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A COMPARISON STUDY OF DIFFERENT METHODS USED IN THE DETECTION OF GIARDIA LAMBLIA ON FECAL SPECIMEN OF CHILDREN

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ABSTRACT:

OBJECTIVE: *The purpose of this study was to compare results obtained using a single fecal specimen for O&P examination, direct immunofluorescent assay (DFA), and two conventional staining methods.*
DESIGN: *Hundered and fifty children fecal specimens were collected and examined by each method. The O&P and the DFA were used as the reference method.*
SETTING: *The study was performed at the laboratory in the Basic Medical Science Institute JPMC Karachi.*
PATIENTS OR OTHER PARTICIPANTS: *The fecal specimens were collected from children with a suspected Giardia lamblia infection.*
MAIN OUTCOME MEASURES: T*he amount of agreement and disagreement between methods.*
1. Presence of giardiasis in our population.
2.The sensitivity and specificity of each method.
RESULTS: *There was 45(30%) positive 105(70%) negative on DFA, 41(27.4%) positive 109(72.6%) negative on iodine and 34(22.6%) positive 116(77.4%) on saline method. The sensitivity and specificity of DFA in comparision to iodine were 92.2%, 92.7% respectively. The sensitivity and specificity of DFA in comparisoin to saline method were 91.2%, 87.9% respectively. The sensitivity of iodine method and saline method in compariosn to DFA were 82.2%, 68.8% respectively. There is mark diffrence in sensitivity of DFA to conventional method.*
CONCLUSION: *The study supported findings of other investigators who concluded that DFA method have the greater sensitivity. The immunologic methods were more efficient and quicker than the conventional O&P method.*

KEY WORDS: DFA = direct immunofluorescent assay; O&P = ova and parasite;

INTRODUCTION:

The intestinal protozoon Giardia, the causative agent of giardiasis, was first described over 300 years ago by Leeuwenhoek. Giardia lamblia is a flagellated protozoon that infects the small intestines of humans and is a major cause of intestinal infection throughout the world [1]. Prevalence rates in children in develop countries are quoted as 2-5 % but up to 20-30% in the developing countries [2].It is much more common in children than in adults, especially in children under 10 years of age, prevalence is 2-7 times greater than in adults [3]. The Giardia trophozoite exhibits a characteristic pear, or teardrop, shape with bilateral symmetry when viewed from the top. It is typically 12-15 ¦Ìm long, 5-10 ¦Ìm wide, and 2-4 ¦Ìm thick. Characteristic features of the stained trophozoite include: two nuclei with central karyosomes, fibrils running the length of the parasite, and median bodies. The large karyosome and lack of peripheral chromatin gives the nuclei a halo appearance. The fibrils are called axonemes and are formed from the proximal regions of the flagella within the body of the trophozoite. The median bodies are a pair of curved rod-shaped structures, which lie posterior to the nuclei. At the ultra structural level the median bodies contain an array of microtubules. The functions of the median bodies are not known, but most believe they are somehow involved with the adhesive disk and its formation. An adhesive disk not always visible by light microscopy, occupies the ventral side of the anterior end. The giardia cyst had four nuclei usually located at the anterior end of the cyst. The flagella and adhesive disk are lost as the cyst matures, but the axonemes and median bodies persist. The distinctive fibrils (i.e., axonemes), which extends across the length of the cyst [4].

Formula:

TP
TP+FN
37
7+4 =41

Specificity:
Specificity is the ability of test to detect people who do not have disease

Formula:

TN
TN+FP
101
101+8 =109

Positive predictive value (PPV):
Formula:

TP
TP +FP
37
37+8 =45

Negative predictive value (NPV):
Formula:

TN
TN +FN
101
101+4 =105

TABLE -1 Fig -1
SENSITIVITY AND SPECIFICITY OF DFA IN COMPARISON TO IODINE FOR *GIARDIASIS*

Test	***Giardiasis***	***Giardiasis***	**Total**
DFA	True positive DFA test	False positive DFA test	
Positive	37	8	37+8=45
Negative	FN -ve 04	TN-ve 101	FN+TN 4+101=105
Total	TP+FN 37+4=41	TN+FP 101+8=109	TP+FP+TN+FN 37+8+101+4=150

ABBREVIATIONS: TP= True Positive, TN=Total Negative,FN=False Negative,FP=False Positive

