A BACTERIOLOGICAL COMPARISON BETWEEN A NORMAL AND AN INFLAMED APPENDIX

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ABSTRACT

Acute appendicitis is a common surgical emergencies problem, in spite of the diagnostic and therapeutic advancement in medicine; there was no specific investigation for diagnose it.

The aim of the study is to improve the diagnostic accuracy in order to reduce the 32% or higher percentage of normal appendix after appendectomy.

A prospective study between a normal and an inflamed appendix wall of 106 patients admitted to Al-Yarmouk teaching hospital and Al-Ramady teaching hospital age range between 15 - 42 years old was done by culturing on specific media, using the API system (analytical profile index) for specific diagnosis then colony counting by standard plat count.

The results showed considerable differences between the histologically normal flora and acutely inflamed appendix in the number of the same bacteria. Escherichia coli, streptococcal species and Bacteroides species, were the common organisms separated and were found in a count of 10³ - 10⁸ organisms / gm. of tissue. The Bacteroides species are the most dominant flora in inflamed and normal appendices.

The increase bacterial colonies in acute inflamed appendices compared to normal appendix make the bacterial infection as a factor in the etiology of the pathogenesis of the appendicitis. Still it was the result of the growth of the already existing bacteria and since some of these bacteria showed a statistically significant with appendicitis, they could be used as a marker for the diagnosis by adding it to the Alvarado Score for Acute Appendicitis.

Keyword: Appendicectomy, Appendicitis, Bacteroides species, Escherichia coli, and streptococcal species

INTRODUCTION

Acute inflammation of the appendix is the most repeated status which needed urgent operation. It needs to have knowledge about the detail of the causative factors. (1) The common cause of acute appendicitis is invasion of Bacteria and conform this to the clinical finding. (2) Acute appendicitis can be obstructive or non-obstructive. (3) The non-obstructive type of acute appendicitis is
caused by invasion of bacteria to the lymphoid tissue in the appendicular wall.\(^4\) Because there was no obstruction to the lumen of the appendix, so less liable to gangrene and the acute attack may spontaneously resolved.\(^5\)

The acute appendicitis diagnosed clinically; the decision to operate depending only on a clinical suspicion can lead to removal of a normal appendix in more than 30% of cases. Many of clinical finding and laboratory investigations have been produced to assist the diagnosis. The score was mostly used. Which is a clinically score system applied for diagnosis of acutely inflamed appendix\(^6\). The Alvarado score have two laboratory measurements with six clinical articles of total ten points. A modified Alvarado score is at present in use\(^7\).

The Alvarado score composed from history of patient, the clinical checking with laboratory investigations:

- Generalized abdominal pain which shifted to right iliac region
- Anorexia
- Nausea or/ and vomiting
- By palpation there was a tenderness in the right iliac fossa region
- Positive rebound tenderness
- Fever with temperature more than 37.3 °C
- Leukocytosis, and white blood cells > 10,000 /\(\mu L\) in serum
- The percentage of neutrophils (Neutrophilia) will be increased in serum,

The most two important factors, are the leukocytosis and tenderness in the right iliac fossa are given (2) points for each, and (1) point for each other (6) factors, for a potential total score of (10) points.

A score of 5-6 points is suitable for the diagnosis of acute inflammation of the appendix. A score of 7-8 points indicates a possible of acute inflammation of the appendix and a score of 9-10 points suggested that a possible of acute inflammation of the appendix.\(^8\)

There was no information about the quantitative flora as a whole from the normal appendicular tissue and the inflamed appendix. This study was undertaken to display the numerically dominant bacterial flora and precisely compare between the floras of the normal histologically appendices and acutely inflamed appendices.

**MATERIAL AND METHODS**

**Patient specimens**

A prospective study of consecutive patients attending the Emergency Department whom were admitted to Al-Yarmouk teaching hospital and Al-Ramady teaching hospital undergoing appendicectomy between 1\(^{\text{st}}\) of April till 25\(^{\text{th}}\) of July 2019 was studied. Six patients were excluded because they obtain antibiotics during two weeks before admission.

