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IMPROVEMENT OF ENZYME IMMUNODETECTION IN THE LABORATORY DIAGNOSIS OF HEPATITIS E VIRUS

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Abstract

Hepatitis E is an RNA virus causing chronic diseases with detrimental effects, such as liver cancer and cirrhosis. Various biochemical tests exist for its diagnosis, but low sensitivity and cross-reactivity led the focus toward improved serological methods: immunoglobulin-based and enzyme immunodetection.

This study aims to determine the effectiveness of improved enzyme immunodetection methods in hepatitis E virus laboratory diagnosis compared with other diagnostic methods.

A systematic literature review was conducted over secondary source databases: Google Scholar, Web of Science, Springer, ScienceDirect, and PubMed. The study followed a Preferred Reporting of Items for Systematic Reviews and Meta-Analysis checklist for conducting the systematic review. Abstracted and full-text peer-reviewed articles were selected, published in English in 2015-2022.

The study employed keywords and set publication dates to search for the most relevant articles. However, these studies were assessed using the risk of bias tools: the Cochrane Risk of Bias for randomized controlled design and the Newcastle-Ottawa Scale for non-randomized controlled design. Critical Appraisal Skills Programme checklist was also used to assess the quality of the studies that cannot be assessed by the Cochrane risk of bias tool and Newcastle-Ottawa Scale. After the selection, the data were synthesized using a qualitative approach to present the results. About 10 articles were identified, including randomized, cohort, qualitative, and diagnostic studies. They found the specificity of an immunoassay to achieve a significantly high specificity (98.3%) and sensitivity (89.5%) for immunoglobulin G-based hepatitis E virus detection. However, they reported that immunoglobulin G and immunoglobulin M detection in the suspected hepatitis E virus patients and exposed groups gave potential results for detecting hepatitis E virus. The method was found as efficient as can be designed as non-invasive and with low risks and challenges.

The results showed that the improved enzyme immune-detective method can assist in providing a reliable, easily accessible, and error-free hepatitis E virus diagnostic method. Thus, future research must focus on exploiting these methods and strategies.

Keywords: *hepatitis E, enzyme immune detection, liver cancer, cirrhosis, biochemical tests, lab diagnosis, IgG, IgM.*

Introduction

Hepatitis E is a single-stranded RNA virus with ~7.2 kb in length has 3 open reading frames and is a non-enveloped virus in the fecal material and bile of the liver, while in the blood, it is covered by a lipid envelope [*Ahmad I et al., 2011*]. This means that the virus has a different structure when it is present in different bodily fluids. The family of Hepeviridae belongs to the genus of Orthohepevirus, which contains four species of Orthohepevirus A and eight genotypes [*LeDesma R et al., 2019*]. Orthohepevirus B is floating in chickens, Orthohepevirus C is found in rats, and Orthohepevirus D is found in bats.

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Orthohepevirus A five members cause infection in human beings [*LeDesma R et al., 2019*]. Genotype 1 of HEV (HEV-1) and Genotype 2 of HEV (HEV-2) are called viruses of humans. They caused adverse infections in people of the Middle East, Mexico, Asia, and Africa and spread through the water droplets' contamination of the feces of humans [*Zuckerman J, 2003*]. Hepatitis E virus-1 and hepatitis virus E-2 cause 3.4 million acute liver infections and 70,000 deaths from acute hepatic failure. In comparison, genotype 3 and genotype 4 infect animals, and pigs, spread infection from pigs to humans, and are categorized as zoonotic viruses [*Wang B, Meng X, 2021*]. Infection can be spread directly by contacting infected animals and/or eating HEV-polluted products or food [*Al-Sadeq D et al., 2018*]. Polluted and contaminated can be used interchangeably in this context to refer to food that is contaminated with HEV, whether through contact with infected animals or other means, including handling by infected food handlers, using contaminated equipment during food preparation, and exposure to contaminated water sources. Hence HEV can cause obligate as well as zoonotic human infections [*Pischke S et al., 2017; Al-Sadeq D et al., 2018*].

Hepatitis E virus is a common worldwide RNA virus that induces acute and chronic hepatic infections, which leads to jaundice, liver cirrhosis, hepatomegaly, and hepatic cancer [*Kartashova E, Sarvilina I, 2018, 2019; Denner J, 2019*]. However, these infections can advance to other body parts like kidneys, brain, heart, pancreas, lymphatic system, and peripheral nervous system and manifest neuropathy, myocarditis, thyroiditis, meningitis, glomerulonephritis, and lymphoma but the pathophysiology of these infections regarding the HEV is still unclear [*Kamar N et al., 2017; Montpellier C et al., 2018*]. The four genotypes of HEV cause acute and chronic infections in humans, including genotypes 1, 2, 3, and 4, i.e., HEV-1, HEV-2, HEV-3, and HEV-4. HEV-1 and HEV-2 have constrained pathogens in humans, while HEV-3 and HEV-4 can spread infection from animals to humans with compromised immune systems due to several medications and disorders [*Sridhar S et al., 2021*]. In many developing countries, HEV can cause epidemics of acute infections of hepatitis due to the contamination of water with human feces. Infections that occur due to zoonotic transmission mode and contaminated blood products can embark to chronic infections of the liver, mostly in those patients whose immune system is already weak due to acquired immune-deficiency syndrome and other diseases [*Zuckerman J, 2003; Turwelis et al., 2022; Zein A et al., 2022*]. Currently, there is no approved medication to treat HEV infections, but various types of research show ribavirin's increased efficacy in HEV-infected patients [*Doting M et al., 2017*]. All these things manifest the complications and the consequences of HEV-caused infections.

