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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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COMPARISON BETWEEN PCR STUDY AND ELISA STUDY AMONG PATIENTS WITH DIARRHEA

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Abstract.

Background: Amoebic dysentery is a common infectious disease that is acquired through contaminated food and water harboring the infective stage of the parasite. *Entamoeba histolytica* is a parasite that is spread globally causing increasing morbidity and mortality in developing countries.

Objectives: To Identify the infection of *Entamoeba histolytica* by PCR and other lab methods among patients attending Kirkuk hospitals. Methods: The current study involved the examination of 220 fecal specimens from children under 15 years during the period of 1st January 2023 until 5th of June 2023. It involved microscopic examination of fecal samples confirmation of diagnosis with two different ELISA tests that capture *E. histolytic*. Also, microscopic positive samples were submitted to nucleic acid detection of *E. histolytic* via Real-Time PCR.

Results: The percentage of positive specimens that were tested with *E. histolytica* / *dispar* ELISA (DRG ELISA), out of 93 stool specimens, 59(63.44%) were positive, while the remaining specimens 34(36.56%) were negative despite being tested positive by microscopy. The DRG stool ELISA revealed sensitivity and specificity (69.28 % and 97.91 %) respectively and a predictive value of (97 %). The sample that was the positive result with DRG ELISA was discriminated via Tech Lab *E. histolytica* ELISA which detects the presence of only *E. histolytica* alone in fecal samples. Out of 93 examined specimens, only 24(25.81%) were positive while the remaining 69(74.19%) were negative. DRG ELISA for *E. histolytica/ dispar* positive results were 63.44%, while TechLab ELISA has produced 25.81% positive *E. histolytica*. Whereases, RT PCR results were only 20.44%. Qi square analysis was applied and yielded a significant difference v=between the method of diagnosis with P=<0.0001.

Conclusion: Microscopy-positive Entamoeba complex is a primitive means of detection of Entamoeba complex and diagnosis should always be confirmed with superior method like ELISA or PCR.

Key words. Amoebic dysentery, *Entamoeba histolytica*, PCR, ELISA.

Introduction.

Amoebiasis is recognized as the second most prevalent cause of mortality among parasitic infections [1-3]. Amoebiasis affects around 500 million individuals annually, with only a minority of approximately 10% manifesting clinical manifestations [4]. The genus Entamoeba encompasses numerous species, including E. histolytica, *E. dispar, E. moshkovskii, E. polecki, E. coli,* and *E. hartmanni*. Among these species, six are found in the human intestinal lumen. It is important to note that *E. histolytica* is the sole species that has been definitively linked to pathological consequences in humans, while the remaining species are generally regarded as nonpathogenic [3,5].

The clinical manifestations caused by *E. histolytica* range from asymptomatic colonization to amoebic dysentery and invasive extra-intestinal amoebiasis, primarily characterized by the occurrence of liver abscesses. An estimated 50 million individuals have invasive illnesses, leading to an annual mortality rate of 100,000 individuals [4,6]. The microscopic identification of the parasite in stool for the diagnosis of amoebiasis is characterized by low sensitivity and an inability to differentiate between the invasive parasite *E. histolytica* and the commensal parasite *E. dispar* [1,6]. Due to the inability of microscopic inspection to differentiate between these two organisms, it is no longer considered a reliable method for diagnosing amoebiasis. Recently, there have been advancements in the development of molecular techniques that are both sensitive and specific in distinguishing between *E. histolytica* and *E. dispar*. These techniques encompass the utilization of an enzyme-linked immunosorbent assay (ELISA) to detect an *E. histolytica* antigen, the application of polymerase chain reaction (PCR) to amplify amoebic DNA, and the cultivation of stool samples followed by isoenzyme analysis [1,7]. Various molecular diagnostic tests have been employed for immunodiagnosis of amoeba, including serological techniques such as indirect haemagglutination (IHA), counter immunoelectrophoresis (CIE), amoebic gel diffusion test, complement fixation (CF), indirect fluorescence assay (IFA), latex agglutination, and enzyme linked-immunosorbent assay (ELISA) [5,8]. In recent times, real-time PCR has emerged as the most sensitive method for detecting *E. histolytica* in stool samples, surpassing the sensitivities of traditional nested PCR and ELISA. These latter techniques have been specifically designed for the detection and distinction of *E. histolytica* and *E. dispar* in clinical samples. The implementation of molecular techniques has prompted a reassessment of the epidemiology of amoeba, specifically in regions characterized by high endemic rates. This revaluation focuses on the prevalence and morbidity associated with amoeba infections [5].

