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# Molecular and Immunological Study of Hepatitis Viral in Children in Ramadi City

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**ABSTRACT**

**Background:** The worldwide prevalence of Viral Hepatitis has been the cause of severe liver diseases and cancers, affecting more than 300 million people. As for the AL-Anbar region in Iraq, this study sheds light on the prevalence rate among children of hepatitis with its three classifications: A, B, and C.

**Materials and Methods**: A retrospective analysis of 127 children aged 6 months to 15 years was performed. Comprehensive diagnostic techniques were utilized on collected blood samples, including biochemical and rapid tests (IgM and IgG detection), ELISA, and rtPCR.

**Results:** Analysis, carried out using IBM SPSS (Statistical Package for the Social Sciences) software, unveiled that Hepatitis A was the predominant infection (39.4%), succeeded by Hepatitis B (33.1%), and Hepatitis C (27.6%). A notable correlation was observed between ELISA and PCR test outcomes for all three types of Hepatitis, reinforcing the efficacy of these diagnostic methods.

**Conclusion:** The substantial prevalence of hepatitis types A, B, and C in the AL-Anbar children's community mandates prompt public health interventions. Emphasizing the need for effective screening, our study highlights the necessity for implementing preventive strategies to curb the spread of these infections.

**Keywords:** Viral Hepatitis in Pediatric, Pediatric, ELISA and PCR.

## 1. Introduction

Viral hepatitis is one of the most important general health problems spread worldwide, and it is one of the chronic diseases that often affect more than 300 million people from different countries of the world (1). Moreover, it is considered one of the common causes of liver cancer and its various diseases (1). Two types of hepatitis viruses that play an important role in prenatally transmitted infections before birth are B and C. In addition, the majority of infection cases of these two types of hepatitis differ according to geographical regions. The effects seem to associate for the harshness of persistent liver illnesses like hepatocellular carcinoma and chronic disorder of the liver marked by degeneration of cells (2). Hepatitis B contagion is a short Deoxyribonucleic acid virus accompanied by uncommon characteristics identical to any intermediate group of RNA viruses that integrates into the host cell a Deoxyribonucleic acid (DNA) version of their genome. It is an infective agent that is typical of the Hepadnaviridae group (3). HBV is labeled into eight genetic constitutions of an individual organism, (from A - H) depending on the sequence comparison. Per genotype has a distinctive geographic allocation (4). The genetic code of HBV has the form of a circle DNA of nearly 3.2 kilobases (kb) couples and is partially double-stranded. The HBsAg, structurally and functionally, can be split into the S, pre-S1, and pre-S2, areas (1). Regarding children less than 15 years old, the transmission dynamics of Hepatitis B virus (HBV) exhibit distinct patterns that warrant meticulous investigation. According to a comprehensive review conducted by Beasley and Hwang 2017 (5), and corroborated by a meta-analysis from Schweitzer (6), vertical transmission, also known as mother-to-child transmission (MTCT), has been determined to be the most significant route of HBV dissemination in this age group. This transmission occurs either in utero or during the perinatal period, with a higher probability of infection in infants born to mothers with hepatitis B surface antigen (HBsAg)-positive, particularly those with elevated viral loads..

Additionally, horizontal transmission through close household contact with infected individuals or through exposure to contaminated objects has been implicated in the spread of the virus among young children (7). These findings underscore the importance of timely interventions, such as vaccination and prenatal screening, in curbing the transmission of HBV among children under 15 years old. HBV can incorporate inside the host genome, and it replicates via an RNA intermediate. The virus can live and survive in diseased cells, by the special markers for the HBV duplication process vest a distinct its capability for life. Both biological and serological tests were designed to diagnose diverse shapes of HBV-linked illness and the ability to therapy chronic hepatitis B infection (8)., A growing number of cases of hepatitis persist for a long time or are constantly recurring was linked to chronic hepatitis C virus (HCV) infection since the discovery of HCV in 1989. An examination of patients deemed to be infected with non-A or non-B hepatitis will reveal this when patients are evaluated before accurate hepatitis C testing techniques become available (9). Hepatitis C contagion is small particles of genetic material (RNA) belonging to the category of any of a group of RNA viruses, mostly having arthropod vectors that play a substantial role in HDV and veterinary medicine, an RNA virus which is dependent upon a co-existing chronic condition with HBV. The LKM-1 (autoantibodies are antibodies that target a specific molecule in the liver called cytochrome P450) and LKM-3 (autoantibodies, target a different molecule in the liver called formiminotransferase cyclodeaminase) autoantibodies have different molecule markers, respectively. Both (LKM-1) and (LKM-3) are connected with liver infection with (2%) and (10%) respectively. Furthermore, HDV infection is associated with autoantibodies (LKM-3) (10). Around 70 million individuals in the world can become infected with a chronic case of HCV, especially when methods involving horizontal blood

