was between iron content of gallstone and bile of mixed stone patients ($r=0.243$), which was insignificant in cholesterol ($r=0.220$) and pigment ($r=0.014$) stone patients. A significantly positive correlation was also found between oxalate of gallstone and bile of mixed stone patients ($r=0.284$), which was negatively significant in pigment stone patients($r=-0.239$) and insignificant in cholesterol stone patients ($r=-0.268$). There was also a significantly positive correlation between copper ($r=0.447$) of stone and bile of pigment stone patients which was insignificant in cholesterol ($r=0.030$) and mixed ($r=-0.155$) stone patients.

There was a significantly positive correlation between copper ($r=0.569$) of serum and bile of cholesterol stone patients, which was in significant in mixed ($r=0.172$) and pigment($r=0.029$) stone patients. There was a

significant negative correlation between magnesium of serum and bile of both cholesterol and mixed stone patients ($r=-0.284$, $r=-0.247$ respectively) but the same was significantly positive in pigment stone patients ($r=0.293$). There was a significant positive correlation between total bilirubin and oxalate of serum and bile of both mixed ($r=0.255$, $r=0.531$ respectively.) and pigment ($r=0.238$, $r=0.267$ respectively) stone patients but was insignificant in cholesterol stone patients($r=0.263$, $r=-0.006$ respectively). There was a significantly positive correlation between sodium of serum and bile of mixed stone patients ($r=0.339$), which was insignificant in cholesterol ($r=0.001$) and pigment ($r=-0.014$) stone patients. There was a significantly positive correlation between potassium of serum and bile ($r=0.297$) of pigment stone patients, which was insignificant in cholesterol ($r=0.072$) and mixed ($r=-0.082$) stone patients. These correlative studies indicate the different etiology and pathogenesis of three types of gallstone. Earlier, Mishra and Dhull (11) found a direct correlation of protein, calcium and cholesterol in bile and various groups of gallstones but no correlation in the level of bilirubin and bile acids in the bile samples and various groups of calculi. The positive correlation between the level of a chemical constituent in two biological materials indicates that it varies constantly in the two in the same direction. On the other hand if there is an increase of level in one biological material associated with the decrease in other biological material, it is a case of negative correlation.

DISCUSSION

Our results as given in Table 2 show significantly positive and negative or insignificant correlation between the chemical constituents of bile and gall stones and serum and bile of all three types of gall stone patients indicating the different aetiology of cholesterol, mixed and pigment stones.

In cholesterol stone patients, a significantly positive correlation between cholesterol and calcium of bile and gallstones indicates that increase in total cholesterol and calcium in bile is followed by their increase in gallstone in cholesterol stone patients. The increase in level of cholesterol and calcium in serum leads to their increase in bile followed by their deposition as calcium salt of cholesterol to form cholesterol stones. This is evident from the presence of calcium salt of cholesterol as a major component of cholesterol stones. There was a significantly positive correlation ($r= 0.569$) between copper content of bile and serum in cholesterol stone patients, which reveals that increase in copper level of serum in these patients is followed by increase in bile. This indicates a role of Cu^{2+} in cholesterol stone formation. It is supported by our observation that copper content of cholesterol stones is about two times higher than that of mixed and

Table 1. Content of various chemical components in stone, bile and serum of different types of gallstone patients

Component Analysed	Cholesterol Stones (n=52)				Mixed Stones (n=76)				Pigment Stones (n=72)			
	Stone (mg/gm)	Bile (mg/dl)	Stone (mg/dl)	Serum (mg/dl)	Stone (mg/gm)	Bile (mg/dl)	Stone (mg/dl)	Serum (mg/dl)	Stone (mg/gm)	Bile (mg/dl)	Stone (mg/dl)	Serum (mg/dl)
Total Cholesterol	597.98±33.2	227.14±8.68	198.14±6.13	0.52±0.03	594.78±24.91	160.35±7.14	204.98±6.04	0.77±0.04	489.85±28.68	182.36±7.17	220.47±5.94	0.97±0.03
Total Bilirubin	2.10±0.32	2.50±0.61	0.52±0.03	0.52±0.03	1.72±0.19	6.53±0.48	0.77±0.04	0.77±0.04	4.87±0.49	3.61±0.35	0.97±0.03	0.97±0.03
Calcium	10.66±1.85	9.83±0.60	9.64±0.12	9.64±0.12	26.52±2.39	13.54±0.92	10.36±0.09	10.36±0.09	27.60±3.13	10.83±0.48	9.35±0.09	9.35±0.09
Oxalate	6.54±0.29	0.59±0.046	0.81±0.061	0.81±0.061	6.44±0.29	0.65±0.049	0.80±0.049	0.80±0.049	7.02±0.25	0.56±0.042	0.83±0.05	0.83±0.05
Inorganic Phosphate	18.43±2.71	17.30±1.95	6.14±0.28	6.14±0.28	11.74±1.45	21.81±1.72	3.52±0.21	3.52±0.21	8.49±1.49	18.84±1.57	5.35±0.33	5.35±0.33
Magnesium	8.48±1.01	15.74±1.89	5.64±0.32	5.64±0.32	8.26±0.98	19.84±1.14	5.93±0.35	5.93±0.35	10.84±1.06	11.97±0.98	5.50±0.209	5.50±0.209
Iron	0.64±0.054	0.12±0.009	0.136±0.009	0.136±0.009	1.09±0.089	0.087±0.003	0.141±0.008	0.141±0.008	0.78±0.058	0.144±0.008	0.218±0.010	0.218±0.010
Copper	0.70±0.104	0.035±0.003	0.115±0.008	0.115±0.008	0.354±0.034	0.026±0.001	0.102±0.008	0.102±0.008	0.348±0.053	0.042±0.002	0.118±0.009	0.118±0.009
Sodium	1.05±0.19	317.57±11.85	327.19±7.58	327.19±7.58	1.19±0.21	284.28±8.28	309.89±6.30	309.89±6.30	3.12±0.38	295.96±7.54	313.29±7.43	313.29±7.43
Potassium	0.229±0.02	40.27±2.14	14.02±0.77	14.02±0.77	0.296±0.029	36.68±1.72	13.59±0.402	13.59±0.402	0.645±0.067	33.79±1.65	15.73±0.82	15.73±0.82
Stone's data given on dry wt basis, Data are given in mean±S.E.												

