

Histochemical study of alkaline phosphatase enzyme in gallbladder containing cholesterol stones

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ABSTRACT

Objective: To study the alterations in the expression pattern of alkaline phosphatase (ALP) in gallbladder mucosa in cases of cholelithiasis (cholesterol type) in order to find its role as an indicator of functional alteration in gall bladder mucosa in these cases.

Materials and methods: A prospective study was conducted over a period of 2 months started from July 1st 2010 to September 1st 2010 at the laparoscopic unit in Al-Jumhori Teaching Hospital, Mosul, Iraq, and the Laboratory of postgraduate studies at the Department of Anatomy, Histology and Embryology, College of Medicine, University of Mosul. Analysis of the sections which were obtained from twenty-two surgically resected gallbladder specimens using laparoscope was done. Mean age was 50.5±2.1 years (range 21-77), while the male:female ratio was 1:6. Clinical details were retrieved from the records. The cholecystectomy specimens that were received were fixed in 10% formalin. The findings on gross examination were noted, and the stones recovered from the gallbladder were analyzed by chemical examination. Multiple sections were taken from each cholesterol stone-containing gallbladder (body, neck, and fundus) for paraffin blocks and were examined after staining with hematoxylin and eosin (H&E). Microscopic examination was done to assess the type of histological changes. To evaluate the enzymatic activity of alkaline phosphatase, sections were stained using alkaline phosphatase stain (Gomori's method- Cobalt).

Results: Light microscopic examination using haematoxylin and eosin (H&E) revealed that 13 (59.09%) out of 22 cases were diagnosed as cholelithiasis, while sections obtained from 3 (13.6%) out of 22 cases showed inflammation. Rokitsansky-Aschoff sinuses were noticed in 6 (27.27%) out of 22 cases. Using alkaline phosphatase stain (Gomori's method- Cobalt), the amount of alkaline phosphatase activity varied considerably among the different areas in the same sections, and often among different areas of the same layer of gallbladder, thus making the assessment difficult. However, this study showed 14 (63.63 %) out of 22 cases with moderate reaction to ALP stain in the mucosa, while 8 (36.36%) cases showed weak to negative reaction to that stain.

Conclusion: The changes in ALP localization patterns may represent functional alterations either preceding or running concurrently with morphological damages in gallbladder with cholesterol stones. Futural studies will be recommended to localize ALP with different gallbladder disorders especially on ultrastructural, immunohistochemical, and biomolecular levels.

Keywords: Gallstones, histochemistry, alkaline phosphatase.

تحليل نسيجي كيميائي لإنزيم الفوسفاتاز القاعدي في الحويصلة الصفراوية في حالات الحصى الصفراوية الحاوية على الكولسترول

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الخلاصة

هدف الدراسة: مناقشة التغييرات في طريقة تعبير إنزيم الفوسفاتاز القاعدي في الطبقة المخاطية للمرارة الحاوية على الحصى (نوع الكوليسترول) لغرض إكتشاف أي دور له كمؤشر للتغيرات الوظيفية في خلايا الطبقة المخاطية في تلك الحالات.

طريقة الدراسة: أجريت الدراسة خلال فترة شهرين من الأول من تموز إلى الأول من أيلول/ ٢٠١٠ في وحدة الجراحة الناضورية في مستشفى الجمهوري التعليمي في الموصل ومختبر الدراسات العليا في كلية الطب، جامعة الموصل. تم أخذ التقارير السريرية والمرضية المفصلة من سجلات المرضى. وقد تم أخذ العينات من الذين أخضعوا لعملية إستئصال المرارة عن طريق ناظور البطن أو فتح البطن. وتم حفظ العينات وهي الحويصلات الصفراوية (المرارة) في الفورمالين بنسبة تركيز عشرة بالمئة وذلك بعد التحليل الكيميائي للحصى. وتم تحضير الشرائح البارافينية من مناطق العنق والجسم والقمع من تلك الحويصلات الحاوية على الكوليسترول. وتم فحصها بالمجهر الضوئي بعد صبغها بصبغتي الهيماتوكسيلين والايوسين وذلك للتعرف على وجود التغييرات النسيجية التي تشمل الإرتشاح الإلتهابي، وتكدس الكوليسترول النسيجي، وخلل التنسج، وحالات من التبدل الكامل (الحوؤل)، ووجود تغييرات ورمية أو شبه ورمية. ولغرض تقييم النشاط الإنزيمي لإنزيم الفوسفاتاز القاعدي تم صبغ الشرائح بصبغة كموري-كوبلت.