**Specimens’ collections:**

The sample of appendix was divided longitudinally and the concentrated material was removed. First half of the appendix was examined histologically, and the other half was subdivided transversely into proximal (appendicular orifice) and distal (core) sections. The single portions was weighed, and putted in transport medium (Todd Hewitt broth, 0-1% cystine hydrochloric acid and 10% glycerol by volume) (recommended for the cultivation of streptococci and other fastidious microorganisms) and homogenized.

**Method**
Sequent recorded dilutions of the homogenate then made and coated on a chosen of media (MacConkey’s, chocolate, vpt (which agar including trimethoprim, polymixin and vancomycin), neomycin, blood and Bacteroides Bile Esculin (gentamycin 100 μg/ml, Trypticase agar plus, 20% bile- inhibits most non-pathogenic Bacteroides spp and 0.1% esculin) then incubated under the suitable microaerophilic, anaerobic and aerobic conditions 25°C for 48hrs or 37°C for 24hrs.

Dominant isolates were purified by sub culturing and identified. Bacteroides species were identified using the Analytical Profile Index20A system (BioMerieux, Inc., Hazelwood, MO) and streptococcal isolates by Lancefield grouping, fermentation of sugars, and growth on special media.

**Calculation**

Counting was performed by standard plat count (is an indirect measurement of cell density and reveals information related only to live bacteria) then Calculate the numbers of microorganism( Colony forming unit CFU) /ml or gm. of sample by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar.

\[
\text{Number of colonies (CFUs)} = \frac{\text{No. of microorganism/} \text{ml or gm.}}{\text{Dilution } \times \text{amount plated}}
\]

Any CFU below 30 or above 300 was disregarded.

Log mean counts for every bacterium were compared between the normal and inflamed groups by t-Test and within individual appendix by the paired t-Test. The isolation rates among these 2 groups were matched by the \(X^2\) test.

**RESULTS**

Total of 100 patients were involved in the study, there ages from 15 -42 years, 68 (68%) patients were confirmed histologically as a cases of appendicitis 10 patients of them found to have perforated appendix. 32 (32%) patients have normal non inflamed appendix.

**Microbial findings**

**Dominant Bacteria**

A total of 18 different bacteria were isolated from both inflamed and normal appendix. Escherichia coli, Bacteroides spp. and Streptococcus spp. were the common isolated microorganisms and they found in a count of \(10^3\) to \(10^8\) microorganisms/gm. of tissue. Table 1 show that Bacteroides species were the most common microorganisms in both normal and inflamed appendices.

**Isolations**

Table (1) showed that the different isolation rates of Bacteroides thetaiotaomicron, Bacteroides distasonis, Bacteroides ovatus and Anaerobic streptococci showed no significant difference between normal and inflamed appendix while the different isolation rates for Bacteroides fragilis, Bacteroides vulgatus and Clostridium welchii were significant \(P\) value <0.05. For Streptococcus spp. the different isolation rates of Streptococcus milleri, Streptococcus salivarius and Streptococcus sanguis showed no significant difference between normal and inflamed appendix while the different isolation rates for Streptococcus mutans, Streptococcus anginosus and Streptococcus agalactiae \(P\) value < 0.05 as shown in table 2.

In table 3 the Gram negative bacteria collected (Escherichia coli and Pseudomonas species) both showed no significant difference only Coliform bacteria had a \(P\) value < 0.05.

In case of Staphylococcus aureus there was a significant difference between normal and inflamed appendix \(P\) value < 0.05.
Table 1: A comparison of the Anaerobic Microorganisms isolated from the tested patients

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Histologically normal appendix No:32</th>
<th>Acute inflamed appendix No:68</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>Counts/gm.</td>
<td>Mean</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>7(21.8)</td>
<td>3.25x10^7-3.6x10^7</td>
<td>3.35x10^7</td>
</tr>
<tr>
<td>Bacteroides thetaotaomicron</td>
<td>12(37.5)</td>
<td>3.42x10^7-1.55x10^8</td>
<td>7.85x10^7</td>
</tr>
<tr>
<td>Bacteroides distasonis</td>
<td>1(3.1)</td>
<td>8.23x10^7</td>
<td>-----</td>
</tr>
<tr>
<td>Bacteroides ovatus</td>
<td>1(3.1)</td>
<td>4.14x10^7</td>
<td>-----</td>
</tr>
<tr>
<td>Bacteroides vulgatus</td>
<td>1(3.1)</td>
<td>4.65x10^7</td>
<td>-----</td>
</tr>
<tr>
<td>Clostridium welchii</td>
<td>10(31.2)</td>
<td>5.53x10^7-1.94x10^8</td>
<td>9.39x10^7</td>
</tr>
<tr>
<td>Anaerobic streptococci</td>
<td>---</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