Biochemical tests have been used to diagnose the HEV infectious virus, but they do not confirm the presence of HEV in the patient's body. These tests can provide useful information about the presence of the virus, but they are not always conclusive and may need to be combined with other diagnostic tests, such as serological tests or nucleic acid amplification tests, to confirm the presence of HEV infection in a patient's body [*Todt D et al., 2018; Oleinik A et al., 2020 a; b*]. Ongoing research focused on developing improved diagnostic techniques for HEV testing in the future [*Todt D et al., 2018; Aslan A, Balaban H, 2020*]. However, there are also molecular techniques to detect HEV infections, including PCR, amplifying viral DNA/RNA to increase the low amount of viral genome, and detecting the desired sequence of a viral gene involved in causing human infection [*Al-Sadeq D et al., 2018; Khayrutdinov M et al., 2022*]. Apart from conventional PCR methods, new types of PCR techniques like multiplex PCR, real-time PCR, and nested PCR, because conventional PCR takes more time and is less efficient than these types [*Al-Sadeq D et al., 2018; Xiang H et al., 2023*]. Various serological methods have also been used to detect HEV through blood, cell cultures and fecal samples [*Khudyakov Y, Kamili S, 2011; Davletova A et al., 2019*]. One study in literature proposed a method in which samples are divided using iodixanol gradients, performed *in vitro* infectivity biochemical assays using chimeric human liver in mice and analysed specific proteins using biochemical strategies. The particles causing acute and chronic HEV infections were analysed through the electron transmission microscopy technique. Hence, all these methods are the detective processes to identify the presence of HEV in the patient's body.

However, some issues are associated with the molecular and biochemical diagnostic methods of HEV detection, including limited sensitivity and specificity, cross-reactivity with other viruses, and difficulty in detecting certain genotypes of HEV [*Hyams C et al., 2014*]. To overcome these issues, the serological process of immune-detective methods for Hepatitis E diagnosis has been developed that detect the immunoglobulins (antibodies) associated with HEV acute and chronic infections, especially immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies were detected in case of HEV infections. There is an increased prevalence of HEV IgM enzyme immunoassay kits that are commercially available to detect the presence of the virus accurately such as Wantai's ELISA test kit for HEV with 99% specificity which indicates greater sensitivity for viral detection [*Shrestha A et al., 2016*]. One study tested approach of microwell plates coated with HEV recombinant immunoglobulins from the open reading frame-2 structural region of HEV-1 and HEV-2 to capture the IgG and IgM antibodies associated with the HEV acute and chronic infections, present in the blood sera of humans [*Al-Sadeq D et al., 2018*]. This shows effective results, suggesting the enzyme immune-detective approach as a novel and efficient method of HEV detection.

Based on the challenges of low sensitivity, and cross-reactivity, the research is focusing on improved serological method of enzyme immune-detection in the lab diagnosis to detect the HEV-associated IgG and IgM with more specificity and efficiency. It will give more precise results than conventional diagnostic methods of HEV diagnosis. Using the recombinant antibodies from a specific region of the HEV that is associated with causing acute and chronic hepatic infections and detecting IgG and IgM from the blood sera of humans.

The aim of the present research is, therefore, to analyze improved enzyme immune-detective methods for laboratory diagnosis of the HEV: particularly in Indonesia and Russia. Based on this, the following study objectives are outlined:

1. To evaluate the effectiveness of the improved enzyme immune-detective method in the diagnosis of HEV, by examining its performance in comparison to other methods used in the diagnosis of the disease.
2. To investigate the process of creating an advanced enzyme immune-detective diagnostic method for detecting HEV. This involves developing a reliable and efficient method that can accurately detect the virus in patient samples.
3. To determine how the improved enzyme immune-detective method can contribute to the diagnosis of HEV, by examining its role in the overall diagnostic process and identifying its strengths and weaknesses.
4. To identify and quantify the factors that affect the accuracy and reliability of the improved enzyme immune-detective method in diagnosing HEV. This includes analyzing various factors such as sample quality, method specificity, and assay sensitivity to evaluate the overall effectiveness of the method.

An existing study highlighted the significance of advancement and optimization of enzyme immunodetective assay to detect the presence of anti-HEV antibodies in the blood serum [*Sridhar S et al., 2021*].

According to this research objectives, the hypotheses of the contemporary study are as follows:

√ *H1:* Adopting improved enzyme immune detection transforms the lab diagnosis procedures for HEV diagnosis.

√ *H2:* Adopting improved enzyme immune detection facilitates the patients, laboratory diagnostic researchers and technicians in the HEV diagnosis process.