Materials and Methods.

Study Population and Design: In the present investigation, spanning from January 1, 2023, to May 20, 2023, a total of 220 pediatric patients under the age of 15, presenting symptoms of diarrhea and/or abdominal discomfort, were enrolled from three healthcare facilities: Pediatric Hospital, Azadi Teaching Hospital, and Kirkuk Teaching Hospital, all located in Kirkuk City. Fecal samples were obtained from these patients for further analysis. The samples that showed positive results under microscopy were subjected to further analysis using DRG ELISA, a technique based on antigen detection of *E. histolytica*. The specimens that tested positive were subsequently screened

using TechLab *E. histolytica* II monoclonal ELISA, another antigen detection method. The submitted samples were positive for amoebae, and they were subjected to DNA extraction and PCR analysis to detect the presence of the *E. histolytica* gene.

Data Collection: Sterile wide-mouth screw cap containers were utilized for the collection of stool samples. Subsequently, fresh samples were subjected to examination under light microscopy, employing a high-power magnification of 40X. A small quantity (1-3 ml) of stool samples was obtained and stored in sterile screw cap containers at a temperature of -20°C until further analysis using ELISA and DNA extraction methods.

Statistical Analysis: The statistical analysis was conducted using the GraphPad Prism analytical program. Comparisons were done, where necessary, using the Chi-square test and t-test. Data were deemed non-significant when the P value exceeded 0.05, however, a significant difference in the data was shown when the P value was less than 0.05.

Results.

Detection of *E. histolytica* / *E. dispar* in stool specimens by ELISA: Table (1) exhibited the percentage of positive specimens that were tested with *E. histolytica* / *dispar* ELISA, these samples were microscopy positive and further confirmed with DRG ELISA. Out of 93 stool specimens, 59(63.44%) were positive, while the remaining specimens 34(36.56%) were negative despite being tested positive by microscopy. The DRG stool ELISA revealed sensitivity and specificity (69.28 % and 97.91 %) respectively and a predictive value of (97 %).

Table 1. *Detection of E. histolytica / dispar antigen in stool specimens by DRG ELISA.*

DRG ELISA E. histolytica/dispar	**No.**	**%**
Positive	59	63.44
Negative	34	36.56
Total	93	100.00
Sensitivity	73.17%	
Specificity	96.42%	
Predictive value	98.94%	

Detection of *E. histolytica* antigen in fecal specimens: Our data demonstrated that the sample that produce a positive result with DRG *E. histolytica* /*E. dispar* ELISA was discriminated via TechLab *E. histolytica* ELISA that detect the presence of only *E. histolytica* alone in fecal samples. Out of 93 examined specimens, only 24(25.81%) were positive while the remaining 69(74.19%) were negative, as depicted in Table 2.

Table 2. *Detection of E. histolytica antigen in fecal specimens via Tech Lab ELISA.*

Tech Lab ELISA	**No.**	**%**
Positive	24	25.81
Negative	69	74.19
Total	93	100.00
Sensitivity	69.28%	
Specificity	97.91%	
Predictive value	98.97%	

Detection of *E. histolytica* gene: The current result illustrated that the entire specimen produced positive results with microscopy in addition to both DRG and TechLab ELISAs were submitted for genetic extraction of amoebic DNA and further amplification of *E. histolytica* specific gene. Out of 93 microscopy-positive specimens, RT PCR showed that only 19(20.44%) were positive for the *E. histolytica* genome, while the remaining 74(79.56%) have no detectable *E. histolytica* DNA (Table 3). The amplification process was set on the FAM channel of the RT PCR machine to allow the detection of amoebic DNA [9].

Table 3. *Detection of E. histolytica gene in fecal specimens via RT PCR amplification*

RT PCR for E. histolytica	**No.**	**%**
Positive	19	20.44
Negative	74	79.56
Total	24	100.00
Sensitivity	74.22%	
Specificity	96.74%	
Predictive value	96.91%	

Comparison of DRG ELISA stool antigen, TechLab ELISA stool antigen, and RT PCR: Our results revealed that different methods of diagnosis of patients infected with amoebiasis could have variable results, Table (4.13). DRG ELISA for *E. histolytica*/*dispar* positive results were 63.44%, while TechLab ELISA has produced 25.81% positive *E. histolytica*. Whereases, RT PCR results were only 20.44%. Qi square analysis was applied and yielded a significant difference v=between the method of diagnosis with P=<0.0001.