exposure such as drugs administered intravenously, unscreened blood transfusions, needle sticks, and sexual practices with a high level of risk are used to transmit the ailment (11).

A viral infection, such as Hepatitis A (HAV) contagion, illustrates mostly a sickness in kids with generalized manifestation and yellowish color jaundice (12). As a rule, kids take part in the transport of the HAV. Globally, HAV contagion is among the most common and leading causes of severe hepatitis (13). Twenty to twenty-five percent of clinical hepatitis which still poses a serious threat in developing countries is generated by HAV contagion (14). In general, the severity of disease is age-dependent, and the illness is self-limited. HAV infection in kids typically does not exhibit symptoms (15). However, when non-immune patients, both adolescents and old men, are exposed to the virus, they suffer serious clinical conditions (16). The state of being infected with serious HAV is transferred via routes involving the feces and the mouth, either through ingestion of contaminated water and food or through direct contact between family members (17). Hepatitis E virus (HEV) is classified as a single-stranded, non-enveloped, four-genotype, sense-positive RNA virus belonging to the genus *Orthohepevirus* from the family Hepeviridae, responsible for causing endemic forms of self-limiting hepatitis E and acute in humans (18). This virus is primarily transmitted via the fecal-oral route, in addition, contaminated water is one of the most important sources of E infection (19). In the last years, HEV has been identified as an emerging health concern worldwide due to its increasing prevalence and potential to cause large-scale outbreaks (20). Although HEV infection is usually asymptomatic or mild in the most cases, it can lead to many serious health complications the most important of these is fulminant hepatic failure, especially for vulnerable populations such as pregnant women and immunocompromised individuals (21). Currently, no specific antiviral treatment is available for HEV, and prevention relies on good sanitation practices and, where available, vaccination (22).

## 2. Material and Methods

In our study, we conducted a retrospective analysis of 127 children living in the AL-Anbar government to assess the prevalence of hepatitis. The current study included a sample of children whose ages ranged from 6 months to 15 years from Ramadi and other cities. Blood samples from a total of 127 participating children were collected by a gel tube, with 3 ml of blood collected for biochemical and rapid tests (IgM and IgG detection). Moreover, the collected blood sample was subjected to ELISA analysis to produce more accurate results. An additional 2 ml of blood was also obtained in EDTA for molecular analysis through rtPCR. Data analysis was done utilizing the 25th version of the SPSS software. Quantitative and qualitative data were represented by mean, standard deviation, frequencies, and percentages, respectively. To compare paired or unpaired groups, the suitable paired t-test was employed. A P-value below 0.05 was considered to be statistically significant, while ($P = 0.01$) suggested a considerably stronger degree of significance (6). The study was approved by the Ethical approval committee (EAC) in the Ministry of higher education and scientific research - University of Anbar / IRAQ.

**Enzyme-linked Immunosorbent Assay tests:** Different ELISA kit was used for the qualitative determination of Hepatitis virus in the patient's serum.

**a. Estimation of Hepatitis A virus:** Anti-HAV IgM in human serum can be detected qualitatively using the Sunlog Human anti-HAV IgM antibody (anti-HAV IgM) ELISA kit. The microplate that comes with this kit has been pre-coated with a solid-phase antigen that is

particular to anti-HAV IgM. The specific antigen is combined with the samples before being added to the Microplate wells.

**b. Estimation of Hepatitis B Surface Antigen:** An in vitro diagnostic kit called Fortress HBsAg is used to identify human serum or plasma HBsAg (hepatitis B surface antigen). The test makes use of a sandwich-based enzyme-immunoassay. Monoclonal anti-HBs (antibody to HBsAg), a solid-phase antibody, has been coated on microtiter wells.