Research significance: The current research is particularly significant in providing an improved laboratory diagnostic method for HEV diagnosis. It provides significant insight to various researchers and laboratory technicians to use improved serological enzyme immuno-detective methods to detect HEV in a patient's body accurately [*Al-Sadeq D et al., 2018; Montpellier C et al., 2018*]. Although the laboratories are currently using many serological methods, this improved enzyme immune-detective method will provide a more accurate, efficient, and less time-consuming process for HEV diagnosis [*Khudyakov Y, Kamili S, 2011*]. Based on the predicted analysis, this method also provides precise quantitative analysis and detection of various immunoglobulins associated with the HEV presence. This method has high specificity, efficiency, and sensitivity and can detect anti-HEV IgG and IgM antibodies accurately and precisely.

Additionally, the improved enzyme immune-detective method can assist in providing a reliable, easily accessible, and error-free HEV diagnostic method which has high diagnostic potential for specifically HEV-associated IgG and IgM antibodies [*Shrestha AC et al., 2016; Nurbekova ZK et al., 2018*]. Because the better immune-detective method for HEV diagnosis provides better immune-reactivity antigen and antibody reactions belonging to different genotypes of HEV, this research paper is an excellent way to assist the diagnostic researchers and lab technicians in developing a better and improved enzyme immune-detective method for HEV diagnosis. The scope of the current study is significant for population, suffering from HEV infections who need effective diagnosis for its confirmed presence. Though many diagnostic interventions are being used for HEV diagnosis, the focus of this research is mainly on improved serological enzyme immune-detective lab diagnosis methods for HEV diagnosis.

Material and methods

Research approach: The present study adopted a qualitative approach to systematically analyse the existing evidence. The study adopted an approach to systematically appraise the knowledge from secondary data. The choice of this approach was based on prepositions that well-constructed knowledge from evidence can contribute to the development of realities that are replicable in progressive knowledge. Most importantly, the choice of this approach allowed for effectively assessing the non-arithmetic information across the area of improved detection methods for HEV. For example, there are many concerns such as analysing the four genotypes HEV-1, HEV-2, HEV-3, and HEV-4 and improvement of enzyme immuno-detection in the laboratory diagnosis of HEV for HEV patients and those that are immunocompromised as well. A step-by-step method guideline has been followed after proposing the research question using a PICO framework [*Tawfik G et al., 2019*].

√ Population – patients suffering from HEV infection.
√ Intervention – improved enzyme-based immune-detection method.
√ Comparison – searching for effectiveness through comparative evaluation.
√ Outcomes – efficient laboratory diagnosis of HEV.

Thus, the question of this research is, how does an improvement in the enzyme immune-detective method transform efficiently the laboratory diagnosis process of the hev?

after developing an effective research question, the research used a preferred reporting of items for systematic reviews and meta-analysis checklist to guide the methodology of systematic review whereby critical steps were involved and these have been discussed sequentially.

Eligibility criteria: The eligibility criteria were based on PICO, such that the study assessed the date, design, and type of included research. The criteria proposed for the current study were divided into two sections: inclusion and exclusion criteria, to retrieve pertinent data related to the research topic [*McCrae N et al., 2015*].

For inclusion, the study chose the journal articles, case studies, full-text scholarly articles from authentic databases, including Google Scholar, Web of Science, PubMed, Science Direct and Springer, whose publication language is English and published within the timeline of 7 years (2015-2022) and whose abstract and other information is related to the aims and objectives of the current study are included to retrieve the most relevant data for the contemporary study. For example, the context relevant to the improved serological method of enzyme immuno-detection for lab diagnosis of HEV.

However, the study excluded all duplicated data, abstract-only articles, and information from grey sources, including blogs, websites, and news press, whose publication language is not English and published before the year 2015 and whose information is not relevant to the research objectives of the proposed study are included in the exclusion criteria to exclude journal articles, case-studies abstracts and full-text of the articles.

Information sources: While researching to retrieve the most effective data, different scholarly research databases as were selected such as Google Scholar, Web of Science, Science Direct, Springer and PubMed [*Tawfik G et al., 2019*]. The articles were searched across all these databases between the dates 01-08-2023 and 30-08-2023. The choice of all these databases for searching was crucial such that these can cover almost all medical and

health articles. Besides, these databases supported the use of Boolean Operators like AND in between the selected keywords for the present study which helped in specification and filtering the results for searching the most relevant articles.

Search strategy: This section further elaborates on how and what were the keywords selected and used in different combinations for searching the articles on databases [*Bramer W et al., 2018*]. The keywords were "enzyme," "immunodetection," "hepatitis E," "laboratory," "diagnosis," "hepatitis E virus," "HEV," "improvement," "immunoassay," "antigen," "IgM," and these keywords were also used as "Improvement of enzyme immunodetection and laboratory diagnosis of hepatitis E virus," "Laboratory diagnosis and HEV", "enzyme immunodetection and hepatitis E", "Hepatitis E virus AND improved laboratory diagnosis." "HEV and IgM detection," and "HEV antigen detection and immunoassays and so on.

Selection process: Before searching the articles and collecting data, the study first decided the selection strategy which is attributed to understanding the criteria for selection at each stage of screening and how many reviewers can review the articles prior to selection. In this regard, the study performed the title and abstract screening during the manual searching. Hereby, two reviewers as per Cochrane guidelines assessed the studies based on their knowledge and understanding. This helped in removing any duplicated results and reading the abstract significantly helped in determining whether the findings are similar to ensure that study achieves the generalizability in terms of reporting the data [*Tawfik G et al., 2019*]. Besides, the reviewers or assessor quickly screened for the full-text accessibility of the articles even though the design and findings are critical but if the study is not accessible in full-text, it will not be suitable to add in the process of selection and therefore, a carefully devised selection process was carried out.