النتائج: تم تحليل النتائج للعينات المأخوذة من ٢٢ مريضاً أجريت لهم عملية إستئصال الحويصلة الصفراوية عن طريق ناظور البطن. كان معدل أعمار المرضى في هذه الدراسة ٢١,٥ ± ٥٠ سنة، وكانت الأعمار تتراوح بين ٢١-٧٧ سنة. وكانت نسبة الذكور إلى الإناث هي ١ : ٦. وبعد الفحص بالمجهر الضوئي للشرائح البارافينية بعد صبغها بالهيماتوكسيلين والايوسين لوحظ تكدس الكوليسترول النسيجي في ١٣ حالة (٥٩,٠٩%) من ٢٢ حالة، بينما كان وجود الإرتشاح الإلتهابي في ٣ حالات (١٣,٦%)، بينما لوحظ جيب روكيتانسكي اشكوف في ٦ حالات (٢٧,٢٧%). من جهة أخرى لوحظ أنه بعد صبغ الشرائح بصبغة كموري-كوبلت إن الفاعلية لإنزيم الفوسفاتاز القاعدي قد تنوعت بين مختلف المناطق في الشريحة الواحدة وكذلك في مختلف مناطق الطبقة الواحدة وهذا ما جعل التقييم صعباً. لقد لوحظ في هذه الدراسة أن هناك ١٤ حالة (٦٣,٦٣%) كان فيها نشاط إنزيم الفوسفاتاز القاعدي متوسطاً بينما كان نشاط هذا الإنزيم ضعيفاً في ثمان حالات (٣٦,٣٦%).

الاستنتاجات: لوحظ في هذه الدراسة وجود تحويرات في التعبير عن إنزيم الفوسفاتاز القاعدي في الطبقة المخاطية للحويصلة الصفراوية الحاوية على الحصى (نوع الكوليسترول)، وهذا يمكن أن يعتبر كعلامة للتغيرات الوظيفية في خلايا الطبقة المخاطية. ومن الممكن توصية دراسات مستقبلية على مستوى الجزئي الحيوي والنسيجي المناعي الكيميائي وكذلك باستخدام المجهر الإلكتروني للاستدلال على دور هذا الإنزيم في حالات حصى الحويصلة الصفراوية.

الكلمات المفتاحية: حصى الحويصلة الصفراوية، كيميائية الأنسجة، إنزيم الفوسفاتاز القاعدي.

INTRODUCTION

Cholesterol gallstones are one of the most prevalent and most costly digestive diseases in the world.^{1,2} The main function of the gallbladder is to concentrate bile by the absorption of water and sodium.^{3,4}

The exact pathogenesis of gallstones remains poorly understood,³ and it has been reported that gallstones disease could be due to multiple causes.^{5,6} Moreover, there is considerable evidence indicating that patients with gallstone have altered gallbladder functions with respect to secretion, absorption and inflammation in the mucus membrane when compared with gallstone-free patients.⁷⁻⁹ There is mounting evidence that oxidative stress or an imbalance in the oxidant/antioxidant activity plays a pivotal role in these functional alterations. The abnormalities of gallbladder epithelial function has been involved in gallstone formation especially cholesterol type.¹⁰⁻¹² The data in several studies showed that the high level of oxidative stress may be related to altered activity of functional enzymes in mucosal cells

including antioxidant enzymes such as catalase, glutathione peroxidase, glutathione transferase and glutathione reductase, in addition to the activity levels of alkaline phosphatase (ALP).^{13,14}

ALP encompasses a group of heterogenous enzymes that catalyze the hydrolysis of monophosphate esters at alkaline pH.¹⁵ ALP is present in the serum in soluble form, but it is also found in many cellular membranes throughout the body.¹⁶

MATERIALS AND METHODS

The study was conducted in Al-Jumhori Teaching Hospital, Mosul, Iraq. It included twenty-two cholecystectomies performed at the laparoscopic unit over a period of 2 months started from July 1st 2010 to September 1st 2010. Clinical details were retrieved from the records. The cholecystectomy specimens that were received were fixed in 10% formalin. The findings on gross examination were recorded. The stones were analyzed by chemical examination as described by Burtis and Aswood in 1999 and Tietz in 1986. Multiple sections were taken from each cholesterol stone-containing

gallbladder (body, neck, and fundus) for paraffin blocks and were examined after staining with hematoxylin and eosin (H&E).

Microscopic examination was done to assess the type of histological changes including inflammatory infiltrate, presence of fibrosis, cholesterolosis, metaplasia, tumor, or tumor-like lesion.

To evaluate the enzymatic activity of alkaline phosphatase, sections were stained using alkaline phosphatase stain (Gomori's method- Cobalt).^{17,18} Appropriate controls for the incubation were also run by omitting the substrates from the respective media.

To ensure maximum resolution of the histochemical reaction products for microscopy and to obtain optimal contrast in photomicrography, counterstaining was done in all the preparations using safranin.

RESULTS

Mean age of the patients was 50.5± 2.1 years (range 21-77), while the male:female ratio was 1:6.

Light microscopic examination of haematoxylin and eosin (H&E) stained sections revealed that 13 (59.09%) out of 22 cases had cholesterolosis (**Figure 1**), 3 (13.6%) showed inflammation, and Rokitansky-Aschoff sinuses was noticed in 6 (27.27%) (**Figure 2**).

The various histological abnormalities in gallbladder containing cholesterol stones with different frequencies are shown in **Table 1**.

By using alkaline phosphatase stain (Gomori's method-Cobalt), the amount of alkaline phosphatase activity varied considerably among the different areas in the same section, and often among different areas of the same layer of gallbladder, thus making the assessment difficult.

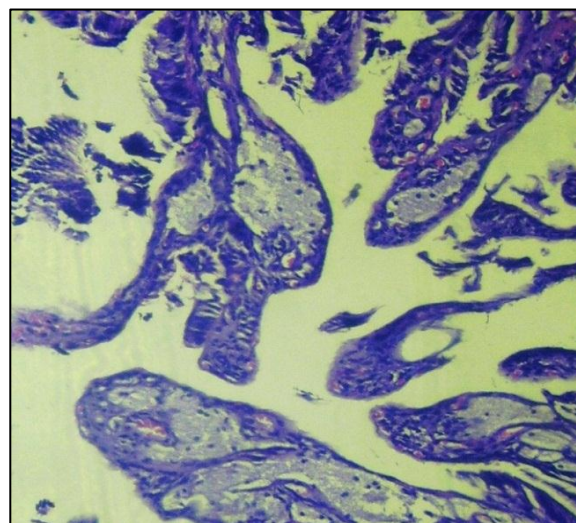
As shown in **Figure 3** there is weak reaction to ALP stain in the mucosa of gallbladder as noticed in 8 (36.36%) out of 22 cases. **Figures 2&4** show moderate reaction to that stain accompanied with Rokitansky-Aschoff sinuses and cholesterolosis, the moderate activity of ALP stain was noticed in 14 (63.63%) cases.

Some cases of cholelithiasis showed moderate to marked reaction of the mucosa and perimuscular layers to ALP stain as shown in **Figure 5**.

Some sections showed moderate reaction to ALP stain in the wall of the blood vessels (**Figure 6**).

The reaction of the various gallbladder layers to ALP stain was shown in **Table 2**.

Figure 1. A photomicrograph of gallbladder containing



cholesterol stone showed cholesterolosis. (H&E x 250).

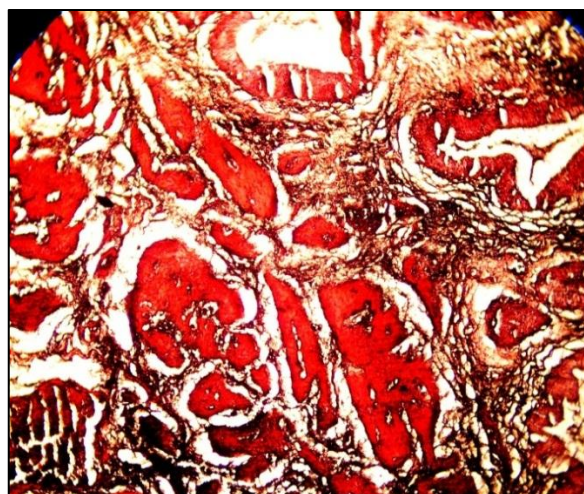


Figure 2. A photomicrograph of gallbladder containing cholesterol stone showed Rokitansky-Aschoff sinuses with moderate reaction to ALP stain. (ALPx400).

Table 1. The frequencies of different histological abnormalities.

Histological Findings	Frequency (No.=22)
Normal	0 (0.0%)
Cholesterolosis	13 (59.09%)
Rokitansky-Aschoff Sinuses	6 (27.27%)
Inflammation	3 (13.6%)

Table 2. The reaction of different layers of gallbladder to ALP stain.

Site	ALP
Mucosa	++/+++
Fibromuscular layer	+ / ++
Perimuscular layer	++

+++ , markedly positive; ++, moderately positive; +, weakly positive; -, negative.

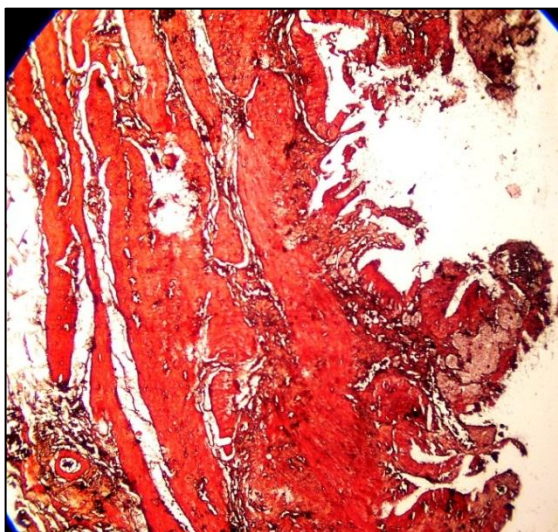


Figure 3. A photomicrograph of gallbladder containing cholesterol stone showed weak reaction to ALP stain. (ALPx250).

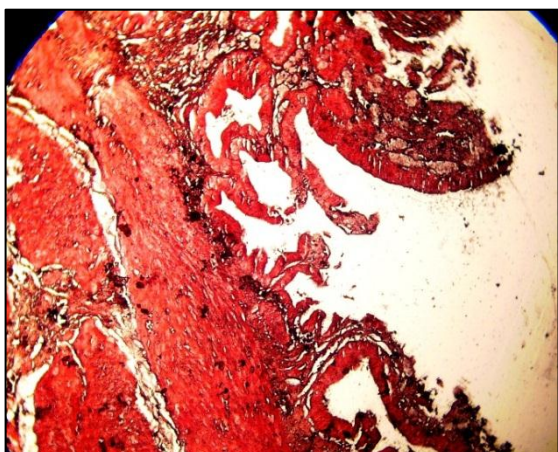


Figure 4. A photomicrograph of gallbladder containing cholesterol stone showed cholesterlosis and moderate reaction to ALP stain. (ALP x 250).

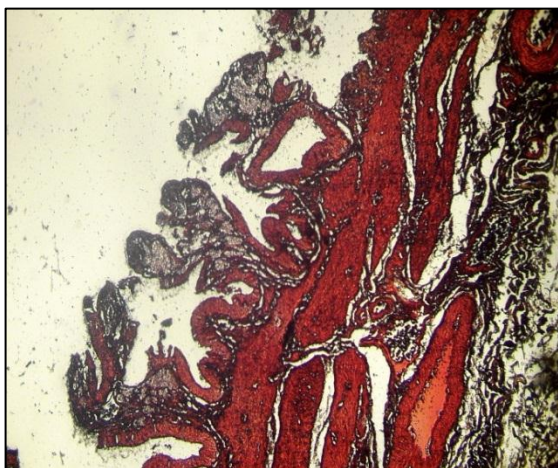


Figure 5. A photomicrograph of gallbladder containing cholesterol stone showed cholesterlosis and moderate to marked reaction to ALP stain in the mucosa and perimuscular layer. (ALP x 250).



Figure 6. A photomicrograph of gallbladder containing cholesterol stone showed moderate reaction to ALP stain in the wall of the blood vessels. (ALP x 400).

DISCUSSION

Alkaline phosphatase has been shown to play an important but ill-defined role in transmembrane transfer mechanism as the enzyme is found primarily in cell membrane where active transport processes take place.¹⁹⁻²¹

The serum ALP is a measure of the integrity of the hepatobiliary system, however, it's wide spread in many tissues and fluids throughout the body gives its broad diagnostic potential.^{16,22,23}

This study revealed that large number of patients was between 35 and 45 years, and male to female ratio was 1:6. These findings are in accord with Schirmer *et al* in 2005,²⁴ Mohan *et al* in 2005,²⁵ and Al-Khayatt *et al* in 2009,²⁶ who have also shown predominance of females among patients with gallstone diseases due to the effects of female sex hormones. Our results are consistent with those of Mohan *et al* in 2005²⁵ who attributed the decrease in activity of cholesterol α reductase, and increase in activity of HMG CoA reductase to increased cholesterol secretion and saturation of bile.

Cholesterlosis was found to be the most frequent lesion mainly in the corpus and fundus, similar to the findings of Shcherbinina *et al* in 2007²⁷ and Mohan *et al* in 2005.²⁵

This study showed that ALP activity varied considerably among the different areas in the same section, and often among different areas of the same layer of gallbladder, thus making the

assessment difficult. These findings are comparable to those of Saxena and Murthy, 2005,²² Mangal *et al* in 2005,²⁰ and Al-Allaf in 2009.²⁹

There are many contradictory views concerning the ALP activity in several disorders. This study showed that the moderate activity of ALP stain was noticed in 14 (63.63%) cases. These findings are consistent with that of Mangal *et al* in 2005²⁰ who reported an increased ALP attributed to tissue ischemia, He suggested that there is considerable increase in lysosomal activity, presumably as a response to tissue ischemia and which by altering the tissue pH of cells, stimulates lysosomal activity which leads to cellular damage and destruction, with the release of ALP from vesicles into cytoplasm. This high intensity of ALP may be due to increased synthesis of this enzyme which could be a compensatory mechanism.^{20,27}

We noticed moderate activity of ALP around the wall of blood vessels, these findings are similar to those of Saxena and Murthy in 2005²² and that of Al-Allaf in 2009.²⁹ They reported that strong activity of ALP was found in the adventitial layers of blood vessels and even capillary endothelium. They reported that the distribution of this enzyme on the cellular surface and cytoplasm, vascular adventitia and capillary endothelium may provide indication that the enzyme might participate in the transport of substances across the membranes.

Weak reaction to ALP stain in the mucosa of gallbladder was noticed in 8 (36.36%) out of 22 cases, which is similar to that of Geetha in 2002³⁰ who reported that the activities of functional enzymes in mucosa such as ALP, Na(+)-K+ (EC 3.6.1.3) and Ca²⁺ (EC 3.6.1.2) adenosine triphosphatase showed significant decrease, however, there are differences in the used staining methods and sample size. In addition, his histopathological observation showed lipid accumulation, dilated blood vessels, necrotic and fibrotic changes and inflammation in the gallbladder mucosa of gall stone patients. He suggested that there is a high level of oxidative stress in the gall bladder mucosa in cases of gall stones, a finding that may be related to a decreased activity of functional enzymes in mucosal cells. Such a condition might result in an altered gallbladder absorption and secretion of bile components such as mucins and glycoproteins.

The resultant increased risk of bile saturation would further contribute to the progress of gall stone formation.^{26,30} In addition, Saxena and Murthy,²² reported that the overall decrease in the ALP activity in some cases indicates an impairment in the cellular transport mechanism. Futural studies will be recommended to define the localization of ALP with different gallbladder disorders especially on the levels of electron microscope, immunohistochemistry, and biomolecular lines.^{31,32}

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