**c. Estimation of Anti- Hepatitis C Virus:** An enzyme-linked immunosorbent test called the Fortress HCV kit is used to identify antibodies to the HCV in plasma or human serum. This test is designed to examine blood donors and test and diagnose people with HCV infection. This kit uses a two-step incubation approach to detect antibodies to HCV using a solid phase, indirect ELISA method. Recombinant, highly immunoreactive antigens matching the core and non-structural sections of HCV were previously encapsulated by polystyrene microwell strips (Fourth generation HCV ELISA). Antibodies against the HCV antigens pre-coated on the solid phase will bind to them during the first incubation stage.

**d. Estimation of Hepatitis D Virus Antigen (HDV-Ag):** An ELISA kit from Sunlog Biotech is used to measure the levels of HDV-Ag in human serum. Sandwich-ELISA is the technique used in this ELISA kit. This kit's micro enzyme-linked immunosorbent test strip plate already contains an HDV-Ag-specific antibody pre-coated on it.

**e. Estimation of Human Hepatitis E virus antibody IgM:** HEV-IgM in the human serum is qualitatively detected using the Sunlog Biotech Human Hepatitis E Virus Antibody IgM (HEV-IgM) ELISA Kit. ELISA is a technique based on EIA (enzyme immunoassay) for the detection and quantification of enzymes and also it is used for qualitative analysis of these enzymes. This kit's microplate has a pre-coated HEV-IgM-specific antigen, also referred to as a solid-phase antigen.

## 3. Results

The age range of the studied children ranged from one month to 15 years, and the mean for this age group was (6.89 ± 4.10) years. The highest percentage of the participants' children (48 children, 37.8%) were within the age group (5-9) years. Regarding gender, there were 76 males (59.8%) versus 51 females (40.2%) with a ratio of 1.49:1. More than half of study children 69 (54.3%) live in rural areas while the remaining 58 (45.7%) live in urban areas (Table 1). The most common symptoms and signs were abdominal pain in 69 patients (54.3%), jaundice in 65 patients (51.2%), and fever in 60 (47.2%) of patients, while loss of nausea, appetite, fatigue, vomiting , pale or clay-colored stool, and dark urine were reported among 40 (31.5%), 39 (30.7%), 36 (28.3%), 32 (25.2%), and 24 (18.9%) of studied patients, respectively.

### Prevalence of Hepatitis Virus Infection

The HAV was detected in 50 (39.4 percent) of 127 youngsters (HAV). The HBV and HCV were found in 42 (33.1%) and 35 (27.5%) youngsters, respectively. HDV-Ag was present in fifty percent of individuals with HBV. In addition, no HEV was found among the youngsters tested (Figure 1).

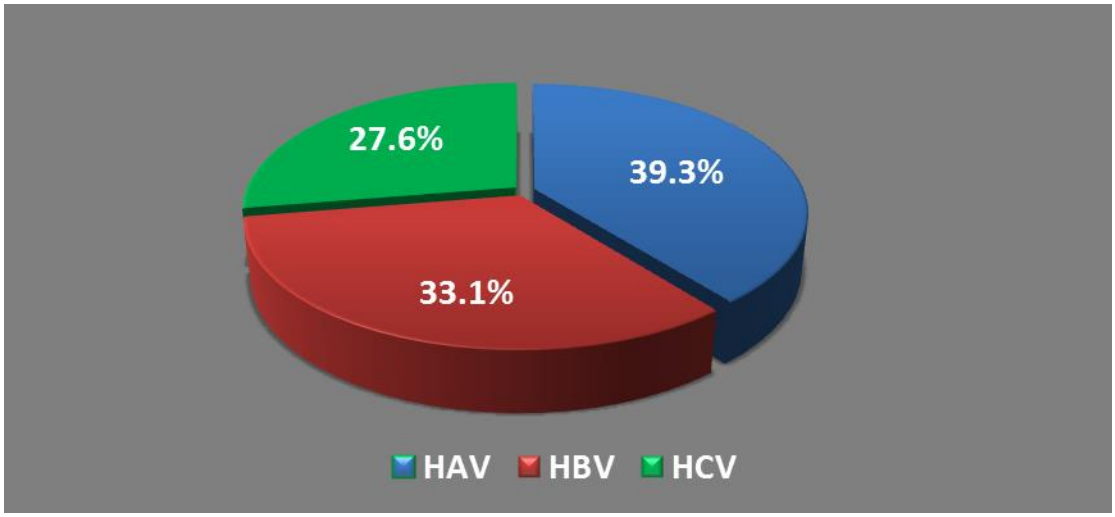


Figure 1: Shows the prevalence of hepatitis (A, B, and C) in the investigated children

