IL-8 is a valuable laboratory marker for detection of amoebiasis severity

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Abstract

Background: Amoebiasis is caused by Entamoeba histolytica and is an important human parasitic disease. IL-8, also known as neutrophils chemotactic factor, induces chemotaxis in target cells. The aim of current study was to assess using IL-8 as a laboratory marker for amoebiasis severity. Methods: Sixty three cases with clinical diagnosis of amoebiasis were selected in addition to 26 apparently healthy controls with negative diagnosis of amoebiasis. For both cases and controls, general stool examination using wet mount and assessment of IL-8 were done using IL-8 ELISA kit (Biosource) according to manufacture instructions. Student’s t-test was used to compare the mean and standard deviation and P value <0.05 was considered statistically significant. Results: This study showed that in patients less than 15 years old, the incidence of amoebiasis was 52.94% in males and 47.06% in females with no statistical significance (P value >0.05) while in those more than 15 years old, incidence of amoebiasis was 82.75% in males and 17.24% in females with statistical significance at P value <0.05). The mean±SD IL-8(pg/ml) was 153.23±28.47 in those who had pus cells <++ (30 pus cell /HPF) while it was (249.58±33.12pg/ml) in those who had pus cells ++++ (30 pus cell /HPF) with statistical significance (P value <0.05). While the mean±SD IL-8(pg/ml) was 168.49±41.47 in patients with mild amoebiasis, it was (233.45±29.66pg/ml) in patients with severe amoebiasis with statistical significance (P value <0.05). Conclusion: Amoebiasis has a male predominance in those more than 15 years old. IL-8 is a valuable laboratory marker that can be used for detection of severity of amoebic colitis and it is a helpful laboratory marker to differentiate mild from severe amoebiasis.

Keywords: IL-8, Entamoeba histolytica, protozoa, ELISA, laboratory marker.

How to cite this article: Aboqader RF, Al-Hasnawy MH (2019): IL-8 is a valuable laboratory marker for detection of amoebiasis severity, Ann Trop & Public Health; 22(9): S252. DOI: http://doi.org/10.36295/ASRO.2019.220922

Introduction

Amoebiasis is caused by Entamoeba histolytica and is an important human parasitic disease. E. histolytica parasitizes approximately 10% of the world population, of which 90% of infections are asymptomatic. It has been estimated that 40-50 million people develop clinical amoebiasis annually, resulting in up to 100,000 deaths (1). E. histolytica infection is estimated to kill more than 55,000 people each year (2).

IL-8, also known as neutrophils chemotactic factor, has two primary functions. It induces chemotaxis in target cells, primarily neutrophils but also other granulocytes, causing them to migrate toward the site of infection. IL-8 also stimulates phagocytosis once they have arrived (3).
It was reported that amoebiasis is highly severe disease and some of cases may need intensive care or urgent surgical interference such as fulminant necrotizing colitis, toxic megacolon and fistulizing perianal ulcerations, especially when diagnosis and treatment were not timely\(^{(4,5)}\) and because there was no quick laboratory methods that can be used to detect the clinical severity of disease. So, we focused on this laboratory parameter to evaluate its use as a severity marker. Therefore, the aim of current study was to assess using IL-8 as a laboratory marker for amoebiasis severity.

**Material and Method**

Sixty three cases with clinical diagnosis of amoebiasis were selected in addition to 26 apparently healthy controls with negative diagnosis of amoebiasis. For both cases and controls, general stool examination using wet mount and assessment of IL-8 were done using IL-8 ELISA kit (Biosource) according to manufacturer's instructions.

**Statistical analysis**

Data were expressed as mean and standard deviation. Student’s *t*-test was used to compare the results. *P* value <0.05 was considered statistically significant.