Data collection: In the initial phase of the primary research approach, 150 research articles were collected from scholarly data sources, including Google Scholar, PubMed, Springer, and Science Direct, and grey study sources, including websites, blogs, and newspapers. Later on, primary screening was conducted, and, in that process, copied and irrelevant abstracts, findings, and outcomes were eliminated, and later on, n = 70 research journals were passed down for the secondary screening process. More journal articles were removed based on their titles, abstracts, methodology, conclusion, language, and timeline of publication, resulting in n = 25 journal articles for the final data collection and systemic analysis procedure. In the end, the content analysis was performed systematically to examine its quality, resulting in n = 16 journal articles for literature probing.

Data items: Some of the important items that were sought in the data from each article were listed as follows: Title, Author, Year, Aims, Methodology, Results, Conclusion/Implication

However, it was also ensured that the study included the targeted participants, and the detection methods were relevant to the aim of the present research design which is to assess the effectiveness of improved enzyme-based immune detection of articles.

Risk of bias: This is a very important phase of the systematic review practice following the Preferred Reporting of Items for Systematic Reviews and Meta-Analysis Checklist 2020. Assessing the risk of bias ensured that the choice of the article is within the best possible interest of achieving the objectives at present. For randomized controlled

Table 1

Representing risk of bias assessment using the Newcastle Ottawa Scale in two selected cohort studies

NOS	*Norder H et al., 2015*	*Scotto G et al., 2015*
Representativeness of exposed cohort	0	5
Selection of non-exposed cohort (suspected)	7	5
Ascertainment of exposure	1	0
Demonstration of outcomes of interest at the start of study	1	6
Comparability of cohort based on design and analysis	0	0
Assessment of outcomes	1	6
Long-term follow up	0	0
Adequacy of follow up if any	0	0
Total score	10	22

designs, the study used a Cochrane risk of bias assessment to assess the quality of studies. However, for non-randomized studies, a Newcastle Ottawa Scale was used (Table 1). Both tools have helped in the quality assessment of selected data.

Data analysis: Data analysis may vary depending on the study design such that thematic analysis and critical analysis can be used for qualitative analysis in qualitative research, whereas statistical, regression, and correlational analyses can be used for quantitative research. However, at present, the study aimed to assess the effectiveness of immune detection of HEV, and therefore, a critical analysis of the key findings from the literature was conducted. Hereby, data about statistics of the method's sensitivity and efficiency of the method was interpreted and discussed.

Results and discussion

For the risk of bias assessment of randomized study designs, the Cochrane risk of bias tool for randomized trials was used. However, assessing the nature of the study design, only one randomized trial was included in the present review which was conducted by Pisanic et al. (2017). Thus, the study developed a selective rating scale as per the Cochrane assessment of bias which was attributed to assessing the possibility of any of the five risks in the selected study. These were assessed for five risks such as bias in the randomization process (D1), bias in intervention (D2), biases in outcomes with missing data (D3), bias in measuring outcomes (D4), and bias in selection of results (D5). The rating was developed as high (+), low (-), and some concerns (?). For the randomization process, there were no specific biases identified but the selection of only potential patients may entail some concerns for those patients who might have needed attention. As far as the bias of intervention is concerned, the study chose the appropriate detection method as oral fluid immunoassay for HEV detection, deliberating the low risk of biases. The risk of bias in getting outcomes, measuring, and reporting the outcomes was low as reflected from the comprehension of results. The choice of study was potential in the review.

Besides, other studies used a Newcastle Ottawa Scale for risk of bias assessment as all are non-randomized study designs. The rating is from 0-9 and as follows; 0-2 (poor quality), 3-5 (fair quality), and 6-9 (good quality). The rating was performed for eight elements as stated in table 2. This was performed in two cohort studies (Table 1). For other studies, there suggested that the risks are to be identified using the CASP checklist which basically determines the quality of the study, however, based on the different nature of the study design, this tool was efficient in assessing quality that can interpret results for determining if any risk of biases are present in Critical Appraisal Skills Programme [*CASP, 2023*].

Descriptions and discussion

Pisanic et al. (2017) conducted research using Wantai's serum based HEV ELISA kits for IgG and IgM to determine immunoassay's sensitivity and specificity for HEV IgG for past HEV infections and HEV IgA for current HEV infection, to develop an immunoassay that utilizes oral fluid as a sample rather than blood serum or blood plasma to check the recent and previous HEV infections. It was found that patients with acute viral hepatitis or without signs of the disease underwent the assay's validation using paired oral fluid and serum samples. The oral fluid-based immunoassay's sensitivity and specificity for detecting HEV-IgG were 98.7% and 98.4%, respectively. The oral fluid-based immunoassay's sensitivity and specificity for HEV IgA were 89.5% and 98.3%, respectively. Due to strong concordance between HEV IgG and IgA and available high-performance serum HEV ELISA kits, i.e., IgG and IgM, population-based surveillance of historical and recent HEV infections may be broadened to better understand its ecology. So, in this manner, this method transforms the lab diagnosis procedures for HEV diagnosis, which is one of the hypotheses of the present research. However, the study's limited sample size of verified HEV IgM positive samples, which undoubtedly harmed how well the EHMIL saliva HEV IgA assay performed, is one of its limitations. Moreover, a comparison study was conducted by Wen et al. (2015) in which a comparison was made between recent infection-related markers of HEV, including the HEV antigen, HEV RNA, anti-HEV IgM, and high ALT levels, which may help to clarify the relevance of HEV antigen detection in acute