Table 4. *Comparison of different techniques for E. histolytica diagnostic.*

Examination method	**Total No.**	**+ve**	**%**	**−ve**	**%**	**P value**
DRG *E. histolytica / E. dispar* stool antigen	93	59	63.44	34	36.56	<0.0001
Tech Lab *E. histolytica II* stool antigen	93	24	25.81	69	74.19	
RT PCR for *E. histolytica*	93	19	20.44	74	79.56	

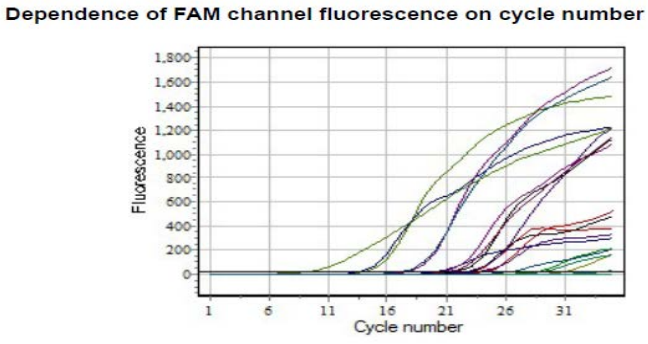

Figure 1. *RT PCR amplification of E. histolytica gene on FAM channel.*

Discussion.

ELISA for *E. histolytica*/*E. dispar*: Our data illustrated that ELISA for *E. histolytica* /*E. dispar* has detected 63.44% positive amoebic form out of 93 specimens. Our results disagree with a Bayoumy et al., study that reported 31.6% of amoebiasis detected via the ELISA method [10]. The result displayed here is differing from another report which indicated that an ELISA-based detection of amoebic form was positive in 31.6% [5]. The variation in the results found in our data and other reports could be because each research has a study population that cover certain criterion as well as the basic tool used in each research for the initial diagnosis of stool amoebiasis which mainly rely on microscopy which greatly influences by the personal skills and experience to detect the parasite stages. In addition to the basic principle of ELISA used in the assessment which could have variable sensitivity and specificity [11]. The basic principle of stool antigen ELISA is proposed to capote either *E. histolytica*/*dispar* or *E. histolytica* alone, in each circumstance, specificity must be high to detect the presence of any amoebic forms [1]. However, compared to microscopy, microscopy is capable of detection of the three common species of Entamoeba, i.e. hsitolytica, and disparandmoshkoviskii [11]. The above-mentioned ELISA are only capable of capturing two of the three species and hence lowering the specificity rate of ELISA dedicated for capturing *E. hsitolytica/ dispar*. Also, it does require the parasite to be at a certain load to allow the kit to detect the presence of amoebic antigen in the tested specimens and in the case of inappropriate or inadequate sample reconstitution false negative results may obtain. Over that, false positive or false negative results could be encountered because the ELISA technique required trained laboratory staff to proceed with ELSA protocol, and inexperienced technicians could significantly increase invalid data generated from ELISA [12,13]. Moreover, each ELISA kit has a specific target to which the antibody coat will react, thus if the amount of antigen in the sample was below a detectable level, then a false negative result will be produced, therefore despite an ELISA test having a credible specificity and reliability, it has some drawbacks for instance, contamination of the wells with samples from other wells due to poor techniques, inaccurate addition of the reagents, sample dilution may increase the possibility of a negative result, inappropriate washing process of the well may result in contamination and false positive data will generated [14].

Our data revealed that the sensitivity of each ELISA applied in the research was 73% and 69% respectively. Our data are in line with Uslu et al. research which conducted a comparison of different methods for the detection of amoebiasis and reported sensitivity for ELISA at 64% [15]. On the contrary, these data conflict with another research that indicated a sensitivity of the ELISA test of 96% compared to microscopy [5]. Hooshyar et al., study reported a sensitivity of 96% for ELISA compared to microscopy [5]. Moreover, Fotedar et al., referred to the ELISA test's sensitivity and specificity at (93% and 75%, respectively) [7]. It has been demonstrated that ELISA is useful for ordinary tasks. According to a study by Solaymani et al., this test has been shown to have good sensitivity and specificity for detecting *E. histolytica* antigen in stool specimens from individuals with amebic colitis and asymptomatic intestinal infection [5].

The variation of the sensitivity in the ELISA dedicated for amoebic detection in each research could be because each ELISA has a sensitivity point at which it can detect the target antigen as well as the parasite load in each sample which can greatly affect the sensitivity of an ELISA test. In addition, another factor that may influence the sensitivity of an ELISA is the inadequate mixing of fecal specimens during sample preparation and processing which can reduce the recovery of the parasite in samples [7].

