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Comparative Study of Sodium and Potassium in Different Types of Gallstones and in Serum of Subjects with Gallstones and Controls

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Abstract

The study comprises evaluation of sodium and potassium in the pathogenesis of human gallstones as well as measurement of the concentration of these elements in gallstones and in sera of 109 gallstone subjects and 100 controls (age and sex matched with no personal or family history of gallstone disease). It was observed that serum concentrations for both sodium and potassium were comparable ($p>0.05$) between gallstone subjects and control subjects. In gallstones the concentration of sodium was significantly higher as compared to potassium ($p<0.05$). Normal sodium to potassium ratio was seen in serum of gallstone subjects, whereas, low sodium to potassium ratio was seen in gallstone carriers. Amongst the different types of gallstones, significantly high ($p<0.05$) concentrations of sodium and potassium were seen in calcium bilirubinate gallstones. The levels for these mineral elements were also raised in serum of pure calcium carbonate gallstone subjects.The results demonstrate that the higher concentration of sodium and potassium in gallstones may involve in both calcium bilirubinate gallstones and in serum of calcium carbonate gallstone subjects, which indicate their association with calcium in the precipitation of calcium bilirubinate and calcium carbonate in bile. Furthermore, low sodium to potassium ratio in gallstones indicates low ratio in bile of gallstone subjects.

Introduction

Gallstone disease is a common surgical problem throughout the world [1–3] but its pathogenesis remains unclear. Many theories have been put forward to explain the mechanism of stone formation.

Bile consists of a mixture of water, lipids, electrolytes (sodium, potassium, calcium, etc) and proteins [4]. The primary lipid components of bile include bile acids, phospholipids and cholesterol. Supersaturation of bile with cholesterol, precipitation, nucleation, and subsequent growth from microliths are known phenomena [5–8]; however, other factors which influence the precipitation of cholesterol are unclear. Possibly, the precipitation is aided by certain promoting agents [9–12]. In addition to the major well-known constituents (cholesterol, bilirubin and phospholipid) gallstones also contain a number of other elements with calcium as the major constituent [13]. Cholesterol gallstones develop when the capacity of bile acids and phospholipids to solubilize cholesterol is exceeded by the amount of cholesterol secreted into the bile [14]. As a result of the lipid imbalance, the micelles become supersaturated with cholesterol, giving rise to the formation of crystals. As the condition progresses, the gallbladder secretes excess mucus, which nucleates the formation of solid cholesterol crystals. The role of calcium and iron in cholesterol gallstone formation has been under discussion for some time [15, 16]. Elements like Cu and Zn were excreted from liver via bile. In addition, it has been reported that the role of some elements is significant (for example calcium) in the formation of gallstones [17]. Role of sodium and potassium in the structure or formation of gallstones is still unclear, although, observations suggest that the

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changes in the serum metal levels could influence the biliary metal level [18], which in turn, may influence the composition of gallstones. It is also known that metal imbalances either marginal or severe are considered as risk factors for several diseases of public health importance [19, 20]. Present study aims to investigate the levels of sodium and potassium in gallstones and serum of gallstone subjects.

Materials and Methods

The concentrations of sodium and potassium in serum of 109 gallstone subjects and 100 controls were determined by Jenway Clinical Flame photometer model PFP7C, UK. The samples for sodium and potassium in serum were prepared as per reported method [21].

Gallstone samples recovered by cholecystectomy from subjects were washed carefully with doubly distilled deionized water (to remove bile and debris) and dried over silica gel. Each gallstone sample was powdered with an agate mortar and pestle and the powder thus obtained was stored in a 5ml sample tube, kept over silica gel in dark cabinet until analyzed for chemical composition by Nicolet Avatar 330 FTIR (Fourier transform infrared) spectrometer [22]. The gallstone samples for sodium and potassium were prepared as previously reported [23].

All glassware were rinsed overnight in 20% HNO_3 and washed three times with double-distilled deionized water. All the reagents used were ultra pure grade supplied by E Merck; Germany. Two series of sodium and potassium standards were prepared for the determination of Na and K in serum and gallstone samples separately.