Table 2

Data findings

Pisanic N et al., 2017
Aim: The study aims to create an immunoassay that utilizes oral fluid rather than serum or plasma to evaluate recent and past HEV infections.
Methodology: Patients with acute viral hepatitis or without signs of the disease underwent the assay's validation using paired oral fluid and serum samples. Calculations were made using Wantai's serum based HEV ELISA kits for IgG and IgM immunoglobulins to determine immunoassay's sensitivity and specificity for HEV IgG for past HEV infections and HEV IgA for current HEV infection.
Results: The oral fluid-based immunoassay's sensitivity and specificity for detecting HEV-IgG antibodies were 98.7% and 98.4%, respectively. The oral fluid-based immunoassay's sensitivity and specificity for HEV IgA were 89.5% and 98.3%, respectively.
Conclusion: The non-invasive oral fluid-based immunoassays, i.e., Due to strong concordance between HEV IgG and IgA and available high-performance serum HEV ELISA kits, i.e., IgG and IgM, population-based surveillance of historical and recent HEV infections may be broadened to better understand its ecology.
Wen G et al., 2015
Aim: A comparison of current HEV infection-related markers, including the HEV antigen, HEV RNA, anti-HEV IgM, and high ALT levels, may help clarify the relevance of HEV antigen detection in acute HEV infection.
Methodology: From a panel of 95 monoclonal antibodies, the high-ability HEV virus-binding Mab 12F12 was chosen as the capture antibody.
Results: Results indicate that this novel antigen detection method agrees with RNA detection and may be a significant diagnostic tool for acute HEV infections.
Conclusion: The new technique developed by the contemporary study for HEV antigen detection will be crucial for diagnosing severe hepatitis E and detecting HEV-positive blood donors, both during the acute phase and after seroconversion to anti-HEV.
Lhomme S et al., 2019
Aim: The research focuses on the most recent features of molecular and cellular tests for HEV diagnosis methods employed for screening.
Methodology: This study uses different lab diagnosis methods for HEV detection, such as IgM assay performance, RT-PCR assays, isothermal amplification assays and genotyping.
Results: Due to the HEV epidemiology finding, blood donors may now be subject to HEV RNA testing to identify transfusion-transmitted diseases. HEV in various industrialized nations has prompted the definition of methods to increase blood safety. However, food exposures may be more dangerous for immunocompromised people than transfusions regarding the risk of HEV infection.
Conclusion: This study concludes that further testing of diagnostic methods will be necessary to determine the best diagnostic techniques for HEV diagnosis.
Kmush B et al., 2015
Aim: The study aims to compare the seroepidemiology by using a novel approach by retesting banked sera from a community serosurvey that was previously assessed by using WRAIR gold standard test.
Methodology: The WRAIR developed an internal enzyme immunoassay to test all individuals for HEV antibodies and overall Ig. By using Wantai anti-HEV IgG ELISA, the study re-evaluated the banked sera of 1,009 subjects in 2014.
Results: In contrast to the Wantai assay, which gave a considerably higher estimated seroprevalence of 46.7% (95% CI: 43.5-49.8) (P 0.001), the WRAIR assay assessed the whole population's seroprevalence to be 26.6% (95% confidence interval [CI]: 24.0, 29.5)
Conclusion: The study concluded that it is mandatory to retest populations with recent immunoassays to develop improved estimates of disease burden at a population level.
Zhao C, Wang Y, 2016
Aim: Based on the substantial advancements in HEV detection, the present study will outline the most prevalent lab methods used to diagnose HEV contamination.
Methodology: The assays used by this study include antigen used in antibody detecting assays, anti-HEV IgG and IgM Assays, total HEV antibody assays, HEV antigen assays, and HEV Nucleic Acid detecting assays.
Results: HEV RNA, antigen, and serum HEV antibody detection are required for the laboratory diagnosis of infection of HEV (IgM and IgG). It was noted that anti-HEV IgG could persist for more than 10 years, indicating remote exposure. Still, anti-HEV IgM can be found during the acute phase of illness and continue for around 4 or 5 months, suggesting a recent infection.
Conclusion: The development has made significant progress, refined various HEV test formats and increased sensitivity and specificity.