It has been demonstrated that the sensitivity and robustness of the REAL-TIME assay are directly equivalent, capable of identifying 10 parasite genomes but not one, and unaffected by the presence of DNA from the host or the other test species. According to the RT-PCR results, the *E. histolytica* genome was confirmed in 19(20.44%) patients, these data were close to Dhamiaa Maki Hamza, et.al, demonstrated that from 300 samples that were thought to be infected, 243 (81%) were shown to be positive using RT-PCR analysis, and this method is what determined the distribution of *E. histolytica* infection.

However, our data are not in agreement with Ngui R, et. al study that recorded the prevalence of 75% of *E. histolytica* in faecal samples using RT PCR [16]. In addition, our result is not in line with another research that differentiated the amoebic species and recorded two *E. histolytica* in faecal specimens out of 1383 samples [17]. Added to this, a group of researchers has conducted a screening and differentiation of amoebic form in stool and recorded 0.14% for the *E. histolytica* genome [18]. Moreover, Dhamiaa, et al. study was conducted in Iraq and discovered the *E. histolytica* genome in 81% of fecal specimens obtained from children with diarrhoea [19]. Additionally, El-Sheikh, et al. reported the prevalence of *E. histolytica* at a rate of 2.2% in 576 fecal samples taken from Saudi Arabian children who had acute diarrhoea [20]. add to this, our findings are strongly comparable to the Zeyrek et al., study where 53.18% of patients with gastrointestinal symptoms in a Turkish endemic location were diagnosed with *E. histolytica* [21]. While another research conducted in Erbil city by Salih et al., indicated a PCR-positive amoebic DNA of (2.33%) [22]. On the other hand, when Fecal samples were taken from children who were thought to have *E. histolytica* infection, and after being examined under a microscope, which revealed 194 positive results, they were then sent for PCR testing. According to the results of RT-PCR, the prevalence of *E. histolytica* was determined to be 64.7% (194/300 samples) versus 81% (243/300) based on microscopy.

Several factors may contribute to the high frequency of *E. histolytica*, including poor hygiene, tainted food and water, and direct transmission, Additionally, environmental, economic, and social reasons as well as malnutrition, which dramatically increases children's vulnerability to Entamoeba histolytica, and lack of sanitary restrooms, are contributing to this situation [19]. The results of the current study differ from those of previous studies, which may be attributable to differences in the effectiveness of the sewer system, personal hygiene, population density, geographic location, the number of tested samples, the length of the study, living habits, and ages.

Parasite from liquid stool is more readily extracted in the process of DNA extraction of parasite genome than semisolid stool consequently less parasite will be obtained in the extraction process from those with semisolid ones. Another factor that may affect the PCR result is the contamination of the reagents dedicated for PCR amplification or inadequate experience in the flow of the PCR steps can significantly alter the result interpretation and generate false data [23,24].

The PCR is considered a gold standard confirmatory method, and it ranks superior to other techniques as it has the high specificity and sensitivity and the method is labour intensive with limited time for generating the results with high accuracy and accendibility. Although this method is specific and sensitive still it has some sophisticated requirements like a PCR machine which is fairly expensive, extraction device and kits, specific primers to amplify the target genome, skilled laboratory staff to apply the master mix for the reaction and programming the thermocycler, in addition, data interpretation does require experts personnel to approve the data [23]. These factors are not abundant and not easily accessible so most of the reports are prone to personal judgement to decide whether the specimen is positive for amoebic form or neglected as a negative sample [22].

Regarding PCR sensitivity, our data revealed a sensitivity for PCR at 74%, this result is close to another study that indicated 88% sensitivity for PCR in amplifying the *E. histolytica* genome [16]. Another report found a sensitivity of PCR detection of *E. histolytica* gene in fecal specimens at about 78% [25]. The variation of the sensitivity of PCR in each study may be explained by the fact that PCR is an accurate method for detection, but it has some limitations on top is the expertise required to achieve reliable results as well as the need to include a large amount of stool sample for extraction in order obtained detectable amount of parasite genome which in turns generate positive results. Some other factors that may affect sensitivity include the sample size of each study as well as the efficacy of microscopic diagnosis that will consequently nominate the specimen for PCR amplification [25].

Conclusion.

Antigen-specific ELISA kits are available to aid in the confirmed diagnosis of the disease. PCR technology is widely used in hospitals and could be applied to help genetic differentiation between the types of amoebas as it is a gold standard method and has high specificity and sensitivity.

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