Table 2. Correlation between various components of gallstone, bile and sera of different types of gallstone patients

Component Analysed	Cholesterol Stones		Mixed Stones		Pigment Stones	
	Stone vs Bile	Serum vs Bile	Stone vs Bile	Serum vs Bile	Stone vs Bile	Serum vs Bile
Total Cholesterol	0.552**	0.145*	-0.139*	0.054*	0.014*	-0.17*
Total Bilirubin	-0.064*	0.263*	-0.089*	0.255**	-0.066*	0.238**
Calcium	0.421**	0.017*	-0.087*	0.053*	-0.024*	-0.049*
Oxalate	-0.268*	-0.006*	0.284**	0.531**	-0.239***	0.267**
Inorganic Phosphate	-0.262*	-0.150*	0.029*	0.038*	-0.176*	0.207*
Magnesium	-0.227*	-0.284***	-0.161*	-0.247***	0.205*	0.293**
Iron	0.220*	-0.045*	0.243**	0.079*	0.014*	0.216*
Copper	0.030*	0.569**	-0.155*	0.172*	0.447**	0.029*
Sodium	0.132*	0.001*	-0.103*	0.339**	-0.086*	-0.014*
Potassium	0.183*	0.072*	0.093*	-0.082*	-0.093*	0.297**
* = Insignificant Correlation, ** = Significant Positive Correlation, *** = Significant Negative Correlation						

pigment stones (Table 1). However there was a significantly negative correlation for Mg^{2+} level between serum and bile which indicates that the increase in Mg^{2+} level of serum is also indicated with its decrease in bile and vice versa. This indicates no role of Mg^{2+} in cholesterol stone formation. Earlier a good positive correlation between total cholesterol and calcium of sera and gallstones of cholesterol stone patients was reported from this laboratory (3).

In mixed stone patients a significantly positive correlation between oxalate and iron content of bile and gallstones and between total bilirubin, oxalate and sodium of serum and bile indicate that increase in level of oxalate and bilirubin in serum leads to its increase in bile followed by its deposition into mixed stone in the form of Ca^{++} salt. This is supported by our observation of a positive correlation between oxalate and bilirubin contents of serum and gallstones (3). Iron also play a role in the mixed stone formation which is evident from comparatively high content of iron in mixed stones than that of cholesterol and pigment stones (Table 1).

In pigment stone patients a significant positive correlation between Cu content of bile and gall stones indicates a positive role in Cu^{2+} in pigment formation. A significantly positive correlation between total bilirubin, Mg, K of serum and bile of these patients reveals the increase in these substances in bile is due to their increase in serum which ultimately leads to precipitation of bilirubin along with Ca to form pigment stone. This is supported by our earlier reported

positive correlation between bilirubin content of sera and pigment stones (3).

A significantly positive correlation between contents of Cu, Na and K of serum and bile of cholesterol stone, mixed stone and pigment stone patients respectively, could be implied to confirm the different types of gall stone patients (Table 2).

REFERENCES

1. Udupa, K.N., Chansouria, J.P.N., Gode, J.D. and Gupta, S. (1968) Studies on etiology of gallstone Ind. J. Surg. 30, 120-128.
2. Nakayama, F. (1967) Quantitative microanalysis of bile J. Lab. Clin. Med. 69, 594-609.
3. Pundir, C.S., Rani, K., Garg, P., Chaudhary, R., Chandran, P. and Kumari, M. (2002) Correlation between chemical composition of biliary calculi and sera of stone formers. Ind. J. Med. Sci. 56 (8), 373-375.
4. Verma, G.R., Pandey, A.K., Bose, S.M. and Prasad, R. (2002) Study of serum calcium and trace elements in chronic cholelithiasis. ANZ. J. Surg. 72(8), 596-599.
5. Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W. and Fu, P.C. (1974) Enzymatic determination of total serum cholesterol. Clin. Chem. 20, 470-475.

6. Gambino, S.R. (1965) In "Standard methods of clinical chemistry". Meiter S. Ed., Academic Press, 5, 55.
7. Satyapal and Pundir, C.S. (1993) Purification and properties of an oxalate oxidase from leaves of grain sorghum hybrid CSH-5. *Biochem. Biophys. Acta.* 1161, 1-5.
8. Young, S.D., Pestaner, L.C. and Gibberman, V. (1975). Effect of drugs on clinical laboratory tests. *Clin. Chem.* 21, 5.
9. Fiske, C.H. and Subba Row, Y. (1925) The colorimetric determination of phosphorous. *J. Biol. Chem.* 66, 375-400.
10. Neill and Neely (1956) Titan yellow method for magnesium. *J. Clin. Pathol.* 9, 162 cited in Hawk's *Physiological Chemistry*. Ed. B.L. Oster, 4th Edition - The Blackiston Division, McGraw Hill Book Co., 1965, p. 1139.
11. Mishra, R.R. and Dhull, J.C. (1991) Quantitative analysis of protein, calcium, cholesterol, bilirubin and bile acid in gall bladder bile and gallstones. A thesis for M.S. (Surgery) Maharshi Dayanand University Rohtak , p 1-70.