Comparison between Specificity and Sensitivity of Intestinal Giardialamblia Assays in AL-Door District, Salahdin Province, Iraq

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Abstract: The Giardia lamblia is known as a considerable cause of diarrhea in human. Difficulties are confronted in the detection of that parasite in patients’ faces because of intermittent excretion of the parasite. In this study, it was determined specificity and sensitivity of floatation method by zinc sulphate solution and ELISA assay for Giardialamblia detection compared with direct iodine stain method. Among 82 patients who were attending AL-Door hospital through period of September to December 2018, the prevalence of Giardiasis was 32.9% and 67.1% was negative. As well this study showed that the percentage of G. lamblia infection was 31.7% by direct iodine stain method, 29.3% was by floatation method, and the sensitivity ratio was 92.3%, specificity was 100%. While 32.9% was positive by ELISA assay and the sensitivity ratio was 92.3%, specificity was 94.6%. Also, our study found that the prevalence for Giardiasis depending on gender in this study was 67% for male, 33.3% for female. The results of study were showed the prevalence of infection was 23.2% in age group 8-25 years, 7.3% in 26-35 years and 2.4% in age group 36-45 years. The distribution of infection depends on residence which was 20.7% for rural and 12.2% for urban.

Keywords: Giardia intestinalis, floatation method, ELISA, AL-Door district, Salahdin province.

I. Introduction

Giardialamblia or G. intestinalis flagellated parasite that infects alimentary tract of a variety of the mammalian hosts involving human(Berrilli et al., 2010). Because of G. lamblia has a fecal-oral circulation and is transmitted by consumption of contaminated food or water or by infected person to person contact, the highest infection rates is present in the regions where hygienic conditions are bad(Shah et al., 2008). The highest averages of infection are observed in developing countries, where infections occur mainly among individuals populating in closed communities, immigrants and travelers coming back from endemic countries(Omar et al., 2013). Infected person could be asymptomatic while other suffer diarrhea, bloating and abdominal pain, malaise as well as weight loss and that is because of themalabsorption (Salim et al., 2013). This cause changes in enteric epithelial function with microvilli shorting (Mank et al., 1997). For technicians, it is difficult to diagnose Giardia because the parasite's cysts are introduced intermittently besides similarity of those cysts with other microorganisms such as yeast (Payne et al., 2005).

In the current study, specificity and sensitivity of techniques (including direct iodine stain method, floatation method by Zinc sulphate solution and enzyme-linked immunosorbent assay for antigenic detection in fecal samples) are compared for G. lamblia diagnosis. Value of each Sensitivity, Specificity and predictive were calculated as following:

\[
\text{Sensitivity} = \frac{TP}{TP+FN} \times 100 = \\
\text{Specificity} = \frac{TN}{TN+FP} \times 100 = \\
\text{Positive predictive value or PPV} = \frac{TP}{TP+FP} \times 100 = \\
\text{Negative predictive value or NPV} = \frac{TN}{TN+FP} \times 100 = \\
\text{Accuracy of the test} = \frac{TP+TN}{TP+TN+FP+FN} \times 100 =
\]

II. Materials and methods

This study carried out in period of September to December 2018, Among 82 patients with abdominal symptoms and diarrhea whose ages ranged from 8 to 45 years, who were attending AL-Door model hospital. From each patient fecal samples were collected at the same time and complete information were revealed in special questionnaire designed for this purpose. Each sample was divided into three parts, the first part was diagnosed by direct microscopical method after preparing 3slides which was stained by lugol iodine stain for immediately trophozoite or cysts detection (Gupta, 1979). The second part was diagnosed via floatation method by zinc sulphate solution (John and Petri, 2006) and the last part was added formalin solution 10% to it and stored for
ELISA assay (for detect trophozoites& cysts of G. lamblia antigens in feces samples (DRG/Germany)) which was done later depending on manufacturer instructions.

III. Results

Among 82 feces samples diagnosed, 67.1% was negative and 32.9% was positive for Giardiasis. Table (1).

### Table (1): Giardiasis among infected & non infected patients and a comparison between three methods for Giardiasis diagnosis.

<table>
<thead>
<tr>
<th>No. of examined samples 82</th>
<th>No. of positive samples</th>
<th>No. of negative samples</th>
<th>Diagnosis method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Direct iodine stain method</td>
<td>27</td>
<td>32.9%</td>
<td>55</td>
</tr>
<tr>
<td>Floatation method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>24</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>2</td>
<td></td>
<td>56</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td></td>
<td>56</td>
</tr>
</tbody>
</table>

The prevalence of Giardiasis depending on gender in this study was distributed to 67% for male and 33.3% for female. However, the prevalence depends on age groups was 23.2% in age group 8-25 years, 7.3% in age group 26-35 years and 2.4% in age group 36-45 years. While the distribution of infection depends on residence was 20.7% for rural and 12.2% for urban. Table (2).

### Table (2): Demographic data of Giardia infected patients

<table>
<thead>
<tr>
<th>Chi-square X2</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.790</td>
<td>0.371</td>
</tr>
<tr>
<td>9.158**</td>
<td>0.01</td>
</tr>
<tr>
<td>10.055**</td>
<td>0.002</td>
</tr>
</tbody>
</table>

The percentage of G. lamblia infection was 31.7% by direct iodine stain method and 29.3% by floatation method. The whole samples which were positive by floatation method were positive by direct iodine stain method and 2 samples which were negative by floatation method were positive by direct iodine stain method. Table (1&3) and fig. (1).

