Role of Immunohistochemical Markers in Diagnosis of Clinically Suspected Cases of Appendicitis and Its Correlation with Histopathology

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1. Introduction

The appendix is a normal true diverticulum of the cecum. Like any diverticulum, it is prone to acute and chronic inflammation, and acute appendicitis is a relatively common entity. Other lesions, including tumors, can also occur in the appendix but are far less common.¹

Acute appendicitis is most common in adolescents and young adults but may occur in any age group. The lifetime risk for appendicitis is 7%; males are affected slightly more often than females. Despite the prevalence of acute appendicitis, the diagnosis can be difficult to confirm preoperatively, and the condition may be confused with mesenteric lymphadenitis (often secondary to unrecognized Yersinia infection or viral enterocolitis), acute salpingitis, ectopic pregnancy, mittelschmerz (pain associated with ovulation), and Meckel diverticulitis.¹

The diagnosis of acute appendicitis being essentially clinical, the decision for operation alone leads to removal of normal appendix in 15-30 percent of cases. The premises that better to remove a normal appendix than to delay diagnosis does not standup to close scrutiny particularly in the elderly. Number of clinical and laboratory based scoring system have been devised to assist diagnosis. The most widely used is the Alvardo score, a score of 7 or more strongly predictive of acute appendicitis.

Patients with clinical signs and symptoms of acute appendicitis, appendectomy relieves symptoms in vast majority of them. Acute appendicitis is one of the most frequent reasons for acute abdominal operation.²

In early acute appendicitis, subserosal vessels are congested, and a modest perivasculaer neutrophilic infiltrate is present within all layers of the wall. The inflammatory reaction transforms the normal glistening serosa into a dull, granular, erythematous surface. Although mucosal neutrophils and focal superficial ulceration often are present, these findings are not specific, and diagnosis of acute appendicitis requires neutrophilic infiltration of the muscularis propria. In more severe cases, focal abscesses may form within the wall (acute suppurative appendicitis), and these may even progress to large areas of hemorrhagic ulceration and gangrenous necrosis that extend to the serosa, creating acute gangrenous appendicitis, which often is followed by rupture and suppurrative peritonitis.¹

The incidence of histologically normal appendix ranges from 8-41 percent. When extensive sectioning is done on histologically normal specimens, it often happens that a focus of inflammation is found in only a few serial section. This condition is known as FOCAL APPENDICITIS-so called because the polymorphonuclear infiltration is confined to a single focus, while the remaining appendix is devoid of any polymorphonuclear cells. It is not clear that all cases of acute appendicitis arise from this focal inflammation; however such inflammatory foci may be the earliest recognisable manifestation of appendicitis in some so called negative appendectomies.

A substantial HNA exhibit increased expression of TNF-alpha and IL-2, messenger RNA’s (sensitive markers of inflammation in appendicitis) in germinal centers, submucosa and the lamina propria. Therefore, appendectomy is recommended in patients with clinically suspected acute appendicitis even when the appendix does not appear inflamed during exploration.
In a study conducted by Laszlo et al. they concluded that there exists a subgroup of appendicitis within so called HNA in which evidence of an inflammatory pathologic condition is only obvious at a molecular level which was done by staining TNFα, IL2, COX-1,2, PGE2, iNOS, MHC-II antigens using IHC technique. The present study aims to find out age and sex incidence in acute appendicitis cases and to correlate histopathological finding and immunohistochemical finding of suspected cases operated for acute appendicitis.

II. Materials And Methods

Study was conducted in Department of Pathology, Rajendra Institute of Medical Sciences, Ranchi and nearby hospitals. Cases were selected on grounds of clinical suspicion of appendicitis and on the basis of history or presenting with complaints of right iliac fossa pain and fever. During the study period, 47 cases which were clinically diagnosed as appendicitis were operated in Surgery Department and its histopathology was done in Department of pathology, Rajendra Institute of Medical Sciences, Ranchi. Cases comprised of patients admitted or managed in RIMS and nearby hospitals. Duration of study was from July 2013 to September 2014

Among these cases which were initially diagnosed by Hematoxylin and Eosin (H&E) stained tissue sections, 47 cases were taken and were studied by using immunohistochemistry using monoclonal antibody to TNFα and IL2 on two separate sections for each individual case.