There is no ¡°Gold standard¡± for the detection of Giardia lamblia. Stool examination is the traditional, safest and easiest method [5].The initial method of diagnosis is by demonstration of the trophozoite or cysts of G lamblia in the stool by microscopy or stool antigen detection by ELISA. Other methods of diagnosis include examination of duodenal contents by aspiration or biopsy with endoscopies. A definitive diagnosis may require repeated stool examinations, fecal immunoassays, or even sampling of the upper intestinal contents [6]. Direct investigations involve stool examination by fluorescent (IFA) or ELISA is also an effective means of diagnosis [2]. Serology and stool culture are generally unnecessary. The fluorescence stating techniques proved more sensitive than other tests routinely used for diagnosis[7]. The direct immunofluorescent-monoclonal antibodies method resulted in a significantly increased detection rate of giardia8. Further more the monoclonal antibody reagents offers increased sensitivity and an excellent alternative to conventional staining methods these reagents are helpful when screening large number of patients or those with minimal symptoms. Problem of false-positive and false ¨Cnegative result with routine staining methods for stool parasites can be eliminated with monoclonal antibody reagents9. Giardia direct immunofluorescence test (MERIFLUOR?) showed 100% sensitivity and specificity. Comparison of two different ELISAs showed sensitivities of 92% and 87% and specificities of 87% and 91% [10].
Effective means of protection against giardiasis exist and include good hand hygiene, avoiding ingestion of surface water, and preferably drinking only bottled water, or, when bottled water is not available, disinfecting/filtrating the drinking water. Such advice should focus on persons traveling in areas with less than optimal hygienic conditions. Special attention, before and after travel, should be given to parents of young children[11].Nitroimidazoles:-The nitroimidazoles class of agents used to treat G. lamblia infection includes metronidazole, tinidazole, ornidazole, and secnidazole. Metronidazol (-hydroxyethyl)-2-methyl-5-nitroimidazole)[12]

MATERIALS AND METHODS

Hundered and fifty fecal specimens from children with a suspected Giardia lamblia infection were collected for Giardia testing. Each specimen was coded and processed separately to eliminate the possibility of observer bias. Specimen were collected in a 10% formalin vial was used to perform the direct wet mount. The traditional O&P method was performed with the specimen from the 10% formalin vial and included microscopic examination of saline and iodine direct wet mounts 13.Testing by immunological method included product from manufacturer(waterborne?)22.The ST102R.Girdi-a-Glo assay utilized the principle of directimmunofluorescence(DFA). The 'detection reagene' contained a mixture of labeled monoclonal antibodies directed against cell wall antigens of Giardia cysts. A smear of the fecal specimen was treated with the detection reagent and then counterstained. The slides were rinsed to remove unbound antibodies, a coverslip was mounted, and the slides were examined for fluorescent apple green color and characteristic morphology of Giardia cysts using a fluorescent microscope.

RESULTS

Giardiasis was positive in 45(30%) and negative in105(70%) using DFA, by using iodine 41(27.4%) positive, 109(72.6%) negative and on saline method 34(22.6%) positive, 116(77.4%) were negative. The male found more positive then female by all three methods. The age group 1-5 years having heighest number of positive cases on all three methods. The mean age were 4 to 5 years by all three methods. the sensitivity of DFA method in comparison to iodine for giardiasis.(Table 1 fig: 1) The sensitivity was 90.2% and specificity were 92.7% and positive predictive value 82.2%, negative predictive value 96.2 %. There were 8 (5.3%) false positive and 4 (2.6%) false negative cases on DFA in comparison to iodine.(Table2 Fig: 2)The sensitivity and specificity of DFA in comparisoin to saline method were 91.2%, 87.9% respectively. Positive predictive value

Sensitivity:

Sensitivity of a test is its ability to detect people who do have disease.

Formula 1

TP
TP+FN
31
31+3 =34

Positive predictive value (PPV):
Formula:2

TP
TP +FP
31
31+14 =45

Specificity:

Specificity is the ability of test to detect people who do not have disease.

Formula:**3**

TN
TN+FP
102
102+14=116

Negative predictive value (NPV):
Formula:4

TN
TN +FN
102
102+3 =105

82.2%, negative pridictive value 92.7%. There were 8 (5.3%) false positive and 4(2.6%) fasle negative in comparison to iodine method. The sensitivity of iodine method and saline method in compariosn to DFA were 82.2%, 68.8% respectively.

DISSCUSSION

In this study 27.3% were positive with iodine method, 22.6 % were positive with saline method and 30% were found positive with directimmunoflouresence assay for giardiasis. In a study conducted by Zimmerman and Needham 14 in 1995 who found6.4% positive cases for giradiasis detected MERIFLUOR direct immunofluorescence (DFA) assay. In 1992 study by Garcia et al[9] found a prevalence rate of 27.0 % with direct immunofluorescent assay which is in accordance with this study. In present study it was found 27.3 % positive cases of giardiasis by conventional method. Shakkoury & Wandy (2005)15 found a prevalence of 78 % in children by conventional method. The study conducted by Al-Mekhlafi et al, (2005) [16]also showed the prevalence rate of 24.9 % in children. In epidemiological meaning the prevalence may not be established because of the limitenumber of cases in these studies.