Table 2: A comparison of the Streptococcus Spp. isolated from the tested patients

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Histologically: Normal appendix No: 32</th>
<th>Histologically: Acute inflamed appendix No:68</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>Counts/gm.</td>
<td>Mean</td>
</tr>
<tr>
<td>Streptococcus milleri</td>
<td>14(43.75)</td>
<td>4.81 x10^7-5.05 x10^8</td>
<td>1.137x10^8</td>
</tr>
<tr>
<td>Streptococcus salivarius</td>
<td>9(28.12)</td>
<td>4.81 x10^7-5.05 x10^8</td>
<td>1.878x10^7</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>5(15.62)</td>
<td>1.1 x10^5-6.09 x10^6</td>
<td>3.1x10^6</td>
</tr>
<tr>
<td>Streptococcus sanguis</td>
<td>2(6.25)</td>
<td>6.98 x10^5</td>
<td>-----</td>
</tr>
<tr>
<td>Streptococcus anginosus</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Streptococcus agalacticae</td>
<td>2(6.25)</td>
<td>5 x10^7</td>
<td>-----</td>
</tr>
</tbody>
</table>

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Table 3: A comparison of the other Microorganisms isolated from the tested patients

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Histologically normal appendix</th>
<th>Acute inflamed appendix</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No: 32</td>
<td>No: 68</td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
<td>Counts/gm.</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>20(62.5)</td>
<td>$2.69\times10^5-1.44\times10^8$</td>
</tr>
<tr>
<td><strong>Helicobacter pylori</strong></td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td><strong>Staphylococcus aurous</strong></td>
<td>6(18.75)</td>
<td>$6.02\times10^5-7.91\times10^6$</td>
</tr>
<tr>
<td><strong>Coliform Bacteria</strong></td>
<td>6(18.75)</td>
<td>$7.23\times10^4-3\times10^6$</td>
</tr>
<tr>
<td><strong>Pseudomonas species</strong></td>
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<td>------</td>
</tr>
</tbody>
</table>

**DISCUSSION**

This study showed that for the Anaerobic bacteria there was a quite significant increase in colony count for the inflamed appendix compared to the normal appendix which could be explained throw the ischemia produced favoring its invasion throw the tissue allowing the bacteria to multiply and expand its environment. Although this becomes clear to exclude Bacteroidesfragilis as the main pathogen in acute appendicitis, it obviously has a significant role as a secondarily invasive organism which gives rise to appendicular sepsis. (9)

The counts of *Escherichia coli*, *Streptococcus milleri*, *Streptococcus salivarius* and *Streptococcus sanguis* which existing in the appendix, does not significantly differ among the normal and acute inflamed appendix, although the ischemic tissue in acute appendicitis would be suspected to favor overgrowth of microaerophilic and anaerobic organisms. The insufficiency of increased counts indicates that the number of microorganisms infesting the wall is low in comparison with *Bacteroides spp*.

For Staphylococcus aurous, coliform bacteria, *Streptococcus agalactiae* which showed a significant association between normal and inflamed appendix the colony count was in a state of decrease due to the overgrowth of the rest of bacteria indicating that they grow on the surface and didn’t invade the ischemic tissue. For *Helicobacter pylori* and *Pseudomonas species* both had no significant association between normal and inflamed appendix.

This all would favor the distribution of the bacteria through the wall of the appendix during the inflammation as a passive process due to loss of viability of the wall of the appendix.

**CONCLUSION**

With all the advancement in medicine still there is a high incidence (15-30%) to do an appendectomy and remove a normal appendix. This is mostly due to the dependence on physical examination and screening test rather than depending on a specific test.

The ability of the anaerobic bacteria to invade the ischemic tissue and reaching the blood could help to decrease this percentage.
That is by creating specific antibodies to the surface antigens of these bacteria and a simple strip of Ag-Ab direct immune response could be of help if added to the Alvarado Score for Acute Appendicitis. This test could include all the bacteria which showed significant association between normal and inflamed appendix.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES