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 cholerterosis, while sections obtained from 3 (13.6%) out of 22 cases showed inflammation. Rokitansky-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.
**INTRODUCTION**

Cholesterol gallstones are one of the most prevalent and most costly digestive diseases in the world. The main function of the gallbladder is to concentrate bile by the absorption of water and sodium. The exact pathogenesis of gallstones remains poorly understood, and it has been reported that gallstones disease could be due to multiple causes. Moreover, there is considerable evidence indicating that patients with gallstone have altered gallbladder functions with respect to secretion, absorption and inflammation in the mucous membrane when compared with gallstone-free patients. There is mounting evidence that oxidative stress or an imbalance in the activity levels of antioxidant enzymes such as catalase, glutathione peroxidase, glutathione transferase and glutathione reductase, in addition to the activity levels of alkaline phosphatase (ALP), encompasses a group of heterogenous enzymes that catalyze the hydrolysis of monophosphate esters at alkaline pH. ALP is present in the serum in soluble form, but it is also found in many cellular membranes throughout the body.

**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 gross examination were recorded. The stones were analyzed by chemical analysis 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, cholesterosis, 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). 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 cholesterosis (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 cholesterosis, 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.

![Image of gallbladder containing cholesterol stone](image)

**Table 1.** The frequencies of different histological abnormalities.

<table>
<thead>
<tr>
<th>Histological Findings</th>
<th>Frequency (No.=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Cholesterosis</td>
<td>13 (59.09%)</td>
</tr>
<tr>
<td>Rokitansky-Aschoff Sinuses</td>
<td>6 (27.27%)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>3 (13.6%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Site</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosa</td>
<td>++++/++</td>
</tr>
<tr>
<td>Fibromuscular layer</td>
<td>+/-</td>
</tr>
<tr>
<td>Perimuscular layer</td>
<td>++</td>
</tr>
</tbody>
</table>
+++ , markedly positive; ++ , moderately positive; + , weakly positive; - , negative.

Figure 3. A photomicrograph of gallbladder containing cholesterol stone showed weak reaction to ALP stain. (ALP x 250).

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.\textsuperscript{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.\textsuperscript{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 \textit{et al} in 2005,\textsuperscript{24} Mohan \textit{et al} in 2005,\textsuperscript{25} and Al-Khayatt \textit{et al} in 2009,\textsuperscript{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 \textit{et al} in 2005\textsuperscript{25} 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 \textit{et al} in 2007\textsuperscript{27} and Mohan \textit{et al} in 2005.\textsuperscript{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
assessments 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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REFERENCES

11. Adfalh NH, Ostrow JD, Koehler R, et al. Interaction of bovine gallbladder mucin and calcium-binding protein:
effects on calcium phosphate precipitation. 