III. Statistical Analysis

**Sensitivity** (also called the true positive rate, or the recall rate in some fields) measures the proportion of actual positives which are correctly identified as such and is complementary to the false negative rate.

Sensitivity relates to the test's ability to identify a condition correctly. Mathematically, this can be expressed as:

\[
\text{Sensitivity} = \frac{\text{number of true positives}}{\text{total number of cases}}
\]

Sensitivity of histopathology = \( \frac{37}{37 + 4} = 90.24\% \)
Histological examination is 90.24% sensitive in diagnosis a suspected cases of acute appendicitis.

IV. Observations And Results

TNFα and IL2 were studied in total 47 samples of specimen of appendix in suspected cases of acute appendicitis during July 2013 to October 2014.

The table shows incidence among various age groups

<table>
<thead>
<tr>
<th>AGE</th>
<th>NUMBER OF PATIENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9 yrs</td>
<td>0</td>
</tr>
<tr>
<td>10-19 yrs</td>
<td>22</td>
</tr>
<tr>
<td>20-29 yrs</td>
<td>20</td>
</tr>
<tr>
<td>30-39 yrs</td>
<td>3</td>
</tr>
<tr>
<td>40-49 yrs</td>
<td>2</td>
</tr>
</tbody>
</table>

Cases were between 0-49 yrs with a peak incidence in 10-19 yrs age group.

The table shows incidence of clinical feature and laboratory finding in suspected cases of acute appendicitis

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>RIF pain</th>
<th>Leukocytosis</th>
<th>Fever</th>
<th>vomiting</th>
<th>Rovsing's sign</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>36</td>
<td>18</td>
<td>12</td>
<td>39</td>
<td></td>
<td>47</td>
</tr>
</tbody>
</table>

The study on 47 suspected cases of acute appendicitis shows 44 case have RIF pain, i.e 93.61% cases have RIF pain.
The table showing results of histological examination of suspected cases of acute appendicitis

<table>
<thead>
<tr>
<th>suspected cases of acute appendicitis</th>
<th>47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histological evidence of acute appendicitis</td>
<td>37</td>
</tr>
<tr>
<td>No evidence of acute appendicitis</td>
<td>10</td>
</tr>
</tbody>
</table>

Out of 47 clinically suspected cases of acute appendicitis only 37 cases showed histological evidence of acute appendicitis. i.e 78.72% cases of acute appendicitis showed histological evidence of acute appendicitis. IL2 and TNFα IHC markers were studied among all the samples.

The table shows findings of IL2 and TNFα IHC markers in all suspected cases of acute appendicitis.

<table>
<thead>
<tr>
<th>IL2 positive</th>
<th>Histological normal</th>
<th>Acute appendicitis histologically</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>TNFα positive</td>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td>IL2 negative</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>TNFα negative</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>47</td>
</tr>
</tbody>
</table>
Total 47 suspected cases were studied, out of which 37 cases were histologically confirmed cases and both IHC markers were studied it showed 35 cases to be positive for both IL2 and TNFα, i.e 94.59% confirmed cases show expression of inflammatory markers in their germinal centre and cytoplasm of inflammatory cells. 10 histologically normal cases out of 47 suspected cases were also studied for inflammatory markers and 4 cases, i.e 40% cases were found to have expression of IL2 and TNFα in their germinal centre of lymphoid follicle and cytoplasm of inflammatory cells.

V. Conclusion

10 histologically normal cases out of 47 suspected cases were also studied for inflammatory markers and 4 cases, i.e 40% cases were found to have expression of IL2 and TNFα in their germinal centre of lymphoid follicle and cytoplasm of inflammatory cells. Appendix removed from patients with suspected appendicitis often appear normal on histological examination. We examined appendix specimens for expression of abnormal amounts of cytokines, an indicator of an inflammatory response and many cases which were histologically normal, showed presence of acute inflammation.

Bibliography

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