**Correlation between ELISA and PCR tests of HBV, HCV, and HDV**

The Pearson correlation analysis revealed a substantial, positive correlation between the ELISA and PCR tests for HBV (r= 0.705, P= 0.001), HCV (r= 0.746, P= 0.001), and HDV (r= 0.479, P= 0.044) (Figures 2 – 4) and HBV, HCV, and HDV (Table 1).

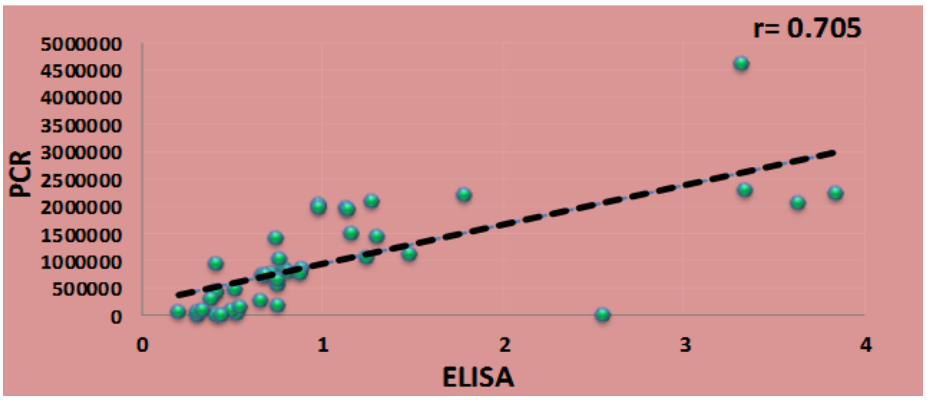


Figure 2: Shows the correlation between the ELISA test and the PCR test of HBV

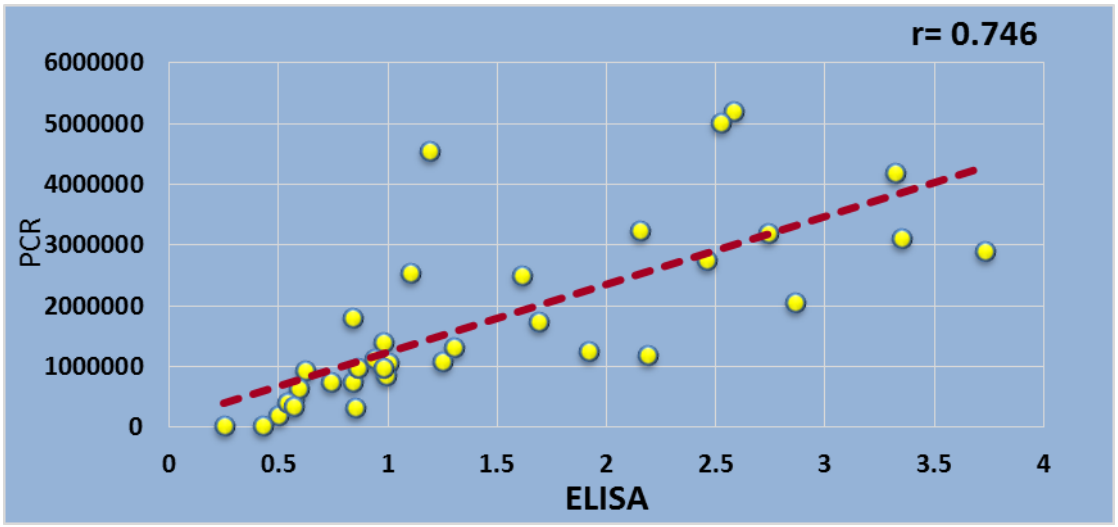


Figure 3: Shows the correlation between the ELISA test and the PCR test of HCV

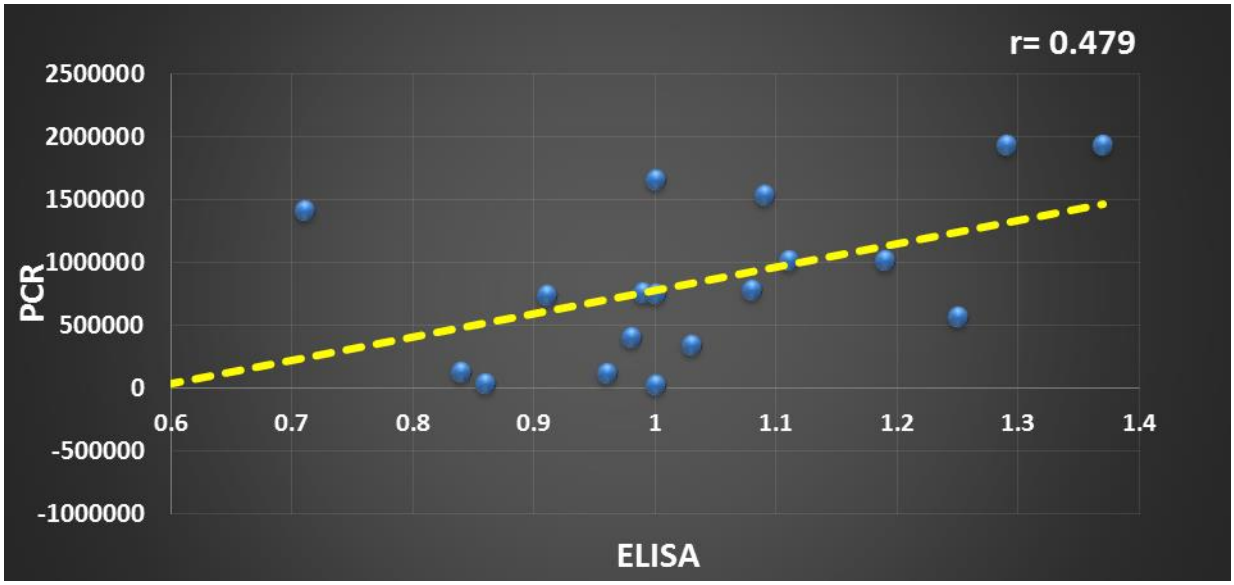


Figure 4: Shows the correlation between the ELISA test and the PCR test of HDV

Table 1: Correlation between ELISA and PCR tests of HBV, HCV, and HDV

| **ELISA Test** | | **PCR- Viral Load** | | |
|---|---|---|---|---|
| | | **HBV** | **HCV** | **HDV** |
| **HBsAg** | **r** | 0.705 | – | – |
| | **P- Value** | **0.001** | – | – |
| **Anti-HCV** | **r** | – | **0.746** | – |
| | **P- Value** | – | **0.001** | – |
| **HDV-Ag** | **r** | – | – | **0.479** |
| | **P- Value** | – | – | **0.044** |

*Correlation is significant at the 0.05 level.

## 4. Discussion

Of 127 children who participated in this study, 39.4% were diagnosed with HAV. The HBV and HCV were detected in 33.1% and 27.6% of the children participating, respectively. Fifty percent of those who had HBV were positive for HDV-Ag. At the same time, no HEV was detected among the children studied. In contrast to other studies, AA Darwish and colleagues enrolled 9352 patients with viral hepatitis and discovered a different result, in that distribution of the type of hepatitis virus reported that HAV infection was the main type of hepatitis virus infection (61.7%) compared to hepatitis B (25.2%), hepatitis C (11.7%), and hepatitis E virus infection (1.2 % ) (23). According to the findings in the study by Zgenç et al., which included 170 children as participants, the total HDV infection was 1.76% percent (3/170); two of the patients had eAg-negative hepatitis B, and one was in the immunoactive

phase (24). Findings from Ezeilo et al. study reported that the rate of HBV infection among the participants was 11.5% (25), which was in accordance with the recent statistical analysis or meta-analysis conducted by Musa and other co-authors, where the results showed the general prevalence of HBV is (11.5%) among children with viral hepatitis (26). By testing 10,044 kids, Solimann et al. estimated the spread of HCV in patients between the ages of 1 and 14. The findings showed that 0.4 percent of people had HCV. HCV antibody seroprevalence was 0.7 percent and viremia was 0.2 percent in children who were male, compared to 0.2 percent and 0.1 percent in those who were female (27). In Das et al study, different results were reported, where HAV was found to be present at a rate of 73.2%, while HEV was present at a rate of 10.7% of cases enrolled (28).

The methodologies used and, in particular, the characteristics of the study population, as well as patient-specific factors like their age at the time of diagnosis—whether mature or premature—the presence or absence of maternal disease transmission, and their vaccination status—are responsible for the observed differences. Receiving blood, hemodialysis, and living with HBV carriers' household contacts are additional factors. Compared to adults, children and teenagers have a lower prevalence and burden of viral hepatitis infection. In small hospital-based cohorts, the prevalence of HCV infection among children and adolescents who had undergone any type of surgery, hemodialysis, or cancer treatment was as high as 20% (29). Compared to adults, testing, treatment, and prevention strategies among children and adolescents have received less attention, in part because until 2017 none of the direct-acting antiviral treatment regimens had been approved for use in those younger than 18 years and there were significant gaps in the evidence to inform pediatric management practices and policies (30). There was a substantial, positive connection between the ELISA and PCR tests for HBV ($r= 0.705$, $P= 0.001$), HCV ($r= 0.746$, $P= 0.001$), and HDV ($r= 0.479$, $P= 0.044$) in this investigation using Pearson correlation analysis.

Despite the availability of numerous markers that can be used to detect HBV, HBsAg remains the primary marker because it appears during the acute phase of infection and disappears during recovery (31). Active chronic or acute Hepatitis B infection is indicated by the presence of HBsAg in serum or plasma. Multiple techniques for detecting HBsAg exist, including immune-chromatography, Enzyme-Linked Immunosorbent Assay (ELISA), and Polymerase Chain Reaction (PCR) (32). Immunochromatography is used to detect HBsAg in developing nations because it is less expensive and does not require specialized laboratory equipment or a trained professional (33). However, numerous studies have demonstrated that immune-chromatography is less sensitive and specific than ELISA (34). Besides, there are also studies reporting that ELISA has lower detection performance than PCR, specifically double step PCR or nested PCR (35). In contrast, researchers reported that the Nested PCR is susceptible to contamination and false positives, particularly during the initial amplification (36). The sensitivity (the method's capacity to report a positive result for someone who is actually infected) and specificity (the method's capacity to report a negative result for someone who is actually not infected) values have an impact on the accuracy of a method used to detect infection. As a result, to reduce false-positive and false-negative results, the method for detecting the infection must have high levels of sensitivity and specificity (37). The positive predictive value (PPV) and negative predictive value (NPV) are additional factors that influence the selection of a detection method in addition to sensitivity and specificity values (NPV). The PPV is the likelihood that someone will be infected when they receive a positive test result, whereas the NPV is the likelihood that they will not be infected when they receive a negative test result. When choosing detection methods, it has been found that high sensitivity and negative predictive values are more crucial factors than specificity

and high positive predictive values (32). Both methods can detect HBV in the specimen, but a study by Naully et al. showed that ELISA had a lower sensitivity value for detecting viral hepatitis (83.33 percent) than PCR did (100 percent) (38). Previous research has shown that ELISA's sensitivity was inferior to that of molecular techniques like traditional PCR. According to a study by Bolad et al., ELISA's sensitivity value for detecting Hepatitis B infection in the blood of healthy donors was much lower than that of conventional PCR (39). Kurdi et al. successfully reported that numerous false-negative ELISA results were discovered in other studies, indicating that the method was less sensitive than the PCR (40). Based on the findings of several research, it can be said that the PCR is a more reliable method for identifying hepatitis B than the ELISA. The method's relationship, value for sensitivity, specificity, and accuracy were all 100%.

## 5. Conclusion

In conclusion, the substantial prevalence of Hepatitis A, B, and C in the pediatric population of the AL-Anbar region necessitates urgent public health interventions. The significant correlation between ELISA and PCR tests validates the use of these methods in diagnosis. These findings underscore the imperative for robust screening, preventive measures, and therapeutic strategies to mitigate the burden of viral Hepatitis in this demographic.

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