Table 2 (continuation)

Talapko J et al., 2021
Aim: The study aimed toward the enhanced certainty of diagnosis of HEV in the target and most vulnerable population, such as pregnant women.
Methodology: Different lab diagnostic tests, such as serological tests, and nucleic acid tests, were used in this study.
Results: IgM anti-HEV antibodies are quickly found using immunochromatographic techniques. In comparison to EIA assay, these tests are cheaper and technically easier. In comparison to ELISA assays, immunochromatographic assays are more sensitive and selective. Due to the imminent requirement for treatment, such quick diagnostics are helpful in pregnant women with suspected HEV infection. Capsid antigen testing is simple and inexpensive compared to RNA detection, making it an effective method for both blood screening and making an early diagnosis of HEV in pregnancy.
Conclusion: The study concluded that preventive measures must be implemented because pregnant women are the most susceptible and at-risk group. The efforts should include testing for HEV RNA, especially because there is currently no effective treatment strategy for pregnant women.
Norder H et al., 2015
Aim: In this study, a comparison has been made between five commercially available assays for detecting anti-HEV IgM and IgG using blood samples and liver samples.
Methodology: Serum samples from 316 patients, 136 of whom had a probable HEV infection, and 500 Swedish blood donors were examined.
Results: In 16% of the blood donor samples and 66% of the patients with probable HEV infection, anti-HEV IgG was found using sensitive tests
Conclusion: The study concluded that there were serum samples from healthy patients with HEV RNA but no serological markers for HEV, despite the excellent sensitivity for anti-HEV detection, so more research is needed to find the implications of this finding.
Abravanel F et al., 2015
Aim: The study's objective is to check the diagnosis worth of a recent HEV IgM rapid test, i.e., immunochromatography assay.
Methodology: Blood samples from 30 acutely affected immunocompetent, and 30 from recently infected immunocompromised patients were taken with HEV RNA in their blood to check the sensitivity and cross-reactivity. The HEV IgM Rapid test and conventional microplate enzyme immunoassay were compared.
Results: Similar to the Wantai microplate assay, the rapid test's sensitivity in immunocompetent patients was 90%. In contrast, in immunocompromised patients, the sensitivity of the rapid test was 73.3%, and that of the microplate assay was 83.3%. The microplate assay method yielded two false positive results of about 3.3%, while the rapid test showed no false positive reactions with samples from HEV RNA-negative patients.
Conclusion: **T**he study concluded that the Wantai rapid test is easy to utilize and is also suitable for rapid testing of acute HEV infection in both immunocompetent and immunocompromised patients.
Wong L et al., 2020
Aim: The aim of the study is to analyze the samples from healthy indigenous populations obtained in 2012 for anti-HEV IgG/IgM.
Methodology: The study performed subjective interviews and observational research to examine the subject's lifestyle choices to get insight into potential causative factors for HEV transmission and disease.
Results: The outcomes showed that six samples of 5.9% from the village of Dusun Kubur contained anti-HEV IgG. Poor dietary habits, inadequate domestic cleanliness, consumption of tainted food and water, direct contact with animal feces, improper residential sewage disposal, and the presence of biodiversity reservoirs may contribute to the spread and acquisition of HEV infection.
Conclusion: The study concluded that although it was higher than the blood donor population in research studies 2 decades ago, the pervasiveness of HEV infection among the limited selection of indigenous populations in this research is lesser than that of the pervasiveness in previous studies.
Scotto G et al., 2015
Aim: This study's objective was to compare the sero-virological pervasiveness of HEV in the general population and HIV patients.
Methodology: A sample of 959 participants, 509 of 53% of whom were HIV-positive cases and 450 from the community, were used to find the sero-pervasiveness of HEV; anti-HEV immunoglobulins were discerned in samples of the blood serum, and western blot testing repeatedly produced positive results. HEV RNA and polymorphisms were also determined in patients who tested positive.
Results: 46 (4.8%) out of 959 analyzed sera samples showed anti-HEV Ig reactivity, verified by western blotting. In patients with HIV infection, the frequency of HEV IgG and IgM was 6.7% compared to 2.7% in the control group. Anti-HEV IgM was detected in 1/12 of the public, 5/34 HIV patients, and 6/46 (13%) serum samples that tested positive for anti-HEV Ig. No patient with HIV had chronic hepatitis from HEV infection.
Conclusion: This study concluded that HEV circulates more frequently in HIV-positive patients while HEV antibodies are less common in the general population. While it was evident in persons with HIV-HEV, co-infection with hepatitis B virus, and hepatitis C virus, chronic hepatitis with HEV alone was missing.

HEV infection. The study chooses a panel of 95 monoclonal antibodies, the high-ability HEV virus-binding Mab 12F12, as the capture antibody. The study results indicate that this novel antigen detection method agrees with RNA detection and may be a significant diagnostic tool for acute infections of HEV. The new technique developed by the contemporary study for HEV antigen detection will be crucial for diagnosing severe hepatitis E and detecting HEV-positive blood donors, both during the acute phase and after seroconversion to anti-HEV. So, these results indicate that hypotheses of the contemporary research i.e., transforms the lab diagnosis procedures for HEV diagnosis and improved enzyme immune detection facilitates the patients have been accomplished.