**Results and discussion**

Figure (1) showed that the frequency of *Entamoeba histolytica* was nearly equal in males and females in those less than 15 year old while there was very high frequency in males in comparison to females in those aged more than 15 year. Most of those more than 15 years old gave history of eating outside home and this practice is seen most commonly in males. In addition to that, male predominance may be due to early cytokine production in response to amoeba infection. It was shown in animal studies that there was interleukin-4-producing cells in male mice but significantly higher numbers of gamma interferon (IFN-γ)-producing cells in female mice in response to amoebiasis. Early IFN-γ production and the presence of functional natural killer cells were found to be important for the control of hepatic amoebiasis as application of an IFN-γ-neutralizing monoclonal antibody or the use of NKT knockout mice (Vα14iNKT, Jα 18−/−) dramatically increased the size of ALA in females\(^{(6)}\).

![Figure (1): Frequency of Entamoeba histolytica in relation to sex and age.](image_url)
It has also been found that the relative iron deficiency or hormonal factors in women of child bearing age may be a protective factor against disseminated disease. The most commonly reported risk factor was travel outside the province and outside the country, and the most frequently reported travel destinations included India and Pakistan. This study was consistent with who showed that the disease was more common in males than females.

Table (1) showed that the IL-8 was significantly high in patients who had pus cells \( \geq ++ \) (30 pus cell /HPF) in their general stool examination in comparison to those had pus cells \( <+ \) (20 pus cell /HPF). So, this meant that IL-8 was strongly activated during severe amoebiasis in relation to pus cells and whenever the IL-8 increases, there will be more pus cells. So, this reflected that in response to IL-8, neutrophils infiltrate the intestinal tract as the first cells of an innate immune response to amoebic invasion.

**Table (1): Mean IL-8 levels in patients with amoebiasis in relation to pus cells**

<table>
<thead>
<tr>
<th>Pus cells</th>
<th>NO.</th>
<th>Mean IL-8 (pg/ml)</th>
<th>Standard deviation</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt;++(30 \text{ pus cell }/\text{HPF}))</td>
<td>39</td>
<td>153.23</td>
<td>28.47</td>
<td>(&lt;0.05)</td>
</tr>
<tr>
<td>(\geq++(30 \text{ pus cell }/\text{HPF}))</td>
<td>24</td>
<td>249.58</td>
<td>33.12</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>26</td>
<td>148.55</td>
<td>25.39</td>
<td></td>
</tr>
</tbody>
</table>

It was reported that *E. histolytica* causes tissue destruction by three main events; direct host cell death, inflammation and parasite invasion which lead to pus cells formation. In addition to that, it was found that *E. histolytica* promotes apoptosis which leads to more activation of IL-8 and more white blood cells chemotaxis and pus cells formation.

As an unusual, but serious, complications such as fulminant necrotizing colitis, toxic megacolon and fistulizing perianal ulcerations can occur, especially when diagnosis and treatment is not timely. Patients who develop necrotizing colitis have a mortality rate of 40% and those with concomitant liver abscess mortality increases up to 89% and some of cases required immediate surgical interference. Also, because there was no clinical and laboratory test that can be used to detect clinical severity, the study of IL-8 might be useful.

Table (2) showed that there was significant increase in IL-8 in patients with severe clinical amoebiasis in comparison to mild form of amoebiasis \( (P \text{ value } <0.05)\). As there was no laboratory test found to detect clinical severity of amoebiasis, the level of IL-8 can be a valuable laboratory marker for assessment of clinical severity of amoebiasis and subsequently we can isolate the cases that need more clinical care than the others.

**Conclusion:** Amoebiasis has a male predominance in those more than 15 years old. IL-8 is a valuable laboratory marker that can be used for detection of severity of amoebic colitis and it is a helpful laboratory marker to differentiate mild from severe amoebiasis.
Table (2): Mean IL-8 level among patients with amoebiasis in relation to clinical state

<table>
<thead>
<tr>
<th>Severity of amoebiasis</th>
<th>No.</th>
<th>Mean IL-8(pg/ml)</th>
<th>Standard deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild amoebiasis</td>
<td>44</td>
<td>168.49</td>
<td>41.47</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Severe amoebiasis</td>
<td>19</td>
<td>233.45</td>
<td>29.66</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>26</td>
<td>148.55</td>
<td>25.39</td>
<td></td>
</tr>
</tbody>
</table>

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

Conflict of Interest
The authors declare that they have no conflict of interest

Funding: Self-funding.

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