While 32.9% was positive by ELISA assay, 24 samples which were positive by direct iodine stain were positive by ELISA assay and 3 samples which were negative by direct iodine stain were positive by ELISA assay. As well as ELISA test failed to detect 2 samples were positive by direct iodine stain Table (1&4) and fig. (1).

### Table (4): Results of ELISA assay against direct iodine stain method

<table>
<thead>
<tr>
<th>Total</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct iodine stain method</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>ELISA assay</td>
<td>27</td>
<td>55</td>
</tr>
</tbody>
</table>

### Table (5): Prevalence of giardiasis depending on diagnostic method

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floatation</td>
<td>92.3%</td>
<td>100%</td>
<td>100%</td>
<td>96.4%</td>
</tr>
<tr>
<td>ELISA assay</td>
<td>92.3%</td>
<td>94.6%</td>
<td>88.9%</td>
<td>96.4%</td>
</tr>
</tbody>
</table>

IV. Discussion

Routine microscopy of recurrent 3 fecal samples is up to the present time being the commended gold standard assay for Giardia lamblia detection, however the sensitivity of that method is still established to be low (Jahan et al., 2014; Beaver and Jung, 1985). Thus, through this study, we estimated the performance of floatation method and ELISA.
assay as diagnostic methods in comparison to traditional microscopy for G. lamblia diagnosis.

Among 82 feces samples diagnosed for Giardiasis, 67.1% was negative and 32.9% was positive depending on gender, the infection in this study was 67% for male, 33.3% for female. Those results differ from that study established by AL-Bayati (AL-Bayati, 2015) and Salmanet. al. (Salman et al., 2014). High activity and frequent exposure to the external environment make male more affected by parasites than female (Salman et al., 2014).

Giardiasis prevalence depending on age groups was higher in age group 8-25. Our results differ from that conducted by butty in Nineveh (Buty, 2011) and AL-Bayati(AL-Bayati, 2015). Usually, younger persons are more infected by Giardiasis because they have more contact with external contaminated land as well as factors related to the immune system (Alam et al., 2011). While the prevalence of infection depending on residence was 20.7% for rural and 12.2% for urban. Several factors cause the spread of Giardiasis among residents of rural areas including: the reduction of educational level, bad experiment in toilet use, contaminated water with parasites, crowded families and lack of insecticides used for killing mechanical transportation of the infected stages of intestinal parasites (Salman et al., 2014).

This study showed that infection of Giardiasis by direct iodine stain method was higher than by floatation method, the whole samples which were positive by floatation method were positive by direct iodine stain method and 2 samples which were negative by floatation method were positive by direct iodine stain method. Those results almost agree with study conducted by salman et al., in Kirkuk city (Salman et al., 2014) and by Gotfred-Rasmussen et. al. (Gotfred-Rasmussen et al., 2016). It also differs from that study established in Tikrit district (Muhsin and Daoud, 2015).

In this study Giardiasis percentage using direct iodine stain method is higher than the rate of infection using floatation method and this may be due to destruction of parasite’s trophozoites by Centrifugation (Salman et al., 2014). However, direct iodine stain method and floatation technique needs proficient staff and is work intense.

While 32.9% of Giardiasis was positive by ELISA assay, 24 samples which were positive by direct iodine stain were positive by ELISA assay and 3 samples which were negative by direct iodine stain were positive by ELISA assay. As well as ELISA test failed to detect 2 samples were positive by direct iodine stain, those 2 false negative results could be related to low Giardia parasite densities and intermittent Giardia excretion with stool (Ali and Hill, 2003).

In comparison with direct iodine stain method, our study found that the sensitivity and specificity of the floatation methodare similar to those of the ELISA test. Those results almost agree with study conducted by Wilson and Hankenson (Wilson and Hankenson, 2010). In addition, the value of each PPV, NPV and accuracy of floatation method are higher than those of ELISA test, those result roughly agree with study conducted by Uchoa and Almosny (Uchoa and Almosny, 2018). Several studies have shown different results for sensitivity, specificity, PPV, NPV and accuracy for the ELISA test (Mohammad and Moawad, 2016; Al-Saeed and Issa, 2010; Ozekinci, 2005). The difference among the previous studies is due to the difference in the number of samples examined in each study, while the ELISA assay sensitivity has been based to be improved against increasing number of samples (Addiss et al., 1991).

V. Conclusions

1. The prevalence of Giardiasis was 32.9% and 67.1% was negative.
2. The percentage of infection was 31.7% by direct iodine stain method, 29.3% was by floatation method (sensitivity ratio was 92.3%, specificity was 100%), While 32.9% was positive by ELISA assay (sensitivity ratio was 92.3%, specificity was 94.6%).
3. The prevalence of infection depending on gender in this study was 67% for male, 33.3% for female. Also the results of study showed the prevalence of infection was 23.2% in age group 8-25 years, 7.3% in 26-35 years and 2.4% in age group 36-45 years. The distribution of infection depending on residence was 20.7% for rural and 12.2% for urban.

VI. Recommendations

1. To conduct laboratory floatation methods for investigating parasites in addition to direct microscopic diagnosis with more than one slide per a sample.
2. To conduct immunological tests such as ELISA to investigate Giardia in the faeces. Laboratory microscopes should be provided with micrometers to accurately diagnose the parasite.
3. Spreading health awareness among the population, especially the rural population, to avoid infection by adhering to sanitary and hygiene conditions, enjoying eating with street vendors and drinking from non-sterilized water.

VII. Reference