Furthermore, Lhomme et al. (2019) conducted research focusing on the most recent features of molecular and cellular diagnostic tests for HEV diagnosis methods employed for screening and diagnosing HEV. The study uses different lab diagnosis methods for HEV detection, which include IgM assay performance, RT-PCR assays, isothermal amplification assays and genotyping. Due to the HEV epidemiology finding, blood donors may now be subject to HEV RNA testing to identify transfusion-transmitted disorders. HEV in various industrialized nations has prompted the definition of methods to increase blood safety. However, food exposures may be more dangerous for immunocompromised people than transfusions regarding the risk of HEV infection. The study concludes that further testing of diagnostic methods will be necessary to determine the best diagnostic techniques for HEV diagnosis. Kmush et al. (2015) also conducted a study which aims to compare seroepidemiology by using a novel approach of retesting banked sera from a community serosurvey that was previously assessed by using the Walter Reed Army Institute of Research (WRAIR) gold standard test. By using Wantai anti-HEV IgG ELISA, he re-evaluated the banked sera of 1,009 subjects in 2014. In contrast to the Wantai assay, which gave a considerably higher estimated seroprevalence of 46.7% (95% CI: 43.5-49.8) (p 0.001), the WRAIR assay assessed the whole population's seroprevalence to be 26.6% (95% confidence interval [CI]: 24.0, 29.5). Retesting populations with recent immunoassays are mandatory to develop improved estimates of disease burden at a population level. Kmush (2015) and Lhomme (2019) and their co-authors concluded that further testing of diagnostic methods which facilitate the lab technicians and researchers and retesting of public thereby facilitating the patients are necessary for providing the improved serological method of HEV lab diagnosis.

Zhao C., Wang Y. (2016) conducted research based on the significant advancements in HEV diagnosis; the present study will outline the most prevalent lab methods used to diagnose HEV contamination. The assays used include antigen-in antibody detecting assays, anti-HEV IgG and IgM assays, total HEV antibody assays, HEV antigen assays, and HEV nucleic acid detecting assays. HEV RNA, antigen, and serum HEV antibody detection are also required for the lab detection of infection of HEV (IgM and IgG). It was noted that anti-HEV IgG could persist for more than 10 years, indicating remote exposure. Still, anti-HEV IgM can be found during the acute phase of illness and continues for around 4 or 5 months, suggesting a recent infection. The development has significantly progressed, refined various HEV test formats, and increased sensitivity and specificity. This research provides a refined HEV lab diagnosis method and confirms the hypotheses of the present study i.e., improved immune detection transforms the lab diagnosis procedures for HEV diagnosis and facilitates the patients, laboratory diagnostic researchers, and technicians in the HEV diagnosis process. Research aimed toward the enhanced certainty of diagnosis of HEV in the target and most vulnerable population, such as pregnant women, was conducted by Talapko et al. (2021). Different lab diagnostic tests, such as serological tests, and nucleic acid tests, were used in this research study. IgM anti-HEV antibodies are quickly found using immunochromatographic techniques. In comparison to the EIA assay, these tests are cheaper and technically easier. In comparison to ELISA assays, immunochromatographic assays are more sensitive and selective. Due to the imminent

requirement for treatment, such quick diagnostics are helpful in pregnant women with suspected HEV infection. Capsid antigen testing is simple and inexpensive compared to RNA detection, making it an effective method for both blood screening and making an early diagnosis of HEV in pregnancy. The study concluded that preventive measures must be implemented because pregnant women are the most susceptible and at-risk group. The efforts should include testing for HEV RNA, especially because there is currently no effective treatment strategy for pregnant women. Thus, it was confirmed that adopting improved enzyme immune detection facilitates the patients [*Talapko J et al., 2021*].

Norder et al. (2015) conducted research in which a comparison was made between five commercially available assays for detecting anti-HEV IgM and IgG using liver samples and blood samples. Examination of serum samples from 316 patients, 136 of whom had a probable HEV infection, and 500 Swedish blood donors were conducted. In 16% of the blood donor samples and 66% of the patients with probable HEV infection, anti-HEV IgG was found using sensitive tests. Norder et al. (2015) concluded that there were serum samples from healthy patients with HEV RNA but no serological markers for HEV, despite the excellent sensitivity for anti-HEV detection, so further research is necessary to determine the implications of this finding. Abravanel et al.'s (2015) objective is to check the diagnostic value of a recent HEV IgM rapid test, i.e., immunochromatography assay for which blood samples from 30 acutely affected immunocompetent and 30 from recently infected immunocompromised patients were taken with HEV RNA in their blood to check the sensitivity and cross-reactivity. The HEV IgM rapid test and conventional microplate enzyme immunoassay were compared. Similar to the Wantai microplate assay, the rapid test's sensitivity in immunocompetent patients was 90%. While in immunocompromised patients, the sensitivity of the rapid test was 73.3%, and that of the microplate assay was 83.3%. The microplate assay method yielded two false positive results of about 3.3%, while the rapid test showed no false positive reactions with samples from HEV RNA-negative patients. It was extracted that the Wantai rapid test is easy to utilize and is also suitable for rapid testing of acute HEV infection in both immunocompetent and immunocompromised patients. So, this study confirms both hypotheses i.e., improved immune detection transforms the lab diagnosis procedures for HEV diagnosis and facilitates the patients, laboratory diagnostic researchers, and technicians in the HEV diagnosis process.

Wong et al. (2020) presented a study to analyse the samples from healthy indigenous populations obtained in 2012 for anti-HEV IgG/IgM. The study performed subjective interviews and observational research to examine the subject's lifestyle choices to get insight into potential causative factors for HEV transmission and disease. The results showed that six samples of 5.9% from the village of Dusun Kubur contained anti-HEV IgG. Poor dietary habits, inadequate domestic cleanliness, consumption of tainted food and water, direct contact with animal feces, improper residential sewage disposal, and the presence of biodiversity reservoirs may contribute to the spread and acquisition of HEV infection. The study concluded that although it was higher than the blood donor population in research studies 2 decades ago, the pervasiveness of HEV infection among the limited selection of indigenous populations in this research is lesser than that of the pervasiveness in previous studies. The study cannot draw any clear conclusions about whether there has been a true decline in seroprevalence among the Aboriginal population or whether modernizing lifestyles have contributed to the decline because the time between the previous and recent study is over 20 years. Both studies have the limitation of small sample sizes. Further, Scotto et al. (2015) aimed to compare the sero-virological prevalence of HEV in human immunodeficiency virus (HIV) patients and the general population. For which samples of 959 participants were collected, 509 of 53% of whom were HIV-positive cases, and 450 from the general community, were used to determine the sero-pervasiveness of HEV, anti-HEV antibodies were detected in serum samples, and western blot testing repeatedly produced

positive results. HEV polymorphisms and RNA were also determined in patients who tested positive. 46 (4.8%) out of 959 analysed sera samples showed anti-HEV Ig reactivity, verified by western blotting. In patients with HIV infection, the frequency of HEV antibodies (IgG and IgM) was 6.7% compared to 2.7% in the control group. Anti-HEV IgM was detected in 1/12 of the public, 5/34 HIV patients, and 6/46 (13%) serum samples that tested positive for anti-HEV Ig. No patient with HIV had chronic hepatitis from HEV infection. This study concluded that HEV circulates more frequently in HIV-positive patients while HEV antibodies are less common in the general population. While it was evident in persons with HIV-HEV, co-infection with hepatitis B virus, and hepatitis C virus, chronic hepatitis with HEV alone was missing. This study confirms hypotheses of the contemporary research i.e., adopting improved enzyme immune detection transforms the lab diagnosis procedures for HEV diagnosis and facilitates the patients, laboratory diagnostic researchers and technicians in the HEV diagnosis process.

Conclusion

The contemporary study aimed to analyse improved enzyme immune-detective methods for laboratory diagnosis of the HEV. The findings determined an improved lab diagnostic serological method for HEV detection such as the examples of oral fluid-based immunoassay and Wantai's microplate methods with their high respective sensitivity and specificity for IgM detection were reported. This oral-fluid-based method was significantly tested for its potential to detect hepatitis C in previous studies. Literature also suggested that such methods in the immunoassay field are highly anticipated because of their rapid and non-invasive nature, which eliminates the risks of diagnostic interventions [*Zmuda J et al., 2001; Shivkumar S et al., 2012*]. Besides, the study also found that in comparison to the standard EIA and ELISA methods, these immunoassay IgM detection for HEV were proved as more feasible and cheaper in diagnostic practices. This is of substantial importance since the current literature is focused on measuring the diagnostic performance of this method for HEV detection [*Krumbholz A et al., 2023; Wen G et al., 2023*]. Currently, testing for IgG in response to HEV, researchers found detecting antigen with 100% specificity whereas for IgM, the specificity was achieved as 88.9% [*Krumbholz A et al., 2023*]. In the present study, the specificity for detecting IgG and IgM for antigen detection by oral-fluid immunoassay was found to be around 98%. Thus, the findings were also crucial in relation to the undergoing research in the field.

The strength of this research lies in using recombinant antibodies from a specific region of the HEV that is associated with causing acute and chronic hepatic infections and detecting IgG and IgM from the blood sera of humans to overcome the issues of conventional diagnostic methods of HEV detection. Moreover, the specificity and efficiency of the serological method of immune enzyme detection in the lab diagnosis to detect the HEV-associated IgG and IgM was improved as it will give more precise results than previous diagnostic methods of HEV diagnosis. Additionally, the improved enzyme immune-detective method assists in providing a reliable, easily accessible, and error-free HEV diagnostic method, which has high diagnostic potential for specifically HEV-associated IgG and IgM antibodies. However, there are also some limitations associated with this study; the main limitation of this research is that the scope of the current study is restricted to Indonesia and Russia. On the other hand, the choice of articles in the present research was based on comprehensive searching and screening but there are some inequalities in the types of research design. For instance, only one study was randomized study design and two were cohort studies whereas the rest had different methodological approaches. This entails the limitation such that limited randomized trials may compromise the generalizability of findings and sensitivity and specificity observing from only limited experimental methods may entail the risk of interpretation biases [*Scotto G et al., 2015; Krumbholz A et al., 2023*]. In relation to the study population, there are still no specific guidelines for managing HEV infections during pregnancy, ne-

cessitating the addition of new research projects and consensus-building strategies to fill gaps in the literature.

Based on the identified limitations, this study encourages improving the selection strategy with more comprehensive search practices and seeking the support of expert evidence-based practice sources. The study also suggests that research must be conducted on improving the guidelines and standards when performing the immunoassay for HEV detection, especially for vulnerable groups like pregnant women and mentally ill patients. This is of prime concern since with even high specificity and sensitivity of test method, there are possible challenges and barriers that may restrict access to seeking HEV diagnostic care advantages.

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