Results

Comparison for serum sodium and potassium levels between gallstone subjects and controls of different groups are shown in Fig. 1 and Fig. 2 respectively. Sodium and potassium levels were not significant in all the groups of gallstone subjects as compared to controls.

In gallstones, sodium was significantly high ($p<0.05$) as compared to potassium in all the groups of gallstone subjects (Table 1). Interestingly, we found no significant variation for sodium and potassium in males of both gallstone subjects and controls.

The ratio between sodium and potassium in serum of gallstone subjects and different types of gallstones was calculated and given in Table 2. Sodium to potassium ratio was normal in serum of gallstone subjects, whereas, in gallstones, low sodium to potassium ratio was noted.

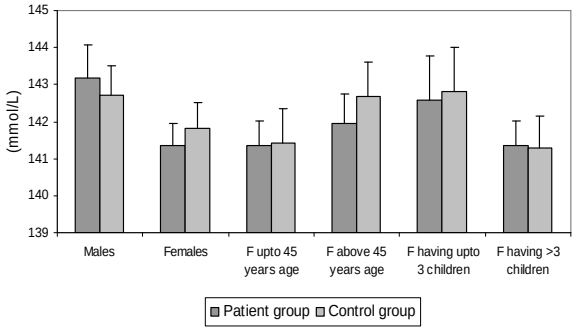

Figure 1. **Comparison of serum sodium levels between gallstone subjects and controls. Each value is expressed in mean.**

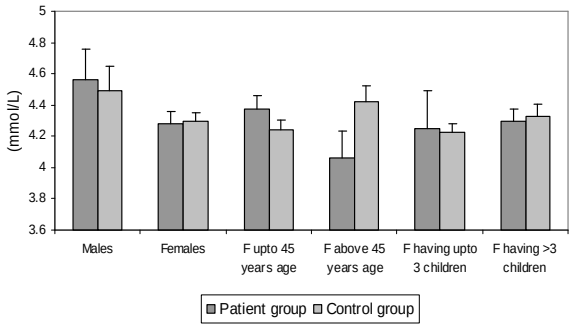

Figure 2. **Comparison for serum potassium levels between gallstone subjects and controls. Each value is expressed in mean.**

Table 1. **Comparison of sodium and potassium contents in gallstones removed from different groups of gallstone subjects.**

Groups	Sodium Mean±S. D. (µg/g)	Potassium Mean±S. D. (µg/g)	p value (≤0 .05)
Male subjects(11)	992 ± 40	1125 ± 15	0.635
Female subjects(N=98)	1828 ± 90	864 ± 49	0.0001
Female subjects of upto 45 years age group (71)	1693 ± 96	950 ± 56	0.002
Female subjects of above 45 years age group (27)	2083 ± 83	693 ± 21	0.003
Female subjects having up to 3 children (81)	1789 ± 39	930 ± 23	0.0001
Female subjects having more than 3 children (17)	1736 ± 29	703 ± 15	0.0001

* p is calculated by student's *t* test

Table 2. **Sodium to Potassium ratio in different types of gallstones and in serum of gallstone subjects**

Type of gallstone	Na : K in Serum (normal 27 – 40)	Na : K in Gallstones
Pure Cholesterol	33:1	2:1
Cholesterol + Ca-carbonate	31:1	4:1
Cholesterol + bilirubin	36:1	2:1
Pure calcium carbonate	33:1	3:1
Calcium bilirubinate	34:1	4:1

Significantly high ($p<0.05$) concentrations of sodium and potassium was observed in calcium bilirubinate gallstones, whereas, increased serum concentration was non-significant in pure calcium carbonate gallstone subjects (Table 3 and 4).

Discussion

Sodium and potassium levels in serum play an important role in diagnosis of different diseases [24]. The base of present study was on the findings of previously published work on metals in gallstones [23]. We found sodium and potassium, in all the gallstones analyzed which indicated a role of these metals in the pathogenesis of human gallstones. Many investigators have suggested that metals especially when present in higher amounts in serum and / or in bile play a significant role in stone formation either in association with structure of conglomerate crystals or in combination with organic molecules [19].

Increased concentrations of sodium in gallstones as compared to potassium in the all groups except in gallstones recovered from males revealed that high concentration of sodium is present in the bile of females. It may be because of certain factors such as hypomotility (due to pregnancy) [25–27] and hypertension [28], etc. Sodium and potassium may be the reason for significant occurrence of gallstones in females as compared to males. Indeed, more thorough investigations are required to clarify the non-significant variation for sodium and potassium in gallstones recovered from male subjects as compared to females.

Sodium forms salt with bile acids which gets accumulated during cholelithiasis. According to Chandran et al. the more the content of bile acid, the more content of sodium, as was observed in calcium bilirubinate compared to other types of gallstones in present study. Furthermore, Chloride ions are always present in biological fluids in human beings including bile, which might get deposited in the form of sodium chloride salt along with major salts of the gallstones. A certain ratio of sodium and potassium is maintained in bile of gall bladder. Higher the sodium content, higher the potassium content [29]. This might be the reason for higher potassium content in calcium bilirubinate stones compared to other types of gallstones (Table 1). A low sodium/potassium ratio in bile is associated with a liver dysfunction, often accompanied by an inadequate secretion of bile acids [30].

Table 3. **Statistical comparison of serum sodium and potassium levels in patients with different types of gallstones**

Metals	Pure calcium carbonate	Cholesterol + bilirubin	Calcium bilirubinate	Pure Cholesterol	Cholesterol + Calcium carbonate	P Values (<0.05)
Sodium	143.18	142.55	141.65	141.35	141.29	N.S
Potassium	4.56	4.29	4.33	4.31	4.49	N.S

p is calculated by ANOVA, N.S.= Non-significant

Table 4. **Statistical comparison of sodium and potassium levels in five different types of human gallstones**

Metals	Pure calcium carbonate	Cholesterol + bilirubin	Calcium bilirubinate	Pure Cholesterol	Cholesterol + Calcium carbonate	P Values (<0.05)
Sodium	6256.54	1200.00	8058.33	1749.22	3325.10	0.01
Potassium	887.88	667.86	2033.33	889.21	893.75	0.01

p is calculated by ANOVA

Gallstones are believed to form, when the concentration of cholesterol exceeded that which can be held in mixed micellar solution with bile acids and phospholipids. Supersaturation of cholesterol is believed to be due to abnormal production of bile from liver. The concept of cholesterol nucleation due to low concentration of bile acids and salts as a basis for gallstone formation has been emphasized for cholesterol stones, which are composed of mainly cholesterol [31]. In present study gallstones show low sodium to potassium ratio as compared to the ratio in serum. These results are in agreement with study of Chandran et al. [32].

Significantly high (<0.05) concentrations of sodium and potassium was observed in calcium bilirubinate gallstones, whereas, non-significant increased serum concentration was noted in pure calcium carbonate gallstone subjects (Table 3and 4). Gall bladder water absorption increases during the early stages of cholelithiasis [33]. The high concentrations of cations found in gallstone samples may be due to this rather than increased secretion of Na^+ and K^+ cations [34, 35]. Apart from that Chandran et al. reported increase in K in bile is due to its increase in serum which ultimately leads to precipitation of bilirubin along with Ca to form pigment stone [32]. Although our observations showed non-significant increased serum concentration of Na and K in pigment stones, whereas, significant increased concentration in pigment gallstones. Hence, we can say that presence of Na and K may be associated with pigment gallstones. Other investigators also reported that the presence of trace metals has been associated with bilirubinate and black pigmented gallstones [36–42]. The presence of significant amounts of sodium and potassium in gallstones as compared to the serum may be a consequence of the presence of abnormal amounts of the Na^+ and K^+ cations in the bile or the co-precipitation process with an organic or inorganic ligand [11].

Conclusion

The results demonstrate that the higher concentration of sodium and potassium in gallstones may involve in both calcium bilirubinate gallstones and in serum of calcium carbonate gallstone subjects, which indicate their association with calcium in the precipitation of calcium bilirubinate and calcium carbonate in bile. Furthermore, low sodium to potassium ratio in gallstones indicates low ratio in bile of gallstone subjects.

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