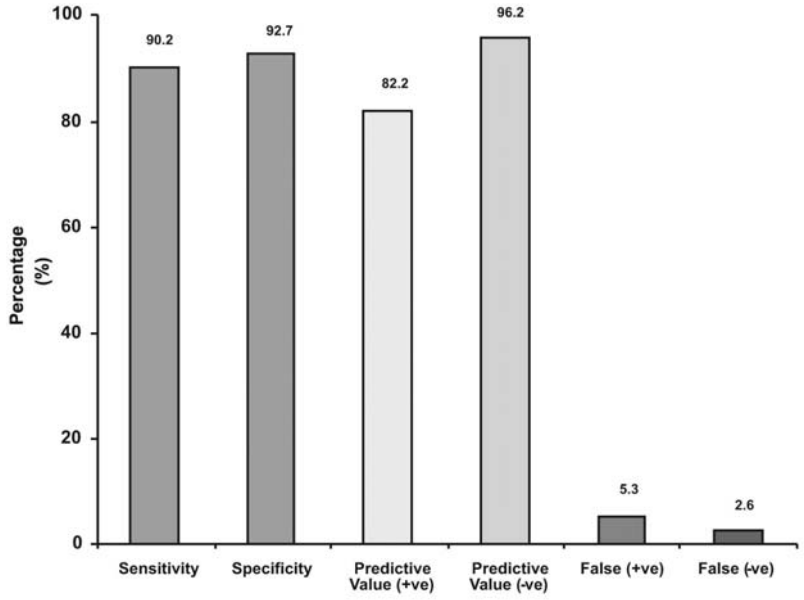
The age prevalence of giradiasis is highest in childhood (Hokelek and Nissen, 2006)[17]. In this study it was found that age group of 1-5 years, having highest number of positive cases on all three methods. This shows that peak age of 4 to 5 years is more vulnerable to giardiasis. Kazi et al (2002)[18] found peak age of four years, which is in agreement with this study. Pennardt (2006)19 reported that giardiasis has a peak rate of 15 to 20 % in children younger than 10 years, which is also in agreement with the results of this study.

In this study it was found that direct immunofluorescence (DFA) microscopy having 90.2% sensitivity and 92.7% specificity in comparison to Iodine method. With saline method sensitivity and specificity were 91.2% and 87.9% respectively. So present study is compareable with Zimmerman and Needham (1995)[15] found the diagnostic sensitivity of MERIFLOUR (DFA) 100% and specificity 99.8% on control cases while in this study only suspected cases of giardiasis were included. In present study if controls would

TABLE -2 Fig -2
SENSITIVITY AND SPECIFICITY OF DFA IN COMPARISON TO SALINE FOR *GIARDIASIS*

Test	***Giardiasis***	***Giardiasis***	**Total**
DFA	True positive DFA test	False positive DFA test	
Positive	31	14	31+14=45
Negative	FN -ve 03	TN-ve 102	FN+TN 3+102=105
Total	TP+FN 31+3=34	TN+FP 102+14=116	TP+FP+TN+FN 31+14+102+3=150

ABBREVIATIONS: TP= True Positive, TN=Total Negative,FN=False Negative,FP=False Positive



have been taken then specificity and sensitivity of DFA could be matched with Zimmmerman and Needham (1995)[14]. Another study conducted by Zell et al, 1990 [20] found 100% sensitivity of DFA on positive donor pool.

Another study conducted by Garcia et al (1992)9 reported that monoclonal antibody reagents offers increased sensitivity and excellent alternative to the conventional staining methods problems of false positive and false negative result with routine staining methods for stool parasites can be eliminated with monoclonal antibody reagents. i-e DFA.

Direct immunofluorescence (DFA) method in addition to having excellent specificity, exhibits vastly improved sensitivity over those of the conventional methods used. These findings supported and supplement the initial experiences of other with this technique. Technologist can become rapidly proficient in the performance of this simple direct immunofluorescence method and allows rapid scanning of stained slides, saving valuable observe time. Additionally the high quality of reagents result in minimal background autofluorescence or non specific staining and enhance the identification of giardia cyst appeared as bright apple green, easy to identify.

False positive and negative cases are reported even by experienced microscopist in conventional method. In this study it was found that 8 false negative and 4 false positive cases resulted by iodine method in comparison to DFA method. There were [14] false negative and 3 false positive cases by saline in comparison to DFA method. This shows that direct immunofluorescnce (DFA) assay is more superior and effective diagnostic tool for identification of giardia.

CONCLUSION

In this study it was found that DFA is superior to conventional methods of detecting giardiasis. Although test is considerably more expensive than conventional staining methods. But the DFA can be replaced over conventional method for diagnosis of giardiasis in routine laboratory to minimize the false positive and false negative cases. The key role of DFA method is its significance in epidemiological and control studies. Because of its high sensitivity and specificity and minimal time taken for scanning the slides.

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